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PREVENTION ET TRAITEMENT DE LA THROMBOSE EN CARDIOLOGIE INTERVENTIONNELLE ET EN PATHOLOGIE THROMBO-EMBOLIQUE VEINEUSE

THÈSE

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ABBREVIATIONS / ABRÉVIATIONS

ACS: Acute coronary syndromes

AST: Acute stent thrombosis

AMI: Acute myocardial infarction

ACC: American College of Cardiology

AHA: American Heart Association

ADP: Adenosine diphosphate

APA: Anti-platelet aggregation

APE: Acute pulmonary embolism

aPTT: activated partial thromboplastin time

ATD: Arterial thrombotic disease

ESC: European Society of Cardiology

BMS: Bare metal stent

CAD: Coronary artery disease

CHD: Coronary heart disease

CD62P: P-selectin

DES: Drug-eluting stent

DD: D-dimers

DIC: Disseminated intravascular coagulation

DVT: Deep vein thrombosis

FVIIa: Activated factor VII

FIB: Fibrinogen

F1+2: Prothrombin fragments 1+2

FDP: Fibrinogen degradation products

ICM: Ischemic cardiomyopathy

LST: Late stent thrombosis

LMWH: Low-molecular-weight heparins

MI: Myocardial infarction

mTOR: Mammalian target of rapamycin

PCI: Percutaneous coronary intervention

PTCA: Percutaneous transluminal coronary angioplasty

PAI-1: Plasminogen activator inhibitor type-1 complexes

PE: Pulmonary embolism

SA : Stable angina

sGPV: Soluble glycoprotein V

SST: Subacute stent thrombosis

ST: Stent thrombosis

STEMI: ST elevation myocardial infarction

TF: Tissue factor

t-PA: Tissue plasminogen activator

UA: Unstable angina

U-PA: Urinary-type plasminogen activator

UFH: Unfractionated heparin

VLST: Very late stent thrombosis

VTE: Venous thrombo-embolism

VTD: Venous thromboembolic disease

A. Prevention and Treatment of Thrombosis in Cardiology Intervention Procedures

I. Introduction

At present, two types of stent have been licensed for clinical practice, namely Bara metal stent (BMS) and Drug-eluting stent (DES). DES, most commonly sirolimus-eluting stents and polymer-based paclitaxel-eluting stents, are now widely used during Percutaneous coronary intervention (PCI), and have largely replaced BMS to treat a variety of native coronary artery and saphenous vein graft lesions.

DES represented a novel approach in stent technology and design. Local drug delivery through the stent is used to inhibit intimal thickening by interfering with different pathways involved in the development of inflammation, cell migration and proliferation and/or secretion of the extracellular matrix. Both the drug and the delivery vehicle must fulfill pharmacological, pharmacokinetic and mechanical requirements. Current successful DES require a polymer coating for drug delivery. Clinical trials examining several pharmaceutical agents, particularly sirolimus and paclitaxel, have demonstrated marked reduction in restenosis following stenting. Sirolimus (rapamycin, Rapamune®), is a natural macrocyclic lactone which acts as an immunosuppressant by blocking calcium-dependent proliferation. Paclitaxel is a taxane produced by *Taxus brevifolia* (Taxol®) which acts as a cytotoxic agent and is used against many tumors. Both compounds block cell cycle progression and thus inhibit smooth muscle cell proliferation.

The development of DES was one of the major revolutions in the field of interventional cardiology. DES dramatically decrease coronary restenosis and the subsequent need for repeat revascularization compared with BMS. One potential mechanism for the reduction of restenosis incidence may be an attenuation of both the local and systemic inflammatory response to PCI. DES effectively inhibit the anti-inflammatory response after balloon induced arterial injury. Although, DES have significantly reduced angiographic restenosis rate and have improved the clinical outcome (Babapulle MN et al., 2004 and Hill RA et al., 2004). However, they have also not been shown to reduce outcomes of death or myocardial infarction (MI) (Versaci F et al., 2002). Late thrombosis and restenosis remains an important subject

of ongoing research (Figure 1).

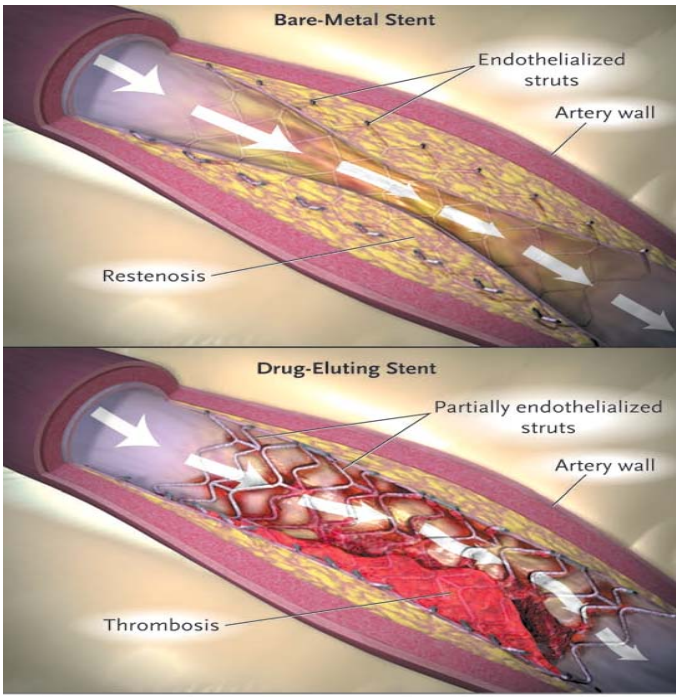

Figure 1. Potential complications of coronary Stenting: Restenosis in a traditional bare-metal stent and late thrombosis in a drug-eluting stent.

I.1 Complication of PCI

The most serious complication of PCI results when there is an abrupt closure of the dilated coronary artery within the first few hours after the procedure. Abrupt coronary artery closure occurs in 5% of patients after simple balloon angioplasty, and is responsible for most of the serious complications related to PCI. Abrupt closure is due to a combination of tearing (dissection) of the inner lining of the artery, blood clotting (thrombosis) at the balloon or stent site, and constriction (spasm) or elastic recoil of the artery at the balloon or stent site.

I.1.1 Stent thrombosis (ST)

Stent thrombosis (ST) is an uncommon but serious complication of coronary artery stents that often presents as death or MI (Cutlip DE et al., 2001 and Orford JL et al., 2002), usually with ST elevation. Coronary ST has remained a serious complication of PCI (Urban P et al., 1998). Although early aggressive anticoagulation schemes were associated with both unacceptably high rates of ST and bleeding complications

(Serruys PW et al., 1994, Fischman DL et al., 1994 and Sigwart U et al., 1987), the advent of dual anti-platelet aggregation (APA) therapy, which associates acetylsalicylic acid (aspirin) with thienopyridines, especially clopidogrel (Plavix®) had salutary effects on both adverse events (Urban P et al., 1998, Schomig A, et al., 1996, Bertrand ME et al., 1998, Leon MB et al., 1998 and Bertrand ME et al., 2000). Despite dual APA therapy, however, ST persists at a rate of 0.5–2% (Cutlip DE et al., 2001, Orford JL et al., 2002, Taniuchi M et al., 2001, Mueller C et al., 2003 and Karrillon GJ et al., 1996) in elective cases, and up to 6% in patients with ACS (Orford JL et al., 2002 and Karrillon GJ et al., 1996). Longer stent length, number of implanted stents (Cutlip DE et al., 2001 and Orford JL et al., 2002), stent malapposition (Uren NG et al., 2002), and residual dissections (Cutlip DE et al., 2001 and Moussa I et al., 1997) have been reported to increase the risk for ST. Reduced (TIMI) flow (Moussa I et al., 1997), individual gene polymorphisms (Kastrati A et al., 2000), and resistance to the APA effects of aspirin (Gum PA et al., 2003) and/or thienopyridines (Barragan P et al., 2003 and Gurbel PA et al., 2003) are additional risk factors.

I.1.1.1 Risk factors for ST at different time points

The classification of ST distinguishes between:

* Acute stent thrombosis (AST) < 24 hours.
* Subacute stent thrombosis (SST) 24 hours to 30 days.
* Late stent thrombosis (LST) >30 days.
* Very late stent thrombosis (VLST) >12 months

AST: AST that usually occurs within minutes to hours after stent implantation, is a rare but severe complication of PCI. Important risk factors include - technical and procedural factors, whether aspirin and clopidogrel (Plavix®) were given early enough, and co-morbidity like diabetes and renal failure. If AST occurs after stent implantation despite a loading dose of clopidogrel, a clinician should consider whether the patient has failed to correctly adhere to the treatment regimen, whether the patient is in a hypercoagulable state, or whether drug resistance is present.

SST: invariably due to anti-platelet regiments, aspirin or clopidogrel resistance. Technical factors play fewer roles than in AST, although stent malposition is a factor. Co-morbidities like previous brachytherapy are important. Accidental withdrawal of clopidogrel is also a risk factor.

LST: usually due to APA regimen mismanagement, and co-morbidities, it is almost unheard of with BMS. Several mechanisms for the LST observed after DES stenting have been postulated: a local drug effect which delays endothelialization or results in the formation of a dysfunctional endothelium, a hypersentivity or inflammatory reaction to the polymer (Virmani R et al., 2004). Other predictors are stent underexpansion and residual reference segment stenosis (Fujii K et al., 2005).

VLST： VLST is more frequent with DES than with BMS. Most VLST are associated with discontinuation of anti-platelet agent because of dental or other non-cardiac surgery.

The reasons for the frequency of LST or VLST in the population treated with DES remain incompletely understood. The presence of endothelial dysfunction and delayed healing were often describedin cases of LST or VLST in DES-treated lesions (Virmani R et al., 2004). The mechanism seems to be related to the delay in the healing process. It may also be related to late hypersensitivity reaction and consequent inflammatory changes predisposing to stent thrombosis even years after initial deployment. These hypotheses are based upon histological characterization of tissue responses in animal studies revealing arrest of the healing process and presence of inflammatory cells as a part of this delayed healing. In addition to this phenomenon, delayed-type hypersensitivity reaction to the polymer and localized hypersensitivity vasculitis within the stented segment could have contributed to the adverse long-term clinical outcome. Some clinical features such as premature discontinuation of anti-platelet therapy, diabetes, lower ejection fraction, bifurcation lesions, and stent under-expansion have been identifiedas independent predictors of VLST (Karvouni E et al., 2005, McFadden EP et al., 2004 and Iakovou I et al., 2005). LST or VLST usually occurs within days to months after aspirin and clopidogrel discontinuation,

and appears more closely related to discontinuation of aspirin (Karvouni E et al., 2005, and Iakovou I et al., 2005).

I.1.1.2 Clinical evidence of ST

Numerous reports have described the occurrence of ST after DES implantation (Pfisterer ME et al., 2006 and Ong AT et al., 2005). However, the true incidence of ST may be underestimated in clinical trials and might occur at substantially higher rates in the "real-world" setting, where more complex lesions are treated. Using DES, the rate of AST and SST seems to be similar to that observed after using BMS. It remains below 1% for both BMS and DES, and the mechanisms underlying the AST and SST after BMS and DES are very similar (Moreno R et al., 2005 and Bavry AA et al., 2005). However, after DES implantation, recent studies have highlighted the increased incidence and potential adverse clinical significance of LST and VLST, compared with those that occur after BMS. In the BASKET-LATE study, the rates of LST were higher in the DES group (2.6% in DES group vs. 1.3% in BMS group) and major adverse cardiac events due to LST were also higher in the DES group (4.9% in DES group vs. 1.3% in BMS group, respectively) (Pfisterer M et al., 2006). In a meta-analysis of 9 trials involving 5,261 pateints, increased rates of VLST were found both for SES (0.6%vs. 0%, p = 0.025) and PES (0.7% vs. 0.2%, p = 0.028), compared with BMS over 4 yr of follow-ups (Stone GW et al., 2007). Similarly, a metaanalysis of 14 trials on 4,958 patients, with follow-ups ranging up to 59 months, showed an increased rate of VLST with SES (0.6% vs. 0.05%, p = 0.02) (Kastrati A et al., 2007).

I.1.2 Pathophysiology of ST

I.1.2.1 Procedure-related factors

Among the procedure-related factors, smaller final lumen dimensions (stent malapposition and/or underexpansion), stent length, persistent slow coronary blood flow, placement of multiple stents, positive remodeling, dissections, geographic miss, and late stent malapposition due to thrombus resolution appear to be most important

for the development of in-stent thrombosis (Cutlip DE et al., 2001, Orford JL et al., 2002, Moussa I et al., 1997, Kereiakes DJ et al., 2004, Schuhlen H et al., 1998, Cheneau E et al., 2003, Chieffo A et al., 2004 and Park DW, et al., 2006). Also, in DES, stent length, stent underexpansion, and residual stenosis have been observed to correlate with an increased risk for ST (Fujii K, et al., 2005 and Park DW, et al., 2006). These factors are of great interest because they can be avoided during the intervention, but they are unlikely to differ between BMS and DES.

I.1.2.2 Patient- and lesion-related factors

Several patient-related factors have been associated with the development of in-stent thrombosis, whatever the type of stent, including low ejection fraction (Moussa I et al., 1997), diabetes mellitus (Silva JA et al., 1999), advanced age (Schuhlen H et al., 1998), and stenting in the setting of an Acute coronary syndromes (ACS) (Park DW et al., 2006). Similarly, in DES, primary stenting in Acute myocardial infarction (AMI), diabetes mellitus, renal failure, and low ejection fraction appear to be associated with an increased risk for ST (Karvouni E et al., 2005, Ong AT et al., 2005, Park DW et al., 2006 and Kuchulakanti PK et al., 2006). In particular, the increased risk in patients with ACS seems to be associated with delayed healing, lack of endothelialization, and presence of a pronounced inflammatory and thrombogenic environment of the exposed necrotic core to flowing blood, accompanied by enhanced platelet reactivity; furthermore, rapamycin and paclitaxel potentiate thrombin-induced expression of tissue factor (TF).

Certain lesion characteristics have been reported to be associated with an increased risk of ST. In DES, this pertains in particular to stenting of bifurcation lesions or in-stent restenosis lesions (Karvouni E et al., 2005, Ong AT et al., 2005 and Kuchulakanti PK et al., 2006). In addition, interventional practice has changed. It now advocates "normal to normal" coronary artery stenting and revascularization of more complex lesions that thus carry a higher risk of ST.

I.1.2.3 APA therapy

Stents are foreign bodies in the vessel wall and thus induce platelet adhesion and activation of the coagulation cascade. Furthermore, high-pressure implantation with noncompliant balloons induces significant vascular injury, with exposure of thrombogenic molecules of the subintima and media (including plaque material) to the blood stream. As a consequence, only potent platelet aggregation inhibition has made the procedure feasible. Hyporesponsiveness to APA drugs has been associated with an increased risk for ST (Wenaweser P et al., 2005). In line with this observation, discontinuation of APA therapy has been observed to be particularly associated with DES thrombosis (McFadden EP et al., 2004, Pfisterer ME et al., 2006 and Eisenstein EL et al., 2006). The appropriate duration of the long-term APA regimen for the prevention of DES thrombosis remains to be assessed in randomized prospective trials; at present, a course of 12 months of dual APA therapy may be considered, especially in high-risk patients treated in the Intensive Care Units of the Departments of Cardiology.

I.1.2.4 Thrombogenicity of the Stent

A predisposition for the development of ST has been observed with certain stent materials; for example, platelet activation was greater during the 30 days after implantation of an open-cell versus a closed-cell stent (Gurbel PA et al., 2002). Stent strut thickness and polymer type and thickness also play an important role. It was shown that the non-erodable polymers of the Cypher and Taxus DES provoked chronic infiltration of the arterial wall by eosinophils, suggestive of hypersensitivity reactions in a small number of cases (Joner M et al., 2006 and Nebeker JR et al., 2006). However, the causal relationship between polymer-induced inflammation and the incidence of LST has only been proven in a minority of patients who had a proinflammatory phenotype. Detailed analysis of the morphological changes in these patients showed a localized immune response, with predominance of CD45-positive lymphocytes and of eosinophils. In fact, Joner et al ((Joner M et al., 2006) reported that all cases of hypersensitivity occur more than 4 months after DES implantation. The preclinical experience in a pig model also showed a progressive development of

granulomatous reactions, including eosinophilic infiltrate, starting at 28 days after Cypher stent implantation: at 1 month, 14%; at 3 months, 43%; at 6 months, 60% (Lüscher TF et al., 2007). One possible explanation of these findings is that the hypersensitivity reaction peaks after the complete release of the drug and is likely related to the polymer. In addition, positive remodeling has been observed in vessels, which evokes a hypersensitivity reaction.

Furthermore, drugs loaded on DES may exert a prothrombogenic effect. Rapamycin (sirolimus) is used on DES, because it is known to inhibit proliferation and migration of vascular smooth muscle cells, important factors in the development of neointima formation and restenosis, through interference with cell cycle regulators (Marx SO et al., 1995 and Poon M et al., 1996). At a subcellular level, rapamycin binds to the FK-506 binding protein 12 and subsequently inhibits the_"mammalian target of rapamycin"(mTOR). mTOR is a downstream component of the phosphatidylinositol-3 kinase pathway, and its blockade influences the regulation of the TF of the coagulation cascade (also called thromboplastin, factor III or CD142) in endothelial cells and monocytes (Steffel J et al., 2006, Steffel J et al., 2005 and Guha M et al., 2002). As a result, rapamycin inhibition of the mTOR increases both thrombin- and tumor necrosis factor (TNF-)-alpha–induced endothelial TF expression and activity at concentrations of rapamycin that are encountered in vivo (**Figure 2**, Steffel J et al., 2005).

Paclitaxel is a lipophilic diterpenoid that binds to the ß-subunit of the tubulin heterodimer, promoting tubulin polymerization, cell cycle arrest, and, eventually, inhibition of vascular smooth muscle cell migration and proliferation (Crown J et al., 2000 and Sollott SJ et al., 1995). In addition, paclitaxel is known to activate c-Jun NH_2-terminal kinase (Wang TH et al., 1998 and Stahli BE et al., 2006), an important mediator of endothelial and monocytic TF induction (Steffel J et al., 2006, Guha M et al., 2002 and Steffel J et al., 2005). Consequently, paclitaxel was also shown to enhance TF expression and activity in endothelial cells (Stahli BE et al., 2006). Again, concentrations used in this in vitro study are comparable with local tissue concentrations of paclitaxel after stent deployment (Finn AV et al., 2005).

In sirolimus-eluting stents, approximately, 80% of the rapamycin has eluted by 30

days, whereas paclitaxel-eluting stents have a biphasic drug release profile in vitro with an initial burst during the first 48 hours after implantation followed by a sustained low-level release for at least 2 weeks (Halkin A et al., 2004). However, both rapamycin and paclitaxel easily penetrate into cells of the vessel wall owing to their lipophilic properties, which leads to chronic retention of the drug in the arterial tissue (Kereiakes DJ et al., 2004, Finn AV et al., 2005 and Suzuki T et al., 2001).

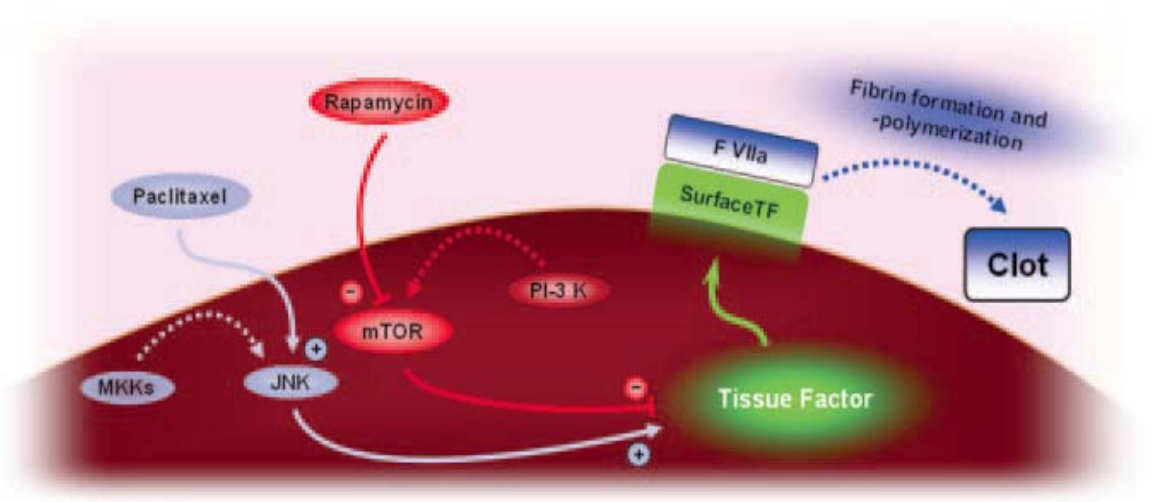

Figure 2. Rapamycin and paclitaxel increase tissue factor (TF) expression. Paclitaxel enhances c-Jun NH2-terminal kinase (JNK) phosphorylation, which in turn leads to an increase in TF protein expression and TF surface activity. The PI3-kinase and its downstream target, the mammalian target of rapamycin (mTOR), inhibit endothelial TF expression; rapamycin inhibits the mammalian target of rapamycin, which leads to a disinhibition of (and thus an increase in) TF expression and surface activity. MKKs indicates map kinase kinases (upstream regulators of JNK); PI-3K, phosphatidylinositol-3 kinase.

Thus, both rapamycin- and paclitaxel-induced TF expression may contribute to a prothrombotic environment after deployment of DES, particularly in the acute and subacute setting and possibly in LST (**Figure 2**). In vitro, rapamycin and paclitaxel not only inhibit proliferation and migration of vascular smooth muscle cells but equally suppress endothelial cells proliferation and migration (Joner M et al., 2006, Marx SO et al., 1995, Poon M et al., 1996, Guba M et al., 2002, Matter CM et al., 2006 and Parry TJ et al., 2005), thereby potentially impeding reendothelialization (**Figure 3**, Steffel J et al., 2005).

Most of the studies regarding the potential pathophysiological events that lead to increased ST after DES stenting have been performed in vitro and/or in animal experiments. Little is known about the relevance of the disclosed mechanisms to the

real situation of stented patients. The aim of our study was thus to examine the level of TF expression in the arterial wall after deployment of DES and its spatiotemporal pattern. The relevance of various hemostasis markers to our purpose will be first discussed as well as the reason why we selected a panel of markers which should allow us to analyse the mechanisms of thrombosis occurring after coronary stenting.

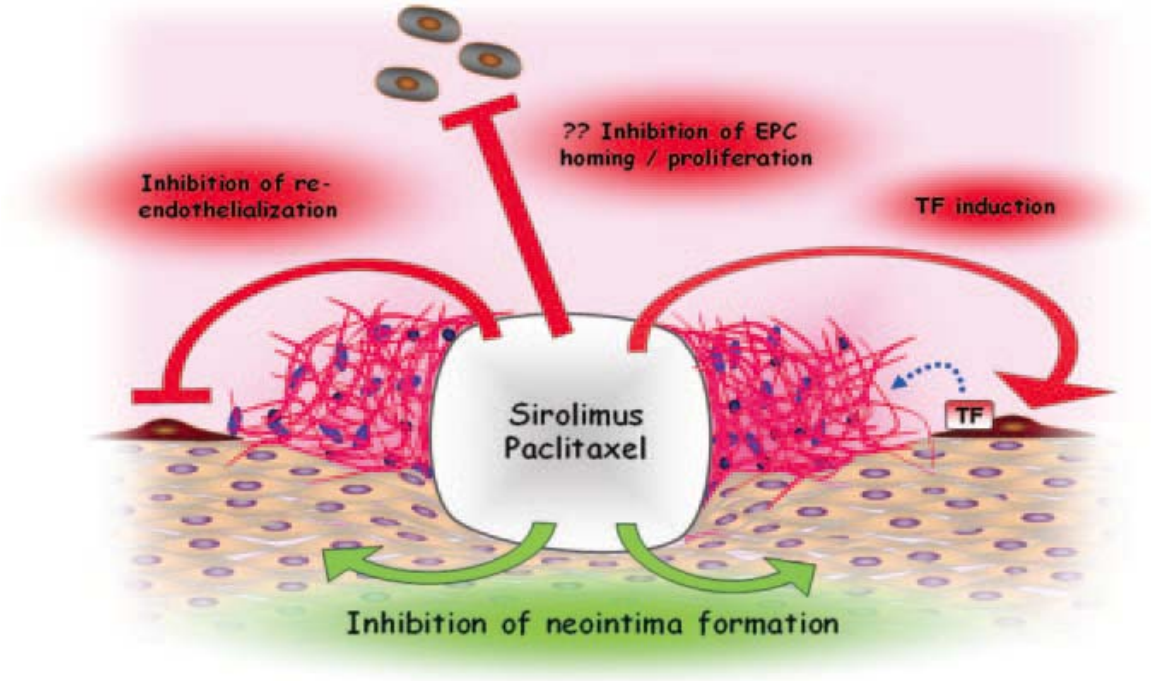

Figure 3. DES reduces neointima formation but may increase stent thrombogenicity. Effect of sirolimus-eluting/paclitaxel-eluting stent strut on the local vessel wall after implantation. Sirolimus/paclitaxel reduces neointima formation by inhibiting vascular smooth muscle migration and proliferation (green arrows). However, the drugs also inhibit reendothelialization, induce tissue factor (TF), and may prevent homing and proliferation of endothelial progenitor cells (EPCs; red arrows/bars).

I.2 Hemostasis in coronary artery disease (CAD) and PCI

Hemostasis is the protective physiological response to vascular injury that results in exposure of blood components to the subendothelial layers of the vessel wall. Arterial thrombosis results from endovascular injury and, to a lesser extent, alterations in hemostatic equilibrium. Although multiple hereditary and acquired hemostatic risk factors have been described in the pathophysiology of venous thrombosis, the degree and type of abnormalities that contribute to arterial thrombosis are less well understood. Markers of haemostatic activation can be used as tools to identify patients

with prethrombotic or thrombotic states, or to analyse the mechanisms of thrombosis occurring in various cardiovascular conditions such as CAD and during PCI procedure.

PCI inevitably causes vessel trauma, with disruption of the endothelium and of the atheromatous plaque. Several studies have demonstrated that vessel injury during percutaneous revascularization exposes underlying collagen and TF, which activate platelets and the extrinsic coagulation cascade (Ong AT et al., 2005, Gasperetti CM et al., 1993, Korovesis S et al., 2000, Borries M et al., 1999, Mizuno O et al., 2001, Namiki A et al., 2004, Inoue T et al., 2000 and Losordo DW et al., 1992). However, most of the previous studies only focused on hemostatic marker changes that occurred in coronary circulation following balloon angioplasty or BMS implantation (Fischell TA et al., 1988, Gasperetti CM et al., 1993, Korovesis S et al., 2000, Borries M et al., 1999, Mizuno O et al., 2001, Namiki A et al., 2004, Inoue T et al., 2000, Losordo DW et al., 1992, Vaitkus PT et al., 1995, Fuster V et al., 1985 and Ip JH et al., 1991). These studies demonstrated that arterial wall injury caused by PCI triggers transient platelet activation leading to localized thrombosis and distal embolisation. Althoughprevious studies reported changes in platelet activation, and/or coagulation or fibrinolysis activation in the coronary circulation during PCI (Gasperetti CM et al., 1993, Korovesis S et al., 2001, Borries M et al., 1999, Mizuno O et al., 2001, Namiki A et al., 2004, Inoue T et al., 2000, Vaitkus PT et al., 1995, Marmur JD et al., 1994, Gregorini L et al., 1997 and Inoue T et al., 1996) data are not very consistent since several authors did not find any changes in local hemostatsis activation in this setting (Oltrona L et al., 1996 and Shammas NW et al., 1994).

The thrombotic event is an acute process thought to be triggered by TF interaction with activated factor VIIa (FVIIa) and almost certainly influenced by haemostatic factors, such as prothrombin fragments 1+2 (F1+2), fibrinogen (FIB), fibrinolytic factors and reactivity of the platelets. Therefore, thrombus formation in the coronary arteries involves several factors including, 1) thrombin formation followed by platelet activation, 2) coagulation activation, and 3) fibrinolytic activation. For this reason, markers of platelet activation, thrombin formation and fibrinolytic activation must be

selected in order to study hemostatic activation during PCI procedure. Hemostasis is closely related to inflammatory processes which may also play a role in occurrence and/or extension of thrombosis; thus, inflammation and its markers must also be taken into account.

I.2.1 Platelet activation

Platelet activation, coronary thrombosis, and ACS are intimately entwined. Platelet activity is noticeably increased following ACS (Trip MD et al., 1990, Sarma J et al., 2002), and PCI (Kabbani SS et al., 2001), and numerous studies have demonstrated that platelet activation plays an important role in thrombus formation in various clinical settings, resulting in unfavorable clinical outcomes (Yip HK et al., 2004 and Schomig A et al., 1996).

I.2.1.1 P-Selectin (CD62P)

Platelet associated P-selectin (CD62P) is a 140-kDa glycoprotein that is present in the α-granules of platelets and translocates rapidly to the cell surface after platelet activation (Hsu-Lin S et al., 1984). CD62P, in healthy individuals, has been suggested to originate from the alternatively spliced form found in endothelial cells and platelets (Johnston GI et al., 1990) and can be found in the plasma as a circulating protein (Dunlop LC et al., 1992). *In vivo*, two main physiological roles are attributed to the integral membrane form of CD62P. First, in inflammation, CD62P is redistributed onto the surface of activated endothelial cells where it mediates the rolling of leukocytes (Mayadas TN et al., 1993). Second, in thrombosis, CD62P expressed on activated platelets present in a thrombus supports the recruitment of leukocytes (Palabrica, T et al., 1992).

Elevated levels of CD62P may reflect platelet activation (Fijnheer R et al., 1997) because P-selectin is proteolytically shed from the plasma membrane *in vivo* shortly after activation (Berger G et al., 1998 and Michelson AD et al., 1996). Therefore, plasma levels of CD62P have been considered to be a useful tool to predict thrombotic consumptive platelet disorders (Chong BH et al., 1994, Blann AD et al., 2001 and Smith A et al., 1999), but they can also reflect endothelial cell activation

(Frijns CJ et al., 1997 and Verhaar MC et al., 1998). As CD62P mediates rolling of platelets on activated endothelial cells and interaction of activated platelets with neutrophils and monocytes, it is generally considered to be the gold standard marker of platelet activation (Michelson AD et al., 1999). An experimental study has shown that CD62P-deficient mice exhibit a slightly prolonged bleeding time, as well as an increased hemorrhagic response in a local Shwartzman reaction (Subramaniam M et al., 1996), suggesting that CD62P could also play a role in hemostasis.

CD62P may thus play a role in thrombosis by promoting platelet aggregation (Merten M et al., 2000) and by inducing a procoagulant state (Andre P et al., 2000). Increased plasma levels of CD62P have been observed in various cardiovascular disorders, including unstable angina where it can be used as a marker of plaque destabilization (Ikeda H et al., 1995). Increased plasma CD62P levels were also observed in acute MI (Shimomura H et al., 1998), and even in patients with coronary artery spasm (Kaikita K et al., 1995), or stable angina (SA) (Furman MI et al., 1998). These findings suggest an important role of CD62P in arterial thrombosis.

I.2.1.2 Soluble glycoprotein V (sGPV)

A new thrombosis marker, soluble glycoprotein V (sGPV), has recently been evaluated in patients with CAD. sGPV is restricted to platelets and is non-covalently linked to the GPIb-IX complex, a receptor for von Willebrand factor (VWF) at the platelet surface (Modderman PW et al., 1992). The exact function of sGPV in primary hemostasis is still unknown but it has been proposed to modulate collagen- and thrombin-dependent platelet responses (Moog S et al., 2001). sGPV is cleaved after exposure of platelets to thrombin, and also by endogenous metalloproteases produced during platelet activation (Rabie T et al., 2005). Although there is no simple relationship between thrombin-induced hydrolysis of sGPV and platelet activation (McGowan EB et al., 1983 and Jandrot-Perrus M et al., 1987), it is suggested that the measurement of sGPV reflects activation of platelets in vivo and may be a useful marker for the diagnosis of thrombosis or prothrombotic states (Fujimura K et al., 1992). sGPV has been evaluated in humans as a thrombosis marker in atherosclerosis

(Blann AD et al., 2001) and MI (Morel O et al., 2004). However, there have been no reports of the early change of sGPV in the coronary circulation after DES or BMS.

I.2.2 Coagulation activation

In the current view of physiological blood coagulation, the formation of fibrin can be initiated through either of two converging cascades: the extrinsic pathway and the intrinsic pathway (**Figure 4**, Wilcox JN et al., 1989). Under pathologic conditions, at sites of vessel injury, bleeding is minimized by the formation of a hemostatic plug consisting of platelets and fibrin. The traditional view of the regulation of blood coagulation is that the initiation phase is triggered by the extrinsic pathway, whereas amplification requires the intrinsic pathway. The extrinsic pathway consists of the transmembrane receptor TF and plasma factor (F) VII/VIIa **,** and the intrinsic pathway consists of plasma FXI, FIX, and FVIII.

Coagulation involves sequential activation of a family of coagulation factors, and results in the conversion of fibrinogen into fibrin monomers; which cross-link to stabilize the platelet-rich thrombus and to form a solid clot. Among coagulation factors, thrombin is a very unstable and easily degraded substance that cannot be measured directly. Conversely, activated TF, FVIIa and Prothrombin fragments 1+2 (F1+2) are which can be selected for our study.

I.2.2.1 Tissue factor (TF)

TF, formerly known as thromboplastin, is a 47-kDa protein expressed in both vascular and nonvascular cells. In the vessel wall, TF is constitutively expressed in subendothelial cells such as vascular smooth muscle cells leading to rapid initiation of coagulation when the vessel is damaged (**Figure 4**). TF has long been known as a key initiator of the coagulation cascade. The coagulation cascade is initiated as soon as TF comes into contact with circulating activated factor VII (VIIa), resulting in the TF-FVIIa complex. This binary complex proteolytically activates factor IX and X, triggering the downstream coagulation pathway (Carson SD et al., 1993). The ultimate events of this cascade are thrombin generation and thrombin-catalyzed events, as well as activa- tion of factors V and VIII, formation of fibrin and activation of

factor XIII, which lead to thrombus formation and stabilization (**Figure 5**).

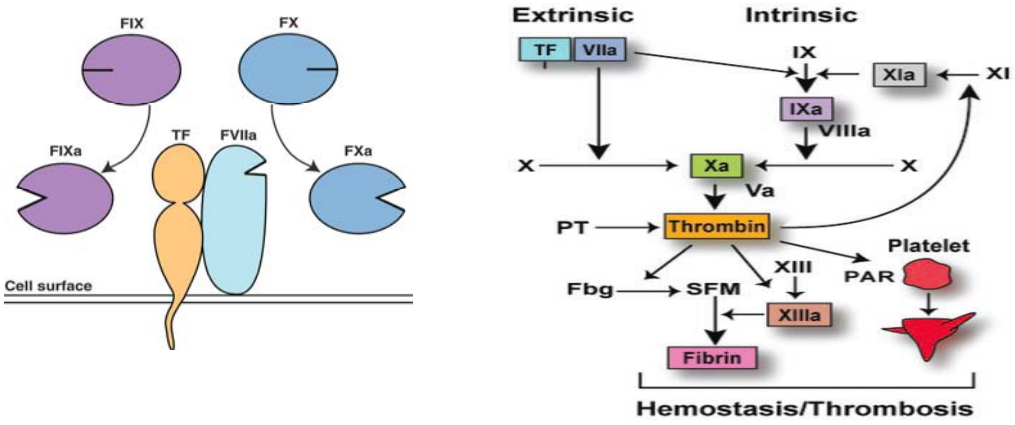

Figure 4. Initiation of the coagulation protease cascade by the TF·FVIIa complex. TF is a transmembrane glycoprotein that localizes FVII/FVIIa to the cell surface. The substrates FIX and FX are proteolytically cleaved by FVIIa to form proteolytically active FIXa and FXa. The prothrombinase complex (FVa:FXa) activates prothrombin (PT). Thrombin activates various proteases and cofactors. Thrombin cleavage of fibrinogen to soluble monomers (SFM), which are cross-linked by FXIIIa, and activation of protease-activated receptors (PARs) on platelets leads to the formation of a clot.

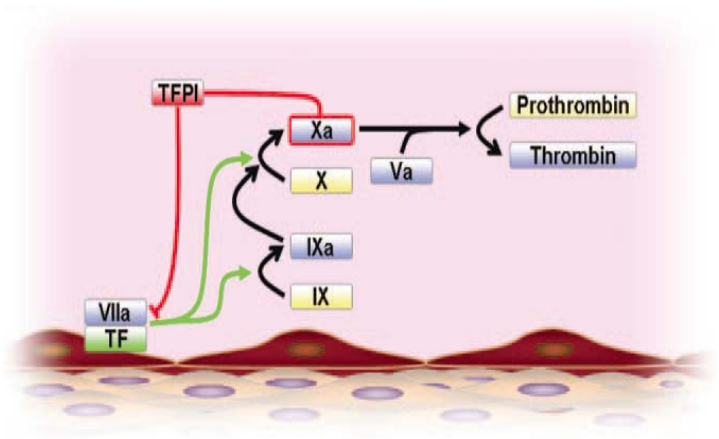

Figure 5. Tissue factor: A key regulator of coagulation. Tissue factor (TF) is a key initiator of the coagulation cascade. Formation of a complex with factor VIIa (FVIIa) leads to activation of factor IX (FIX) and factor X (FX), resulting in thrombin generation and, ultimately, clot formation. Tissue factor pathway inhibitor (TFPI), the endogenous inhibitor of TF activity, is synthesized and secreted mainly by endothelial cells. TFPI binds to FXa and thereby inhibits TF/FVIIa activity.

In atherosclerosis, TF is expressed by macrophage-derived foam cells within atherosclerotic plaques. (Wilcox JN et al., 1989). Moreover, TF expression is higher in atheroma from patients with unstable angina compared to those with stable angina (Annex BH et al., 1995). These results strongly suggest that high levels of TF exposed upon plaque rupture trigger thrombosis and myocardial infarction (**Figure 6**). Inhibition of TF would thus be expected to reduce thrombosis associated with a variety of diseases.

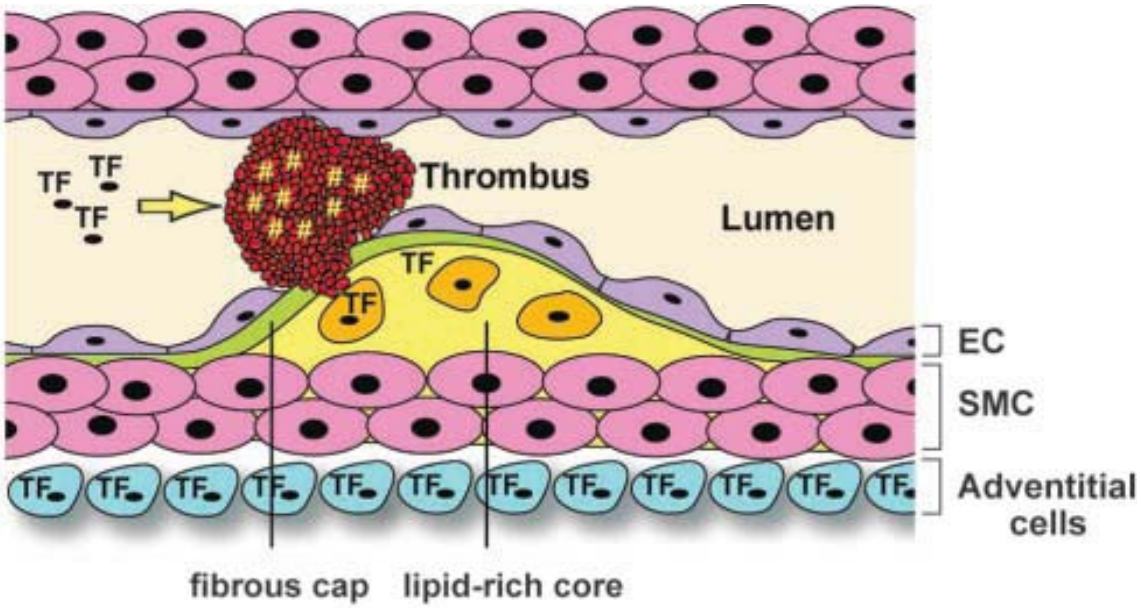

Figure 6. Role of TF in thrombus formation after rupture of an atherosclerotic plaque. TF expressed by foam cells (orange) and in the necrotic core (yellow) of the plaque would be exposed to clotting factors in the blood and initiate clotting after plaque rupture. In addition, blood-borne TF may contribute to thrombus propagation. TF is constitutively expressed by adventitial cells (blue). EC, endothelial cells; SMC, smooth muscle cells.

It is unclear whether or not TF plasma levels are increased after PCI, as some groups found an increase whereas others did not (Tutar E et al., 2003). In one clinical study, PCI in patients with stable or unstable coronary disease resulted in elevated soluble TF levels measured in the coronary sinus after 4 h. This was accompanied by increased levels of thrombin–antithrombin complexes after 24 h (Mizuno O et al., 2000). It is conceivable that plaque dissection caused by balloon dilation may lead to

exposure of the plaque content to the blood stream and thereby increase TF plasma levels. TF induction in vascular smooth muscle cells may importantly contribute to thrombosis associated with arterial intervention. However, this increase was not observed in the study by Marco et al., who did not find significant changes in plasma levels of TF and thrombin–antithrombin complexes after angioplasty in patients with stable and unstable angina (Marco J et al., 2000).

DES are covered with pharmacological agents, which, once released into the coronary artery after stent deployment, inhibit vascular smooth muscle cell proliferation and thereby restenosis. As shown above, and in contrast to reduced restenosis rates, however, the frequency of ST has not decreased with DES as compared with BMS (Iakovou I et al., 2005). Rapamycin, used for stent coating, increases endothelial TF expression, which suggests a potential role for this drug in the development of SST (Steffel J et al., 2005). As this effect on TF expression is not observed with FK-506, another agent used on DES, at least in vitro, application of FK-506-eluting stents may provide a more favorable environment for the prevention of in-stent thrombosis (Steffel J et al., 2005). However, additional studies are needed to assess the implications of these findings in vivo. Platelet activation is a crucial event in the pathogenesis of thrombus formation. The use of platelet receptor antagonists such as clopidogrel has indeed reduced the incidence of ST, whereas withdrawal of APA therapy favors thrombus formation (Mizuno O et al., 2000). Clopidogrel inhibits the release of TF from aggregating platelets, which is of particular importance, as platelet aggregation and secretion are increased in human platelets treated with rapamycin (Leon C et al., 2004). Therefore, it will be of great interest to study the dynamic interaction between rapamycin and platelet activation as well as the spatio-temporal pattern of TF expression in the arterial wall after deployment of DES.

I.2.2.2 Activated factor VII (FVIIa)

FVII is a vitamin K–dependent protein that plays an important role in the initiation of TF-induced coagulation. Most FVII circulates in the plasma as a zymogen of the

serine protease FVIIa (Osterud B., 1984, Fair DS., 1983 and Hagen FS et al., 1986). In the presence of TF, native FVII is converted into its activated two-chain form, FVIIa. This reaction can be catalyzed by several coagulation proteases, including FXa, FIXa, FXIIa, thrombin, and FVIIa. The TF-FVIIa complexes rapidly cleave FIX and FX in their active forms and may cause thrombin generation and fibrin clots. Therefore, when cell-surface TF is exposed to plasma, low levels of FVIIa may serve a "priming" function for "triggering" the clotting cascade (Morrissey JH., 1995).

There is increasing evidence of an important role for TF and FVIIa in the initiation of blood coagulation via the so-called extrinsic coagulation pathway (**Figure 4**). However, FVIIa possesses very little activity unless it is complexed with TF, its essential protein cofactor (Nemerson Y., 1988). Because FVII must be proteolytically activated to be functional, attention has been focused on its mechanism of activation during initiation of the clotting cascade. A variety of plasma proteases, including factors IXa, Xa, XIIa, and thrombin, are capable of converting FVII to FVIIa, and the rate of FVII activation is enhanced dramatically in the presence of TF (Nemerson Y et al., 1985). Recently, FVIIa itself has been reported to activate FVII in the presence of TF (Nakagaki T et al., 1991). Termed “FVII autoactivation”, this process has been proposed as an additional means by which active TF-FVIIa complexes can be generated (Nakagaki T et al., 1991 and Neuenschwander PF et al., 1992).

Therefore, FVIIa is a mediator of biological importance (Scarabin PY et al., 1996), particularly at sites where blood may come into contact with TF, e.g., atherosclerotic plaques (Wilcox JN et al., 1989) or prothrombotically activated endothelium (Colucci M et al., 1983). Clinical and epidemiological data suggest that FVII may be involved in the pathogenesis of CAD. Plaque disruption followed by exposure of TF to blood and further binding of TF to circulating FVII resulting in activated FVIIa and subsequent initiation of the coagulation cascade, is considered to be the major cause of thrombosis in acute MI (Fuster V et al., 1992).

I.2.2.3 Prothrombin fragment 1+2 (F1+2)

Prothrombin is cleaved by the prothrombinase complex into two peptides, the active

thrombin and the prothrombin fragment 1+2 (F1+2). Since we cannot measure thrombin itself, then measurement of F1+2 would be an excellent marker of thrombin generation, since F1+2 is not generated in vivo by any other mechanism. Fragment 1+2 has a half-life of about 1 hour and is cleared from the bloodstream by the liver. An ELISA assay for F1+2 is available which allows the laboratory to provide data in a timely manner and thus may actually helpclinicians to determine if a patient is in a hyper coagulable or prethrombotic state. As a sensitive marker of intravascular thrombin formation, F1+2 has clinical utility for assessing thrombotic risk and monitoring efficacy of anticoagulant therapy (Pelzer H et al., 1991 and Hafner G et al., 1992). Monitoring of Fl+2 has proven useful in assessing risk for cardiovascular disease, and F1+2 is increasingly used as a hemostatic marker in multicenter epidemiologic investigations. The serum F1+2 concentration exceeds normal range only in severe hypercoagulable states (Oltrona L et al., 1996 and, Marmur JD et al., 1994) and Haude *et al (*Haude M et al., 1995) showed that the F1+2 concentration was the most specific and useful predictor of SST. In addition, if a patient with elevated values is treated with anticoagulant drugs (heparin or coumadin), F1+2 measurement can be used to follow the efficiency of the treatment. After discontinuation of therapy, F1+2 serial measurements (e.g.: at 2 wks, 4 wks, 8 wks) may detect recurrence of hypercoagulability in such patients.

I.2.3 Fibrinolysis activation

The fibrinolytic system is of considerable interest in CAD as part of the natural defense against thrombosis as well as a putative factor in the atherosclerotic process (Hamsten A et al., 1994). Impaired fibrinolysis is considered to be a risk factor for CAD and MI (Hamsten A et al., 1994). Disturbances of fibrinolysis have been found in cross-sectional studies of patients with MI (Johnson O et al., 1984) and angina pectoris (ECAT Angina Pectoris Study Group., 1993).

Contributing to the pathophysiology of thrombus formation is an altered balance between the fibrinolytic and procoagulant systems (**Figure 7**, Kohler HP et al., 2000). This hemostatic imbalance may be a consequence of an impaired fibrinolytic system

that acts in concert with either enhanced procoagulantor impaired anticoagulant forces to form a thrombus. Fibrin deposition and lysis must be balanced to maintain and remold the hemostatic seal during repair of an injured vessel wall. The fibrinolytic system dissolves fibrin by means of plasmin, a proteolytic enzyme. Fibrinolysis is activated by plasminogen activators released from vascular endothelial cells. Plasminogen activators and plasminogen from plasma bind to fibrin. Plasminogen activators catalyze cleavage of plasminogen, creating plasmin (**Figure 7**).

The suppression of fibrinolysis due to high plasma concentrations of plasminogen-activator inhibitor type 1 (PAI-1) and increased plasma concentrations of factor VII, FIB, and von Willebrand factor are associated with the development of MI (Thompson SG et al., 1995). In addition, high concentrations of tissue plasminogen activator (t-PA) and D-dimer (DD) increase the risk of MI (Ridker PM et al., 1994). PAI-1 is a fast-acting inhibitor of plasminogen activation. It is produced by the vascular endothelium but is also present in platelets and is considered to be an important regulatory element in fibrinolysis (Kruithof EKO et al., 1987) (**Figure 7**).

I.2.3.1 D-dimers (DD)

D-dimers are (DD) produced as a result of plasmin activity on cross-linked fibrin polymers. Thus, DD indicate the activity of both thrombin and plasmin and are specific for fibrinolysis (Stokol T et al., 2003). DD are effective in detecting both intravascular and extravascular cross-linked fibrin by-products (Berghaus G et al., 1999 and Wada H et al., 1999). It has been suggested that DD are a more sensitive and specific diagnostic test for disseminated intravascular coagulation (DIC) than the traditional fibrinogen degradation products (FDP) assays as they detect the presence of freshly formed fibrin clots and concurrent proteolytic degradation of particulate clots (Asakura H et al., 2006, Gupta PK et al., 2005, Berghaus G et al., 1999, Bick R et al., 1992, Griffin A et al., 2003 and Dempfle C., 1991). DD assays are readily available and considered to be economical, non-invasive, rapid, and easy to perform (Berghaus G et al., 1999, Bick R et al., 1992, Dempfle C., 1991 and Stokol T et al., 2000).

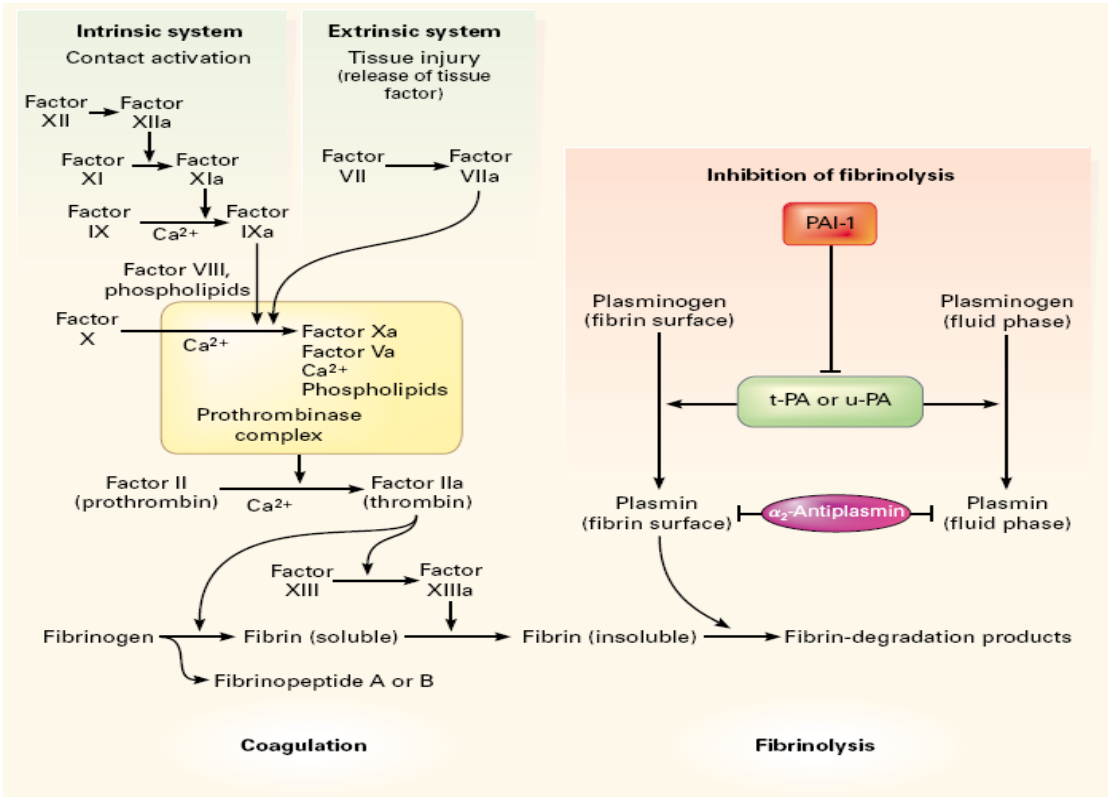

Figure 7. The Coagulation and Fibrinolytic Pathways. The main coagulation reactions are divided into the intrinsic and extrinsic systems. Activation of factor XII on contact with a negatively charged surface initiates the intrinsic coagulation system. (The activated form of the factor is indicated by "a.") The extrinsic coagulation system induces the formation of a complex composed of factor VII and tissue factor, which is released after tissue injury. Some of these reactions depend on calcium ions. Thrombin is formed by an enzyme complex called prothrombinase, composed of factor X, factor V, negatively charged phospholipids, and calcium ions. Intrinsic and extrinsic activation of the coagulation cascade leads to the generation of thrombin, the activation of fibrinogen, the release of fibrinopeptides, the formation of soluble fibrin, and finally, the formation of factor XIII–mediated, cross-linked, insoluble fibrin. The main fibrinolytic reactions involve the inhibition of fibrinolysis by plasminogen-activator inhibitor type 1 (PAI-1) and a_2-antiplasmin. Fibrinolysis is initiated by tissue plasminogen activator (t-PA), urinary-type plasminogen activator (u-PA), and plasmin. Plasmin bound to the surface of fibrin initiates the lysis of insoluble, cross-linked fibrin, with the subsequent generation of fibrin-degradation products. Plasmin bound to the surface of fibrin is better protected from inhibition by a_2-antiplasmin than is plasmin generated in the fluid phase.

In addition to this, they can be run on citrated blood, thus decreasing the number of venipunctures and amount of blood drawn from individuals suspected of a coagulopathy (Stokol T., 2003). As stated above, they are a sensitive and specific indicator of DIC, however, it must be kept in mind that DD are also detected in patients with localized thrombosis, internal hemorrhage, MI, and ischemic stroke, as well as post-operatively (Nelson OL et al., 2003, Haapaniemi E et al., 2008, Koch HJ et al., 2005, Gutiérrez A et al., 2001 and Ridker PM et al., 1994).

Recently, fibrin DD, the degradation product of crosslinked fibrin, has gained increasing interest for several reasons. First, it can be considered as a global marker of the turnover of cross-linked fibrin and of activation of the hemostatic system (Lowe GDO et al., 1999). Second, in contrast to several other markers of hemostasis, DD assays are more stable and more practical to measure and therefore may be more suitable for routine clinical and epidemiological purposes (Lip GYH et al., 1995).

I.2.3.2 Fibrinogen (FIB)

FIB, a dimeric glycoprotein synthesized by the liver, is a major constituent of platelet aggregate and is converted by thrombin to form the fibrin clot. FIB may contribute to atherosclerrosis and thrombosis by increasing blood coagulabity, plasma viscosity, platelet aggregability, and by promoting fibrin deposition in the vessel wall (Andreotti F et al., 1999).

Elevated FIB levels are considered to be an important risk factor for the development of cardiovascular events (Wilhelmsen LSK et al., 1984, Kannel WBWP et al., 1987 and Yarnell JW et al., 1991). There is also a relationship between FIB levels and the extent of angiographically evaluated coronary atherosclerosis (ECAT angina pectoris study., 1993, Broadhurst P et al., 1990 and Handa K et al., 1989). The prognostic influence of elevated FIB levels in ACS has already been identified (Toss H et al., 1997, Haines AP et al., 1983 and Cristal N et al., 1983). Furthermore, atherosclerosis is considered to be a chronic inflammatory disease of the vessel wall (Harker LA et al., 1978) and FIB belongs to the inducible “acute phase proteins of inflammation”. These observations suggest that FIB may also playan important role as

an inflammatory factor in the development of CAD. The links between coagulation/fibrinolysis and inflammation, and their subtle interplay in CAD and its treatment are examined below. Because of this ambiguous role of FIB, the mechanisms by which it contributes to atherogenesis remain unclear and hypothetical. They may be related to fibrin formation, platelet aggregation, inflammation, migration, and or the proliferation of vascular smooth muscle cells (Schwartz RS et al., 1994, Schwartz SM et al., 1995, Raymond J et al., 1999 and Kawasaki T et al., 2001). Interestingly and elegantly, Kawasaki et al (Kawasaki T et al., 2001) showed that early or delayed FIB depletion reduced neointimal formation using a mouse carotid artery ligation model and suggested that the persistent presence of fibrin remains crucial for neointimal formation. Pathologically, even 64 days after coronary stenting in patients with CAD, the lesion showed traces of fibrin. This fibrin was considered to be a remnant of a previous thrombus (within the neointima close to the luminal surface) (Komatsu R et al., 1998). This observation is a good illustration of the persistent presence of fibrin. Although elevated FIB levels after coronary balloon angioplasty have been reported as a risk factor for the development of restenosis (Montalescot G et al., 1995 and Benchimol D et al., 1993), there is little clinical data available concerning the relationship between FIB levels at the time of the procedure ("procedural FIB levels") and intracoronary thrombosis events before and after coronary stenting. Therefore, among the objectives of this study, we intended to evaluate the relationship between procedural FIB levels, which is considered to be an important factor in the processes of coagulation in SA patients undergoing PCI, and further occurrence of stenosis.

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I.2.3.3 Plasminogen activator inhibitor type-1 complexes (PAI-1)

PAI-1, a member of the SERPIN family, is the main inhibitor of tissue- and urokinase- type plasminogen activators and one of the major determinants of fibrinolysis (Pannekoek H et al., 1986) **(Figure 8,** Kohler HP et al., 2000). The main fibrinolytic components of plasma are plasminogen, α_2-antiplasmin, t-PA, and urinary-type plasminogen activator (U-PA) (Kohler HP et al., 2000) (**Figure 8**). When

platelets are stimulated by thrombin, PAI-1 is released on the platelet surface, protecting a blood clot from premature lysis. This mechanism causes a rapid local increase in the PAI-1 concentration in the circulation (Sprengers ED et al., 1986). Thrombin also stimulates the synthesis of PAI-1 in endothelial cells (Gelehrter TD et al., 1986). Platelet-rich arterial thrombi are more readily lysed by t-PA in mice with a deficiency of PAI-1 than in normal mice, suggesting that the inhibition of PAI-1 may improve the outcome of thrombolysis (Zhu Y et al., 1999).

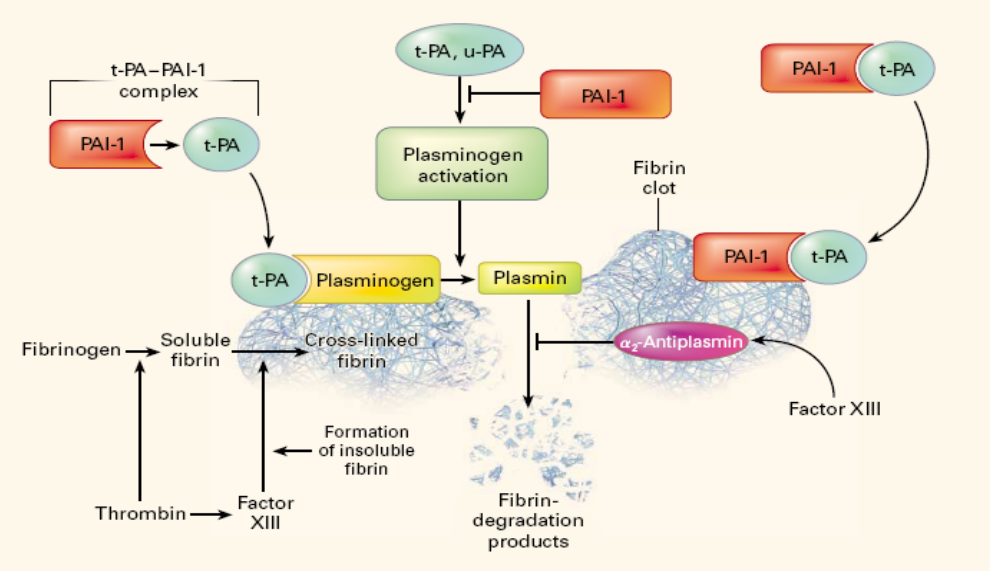

Figure 8. Activation and inhibition of the fibrinolytic pathway. t-PA circulates in plasma as a complex with PAI-1 in a 1:1 ratio. The fibrin clot provides the surface on which the reactions occur. Plasminogen is activated by t-PA or u-PA. Plasminogen, t-PA, and fibrin form a ternary complex that promotes the formation of plasmin and the subsequent lysis of cross-linked fibrin into low-molecular-weight fragments (fibrin-degradation products). PAI-1 also binds to fibrin and, when bound, retains its inhibitory activity against t-PA. α_2-Antiplasmin is cross-linked to fibrin by factor XIII.

The importance of the fibrinolytic system as a regulator of fibrin deposition in the vessel wall raises the question of the role of perturbations in this system in the development of vascular disease. In theory, at least, a decrease in fibrinolysis due to high plasma PAI-1 concentrations might be expected to result in an increase in the

deposition of fibrin and subsequent formation of a thrombus. High plasma PAI-1 concentrations are indeed associated with various thrombotic disorders (Margaglione M et al., 1994 and Thögersen AM et al., 1998) and are an independent risk factor for reinfarction in patients who have had a first MI before the age of 45 years (Hamsten A et al., 1985). There is an association between the presence of CAD and low plasma fibrinolytic activity due to increased plasma PAI-1 concentrations (Francis RB Jr et al., 1988).

I.2.3.3.1 PAI-1 and the progression of vascular disease

There is evidence that high plasma PAI-1 concentrations are associated with the progression of coronary syndromes and the development of MI. High plasma PAI-1 concentrations predict subsequent MI in patients with SA (Held C et al., 1997) and are associated with angiographic evidence of progressive CAD in young men with a history of MI (Bavenholm P et al., 1998). In addition, a genetic polymorphism in the promoter region of the PAI-1 gene has been associated with both high plasma PAI-1 concentrations and unstable angina (UA) (Iwai N et al., 1998).

I.2.3.3.2 PAI-1 in vessel walls and plaques

Atheromatous material obtained at the time of coronary atherectomy in patients with type 2 diabetes mellitus contains more PAI-1, as detected by immunohistochemical studies, than atheromatous material from patients without diabetes (Sobel BE et al., 1998). Similarly, levels of PAI-1 messenger RNA (mRNA) are higher in severely atherosclerotic arteries than in normal arteries (Schneiderman J et al., 1992). These findings indicate that increased expression of the PAI-1 gene in the arterial wall, leading to increased amounts of PAI-1 in plaques, may facilitate thrombotic events after plaques rupture.

I.2.3.3.3 Relationship between PAI-1 and cardiovascular risk factors

That the fibrinolytic system in general, and PAI-1 in particular, play a role in the development of CAD is supported by the biologic characteristics of PAI-1 and the association between high plasma PAI-1 concentrations and other cardiovascular risk

factors. Plasma concentrations of PAI-1 are lower during the day than at night (Angleton P et al., 1989), and it has been proposed that the higher incidence of MI in the early morning hours could be due to higher plasma PAI-1 concentrations, and therefore lower fibrinolytic activity, at night (Andreotti F et al., 1988).

I.2.3.3.4 Relationship between PAI-1 and PCI procedure

In principle, elevated PAI-1 would favour reduced action of tPA, in the vessel wall both during disease development and fibrin deposition. There is evidence from experimental and interventional studies (Anderson KM et al., 1991 and Sobel BE et al., 1998) that increased PAI-1 levels are associated with atherosclerotic progression. In addition, PAI-1 and t-PA directly influence thrombus formation and degradation and thus risk for arterial thrombosis. Several studies have suggested the existence of an increase in PAI-1 levels immediately after PCI (Muldowney JA 3rd et al., 2007 and Huber K et al., 1992), although other studies reported a decrease of PAI-1 in this setting (Prisco D et al., 2001 and Maresca G et al., 1999).

I.2.3.4 Tissue plasminogen activator (t-PA)

Activation of the fibrinolytic system, which is responsible for the dissolution of a fibrin clot through the conversion of plasminogen into the active protease plasmin, also depends on another key proteins, t-PA, a pivotal activator of plasminogen, the main endothelial cell derived blood activator of the fibrinolytic system. Intravascular fibrinolysis is initiated by t-PA that converts plasminogen to plasmin. The main physiological regulator of the activity of t-PA is PAI-1, which resides in the circulation both in plasma and in platelet alpha-granules.

Both retrospective and prospective studies have found associations between high t-PA levels and MI (Ridker PM et al., 1993, Carter AM et al., 1998 and van der Bom JG et al., 1997). Other studies have found similar links with CAD as well as with stroke (Ridker PM et al., 1993, Carter AM et al., 1998). Although it was initially thought that high t-PA antigen levels may be related to a reduced risk, it is now considered that these conflicting findings may reflect the complex pathogenesis of CAD/MI and that high t-PA levels are definitely associated with an increased risk

(van der Bom JG et al., 1997 and Jansson JH et al., 1993). Increased t-PA plasma levels have also been observed in patients with a history of venous thrombo-embolism (VTE) (Gram J et al., 1995).

I.2.4 Polymorphisms in fibrinolytic system genes

Hemostasis does play a role in arterial thrombotic disease (ATD). However, establishing which of the factors are actually "risk factors" has proven surprisingly difficult. Because of its technical simplicity and digital nature, the study of the polymorphisms of the genes which code for coagulation/fibrinolysis proteins as risk factors has grown in popularity. In addition, there is currently great interest in defining the genetic influences in ATD. Knowledge of genetic risk factors will help define the mechanisms of disease and could ultimately assist in the rational design of selective prophylaxis or therapy.

Family studies suggest that plasma PAI-1 and t-PA levels are influenced by genetic factors (Pankow JS et al., 1998). Previous studies usually investigated the effects of non-genetic and genetic factors individually on plasma levels of t-PA or PAI-1 in relatively small populations at high-risk for thromboembolic events (Mansfield MW et al., 1995, Juhan-Vague I et al., 1993 and Toft I et al., 1997). In addition to clinical and angiographic characteristics, genetic factors may contribute to the risk of in-stent thrombosis. Comparing local fibrinolysis activation following stent implantation and testing for possible associations between PAI-14G/5G gene polymorphism and thrombotic events after coronary artery stenting might be helpful in analyzing the thrombogenicity of the stent and its coating.

The human PAI-1 gene is located on chromosome 7 and contains nine exons and eight introns (Strandberg L et al., 1988). Several polymorphisms have been described within the gene, including a cytosine-adenine (CA)n dinucleotide repeat, a HindIII restriction-fragment–length polymorphism, and a common single-base-pair polymorphism (four or five guanine bases) in the promoter region of the gene, 675 bp upstream of the transcriptional start site (4G/5G) (Dawson S et al., 1991). Subjects who are homozygous for the 4G allele (4G/4G genotype) have plasma PAI-1

concentrations that are approximately 25 percent higher than those of subjects who are homozygous for the 5G allele (5G/5G genotype) (Eriksson P et al., 1995). In vitro studies have identified differential binding of transcription-regulating proteins at this site. Increased gene transcription is associated with four guanine bases (the 4G allele), and results in the binding of a transcriptional activator alone, whereas with five guanine bases (the 5G allele), there is also binding of a repressor protein that decreases the binding of the activator (Dawson S et al., 1991 and Eriksson P et al., 1995) (**Figure 9,** Kohler HP et al., 2000). The transcriptional regulation of the PAI-1 4G allele by triglyceride (Eriksson P et al., 1998) may account for the involvement of the PAI-1 genotype in the pathogenesis of CAD. Studies of the relation between the PAI-1 genotype and MI have provided some hints that this may be the case, although overall, the relation is weak. In a study of patients undergoing coronary angiography, the 4G allele was significantly associated with high plasma PAI-1 concentrations, and the 4G allele was most strongly associated with previous MI in the patients with established atheroma (Ossei-Gerning N et al., 1997). In a study of 1179 normal subjects and their firstdegree relatives, the 4G allele was associated with a significant risk of MI (Margaglione M et al., 1998 and Onalan O et al., 2008). A small study found that the 4G allele was related to the development of ACS (Iwai N et al., 1998); however, two large studies, the Etude Cas-Témoin de l'Infarctus du Myocarde (Ye S et al., 1995) and the Physicians' Health Study (Ridker PM et al., 1997) found no relation between genotype and MI. In a study of 2565 subjects who underwent coronary angiography for diagnostic purposes, the PAI-1 genotype was related to the presence of atheroma in the overall sample and to the severity of atheroma in a subgroup of high-risk subjects (Gardemann A et al., 1999), although there was no relation between the PAI-1 genotype and a history of MI. These findings must be considered in the context of the difficulties in estimating the atheroma burden accurately by standard angiographic techniques. The functional characteristics of the 4G/5G polymorphism and its apparent interaction with serum triglyceride concentrations suggest that the 4G allele is more likely to contribute to MI in the presence of hypertriglyceridemia associated with insulin resistance than in its absence.

A recent meta-analysis of all the relevant studies showed a marginally significant effect of PAI-1 genotype on MI (Iacoviello L et al., 1998). In addition, the 4G/5G genotype has been related to the risk of in-stent restenosis after coronary stent implantation (Sakata K et al., 1996).

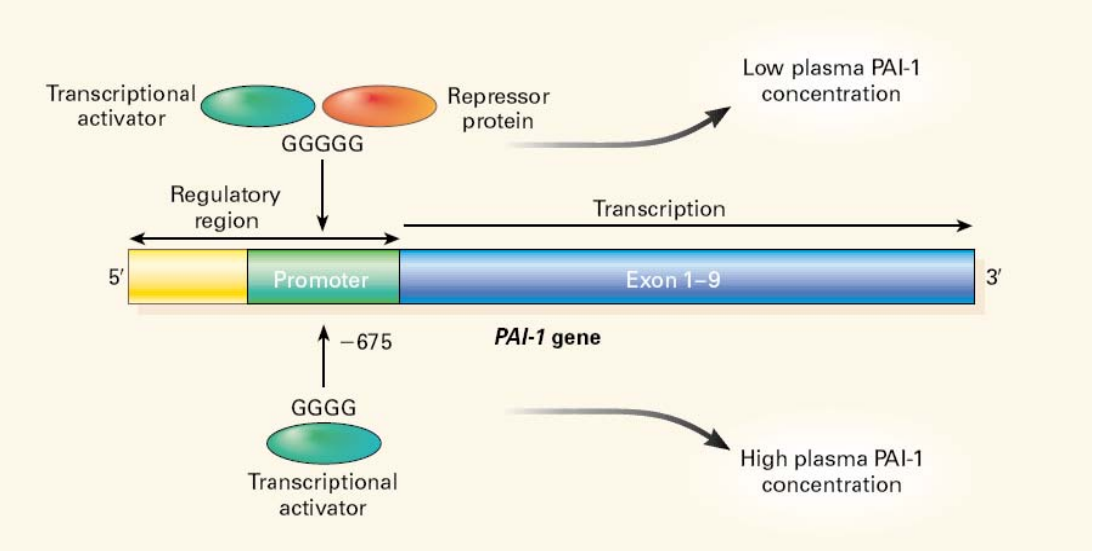

Figure 9. Structure of the gene for Plasminogen-Activator Inhibitor Type 1 (PAI-1) and of the site of the 4G/5G polymorphism in the promoter region. The 4G/5G polymorphism at position -675 influences transcription and therefore plasma PAI-1 concentrations. Differential binding of transcription-regulating proteins at this site has been identified. Increased gene transcription is associated with four guanine bases (the 4G allele) and results in the binding of a transcriptional activator alone, whereas with five guanine bases (the 5G allele), there is also binding of a repressor protein that decreases the binding of the activator.

II. Early changes in local hemostatic activation following PCI: a comparison between DES and BMS

II.1 Objectives of the study

An increased risk of ST has previously been reported following DES implantation as compared to BMS (Iakovou I et al., 2005, Daemen J et al., 2007, Jeremias A et al., 2004, Babapulle MN et al., 2004, Stone GW et al., 2007, Moreno R et al., 2005 and Bavry AA et al., 2005). The incidence of AST ranges from 0.1% to 3% (Chieffo A et al., 2004, Alfonso F et al., 2004 and Scheller B et al., 2001). Despite the increasing use of less thrombogenic stent surfaces, combined with the administration of dual APA therapy, AST has been reported to be responsible for 60 to 70% of death and 20 to 25% or more of nonfatal MI (Daemen J et al., 2007, Babapulle MN et al., 2004, Stone GW et al., 2004, Moreno R et al., 2005 and Bavry AA et al., 2005). Hence, even if LST has gained increasing interest in the DES era, the importance of AST remains high, as patients with early ST are at risk of recurrent MI and ST, predictive of late major cardiac events. For this reason, our study focused on AST.

The mechanisms of AST are not fully understood. PCI, that damages endothelium and fractures atheromatous plaque, activates local hemostasis, leading to platelet activation and thrombin formation. Potential enhanced platelet aggregation might be responsible for DES thromboses that occur early after stent implantation (Gyöngyösi M et al., 2006 and Babinska A et al., 1998). This may be due to retention of eluted drugs in the arterial tissue (Lüscher TF et al., 2007). In addition, several factors are involved in the pathogenesis of in-stent thrombosis. These include procedure-related factors such as mechanical vessel injury or incomplete stent apposition, patient-related factors such as vessel size or coagulation activity, and finally, the thrombogenicity of the stent itself (Honda Y et al., 2003). However, early changes in hemostatic markers following DES implantation have never been reported. Whether the drugs used for stent coating could be involved in the development of in-stent thrombosis has not been explored either (Honda Y et al., 2003). Thus, comparison of local hemostasis activation following DES and BMS implantation might be helpful in analyzing the

thrombogenicity of the stent and its coating.

Family studies suggest that plasma PAI-1 and t-PA levels are influenced by genetic factors (Pankow JS et al., 1998). Previous studies usually investigated the effects of non-genetic and genetic factors individually on plasma levels of t-PA or PAI-1 in relatively small populations at high-risk for thromboembolic events (Mansfield MW et al., 1995, Juhan-Vague I et al., 1993 and Toft I et al., 1997). The 4G/5G genotype has been related to the risk of MI (Boekholdt SM et al., 2001), sudden cardiac death (Anvari A et al., 2001), transplant CAD (He JQ et al., 2002), and in-stent restenosis after coronary stent implantation (Ortlepp JR et al., 2001). Recently, an Alu-repeat insertion/deletion (I/D) polymorphism in the t-PA gene was found to be associated with increased MI risk (van der Bom JG et al., 1997). In addition to clinical and angiographic characteristics, genetic factors may contribute to the risk of in-stent thrombosis. However, no data are available on a potential risk of coagulation/fibrinolysis gene polymorphisms in this situation. Thus, there is currently great interest in defining the genetic influences in ATD, knowledge of genetic risk factors will help define the mechanisms of disease and could ultimately assist in the rational design of selective prophylaxis or therapy.

Therefore, we investigated the early changes in local intracoronary hemostasis following DES and BMS implantation in patients with SA or silent ischemia under dual antiplatelet and anticoagulant pretreatment. In addition, the present analysis was also performed to test for possible associations between the PAI-14G/5G gene polymorphism and early change in local intracoronary hemostasis following PCI procedures. Local levels of hemostasis markers were assayed at various time points before and after stent implantation. In addition, early local hemostasis response was compared before and after DES and BMS implantation to assess whether DES would induce earlier thrombogenicity than BMS.

II.2 Results and personal publications

The results obtained in this prospective study of intra-coronary hemostatic changes after DES and BMS stenting are given in the publication "***Early changes in local***

hemostasis activation following percutaneous coronary intervention in stable angina patients: a comparison between drug-eluting and bare metal stents" (*J Thromb Thrombolysis*, DOI 10.1007/s11239-008-0266-2).

The relationship between gene polymorphism of PAI-1 and changes in intra-coronnary hemostasis parameters in the same patients is given in the publication "***Relationship between plasminogen activator inhibitor-1 (PAI-1) 4G/5G gene polymorphism and early local hemostatic activation in patients with percutaneous coronary intervention procedures***" [*J Intervent Radiol*, 2007;16(9):584-588 *(in Chinese)*]

Other personal publications from the PhD thesis studies:

"***Early Local Intracoronary Platelet Activation after Drug-Eluting Stent Placement"*** [*Chin Med J*, 2007;120(22):1986-1991].

"***Comparison of early effects of baremetal stent and drug-eluting stent implantation on intra-coronary loca l tissue factor levels following percutaneous coronary intervention for stable angina***" [*Chin J Intervent Cardiol*, June 2008, Vol 16, No1 3 (*in Chinese*)].

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Early changes in local hemostasis activation following percutaneous coronary intervention in stable angina patients: a comparison between drug-eluting and bare metal stents

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Abstract *Background* Early change in local intracoronary hemostasis following drug-eluting (DES) and bare metal stent (BMS) implantation has never been assessed in stable angina patients. *Methods* Markers of local platelet activation (soluble glycoprotein V [sGPV] and P-Selectin [CD62P]), coagulation activation (tissue factor [TF], prothrombin fragments 1 + 2 [F1 + 2] and activated factor VII [FVIIa]) and fibrinolysis markers (D-dimers [DD], fibrinogen [FIB], tissue plasminogen activator [t-PA], and plasminogen activator inhibitor type-1 complexes [PAI-1]) were determined in 20 patients with stable angina who underwent percutaneous coronary intervention (PCI). All patients were pretreated with clopidogrel, aspirin, and enoxaparin. Systematic balloon predilation was performed before DES (9 patients) and BMS (11 patients) implantation. All blood samples were drawn 10–20 mm distal to the lesion site. *Results* No significant changes in levels of platelet activation markers occurred during PCI. There was a transient significant increase in TF (14%; $P = 0.004$), in F1 + 2 (40%; $P = 0.001$), and FVIIa (31%; $P = 0.007$) following angioplasty. Similarly, a significant 43% increase was observed in DD levels following balloon predilation, associated with an increase of 46%, 60%, and 70% in FIB, t-PA and PAI-1 levels, respectively (all $P < 0.0001$). All these markers returned to baseline values after stent implantation. No difference was observed between DES and BMS. *Conclusions* Early changes in local hemostasis activation following PCI, were related to balloon predilation. Neither DES nor BMS increased markers of platelet activation, coagulation, or fibrinolysis, under dual antiplatelet and anticoagulant pretreatment.

Keywords Stents · Platelet · Coagulation · Fibrinolysis · Balloon

Introduction

Acute stent thrombosis (AST) is a rare but a severe complication of percutaneous coronary intervention (PCI), occurring usually within minutes to hours after stent implantation, with an incidence ranging from 0.1% to 3% [1–3]. Although it is not a common phenomenon, AST is associated with a highly unfavorable prognosis [2, 4–7].

An increased risk of stent thrombosis has previously been reported following drug-eluting stent (DES) implantation as compared to bare metal stents (BMS) [7–14]. Potential enhanced platelet aggregation might be responsible for DES thrombosis that occurred early after stent implantation [15, 16]. This may be due to retention of eluted drugs in the arterial tissue [17]. However, early changes in hemostatic markers following DES implantation have never been reported. Comparison of local hemostasis activation following DES and BMS implantation might be helpful in analyzing the thrombogenicity of the stent and its coating.

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Therefore, we investigated the early changes in local intracoronary hemostasis following DES and BMS implantation in patients with stable angina or silent ischemia under dual antiplatelet and anticoagulant pretreatment. Local levels of hemostasis markers were assayed at various time points before and after stent implantation. In addition, early local hemostasis response was compared before and after DES and BMS implantation to assess whether DES would induce earlier thrombogenicity than BMS.

Methods

Patient population

Patients with stable angina pectoris or documented silent ischemia, and undergoing elective stenting of a significant coronary artery stenosis, were prospectively enrolled in the study. Exclusion criteria included prior history of bleeding diathesis, stroke within the last 3 months, accompanying valvular disease, an ejection fraction <30%, severe heart failure (New York Heart Association III, IV), acute coronary syndrome (ST and non ST elevation myocardial infarction) within the last 7 days, unstable angina, bifurcation lesion, multiple stent implantation, renal insufficiency with creatinine levels of >3.5 mg/dl, active neoplasia, and prior GP IIb/IIIa inhibitor therapy. The study protocol was approved by the local Ethics Committee and informed consent was obtained for all patients enrolled in the study.

Medication

All patients had received 75 aspirin daily for at least 4 days before the procedure. Clopidogrel was administered at a 300 mg loading dose the day before the procedure. Low molecular weight heparin (enoxaparin: 0.75 mg per kg of body weight) was administered intravenously at the beginning of the procedure.

Coronary angiography and PCI

PCI was performed by two experienced operators according to standard practice using the Judkins approach following right femoral artery puncture. With the patient under local anesthesia obtained with 1% xylocaine, a femoral artery was cannulated with a 6F sheath. Selective coronary angiography was performed after the intracoronary administration of nitroglycerin and at least five standardized views of the left coronary artery and three views of the right coronary artery were obtained. Patients with diabetes mellitus, proximal left anterior descending stenosis, or reference vessel diameter <3.0 mm by visual estimate were assigned to receive DES. All other patients received BMS. Systematic high-pressure balloon predilation was performed before DES or BMS implantation. The stent diameter was recorded as the maximum final balloon diameter as specified by the manufacturer. Stents were deployed at ≥12 atmospheres.

Blood sampling

To detect local hemostasis activation at the lesion site during PCI, a 6F EXPORT® aspiration catheter (Medtronic, Inc., USA) was advanced by monorail technique along the guidewire and positioned 10–20 mm distal to the coronary stenosis. Sets of blood samples were drawn from the coronary ostium through the guiding catheter, before and at the end of the procedure, and from the aspiration catheter placed distal to the lesion before balloon angioplasty, 15 min after balloon dilation, and 15 min after stent placement. This procedure was accomplished in 45–50 min in all patients. All blood samples were drawn slowly in all cases to minimize hemostatic activation and were collected in a vacutainer tube containing 3.8% trisodium citrate. All samples were immediately put on ice and centrifuged at 3,500 rpm for 10 min at 4°C to obtain platelet-poor plasma, then frozen at −80°C until the assays were performed. Initial studies have shown that the catheter itself does not cause artificial increase in levels of hemostasis markers [18]. Measurement of these markers in coronary artery circulation has been shown to be much more sensitive in detecting hemostasis activation than in venous blood [19, 20].

Biochemical determinations

To assess the level of platelet activation, levels of P-Selectin (CD62P) and soluble glycoproteine V (sGPV) were measured. Activated factor VII (FVIIa), tissue factor (TF), and prothrombin fragments 1 + 2 (F1 + 2) were measured to assess coagulation activation. Similarly, levels of D-dimers (DD), fibrinogen (FIB), tissue plasminogen activator (t-PA), and plasminogen activator inhibitor type-1 complexes (PAI-1) were measured to assess fibrinolysis activation.

Measurement of CD62P was performed by quantitative sandwich immunoassay technique (R&D Systems human sP-Selectin, GmbH, Germany), while sGPV was measured by enzyme-linked immunosorbent assay (ELISA, Asserachrom sGPV kit, Diagnostica Stago, Asnieres, France). The normal reference ranges for these two markers were 18–40 ng/ml and 10–60 ng/ml, respectively.

FVIIa was measured by a prothrombin time-based clotting test (using reagents from STACLOT® VIIa-rTF, Diagnostica Stago, Asnieres, France; normal reference range: 30–170 mU/ml). Plasma levels of TF were

measured by sandwich ELISA (IMUBIND® Tissue Factor ELISA Kit, American Diagnostica Inc (AD), France) of 1:4 diluted plasma samples. Our local laboratory considers the normal range for TF antigen in healthy subjects to be 9.5 ± 11.5 pg/ml [21]. Determination of F1 + 2 was performed by sandwich ELISA (Enzygnost® F1 + 2 micro, Dade Behring, Germany) of diluted plasma samples (1:2) to quantify the actual amount of thrombin formed. The reference range for F1 + 2 was 0.4–1.1 nmol/l.

DD were measured using a VIDAS® D-dimer Exclusion technique, a double-sandwich enzyme-linked fluorescent assay (bioMérieux® SA, Lyon, France). A DD level <500 ng/ml was considered negative. FIB was determined using the clotting method of Clauss (The STA®-Fibrinogen⑤ kit, Diagnostica Stago, Asnieres, France; normal reference range: 2.0–4.0 g/l). PAI-1 and t-PA were measured by enzyme-linked immunosorbent assay (ELISA, Asserachrom PAI-1 kit and Asserachrom t-PA kit, Diagnostica Stago, Asnieres, France; normal reference range for PAI-1 and t-PA were 4–43 ng/ml and 1–12 ng/ml, respectively). All reference values were established in larger groups of healthy individuals at the time of the standardization of the assays.

Statistical analysis and power calculation

The primary hypothesis in the study was an expected doubling of the TF levels following stent implantation as compared to baseline values. This would require a minimum of 20 patients, assuming a TF baseline value of 9.5 ± 11.5 pg/ml, and using a 5% one-sided significance level and a beta-error of 0.20. This sample size would allow the detection of a significant increase of 13.5 pg/ml between the groups of DES and BMS.

Data were analyzed using the SAS System (version 8.2, SAS Institute, Cary, North Carolina). Normality of response was verified for all data. Data are expressed as mean ± sd. Differences in baseline characteristics were analysed using the Mann Whitney test for continuous variables. Categorical variables were characterized as percentage and were compared using chi-square or Fisher's exact test. Comparisons for different sites and time points were done using a paired *t*-test and ANOVA with repeated measures for multiple comparisons. All tests were two-sided. A value of $P < 0.05$ was considered statistically significant.

Results

Baseline and clinical characteristics of study population

We prospectively screened 30 consecutive patients suffering from stable angina and undergoing elective stenting of a significant coronary stenosis, between September 2006 and January 2007. Four patients were excluded because they received multiple stents to treat a large dissection. The final analysis included 20 patients, nine of whom were treated with a DES (four paclitaxel-eluting stents [Taxus®, Boston Scientific Corp, Natick, Massachusetts], and five sirolimus-eluting stents [Cypher®, Cordis-Johnson and Johnson, Miami Lakes, Florida]) and 11 of whom received BMS [Liberté™, Boston Scientific Corp, Natick, Massachusetts]. The demographic and clinical characteristics of the patients are summarized in Table 1.

Age, gender distribution, prior history of coronary artery disease, angina class, and left ventricular ejection fraction were similar in the DES and BMS groups. There were no significant differences in cardiovascular risk factors between the two groups, except for diabetes mellitus, since all diabetic patients were treated with DES per protocol.

Table 1 Baseline clinical characteristics of the study population

Clinical characteristics	All patients ($n = 20$)	DES ($n = 9$)	BMS ($n = 11$)	*P* value
Age, years	65.7 ± 11.7	59.3 ± 9.89	65.4 ± 12.7	0.261
Male, *n* (%)	17 (85)	8 (89)	9 (82)	0.659
Diabetes mellitus, *n* (%)	6 (30)	6 (67)	0 (0)	0.001
Hypercholesterolemia, *n* (%)	15 (75)	7 (78)	8 (73)	0.194
Hypertension, *n* (%)	13 (65)	6 (67)	7 (64)	0.887
Current smokers, *n* (%)	11 (55)	5 (56)	6 (55)	0.964
Family history of CAD, *n* (%)	5 (10)	2 (22)	3 (27)	0.795
Previous MI, *n* (%)	2 (10)	1 (11)	1 (9)	0.881
Previous PTCA, *n* (%)	5 (25)	2 (22)	3 (27)	0.795
Previous CABG, *n* (%)	2 (10)	1 (11)	1 (9)	0.881
Stable angina, *n* (%)	11 (55)	5 (56)	6 (55)	0.964
Silent ischemia, *n* (%)	9 (45)	4 (44)	5 (46)	0.964
LVEF, %	60.2 ± 11.2	64.1 ± 8.50	56.9 ± 12.4	0.157

CAD = Coronary artery disease; MI = myocardial infartion; PTCA = percutaneous transluminal coronary angioplasty; CABG = coronary artery bypass graft; LVEF = left ventricular ejection fraction. DES = drug-eluting stent; BMS = bare metal stent

Angiographic characteristics of study patients

The angiographic characteristics of the study patients are displayed in Table 2. Stents were successfully implanted in all patients. Stent length was 16.9 ± 4.5 mm, and stent diameter was 3.0 ± 0.4 mm in the overall population. No acute or subacute stent thrombosis was observed during the hospital stay. All parameters related to PCI were similar in both groups.

Detection of procoagulant activity in coronary circulation

Except for TF values, baseline levels of all hemostatic markers were within the normal reference range, both in the coronary ostium and distal to the lesion. Baseline TF values were found to be three times higher than the upper limit of the normal range. However, there were no significant differences when comparing baseline values at both sites (Table 3).

Changes in hemostatic markers during PCI procedure (Table 4)

In the whole study population ($n = 20$), the levels of CD62P and sGPV did not change significantly after either balloon predilation or stent implantation, when compared with baseline values (Fig. 1).

Plasma levels of FVIIa, TF, and F1 + 2 significantly increased distal to the lesion, 15 min after balloon predilation, when compared with baseline levels. There was a significant 31% increase in FVIIa levels ($P = 0.007$) following balloon angioplasty. Similarly, TF and F1 + 2 levels significantly increased by 14% ($P = 0.004$) and 40% ($P = 0.001$), respectively. All three markers decreased and returned to baseline values 15 min after stent implantation (FVIIa: 31%, $P = 0.002$, TF: 13%, $P = 0.005$ and F1 + 2: 38%, $P = 0.004$, respectively) (Fig. 2).

Similarly, the levels of all fibrinolysis markers increased significantly distal to the lesion 15 min after balloon dilation. There was a significant 43% increase in DD levels following balloon predilation, associated with a significant increase of 46%, 60%, and 70%, in FIB, t-PA and PAI-1 levels, respectively (all $P < 0.0001$). Again, all these markers decreased and returned to baseline 15 min after stent implantation (DD: 42%, $P < 0.0001$; FIB: 31%, $P = 0.0003$; PAI-1: 60%, $P = 0.0001$; t-PA: 45%, $P = 0.0001$; respectively) (Fig. 3).

Changes in hemostatic markers: comparison between DES and BMS

There were no significant differences in the levels of local hemostatic markers between the DES and BMS groups (Table 5). Markers of platelet, coagulation, or fibrinolysis activation showed similar levels, whatever the stent

Table 2 Angiographic characteristics of the study population

Angio characteristics	All patients ($n = 20$)	DES ($n = 9$)	BMS ($n = 11$)	P value
Target coronary artery *n* (%)				
LAD	4 (20)	2 (22)	2 (18)	0.678
RCA	7 (35)	3 (33)	4 (36)	0.843
LCx	9 (45)	4 (44)	5 (45)	0.963
Lesion type*				
A	3 (15)	2 (22)	1 (9)	0.578
B	14 (70)	6 (67)	8 (72)	0.647
C	3 (15)	1 (11)	2 (18)	0.562
Preprocedure				
Lesion length (mm)	9.41 ± 4.50	9.61 ± 4.23	9.25 ± 4.94	0.560
Stent length (mm)	16.9 ± 4.50	17.5 ± 6.20	16.4 ± 4.61	0.487
MLD (mm)	1.00 ± 0.28	1.02 ± 0.21	0.99 ± 0.34	0.185
RVD (mm)	2.63 ± 0.60	2.49 ± 0.51	2.74 ± 0.67	0.672
DS (%)	60.7 ± 6.46	58.8 ± 5.27	62.4 ± 7.12	0.532
Postprocedure				
MLD (mm)	2.64 ± 0.40	2.61 ± 0.45	2.68 ± 0.37	0.753
RVD (mm)	2.88 ± 0.42	2.87 ± 0.46	2.88 ± 0.40	0.975
Balloon inflation pressure (atm)	13.3 ± 2.30	13.2 ± 1.81	13.5 ± 1.91	0.573
Stent inflation pressure (atm)	14.2 ± 2.00	14.4 ± 1.71	14.3 ± 1.90	0.450
DS (%)	8.89 ± 3.17	9.12 ± 4.28	8.69 ± 2.08	0.289

LAD = left anterior descending artery; RCA = right coronary artery; LCx = left circumflex artery; MLD = minimum lumen diameter; RVD = reference vessel diameter; DS = diameter stenosis. DES = drug-eluting stent; BMS = bare metal stent

* = According to the classification of the American College of Cardiology-American Heart Association

Table 3 Baseline levels of hemostatic activation markers

	Ostium preballoon ($n = 20$)	Lesion preballoon ($n = 20$)	P value
Platelet activation			
CD62P (ng/ml)	28.9 ± 8.47	29.5 ± 9.02	0.314
sGVP (ng/ml)	51.2 ± 12.7	49.7 ± 10.2	0.313
Coagulation activation			
FVIIa (mU/ml)	33.2 ± 12.9	33.4 ± 12.9	0.519
TF (pg/ml)	31.5 ± 7.05	31.4 ± 7.30	0.748
F1 + 2 (nmol/l)	0.75 ± 0.28	0.77 ± 0.31	0.145
Fibrinolysis activation			
DD (ng/ml)	499 ± 293	493 ± 294	0.498
FIB (g/l)	3.03 ± 0.62	2.96 ± 0.50	0.419
PAI-1 (ng/ml)	10.9 ± 3.22	10.7 ± 3.21	0.689
t-PA (ng/ml)	10.4 ± 3.10	10.3 ± 2.92	0.737

Ostium preballoon = before balloon angioplasty at coronary ostium; Lesion preballoon = before balloon angioplasty 10–20 mm distal to the lesion; CD62P = antibody P-selectin; sGPV = glycoprotein V; FVIIa = activated factor VII; TF = tissue factor; F1 + 2 = prothrombin fragments 1 + 2; DD = D-dimers; FIB = fibrinogen; t-PA = tissue plasminogen activator; PAI-1 = plasminogen activator inhibitor type-1 complexes

implanted. Particularly, values obtained 15 min after stent placement did not differ between the two stent categories.

Discussion

Local hemostasis activation following PCI procedure

To the best of our knowledge, this study is the first investigation of local hemostasis response to BMS and DES implantation. The main finding of our study is a transient, significant increase in markers of local coagulation and fibrinolysis following balloon induced vascular injury, without a corresponding increase in platelet activation. This confirms previous studies showing that arterial wall injury caused by PCI triggers transient hemostatic activation, leading to localized thrombosis and distal embolisation, although data are not consistent [22–25]. There is a strong and early activation of the hemostatic system, possibly related to endothelium and atheromatous plaque disruption in response to balloon arterial wall trauma. This phenomenon activates prothrombotic factors leading to local hemostatic activation [15, 23, 26]. Changes in these markers were not due to a systemic response, which requires a longer time to occur. Importantly, all markers of local coagulation and fibrinolysis returned to baseline values after stent implantation. These results are consistent with previous studies showing that monocyte TF activity is not increased after stent implantation in patients with stable angina [27]. Subsequent coronary artery stenting following high-pressure balloon dilation could in turn limit the hemodynamic and chemical stimuli for platelet adhesion and aggregation, reflecting local plaque stabilization and quiescence, and thus reducing hemostatic activation. Although the stent itself may probably induce hemostasis activation, this response appears negligible compared to that of balloon angioplasty. It is likely that the presence of a stent minimizes flow-limiting phenomena such as dissection or plaque rupture, known to be stimuli of local hemostasis activation. These results would need to be confirmed by larger studies, before advocating the use of direct stenting [28].

In our study, a dual antiplatelet regimen seemed to control sufficiently platelet activation in the setting of acute

Table 4 Changes in the serum concentration of the hemostatic activation markers during PCI procedure

	Ostium preballoon ($n = 20$)	Lesion preballoon ($n = 20$)	Lesion postballoon ($n = 20$)	Lesion poststent ($n = 20$)	Ostium poststent ($n = 20$)	P value (ANOVA)
Platelet activation						
CD62P (ng/ml)	28.9 ± 8.72	29.5 ± 9.02	32.4 ± 7.96	31.1 ± 9.86	28.7 ± 8.89	0.197
sGVP (ng/ml)	51.2 ± 12.7	49.7 ± 10.2	53.2 ± 15.3	52.4 ± 13.5	47.1 ± 17.2	0.173
Coagulation activation						
FVIIa (mU/ml)	33.2 ± 12.9	33.4 ± 12.9	43.7 ± 17.8	33.4 ± 12.5	32.6 ± 13.3	0.010
TF (pg/ml)	31.5 ± 7.05	31.4 ± 7.30	35.7 ± 8.05	31.7 ± 6.77	30.6 ± 6.96	<0.001
F1 + 2 (nmol/l)	0.75 ± 0.28	0.77 ± 0.31	1.08 ± 0.51	0.78 ± 0.30	0.75 ± 0.29	0.025
Fibrinolysis activation						
DD (ng/ml)	499 ± 293	493 ± 294	705 ± 338	495 ± 296	494 ± 297	0.0002
FIB (g/l)	3.03 ± 0.62	2.96 ± 0.50	4.31 ± 1.23	3.28 ± 0.84	2.93 ± 0.67	0.001
PAI-1 (ng/ml)	10.9 ± 3.22	10.7 ± 3.21	18.2 ± 7.47	11.4 ± 4.27	12.2 ± 4.12	0.002
t-PA (ng/ml)	10.4 ± 3.10	10.3 ± 2.92	16.5 ± 6.98	11.4 ± 4.23	11.7 ± 3.60	0.0007

Lesion postballoon = 15 min after balloon angioplasty 10–20 mm distal to the lesion; Lesion poststent = 15 min after stent implantation 10–20 mm distal to the lesion; Ostium poststent = 15 min after stent placement at coronary ostium

Other abbreviations as in Table 3

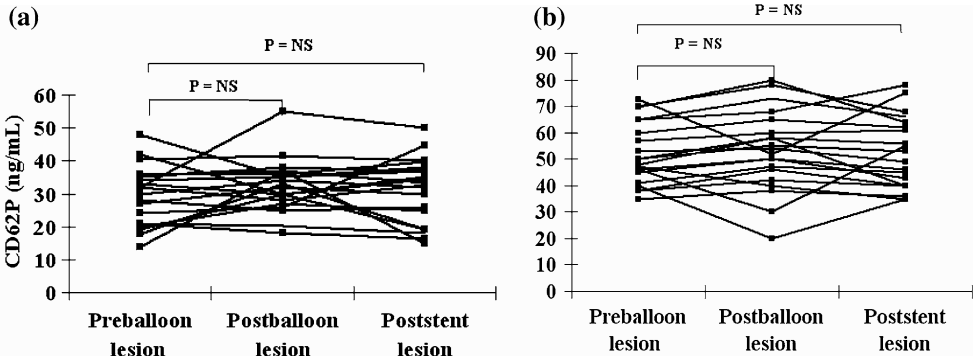

Fig. 1 Individual markers of platelet activation CD62P and sGPV. The levels of CD62P (panel A) and sGPV (panel B) were not significantly different after balloon predilation and after stent implantation when compared with baseline. NS = non significant

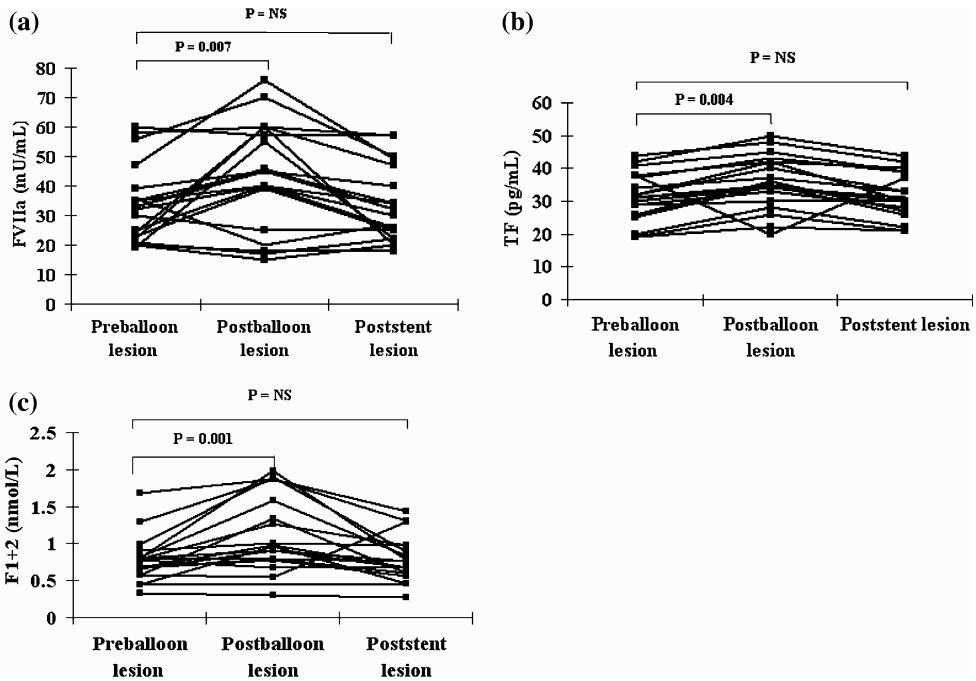

Fig. 2 Individual markers of coagulation activation FVIIa (Panel A), TF (Panel B) and F1 + 2 (Panel C). NS = non significant

vascular wall trauma after PCI. However, administration of enoxaparin before PCI does not appear to have prevented thrombin generation and fibrinolysis activation caused by high-pressure balloon injury. This result is consistent with that of Mizuno et al. [29], who demonstrated elevated levels of TF antigen in the coronary sinus blood after percutaneous transluminal coronary angioplasty (PTCA) in patients with ischemic heart disease (both stable and unstable angina). Similarly, Marmur et al. [18] reported rapid increase in TF procoagulant activity in animal models after balloon injury.

Several observations suggest that the fibrinolytic system plays an important role in hemostatic response to artery wall injury. Clinical studies investigating plasma levels of components of the plasmin activation system before and/or after PCI showed conflicting results. Several studies have suggested the existence of an increase in PAI-1 levels immediately after PCI [30, 31], although other studies reported a decrease of PAI-1 in this setting [32, 33]. FIB has been reported to be significantly related to major adverse clinical events after PCI [34]. In contrast to other markers of hemostasis, DD assays are more stable and useful for determining not only the activation of fibrinolysis but also the severity of a hypercoagulable state [35]. Our results confirm that these four markers are sensitive to vascular wall trauma as reflected by the increased levels we observed after balloon predilation. However, the implantation of a stent resulted in a return to baseline of all these values.

Comparison between DES and BMS

DES have been developed to minimize in-stent restenosis through the time-controlled release of antiproliferative and antiinflammatory medication. Despite reducing restenosis rates, the frequency of in-stent thrombosis has not decreased

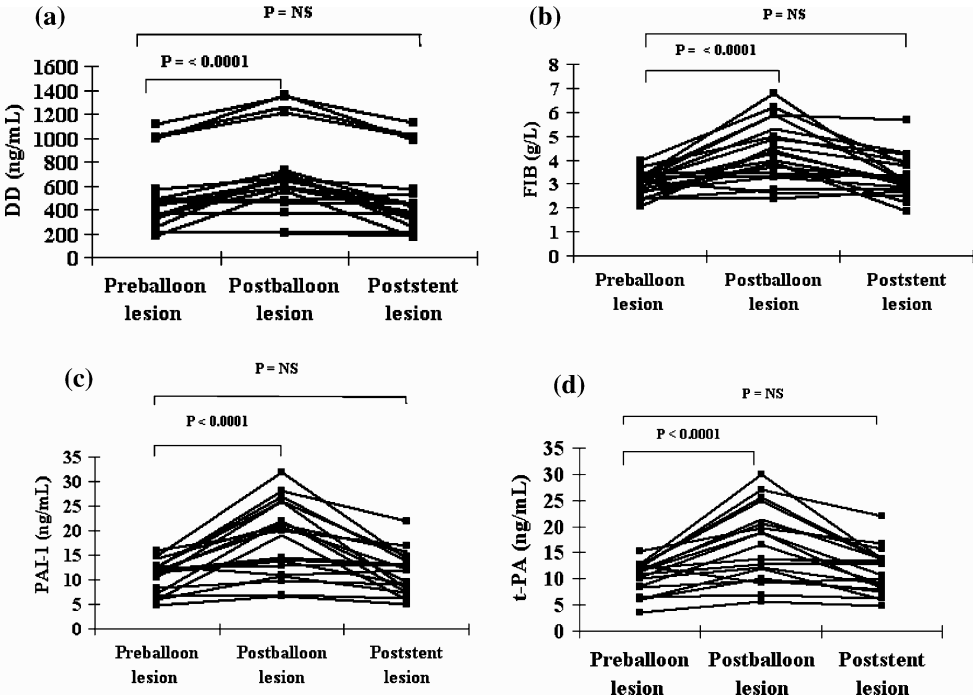

Fig. 3 Individual markers of fibrinolysis activation DD (Panel A), FIB (Panel B), PAI-1 (Panel C), and t-PA (Panel D). NS = non significant

Table 5 Changes in hemostatic markers during PCI: comparison between DES and BMS

	CD62P (ng/ml)	sGVP (ng/ml)	FVIIa (mU/ml)	TF (pg/ml)	F1 + 2 (nmol/l)	DD (ng/ml)	FIB (g/l)	PAI-1 (ng/ml)	t-PA (ng/ml)
Ostium preballoon									
DES	30.8 ± 9.18	50.4 ± 12.8	34.2 ± 12.3	31.2 ± 6.05	0.74 ± 0.28	506 ± 297	2.95 ± 0.53	11.0 ± 2.90	10.5 ± 2.52
BMS	27.3 ± 8.41	51.7 ± 13.3	26.6 ± 10.6	31.6 ± 8.07	0.76 ± 0.31	495 ± 304	3.10 ± 0.70	10.8 ± 9.82	10.3 ± 3.63
P value	0.425	0.939	0.676	1.000	0.849	0.879	0.676	0.790	0.939
Lesion preballoon									
DES	31.0 ± 8.85	51.4 ± 12.0	34.1 ± 12.9	31.0 ± 6.08	0.74 ± 0.27	501 ± 303	3.02 ± 0.44	10.9 ± 2.83	10.3 ± 2.45
BMS	28.2 ± 9.39	52.5 ± 11.9	28.2 ± 15.2	31.6 ± 8.45	0.79 ± 0.33	487 ± 303	2.92 ± 0.56	10.7 ± 3.63	10.3 ± 3.38
P value	0.403	0.723	0.790	0.819	1.000	1.000	0.820	1.000	0.790
Lesion postballoon									
DES	31.8 ± 6.47	53.1 ± 17.2	45.0 ± 17.9	35.8 ± 5.47	1.08 ± 0.58	740 ± 360	4.14 ± 0.89	16.8 ± 6.99	15.4 ± 6.39
BMS	32.9 ± 9.29	53.3 ± 14.3	51.7 ± 23.9	35.6 ± 9.96	1.08 ± 0.47	676 ± 334	4.47 ± 1.48	19.4 ± 7.97	17.4 ± 7.62
P value	1.000	0.939	0.879	0.970	0.761	0.362	0.761	0.494	0.648
Lesion poststent									
DES	31.5 ± 8.61	52.4 ± 14.7	33.3 ± 12.8	31.1 ± 5.39	0.70 ± 0.29	490 ± 303	3.46 ± 0.64	10.9 ± 3.33	10.8 ± 3.35
BMS	30.8 ± 11.2	52.3 ± 13.1	34.8 ± 15.3	32.1 ± 7.96	0.84 ± 0.31	499 ± 306	3.15 ± 0.99	11.8 ± 5.04	11.9 ± 4.95
P value	0.676	0.970	0.939	0.789	0.361	0.732	0.239	0.985	0.909
Ostium poststent									
DES	28.3 ± 8.26	46.2 ± 17.9	33.8 ± 12.9	29.4 ± 5.90	0.71 ± 0.27	498 ± 307	2.82 ± 0.55	12.6 ± 3.87	12.2 ± 2.78
BMS	29.0 ± 9.76	47.7 ± 17.5	28.5 ± 17.2	31.5 ± 7.88	0.78 ± 0.32	492 ± 305	3.03 ± 0.79	11.9 ± 4.49	11.3 ± 4.24
P value	0.879	1.000	0.732	0.620	0.543	1.000	0.970	1.000	0.621

Abbreviations as in Tables 3 and 4. DES = drug-eluting stent; BMS = bare metal stent

with DES compared with BMS [12–14]. Although several experimental studies have examined the local pharmacokinetics of stent-based drug delivery [36], the influence of DES on hemostasis in the coronary circulation is not fully understood. The relative interplay of drug and stent polymer in the hemostasis responses to DES remains unclear [16, 17, 31, 37,

38]. The question arises as to whether drug elution, the stent polymer or the stent itself affects early hemostasis activation. Our results however, did not report any difference in local hemostatic response following DES or BMS placement. The intracoronary hemostatic activation observed after balloon predilation is minimized to a similar extent by DES and BMS. These results do not support hypotheses generated from earlier clinical studies, [6, 8, 12, 39] indicating a higher risk of AST in DES implanted patients. Moreover, there is no direct evidence that drug and polymer may influence the early local hemostatic response in patients under dual antiplatelet therapy and anticoagulant pretreatment. Early stimuli for intracoronary hemostasis activation may be the injured vessel wall rather than the surface material of the stent [40]. Our results however, do not exclude the possibility of a higher risk of late stent thrombosis in DES treated patients, related to delayed endothelialization, incomplete stent deployment or enhanced platelet aggregation after clopidogrel interruption [4, 5, 7, 9, 41]. However, diabetic patients have a different pattern of systemic inflammation than non diabetic patients after PCI. In this context, the response to DES vs. BMS stenting may be outweighed by the overall temporal response to angioplasty.

Study limitations

This study suffers from several limitations. Firstly, this was not a randomized trial. As a result, because of the lack of control arm, we cannot exclude the possibility that the normalization of hemostatic markers, which occurred after stent implantation, may have similarly occurred spontaneously within the same delay in a putative control group. Secondly, there was a significant difference in diabetes mellitus between the two stent groups, since all diabetic patients were treated with DES per protocol. Thirdly, the sample size was small, and we examined only biological criteria, without any follow-up of clinical events. Fourthly, our study included only patients with stable angina or silent ischemia; thus the results may not be applicable to other subsets of patients, such as unstable angina or acute coronary syndromes. Furthermore, we concentrated on acute local hemostasis activation, and we can draw no conclusions about the possible risk of sub-acute or late thrombosis. Moreover, it is possible that pretreatment or other successful risk factor interventions initiated before the coronary intervention may have altered the plasma levels of the hemostasis markers. In this context, we found that baseline levels of TF were actually above normal values, which may be explained by the fact that all patients had documented coronary artery disease. Lastly, we did not assess markers of CRP to study the relationship between early local inflammatory response after PCI and hemostasis activation.

Conclusion

This study shows that early changes in local hemostasis activation following PCI were related to balloon predilation. Neither DES nor BMS increased markers of platelet activation, coagulation or fibrinolysis. Although this hemostatic response occurred after balloon induced vessel injury, all values returned to baseline following stent implantation under dual antiplatelet and anticoagulant pretreatment. These results would need to be confirmed by larger studies, before advocating the use of direct stenting.

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·心脏介入 Cardiac intervention·

经皮冠状动脉介入术中止血活性的早期改变及与纤溶酶原激活剂抑制物-1 基因多态性的相关性

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【摘要】 目的 探讨经皮冠状动脉介入(PCI)术中冠状动脉内血浆纤溶酶原激活剂抑制物-1(PAI-1)、D-二聚体(D-D)、凝血因子Ⅶ(FⅦa)及可溶性 P 选择素(CD62P)活性在球囊扩张和支架植入前后的早期改变及其与 PAI-1 基因多态性的相关性,评估其对急性支架血栓形成的预测价值。方法 选择稳定型心绞痛患者 20 例,按标准方法行冠状动脉造影且证实冠状动脉狭窄均在 70%以上。术中冠状动脉内血样采集顺序依次为:球囊扩张前冠状动脉入口处(Ostium)用引导导管,球囊扩张 15 min 以后及支架植入后 15 min 通过血栓吸引器穿过病灶在病变远端采血。结果 PAI-1 基因多态性在本组中分布为 4 G/5 G 型最多(12 例,60%),4 G/4 G 型其次(6 例,30%),5 G/5 G 型最少(2 例,10%)。4 G 和 5 G 等位基因频率分别为 60%和 40%。具有 PAI-1 4 G/5 G 基因型患者冠状动脉内血浆 PAI-1、D-D 以及 FⅦ活性在球囊扩张后较球囊扩张前明显升高且有统计学意义(P 均为 0.01),然而这些指标在球囊扩张前与支架植入后比较无显著性差异。结论 球囊扩张较支架植入更易损伤血管内皮并导致冠状动脉内局部、早期止血活性的一过性增高,具有 PAI-1 4 G/5 G 基因型患者对这种反应较为敏感。PCI 术前双联抗血小板药物可以有效抑制血小板活性。

【关键词】 经皮冠状动脉介入术;基因,多态性;纤溶酶原激活剂抑制-1;凝血因子Ⅶ;可溶性 P 选择素;D-二聚体

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Relationship between plasminogen activator inhibitor-1 gene polymorphism and early local hemostatic activation in patients with percutaneous coronary intervention procedure

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【Abstract】 Objective To investigate the relationship between plasminogen activatorinhibitor-1(PAI-1) 4 G/5 G gene polymorphism and local homeostatic activation of PAI-1, D-dimers(DD), activated factor Ⅶ (FⅦa) and P-Selectin(CD62P), on patients under percutaneous coronary intervention(PCI) procedures, and to evaluate its prognostic value on acute stent thrombosis by gene polymorphism analysis. Methods 20 stable angina patients with a 70% diameter stenosis by visual estimation during angiography and a clinical indication for revascularization were selected. Lesions were treated with the use of standard interventional techniques, both stents implantation underwent with adjunctive balloon angioplasty. Simultaneous blood samples were drawn in sequence from the ostium of the coronary artery before balloon angioplasty through guiding catheter, from the distal coronary artery just beyond the dilated segment after balloon angioplasty and after stent implantation,

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through aspiration catheter. Markers of PAI-1 and CD62P were measured by ELISA. Markers of FⅦ and DD were measured by technique chronometrique and ELISA VIDAS respectively. Prevalence of the 4 G/5 G polymorphism was investigated using DNA analysis. Results The distribution of PAI-1 genotypes in French people was as follows: 4 G/4 G in 30.0%, 4 G/5 G in 60.0% and 5 G/5 G in 10.0%. Among the patients, the frequency of the 4 G and 5 G allele were 0.60 and 0.40 respectively. In patients with the 4 G/5 G polymorphism of PAI-1 gene, the activities of the PAI-1, DD and FⅦa in the coronary circulation were significantly increased after balloon angioplasty in comparing with those before balloon angioplasty (P = 0.01, respectively). However, there were no significant differences between the levels of hemostatic activation at ostium before balloon angioplasty and distal to lesion after stent implantation in patients with the 4 G/5 G genotype. Conclusions Balloon angioplasty more easily induces vessel shrinkage and arterial wall injury and transient local haemostatic activation in comparing with stent implantation. This reponse would be more obvious in patients with 4 G/5 G polymorphism of the PAI-1 gene. Pretreatment with double chain antiplatelets might effectively control the early local activation of platelet in patients undergoing PCI procedure. (J Intervent Radiol, 2007, 16: 584-588)

【Key words】 Percutaneous coronary intervention; gene, polymorphism; plasminogen activator inhibitor-1; Factor Ⅶ; P-selectin; D-Dimer

众多研究表明，冠心病(CHD)患者体内血小板、凝血及纤溶系统处于不同的活化状态，血浆中血浆纤溶酶原激活剂抑制物-1 (PAI-1)、D二聚体(DD)、凝血因子Ⅶ(FⅦa)和可溶性P选择CD62P素水平增高，促进血液高凝状态及血栓形成，是CHD独立危险因素[1-4]。经皮心脏介入术(PCI)时，球囊扩张和支架植入均可激活止血系统并会影响支架内血栓和在狭窄的发生率。早年研究发现PAI-1基因启动因子序列675位存在4 G/5 G多态性，这种多态性与冠心病、心肌梗死、PCI术前后与血小板、凝血及纤溶活性的相关性已有很多报道，但各个研究结果之间存在明显差异[5-8]。本研究通过在PCI术中于冠状动脉不同部位及球囊扩张和支架植入前后不同时间采样，检测PAI-1基因多态性，选择PAI-1及DD指标做为反映纤溶系统活性，CD62P反映血小板活性以及FⅦ反映凝血系统活性，分别测定其血浆水平，以期揭示球囊扩张和支架植入对冠状动脉内早期止血活性的影响，对血栓形成的分子生物学机制作初步探讨。

1 材料与方法

1.1 对象

选取稳定型心绞痛冠心病患者20例，平均年龄(65 ±11)岁。按标准方法行冠状动脉造影、球囊预扩张和支架植入术。入选患者均经冠状动脉造影证实有冠状动脉狭窄，且均在70%以上，至少于手术前1周口服阿司匹林75～250 mg/d，术前4 d口服75 mg/d或者当日术前顿服300 mg氯吡格雷。在支架植入前，对全部病变血管实施球囊预扩张。PCI术前均给予低分子量肝素。排除有出血倾向、肾功能不全(血肌酐 > 309.4 μmol/L)、瓣膜病、左室射血分数30%以下(心力衰竭根据纽约分级Ⅲ、Ⅳ级)、48 h内急性心肌梗死(AMI)、不稳定型心绞痛、冠状动脉分叉病变以及手术前使用血小板膜糖蛋白(GPⅡb/Ⅲa)受体拮抗剂者。

1.2 方法

1.2.1 标本采集 采血在PCI手术操作中按以下步骤进行：①球囊扩张前，在冠状动脉入口处(Ostium)用引导导管。②球囊扩张后15 min，利用血栓吸引器穿过病变处在病灶远端采样。③支架植入后15 min，血栓吸引器再次穿过病处在病灶下方采样。每次用血栓吸引器抽取15 ml动脉血，分别装入3支4.5 ml(vacutainer tube containing 3.8% trisodium citrate)真空管，立即以3 500 g, 4℃离心10 min，分离血浆并置于-80℃待测。

1.2.2 4 G/5 G多态性分析

1.2.2.1 DNA提取：应用2%乙二胺乙酸(EDTA)抗凝，低渗法分离白细胞，用酚、氯仿、异戊醇法提取DNA，采用等位基因特异性聚合酶链反应(allele specific polymerase chain reaction, ASPCR)，扩增目的基因片段。

1.2.2.2 PAI-1基因启动子多态性分析：引物参照Falk等[9]设计：上游对照引物：5'-AAG CTT TTA CCA-TGGTAACCCTGGT-3'，4 G或5 G基因特异性引物分别为：5'-AGAGTCTGG-ACACGTGGGGA-3'或5'-AGAGTCTGGACACGTGGGGG-3'，共同的下游引物

5'-TGCAGCCAGCCACGTGATTGTCTAG-3'。每一标本都分别进行 2 次 PCR 扩增以检测 PAI-1 启动子基因型中的 4 G 及 5 G 等位基因。

1.2.2.3 PCR 扩增：PCR 扩增总体系 25 μl，10 × PCR 缓冲液 2.5 μl，1 ng/μl DNA，50 μmol/L dNTP (Gibco)，taq DNA 聚合酶 0.4 U，上游引物 200 nmol/L，下游引物 400 nmol/L，$MgCl_2$ 1.1 mmol/L，KCl 50 mmol/L，双蒸馏水补足加至 25 μl，在 PCR 合成仪上合成 DNA 片段。PCR 循环参数：94℃ 5 min 预变性后进入 30 轮循环：94℃变性 35 s，65℃退火 35 s，72℃延伸 70 s。最后 72℃再延伸 5 min。

1.2.2.4 电泳：取 PCR 扩增产物，放入 2%的琼脂糖凝胶电泳，电压 110V，时间 1 ～2 h，置紫外灯下显影，获得 3 种基因型：4 G/4 G、5 G/5 G、4 G/5 G。

1.2.3 血浆 PAI-1、CD62P、FⅦ以及 D-D 活性测定 PAI-1 及 CD62P 采用 ELISA 法，PAI-1 试剂盒由 Asserachrom PAI-1 kit, Diagnostica Stago, Asnières, France 提供，CD62P 试剂盒由 R&D Systems GmbH, Germany 提供。FⅦ采用 Technique chronometrique 方法，DD 采用 ELISA D-dimer assay Vidas® 方法，试剂盒均由 Diagnostica Stago, France 提供。

1.3 统计学处理

1.3.1 样本量的估计 为保证研究结论具有一定可靠性，在课题设计阶段用统计学方法估计了最少受试对象数。以 CD62P 在支架植入后增加 2 个标准差做假设，总体标准差为(18 ±9.0) ng/mL，容许误差为 18 ng/ml，取单侧 $\alpha = 0.05$，$\beta = 0.10$(检验功效为 90%)，共需要 20 例冠心病患者。

1.3.2 所有数据用 SAS (version 9.1, SAS Institute, Cary, North Carolina) 软件处理 首先对全部数据做正态性检验。计量资料采用重复测量资料方差分析及配对 t 检验，计数资料采用卡方检验(χ^2)，结果以均数 ±标准差表示，$P < 0.05$ 为差异有统计学意义。根据 Hardy Weinberg 平衡定律计算各基因型个体数的期望值，以 χ^2 检验进行 Hardy Weinberg 平衡吻合性检验。

2 结果

2.1 PAI-1 4 G/5 G 基因型分布与等位基因频率

PAI-1 基因多态性在本组患者中分布 4 G/5 G 型 12 例(60%)，4 G/4 G 型 6 例(30%)，5 G/5 G 型 2 例(10%)。4 G 和 5 G 等位基因频率分别为 60% 和 40%。通过 Hardy-Weinberg 遗传定律证实样本人群 PAI-1 的基因变异频率符合遗传平衡。

2.2 在 PCI 术中冠状动脉内早期止血活性的改变及其与 PAI-1 4 G/5 G 基因多态性的相关性

术后未发生支架内血栓。在 PCI 手术过程中，球囊扩张前 Ostium 处 FⅦa、PAI-1、D-D 及 CD62P 活性与球囊扩张后 15 min 及支架植入后 15 min 比较，球囊扩张后各个指标均增高，其中 FⅦa、PAI-1、D-D 变化差异有统计学意义(表 1)。各种止血活性的基线水平 (球囊扩张前在 Ostium 处)，4 G/4 G 基因型者最高，4 G/5 G 其次，5 G/5 G 最低，但 3 种基因型间比较无统计学差异 (表 2)。具有 PAI-1 4 G/5 G 基因型患者血浆 FⅦa、PAI-1 和 D-D 活性在球囊扩张后明显升高，与球囊扩张前比较有统计学差异($P = 0.03$, $P = 0.006$, $P = 0.02$)。CD62P 的活性在球囊扩张前后无明显改变 ($P = 0.15$)。同样，这些指标在球囊扩张前处与支架植入后及各种基因型之间比较无统计学差异(表 1, 2)。

表 1 PCI 术中血浆止血活性在不同时间的改变($n = 20$)

检测指标	球囊扩张前	球囊扩张后	支架植入后
凝血活性			
FⅦa(mu/ml)	33.2 ± 12.9	43.7 ± 17.8*	33.4 ± 12.5
纤溶活性			
D-D(g/L)	499 ± 293	705 ± 338*	495 ± 296
PAI-1(ng/ml)	10.9 ± 3.22	18.2 ± 7.47*	11.4 ± 4.27
血小板活性			
CD62P(ng/ml)	28.9 ± 8.72	32.4 ± 9.86	31.1 ± 9.86

* 用配对 t 检验比较不同时间的各种止血活性，球囊扩张前后比较 FⅦa、D-D 及 PAI-1 有显著性差异 ($P = 0.0001$; $P < 0.0001$; $P = 0.0002$)

表 2 在不同时间止血活性与 PAI-1 基因多态性

检测指标	4G/4G($n = 6$)	4G/5G($n = 12$)	5G/5G($n = 2$)
球囊扩张前			
FⅦa(mu/ml)	41.8 ± 32.3	27.6 ± 12.5	22.0 ± 4.24
CD62P(ng/ml)	31.6 ± 15.8	27.9 ± 7.31	20.8 ± 4.53
PAI-1(ng/ml)	13.6 ± 6.01	12.6 ± 6.05	7.85 ± 6.59
D-D(g/L)	543 ± 547	475 ± 482	398 ± 146
球囊扩张后			
FⅦa(mu/ml)	50.0 ± 21.9	50.2 ± 24.9*	48.9 ± 22.5
CD62P(ng/ml)	32.8 ± 7.91	32.2 ± 8.04	31.3 ± 7.14
PAI-1(ng/ml)	13.3 ± 9.36	20.6 ± 7.46*	12.8 ± 7.91
D-D	755 ± 517	797 ± 464*	715 ± 447
支架置入后			
FⅦa(mu/ml)	31.3 ± 17.2	33.2 ± 15.3	30.0 ± 2.83
CD62P(ng/ml)	30.8 ± 13.5	30.6 ± 10.5	37.7 ± 20.2
PAI-1(ng/ml)	11.9 ± 11.8	12.3 ± 5.73	12.2 ± 4.74
D-D(g/L)	588 ± 688	466 ± 433	435 ± 131

* 用配对 t 检验对球囊扩张前后各种止血活性与不同基因型分析，在 4 G/5 G 基因型中 FⅦ、PAI-1、及 D-D 有统计学差异($P = 0.006$; $P = 0.03$; $P = 0.02$)

3 讨论

本研究通过对 20 例法国 CHD 患者冠状动脉循环血液基因检测，发现 PAI-1 4 G/5 G 基因型分布为4 G/5 G 型最多，4 G/4 G 型其次，5 G/5 G 型最少，4 G 和 5 G 等位基因频率分别为 60%和40%，4 G/5 G 型发生 CHD 的频率较其他两型高，提示遗传因素在动脉硬化及血栓性疾病中可能起重要作用：具有 PAI-1 4 G 等位基因频率较 5 G 等位基因频率的人罹患 CHD 的概率高，PAI-1 基因 4 G/5 G 多态性可能部分决定了 CHD 发病的倾向性。我们还发现 CHD 患者血浆 PAI-1、D-D、FⅦa 及 CD62P 基线活性在 4 G/5 G 及 4 G/4 G 型明显高于 5 G/5 G 型，这与 Eriksson 等[10]，Johan-Vague 等[5]，ten Boeked 等[11]报道结果一致，提示多基因遗传性疾病的遗传基础，PAI-1 基因 4 G/5 G 多态性是血浆 PAI-1，D-D，FⅦa 及 CD62P 的重要影响因素，同时也说明这些止血活性存在基因依赖的密切相关性，PAI-1 基因的 4 G 纯合子个体及 PAI-1，D-D，FⅦ及 CD62P 活性高者可能存在 CHD 易患倾向。关于 PAI-1 基因 4 G/5 G 多态性对这些止血活性的影响机制以及在 CHD 发病中的作用，PAI-1 基因 4 G/5 G 多态性是否为 CHD 的独立危险因素，是否与其他危险因素共同参与了该病的发生发展需要进一步大样本研究。

CHD 是一种血栓性多基因遗传病。血栓性疾病患者本身已存在血小板、凝血及纤溶系统间功能失调，血小板及凝血功能亢进，纤溶活性受损和功能失调。PCI 手术中当球囊在动脉硬化狭窄节段加压扩张时，进行性增加的扩张力施加在动脉粥样硬化斑块和血管壁上，斑块会在最薄弱处或与正常组织交界处撕裂，血管中层及外膜伸展以适应扩张球囊的大小，由于球囊扩张和支架对血管壁的损伤和牵拉作用，引起血管内膜急性损伤反应，从而进一步加重局部乃至全身凝血-纤溶系统平衡，易导致局部急性或亚急性血栓形成。本研究显示 CD62P 活性尽管在球囊扩张后也轻度升高，但无统计学差异，说明是术前双联抗血小板药物对 CD62P 活性的抑制作用，由于 CD62P 最灵敏，是反映血小板活化与释放最特异的‘金标准’标志物，在形成局部血栓和参与止血及动脉硬化的发生中起中心环节作用[12]，因此可以排除这个代表血小板活性的指标对球囊扩张机械性损伤的不敏感性。我们的研究还显示 PCI 手术过程中，球囊扩张后 PAI-1、D-D 以及 FⅦa 活性比球囊扩张前明显升高且有显著性差异，但球囊扩张前后与支架植入后相比无明显改变，提示球囊扩张较支架植入更易导致人为的内皮细胞及动脉中层弹力纤维的机械性撕裂、粥样斑块挤压破裂、内皮下促凝，从而激活凝血及纤溶系统[12]，而且这种改变可能是一过性和可逆性的。此外还提示手术前预防性双联抗血小板药物不能完全抑制由球囊扩张后对 PAI-1、D-D 代表的纤溶活性和 FⅦ代表的凝血活性，但这种推测需要与未用药的对照组比较以深入探讨。

本研究最重要的发现就是当我们比较 3 种基因多态性中所有止血活性，其中 CD62P 在球囊扩张前后及支架植入后均无改变，提示术前联合抗血小板治疗对具有 3 种基因型的患者血小板活性有相同的抑制作用。4 G/5 G 基因型患者血浆 PAI-1、D-D、FⅦa 活性在球囊扩张后最高，说明具有 4 G/5 G 基因型的患者血管内皮，凝血及纤溶活性可能对这种人为的机械性刺激较为敏感，这也许是造成极少数患者发生急性与亚急性支架内血栓形成的原因之一。直接冠状动脉支架植入术可能会减少一系列止血活性改变，同时可以减少球囊扩张后引起内膜撕裂的发生率及预扩张时心肌缺血事件的发生率，但有待进一步大样本临床研究。尽管在球囊扩张后各种止血活性一过性升高有可能预示随后发生急性支架内血栓的危险性增加，但支架植入后这些止血活性无明显继续升高说明支架具有一定的稳定血管内皮作用，这个作用可能与药物支架的抗炎作用及支架本身对损伤血管内皮的支撑、压迫作用有关，具体原理还需要进一步探讨。此外，手术前抗血小板治疗是关系到手术时使药物起效峰值的关键，早期手术前预防性联合抗血小板及抗凝治疗可以有效抑制冠状动脉内止血活性，降低急性支架血栓的发生率。因此尤其对具有 4 G 等位基因频率的 CHD 高危人群及需要行 PCI 者，在 PCI 中轻柔操作，进行预防性的抗血小板、抗凝、增强纤溶活性以及保护内皮治疗是预防急性与亚急性支架血栓的关键。

综上所述，本研究主要针对 PCI 术前给予双联抗血小板及抗凝治疗的稳定型心绞痛患者，在冠状动脉内不同部位采样，直接评估冠状动脉内病变灶局部因 PCI 术导致的血小板、抗凝及纤溶活性的早期改变以及与 PAI-1 基因多态性的相关性。尽管样本量少，但经过统计学分析得出，研究结果有一定的可靠性。全部血样来自冠状动脉不同部位（冠状动脉入口及病变灶下段），4 个不同时间（球囊预扩

张前、后，支架植入前、后）共抽取了 120 例（份）血样，每例患者为自身对照，配对分析。由于入选条件严格，操作困难等原因限制了基因研究的样本量，因此有待进一步的大样本研究。

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Original article

Early local intracoronary platelet activation after drug-eluting stent placement

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Keywords: *stents; platelet activation; thrombosis; P-selectin; soluble glycoprotein V*

Background Early local platelet activation after coronary intervention identifies patients at increased risk of acute stent thrombosis (AST). However, early changes in platelet activation in coronary circulation following drug-eluting stent (DES) implantation have never been reported.

Methods In a prospective study of 26 consecutive elective stable angina patients, platelet activation was analyzed by measuring soluble glycoprotein V (sGPV) and P-selectin (CD62P) before and after implantation of either DES or bare metal stent (BMS). All patients were pretreated with clopidogrel (300 mg loading dose) and aspirin (75 mg orally) the day before the procedure. Blood samples were drawn from the coronary ostium and 10 − 20 mm distal to the lesion site.

Results Consistent with the lower baseline clinical risk, the levels of CD62P and sGPV were within normal reference range, both in the coronary ostium and distal to the lesion before percutaneous coronary intervention (PCI) procedure. The levels of CD62P and sGPV did not change significantly (CD62P: (31.1 ± 9.86) ng/ml vs (29.5 ± 9.02) ng/ml, P=0.319 and sGPV: (52.4 ± 13.5) ng/ml vs (51.8 ± 11.7) ng/ml, P=0.674, respectively) after stent implantation when compared with baseline. Changes in these platelet activation markers did not differ between stent types.

Conclusions Intracoronary local platelet activation does not occur in stable angina patients before and immediately following DES implantation when dual anti-platelet is administered.

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Despite reduced restenosis rates, the use of drug-eluting stent (DES) did not reduce the occurrence of stent thrombosis as compared with bare metal stent (BMS).[1-4] Acute stent thrombosis (AST) is a rare but severe complication of percutaneous coronary intervention (PCI). Platelet activation is known to play a key role in the development of AST. The implantation of coronary stents induces vascular wall damage with an altered local flow, and consequently, activates the intrinsic pathway of the coagulation system, which is an important pathophysiological element of subsequent acute and subacute arterial thrombosis that limits the immediate and short-term success of PCI. Previous studies have examined changes in platelet activation in the coronary circulation during PCI.[5-7] However, consistent results have not been obtained. In addition, these studies only focused on changes in platelet activation markers in coronary circulation following balloon angioplasty and/or BMS implantation. Recent studies have shown that both rapamycin- and paclitaxel-induced tissue factor expressions may contribute to a prothrombotic environment after deployment of DES.[8-10] Thus, the question arises as to whether rapamycin- and paclitaxel-eluting also affects platelet activation, especially immediately after DES implantation.

To investigate possible differences in terms of early local platelet response among patients implanted with different types of coronary stents, we assayed in this study the levels of sensitive markers P-selectin (CD62P) and soluble glycoprotein V (sGPV) in the coronary ostium and beyond the lesion both before and 15 minutes after stent placement in stable angina patients with dual antiplatelet and anticoagulant pretreatment and implanted with DES or BMS.

METHODS

Study population

In a protocol approved by the local Ethics Committee, twenty-six consecutive stable angina or silent ischemia patients undergoing coronary stenting were enrolled in a prospective registry after written informed consent had been obtained. Patients with diabetes mellitus, proximal left anterior descending stenosis, or reference vessel

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diameter < 3.0 mm by visual estimate were assigned to receive DES. Among them, 15 of whom were treated with a DES (10 paclitaxel-eluting stents (Taxus®, Boston Scientific Corp., Natick, Massachusetts, USA), and 5 sirolimus-eluting stents (Cypher®, Cordis-Johnson and Johnson, Miami Lakes, Florida, USA)) and 11 of whom received BMS (LiberteTM, Boston Scientific Corp.). Exclusion criteria included prior history of bleeding diathesis, stroke within the last 3 months, accompanying valvular disease, an ejection fraction below 30% (severe heart failure (New York Heart Association III, IV)), acute coronary syndrome (ST and non ST elevation myocardial infarction) within the last 7 days, unstable angina, bifurcation lesion, multiple stents implantation, renal insufficiency with creatinine levels of > 3.5 mg/dl, active neoplasie, and prior GP IIb/IIIa inhibitor therapy.

Procedures and relevant medications

All procedures were performed with standard interventional techniques. Systematic high-pressure balloon predilation was performed before DES or BMS implantation. The stent diameter was recorded as the maximum final balloon diameter as specified by the manufacturer. Stents were deployed at ≥ 1215.9 kPa (12 atmospheres).

All patients were treated with aspirin 75 mg daily for at least 4 days before the procedure. Clopidogrel was administered at a 300 mg loading dose the day before the procedure. Low molecular weight heparin (enoxaparin: 0.75 mg per kilogram of body weight) was administered intravenously at the beginning of the procedure, without anticoagulation monitoring. All patients were on statin therapy. Drugs other than aspirin, clopidogrel and statin, considered acceptable before blood sampling were beta-blockers, calciumchannel blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor blocker.

Blood sampling

Sets of blood samples were drawn from different sites and at different time points. To detect local hemostasis activation at the lesion site during PCI, a 6F EXPORT® aspiration catheter (Medtronic, Inc., USA) was advanced by monorail technique along the guidewire and positioned 10–20 mm distal to the coronary stenosis. Sets of blood samples were drawn consequently from the coronary ostium through the guiding catheter, before and at the end of the procedure, and from the aspiration catheter placed distal to the lesion before and 15 minutes after stent placement. This procedure was accomplished in 45 to 50 minutes in all patients. All blood samples were drawn slowly in all cases to minimize hemostatic activation and were collected in a vacutainer tube containing 3.8% trisodium citrate. All samples were immediately put on ice and centrifuged at 3500 r/min for 10 minutes at 4°C to obtain platelet poor plasma, then frozen at –80°C until the assays were performed.

Biochemic determinations

Measurement of CD62P was performed by quantitative sandwich immunoassay technique (R&D Systems Human sP-selectin, GmbH, Germany), while sGPV was measured by enzyme-linked immunosorbent assay (ELISA, Asserachrom sGPV kit, Diagnostica Stago, Asnières, France). The normal reference ranges for these two markers were 18 to 40 ng/ml and 10 to 60 ng/ml, respectively.

Statistical analysis

We hypothesized that values of CD62P would be increased by two standard deviations (18 ng/ml) between the time points of the study in patients undergoing DES implantation. This would require a minimum of 20 patients to complete the study with a power of 90% and a significance of < 0.05.

Data were analyzed using the SAS System (version 8.2, SAS Institute, Cary, North Carolina, USA). Normality tests were performed on all data. Data are expressed as mean ± standard deviation (SD). Differences in baseline characteristics were analysed using the Mann Whitney test for continuous variables. Categorical variables were characterized as percentages and were compared using chi-square or Fisher's exact test. Comparisons for different sites and time points were done using a paired *t* test and analysis of variance (ANOVA) with repeated measures for multiple comparisons. All tests were two-sided. A value of $P<0.05$ was considered statistically significant.

RESULTS

Demographics and clinical characteristics of study patients

The demographic and clinical characteristics of the patients are summarized in Table 1. Age, gender distribution, prior history of coronary artery disease, angina class and left ventricular ejection fraction were similar in the DES and BMS groups. There were no significant differences in cardiovascular risk factors between the two groups, except for diabetes mellitus, since all diabetic patients were treated with DES.

Angiographic characteristics of study patients

The angiographic characteristics of the study patients are displayed in Table 2. Stents were successfully implanted in all patients. Stent length was (16.9 ± 4.5) mm, and stent diameter was (3.0 ± 0.4) mm in the overall population. No acute or subacute stent thrombosis was observed during the hospital stay. All parameters related to the PCI were similar in the DES and BMS groups.

Detection of procoagulant activity in coronary circulation

At baseline, the levels of CD62P and sGPV were within normal reference range, either in the coronary ostium or distal to the lesion. There were no significant differences

Table 1. Baseline clinical characteristics of the study population

Clinical characteristics	DES (n = 15)	BMS (n = 11)	P value
Age (years)	59.3 ± 9.9	65.4 ± 12.7	0.261
Male (n (%))	13 (86.6)	9 (81.8)	0.259
Diabetes mellitus (n (%))	10 (66.6)	0 (0)	0.001
Hypercholesterolemia (n (%))	11 (73.3)	8 (72.7)	0.990
Hypertension (n (%))	9 (60)	7 (63.6)	0.756
Current smokers (n (%))	8 (53.3)	6 (54.5)	0.864
Family history of CAD (n (%))	4 (26.6)	3 (27.2)	0.895
Previous MI (n (%))	2 (13.3)	1 (9)	0.881
Previous PTCA (n (%))	4 (26.6)	3 (27.2)	0.895
Previous CABG (n (%))	2 (13.3)	1 (9)	0.881
Stable angina (n (%))	8 (53.3)	6 (54.5)	0.964
Silent ischemia (n (%))	6 (40)	5 (45.4)	0.749
LVEF (%)	64.1 ± 8.50	56.9 ± 12.4	0.157
Medications			
Aspirin (n (%))	15 (100)	11 (100)	1.000
Clopidogrel (n (%))	15 (100)	11 (100)	1.000
Enoxaparin (n (%))	15 (100)	11 (100)	1.000
β-blockers (n (%))	6 (40)	4 (36.3)	0.930
ACE inhibitors (n (%))	9 (60)	8 (72.7)	0.194
Calcium-channel blocker (n (%))	5 (33.3)	3 (27.2)	0.123
Statin (n (%))	13 (86.6)	9 (81.8)	0.876
Angiotensin II receptor bloker (n (%))	4 (26.6)	3 (27.2)	0.941

Values are presented as number (percentages) or mean ± SD; CAD = coronary artery disease; MI = myocardial infartion; PTCA = percutaneous transluminal coronary angioplasty; CABG = coronary artery bypass graft; LVEF = left ventricular ejection fraction. DES = drug-eluting stent; BMS = bare metal stent; P value = drug-eluting stent compared with bare metal stent.

Table 2. Angiographic characteristics of the study population

Angio characteristics	DES (n=15)	BMS (n=11)	P values
Target coronary artery (n (%))			
LAD	3 (20.0)	2 (18.2)	0.678
RCA	5 (33.3)	4 (36.3)	0.243
LCX	7 (46.6)	5 (45.4)	0.864
Lesion type* (n (%))			
A	3 (20.0)	2 (18.2)	0.678
B	9 (60.0)	8 (72.0)	0.147
C	3 (20.0)	2 (18.2)	0.678
Preprocedure			
Lesion length (mm)	9.61 ± 4.23	9.25 ± 4.94	0.560
Stent length (mm)	17.50 ± 6.20	16.4 ± 4.61	0.487
MLD (mm)	1.02 ± 0.21	0.99 ± 0.34	0.185
RVD (mm)	2.49 ± 0.51	2.74 ± 0.67	0.672
DS (%)	58.80 ± 5.27	62.40 ± 7.12	0.532
Postprocedure			
MLD (mm)	2.61 ± 0.45	2.68 ± 0.37	0.753
RVD (mm)	2.87 ± 0.46	2.88 ± 0.40	0.975
Balloon inflation pressure (kPa)	1337.49 ± 183.40	1367.89 ± 193.53	0.573
Stent inflation pressure (kPa)	1459.08 ± 173.27	1448.95 ± 192.52	0.450
DS (%)	9.12 ± 4.28	8.69 ± 2.08	0.289
Total stent implanted (n (%))	15 (100)	11 (100)	0.056

LAD = left anterior descending artery; RCA = right coronary artery; LCX = left circumflex artery; MLD = minimum lumen diameter; RVD = reference vessel diameter; DS = diameter stenosis. *According to the classification of the American College of Cardiology-American Heart Association. Other abbreviations as in Table 1. 1 kPa = 0.009869 atm.

(CD62P: (28.9 ± 8.7) ng/ml vs (28.4 ± 9.1) ng/ml, P = 0.639 and sGPV: (51.2 ± 12.7) ng/ml vs (51.7 ± 11.6) ng/ml, P=0.312, respectively) when comparing baseline values at both sites (Table 3).

Changes in platelet activation markers after stent implantation

In the whole study population (n=26), the levels of CD62P and sGPV did not change significantly when compared pre- and post-stent implantation at beyond the lesion. Similarly, values obtained 15 minutes post-stent placement at ostium did not significantly differ when compared with pre-stent placement at ostium. In addition, the level of sGPV decreases post-stent implantation when compared with pre-stent placement at ostium (Table 3).

Table 3. Changes in platelet activation markers before and after stent implantation (n=26)

Platelet activation (ng/ml)	Pre-stent		Post-stent		P values
	Ostium	Lesion	Lesion	Ostium	
CD62P	28.9±8.7	28.4±9.1	29.3±10.0	28.7±8.9	0.197
sGVP	51.2±12.7	51.7±11.6	52.2±13.3	47.0±17.2	0.070

Ostium pre-stent = before stent implantation at coronary ostium; Lesion pre-stent = before stent inplantation at 10 to 20 mm distal to the lesion; Lesion post-stent = 15 minutes after stent inplantation at 10 to 20 mm distal to the lesion; Ostium post-stent = 15 minutes after stent inplantation at coronary ostium; CD62P = antibody P-selectin; sGPV = glycoprotein V. Other abbreviations as in Table 1.

Changes in platelet activation markers between DES and BMS placement

Markers of CD62P and sGPV were measured at similar levels, whatever the stent implanted. Particularly, values obtained 15 minutes post-stent placement at beyond the lesion and ostium did not significantly differ between the two stent categories when compared with baseline respectively (Fig).

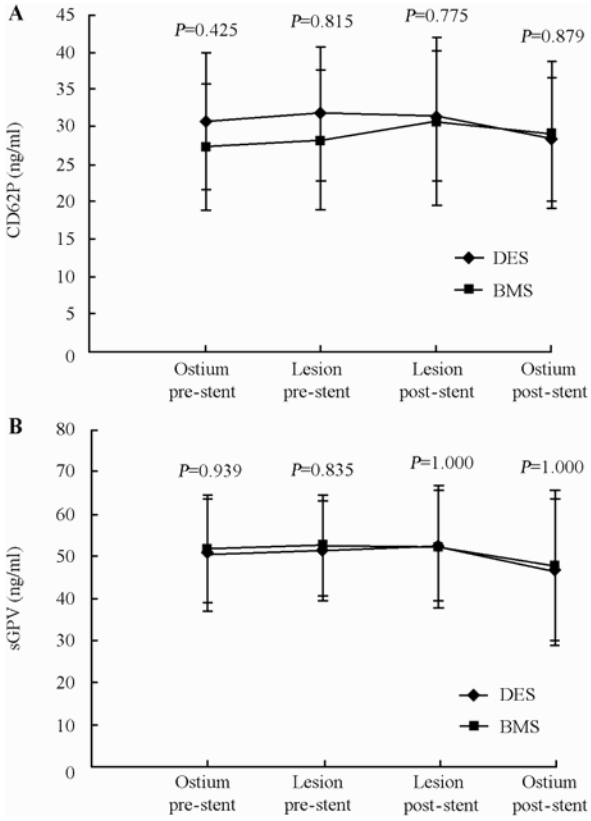

Fig. Changes in local platelet activation markers during PCI: comparison between DES and BMS.

DISCUSSION

To our knowledge, this study is the first investigation of

the early local platelet response to DES implantation in stable angina patients with dual antiplatelet and anticoagulant pretreatment. PCI, especially with stenting, leads to significant platelet activation at the site of intervention.[11] The activated platelets contribute to the risk of thromboembolism at the time of procedure and increase the risk of acute or subacute thrombosis after stenting.[12] However, clopidogrel combined with aspirin reduces the early risk of stent thrombosis, and a clopidogrel pre-treatment strategy is associated with a better outcome.[13,14] Although thrombin activity and increased platelet reactivity may be evident in blood from the coronary circulation, optimal antiplatelet and anticoagulant therapy must suppress both of these, at the site of injury and also in the circulation blood pool. In fact, efficacy of treatment is likely to be most closely reflected by demonstrable suppression of pro-thrombotic activity in the immediate vicinity of the site of plaque rupture.[15] Therefore there is a great interest in the measurement of local platelet activation in coronary circulation undergoing DES or BMS in patients with antithrombotic pretreatment.

Platelet associated P-selectin (CD62P) is a 140-kD glycoprotein which is present in the a-granules of platelets and translocates rapidly to the cell surface after platelet activation.[16] CD62P mediates rolling of platelets on activated endothelial cells and interaction of activated platelets with neutrophils and monocytes, and is generally considered to be the gold standard marker of platelet activation.[17] Increased levels of CD62P have been observed in various cardiovascular disorders, including unstable angina[18] (it could be used as a marker of plaque destabilization), acute myocardial infarction,[19] even in patients with coronary artery spasm,[20] stable angina.[21] These findings suggest an important role of CD62P in arterial thrombosis.

A new thrombosis marker, sGPV, has recently been evaluated in patients with coronary artery diseases. The exact function of sGPV in primary haemostasis is still unknown but it has been proposed to modulate collagen and thrombin dependent platelet responses.[22] sGPV is cleaved after exposure of platelets to thrombin, and also by endogenous metalloproteases produced during platelet activation.[23] sGPV has been evaluated in experimental thrombosis in man as a thrombosis marker in atherosclerosis[24] and myocardial infarction.[25] However, there have been no reports of the early change of DES or BMS for coronary intervention on sGPV in the coronary circulation.

In the literature there is no evidence of any correlation between DES and hemostasis disorders, except tissue factor expressions.[8,10] The principal finding of this study is that both CD62P and sGPV levels do not change immediately in patients with stable angina pectoris undergoing elective coronary DES for *de novo*, single coronary artery lesion. This response in PCI appears similar in patients treated with BMS. Our finding suggests that there is no direct evidence that either the drug or the polymer may influence the early local platelet response. Thus, we can assume that the risk of AST was less and similar in both the DES and BMS implantations in patients with stable angina and pretreatment with dual anti-platelet medication. However, our result, which might be explained by the early blood sampling at local injury site, was not due to a systemic response, which would require a longer period of time to occur. Consistent with our results, the preclinical experience in a pig model also shows that implantation of DES did not prove to be more thrombogenic in the first 24 hours after the intervention as compared with BMS.[26]

Anti-thrombotic and anticoagulant therapies minimize thrombotic complications after PCI. The baseline platelet characteristics are predictive of future platelet activity and thus may identify patients who would be most likely to benefit from more aggressive antiplatelet and anticoagulant regimens.[27] In this study, the three-drug anti-thrombotic regimen maintained the hemostasis activation at the low state achieved before the procedure. Our results demonstrated that detection of CD62P and sGPV levels in the coronary ostium and in the coronary artery beyond lesion were similar before PCI procedure and they were within normal reference range. This finding suggests that the baseline platelet characteristics are predictive of a low risk of stent thrombosis in patients with stable angina.

Acetylsalicylic acid, commonly referred to as aspirin, is a widely used and very well documented antiplatelet drug that lowers cardiovascular morbidity and mortality.[28,29] The antiplatelet effects of aspirin are related to irreversible inhibition of platelet cyclooxygenase (COX), and reduced production of the potent vasoconstrictor and platelet activator thromboxane A2 (TxA2). Recent placebo-controlled trials have shown excellent protection with 75 mg aspirin daily in patients with stable[30] or unstable[31] angina pectoris. More recently, the Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) study demonstrated that the addition of clopidogrel to aspirin was associated with a further 20% reduction in the risk of death, myocardial infarction or stroke in patients with acute coronary syndromes.[32] Similarly, some recent studies have shown that clopidogrel, particularly as a loading dose of 300 mg, can rapidly and significantly inhibit platelet aggregation in both healthy subjects and patients with atherosclerotic disease, even after just 2 hours.[33] Enoxaparin is being used more frequently in patients undergoing percutaneous coronary intervention. Theoretical advantages of low-molecular-weight heparin versus unfractionated heparin include a higher ratio of anti-Xa to anti-IIa activity (3:1 for enoxaparin), a more predictable dose response that precludes the need for frequent monitoring, and the convenience of subcutaneous administration. Several retrospective observational studies have suggested that pretreatment

with statins might reduce the incidence of myocardial infarction after coronary intervention.[34] In addition, a previous study has demonstrated that angiotensin II receptor inhibitor has anti-inflammatory properties and inhibits platelet aggregation.[35]

Our study demonstrated that during PCI, no changes occurred in levels of platelet activation markers in patients under dual anti-platelet therapy pretreatment. This finding suggests the rapid effect of dual anti-platelet pretreatment in attenuating intra-coronary platelet activity. Our results also suggested that dual anti-platelet therapy (aspirin and clopidogrel) pretreatment sufficiently inhibited platelet activation in patients with angina pectoris who underwent DES or BMS. Corroborating our results, Mizuno et al[36] reported no changes in platelet activation in the balloon angioplasty only and stent implantation groups when aspirin and ticlopidine administration; in contrast, the levels of platelet activation markers significantly increased immediately after percutaneous transluminal rotational coronary atherectomy in patients without using antiplatelet therapies. Similarly, Gregorini et al[6] reported that the combined use of ticlopidine, aspirin, and heparin effectively inhibited platelet activation in patients with angina pectoris who underwent PTCA. In addition, in the present study, the widespread use of statins, angiotensin converting enzyme inhibitors and angiotensin receptor blockers maybe also has played important roles in the inhibition of platelet activation.

In summary, this study shows, first, that neither DES nor BMS increased marker levels of platelet activation when dual anti-platelet and anti-coagulant pretreatment is administered to patients with stable angina. Second, platelet activation markers such as CD62P and sGPV seem to be helpful in estimating anti-platelet treatment efficiency. Thus, our findings might have important therapeutic implications. If so, pretreatment with anti-thrombotic medications in patients with stable angina could be effectively used to prevent or limit the initial injury following coronary DES or BMS implantation.

Although the study population was relatively small, we have fulfilled conditions for statistical power calculation. The level of each marker was sampled for comparison at 4 different sites and 2 time points (before and after stent implantation) in each patient, thus 80 time periods were available for comparison for each of the 2 activation platelet markers. In our study, first, we examined only biological criteria, without any follow-up of clinical events. Second, our study included only patients with stable angina or silent ischemia; thus the results may not be applicable to other subsets of patients, such as unstable angina or acute coronary syndromes. Furthermore, we concentrated on acute local hemostasis activation, and we can draw no conclusions about the possible risk of sub-acute or late thrombosis. Last, we have no data about a control group to compare with the pretreatment group, and this comparison needs further investigation.

DES does not increase early local platelet activation, at least under dual anti-platelet pretreatment, which appears similar in patients treated with BMS.

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·临床研究·

药物涂层支架与金属裸支架对稳定型心绞痛患者局部冠状动脉循环组织因子水平早期影响的比较

艾力曼·马合木提 Nicolas Meneveau 刘品明 Francois Schiele Jean-Pierre Bassand

【摘要】 **目的** 比较药物涂层支架(DES)与金属裸支架(BMS)置入前后冠状动脉循环内局部血浆组织因子(TF)水平的变化,探讨DES对血浆TF水平的早期改变及其对急性支架内血栓(AST)形成的意义。**方法** 入选稳定型心绞痛患者26例,按标准方法行冠状动脉造影证实有冠状动脉狭窄均在70%以上。其中15例置入DES(DES组),11例置入BMS(BMS组)。全部患者术前给予阿司匹林、氯吡格雷口服,支架置入前静脉给予低分子质量肝素。PCI术中冠状动脉内血样采集顺序依次为:支架置入前后冠状动脉入口处(ostium)用引导导管,支架置入后15 min通过血栓吸引器穿过病灶在病灶下方(beyond the lesion)采血。血浆TF水平检测采用酶联免疫双抗体夹心法(ELISA)。**结果** PCI术前26例患者在冠状动脉入口处与病灶下方冠状动脉循环内的TF基线水平比较差异无统计学意义(31.50±7.05 ng/L比31.40±7.30 ng/L, $P=0.748$),但高于正常参考值3倍;支架置入后15 min在冠状动脉入口处(29.60±6.96 ng/L比31.50±7.05 ng/L, $P=0.135$)与病灶下方(30.70±7.70 ng/L比31.40±6.30 ng/L, $P=0.230$)冠状动脉循环内的TF水平与术前比较,差异亦无统计学意义。术后15 min,DES组和BMS组冠状动脉入口处(31.20±4.37 ng/L比30.70±5.39 ng/L, $P=0.674$)及病灶下方(31.60±5.39 ng/L比29.00±7.96 ng/L, $P=0.789$) TF水平差异均无统计学意义。**结论** 稳定型心绞痛患者冠状动脉循环血内存在大量的TF。DES和BMS两种支架均不引起冠状动脉内局部、早期血浆TF水平的改变。

【关键词】 血管成形术,经腔,经皮冠状动脉;支架;冠状动脉血栓形成;组织因子

Comparison of early effects of bare metal stent and drug-eluting stent implantation on intra-coronary local tissue factor levels following percutaneous coronary intervention for stable angina

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【**Abstract**】 **Objective** To investigate the early changes in intra-coronary circulatory tissue factor (TF) following drug-eluting stent (DES) or bare metal stent (BMS) implantation in patients with stable angina under dual antiplatelet and anticoagulant pretreatment, and to evaluate their prognostic value on acute stent thrombosis formation. **Methods** The study consisted of twenty-six stable angina patients with an over 70% diameter stenosis lesion by visual estimation during angiography and following revascularization was administered. Blood samples were drawn in sequence from the ostium of the coronary artery through guiding catheter before balloon angioplasty, and from the coronary artery just beyond the dilated segment after stent implantation through aspiration catheter. Plasma levels of TF were measured by sandwich ELISA. **Results** In the whole study population, baseline TF values were found 3 times higher than normal reference range either in the coronary ostium or within the segment distal to the lesion. However, there were no differences when comparing baseline values at both sites ($P=0.748$). There were no significant differences in the levels of local TF marker among the DES and BMS groups either in the coronary ostium or within the coronary segment distal to the lesion ($P=0.789$, $P=0.674$, respectively). **Conclusion** There is a significant

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increase of intra-coronary circulatory TF in patients with stable angina before PCI procedure. Neither DES nor BMS implantation increases the early local intra-coronary circulatory TF activation following PCI procedure.

【**Key words**】 Angioplasty, transluminal, percutaneous coronary; Stent; Coronary thrombosis; Tissue factor

目前药物涂层支架(DES)内血栓形成是全球心血管医师讨论的热点话题。尽管双重抗血小板及低分子肝素抗凝治疗,但 DES所致的急性支架内血栓(acut stent thrombosis, AST)没有比普通金属裸支架(BMS)减少[1-5]。AST的发生率在 0.1%～3%[6-8],发病机制尚未完全阐明,有学者提出由于经皮冠状动脉介入术(PCI)导致的冠状动脉血管内膜急性损伤反应,引起血小板聚集及凝血活性的增高,从而进一步加重局部乃至全身凝血-纤溶系统平衡,形成局部急性或亚急性支架内血栓[9-10]。最新研究表明,置入 DES后,西罗莫司(Rapamycin)和紫杉醇(Paclitaxel)均可以使血液中组织因子(TF)表达增高而导致血栓前状态[11-12]。由此产生了药物涂层支架是否增加早期止血活性的问题。本研究通过 PCI术中在冠状动脉不同部位及支架置入前后不同时间采样,比较 DES与 BMS置入前后稳定型心绞痛患者早期冠状动脉循环内局部 TF活性的变化,试图揭示 DES及抗栓治疗对 TF血浆水平的早期影响,对 AST形成的生物学机制作初步探讨。

资料与方法

一、对象

选取自 2005年 9月至 2006年 7月间来自法国 Franche-comte地区,在 Besancon, Jean-Minjoz医院心脏中心进行 PCI手术的 1 100例患者,其中能够达到本课题入选条件的 30例患者,平均 65±11岁。按标准方法行冠状动脉造影和支架置入术。入选患者经冠状动脉造影证实有冠状动脉狭窄均在 70%以上,均为稳定型心绞痛且至少手术前 1周口服阿司匹林 75～250 mg/d,术前 4日口服 75 mg/d或者当日术前顿服 300 mg氯吡格雷(clopidogrel)。PCI术前均静脉给予低分子质量肝素(enoxaparin)。入选的 30例患者中,4例由于同时置入 DES及 BMS从统计学分析中剔除。排除有出血倾向、肾功能不全(血肌酐 >3.5 mg/dL)、瓣膜病、射血分数 30%以下(心力衰竭根据纽约分级 Ⅲ、Ⅳ级)、48 h内急性心肌梗死、不稳定型心绞痛、冠状动脉分叉病变以及手术前使用血小板膜糖蛋白(GP) Ⅱb/Ⅲa受体拮抗剂者。

二、方法

1. 标本的采集:采血在 PCI手术操作中按以下步骤进行:(1)支架置入前后 15 min在冠状动脉入口处(ostium)用导引导管采血。(2)支架置入前后 15 min,利用血栓吸引器(aspiration catheter)穿过病变处在病灶下方(beyond the lesion)采样。每次用吸取 15 mL动脉血,分别装入 4个 4.5 mL(vacutainer tube containing 3.8% trisodium citrate)真空管,立即以 3 500 r/min速度,4 ℃下离心 10 min,分离血浆并放于 -80 ℃的低温冰箱等待测定。

2. 血浆 TF活性测定:采用酶联免疫双抗体夹心法(ELISA)试剂盒由 IMUBIND tissue factor ELISA kit, American Diagnostica Inc (AD), France提供。本实验室通过检测 30例健康人,测定 TF正常值在 9.5±11.5 ng/L范围。

3. 统计学处理:(1)样本量的估计　为保证研究结论具有一定可靠性(精度和检验效能),本研究在课题设计阶段用统计学手段估计了最少的受试对象数:以 TF在置入 DES后增高两个标准差为假设基础,取单侧 $\alpha = 0.05$, $\beta = 0.20$(检验功效为 80%),总体标准差为 9.5±11.5 ng/L, 容许误差为 13.5 ng/L, DES和 BMS两组共需 20例冠心病患者。实际操作中,我们对 2个组(DES组和 BMS组),2个不同部位(冠状动脉入口及病变灶下段)及 2个不同时间(支架置入前后)共抽取了 120例血样,每个患者均为自身对照,剔除 4例同时置入 DES及 BMS的患者,对合乎条件的 104例给予统计学分析。(2)所有数据用 SAS(Version 9.1, SAS Institute, Cary, North Carolina)软件处理。首先对全部数据做正态性检验,结果以均数±标准差表示。计量资料采用配对 t检验,计数资料采用卡方检验,以 $P<0.05$为差异有统计学意义。

结　果

1. DES及 BMS两组患者临床指标分析:26例入选患者,15例置入 DES[10例置入紫杉醇支架(Taxus, Boston Scientific Corp, Natick, Massachu-

setts), 5例置入西罗莫司支架 (Cypher, Cordis-Johnson and Johnson, Miami Lakes, Florida)], 11例置入 BMS。除了糖尿病 (P =0.001),两组患者的年龄、性别、病史、射血分数及危险因素差异均无统计学意义 (糖尿病患者均置入 DES)。

2. PCI结果分析:全部患者的支架成功置入。术后无一例 AST发生。支架长度为 16.9 ±4.50 mm,直径为 3.0 ±0.4 mm。有关 PCI术的其他指标在两组中均相等。

3. 冠状动脉循环内血浆 TF水平改变: (1)两组患者 (n =26) PCI术前冠状动脉循环内的 TF基线水平在冠状动脉入口处 (31.5 ±7.05 ng/L)与病灶下方 (31.4 ±7.30 ng/L)比较,差异无统计学意义 (P =0.748),但这两处血浆 TF水平均高于正常参考值 3倍。 (2)在冠状动脉入口处 (n =26),支架置入 15 min后 TF水平轻度下降 (29.6 ±6.96 ng/L),但与术前基线水平 (31.5 ±7.05 ng/L)比较,差异无统计学意义 (P =0.135)。 (3)在冠状动脉病灶下方 (n =26),支架置入前 (31.4 ±6.30 ng/L)与支架置入 15 min后 (30.7 ±7.70 ng/L)比较,差异亦无统计学意义 (P =0.230)。 (4)同样,在冠状动脉循环病灶下方 DES组 (n =15)血浆 TF水平 (31.6 ±5.39 ng/L)与 BMS组 (n =11)血浆 TF水平 (29.0 ±7.96 ng/L)比较,差异无统计学意义 (P =0.789)。此外,在冠状动脉入口处, DES组 (n =15)血浆 TF水平 (31.2 ±4.37 ng/L)与 BMS组 (n =11)血浆 TF水平 (30.7 ±5.39 ng/L)比较,差异亦无统计学意义 (P =0.674,图 1)。

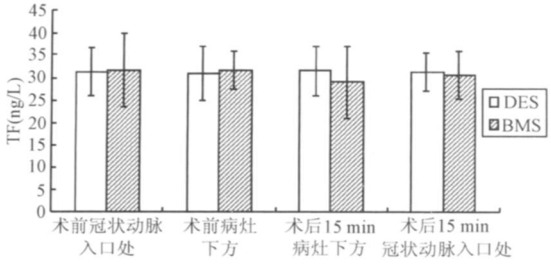

图 1 在不同支架置入前后冠状动脉入口和病灶下方血浆 TF水平的早期改变

讨 论

AST总体发生率很低,但是一旦发生预后很差,近期死亡率 20%～25%,其中 60%～70%的患者出现急性心肌梗死[13-16]。最近一些临床研究表明 DES在减少血管再狭窄的发生率同时增加了支架内血栓的发生[1-5],尽管这些患者使用了抗栓药物。由于 AST的发生率较晚期支架内血栓 (late stent thrombosis, LST)少,因此目前许多临床及基础研究主要以 LST为主。DES引起的 AST形成机制尚不清楚,且处于初步研究阶段,尚未有大量的临床研究。近来一些学者提出 DES可能增加止血活性,尤其导致血小板聚集和 TF活性增高[11,12,17],然而这些止血活性的改变是否在 DES置入后立刻出现,是药物涂层还是支架本身导致这种止血活性的改变等问题还需要探讨。

PCI术后 TF水平的改变有不同报道: Mizuno等[18]报道 43例稳定型和不稳定型心绞痛患者 TF水平在球囊成形术后增高且与血管再狭窄相关。Tutar等[19]报道 PCI治疗前全血中高 TF水平的凝血前状态是球囊成形术和支架置入后血管再狭窄的预测因素,然而 TF水平在球囊成形术和支架置入后并未增高。同样 Agraou等[20]在早期研究中提出稳定型心绞痛患者在支架置入后不引起 TF水平增高。相反 Shammas等[21]对 11例球囊成形术中稳定型心绞痛患者测定冠状动脉循环内止血活性,发现在高剂量抗凝及抗血小板药物使用前提下,球囊成形术不会导致冠状动脉内止血活性的改变。以上这些研究主要针对球囊成形术或者 BMS置入,但有关 DES与止血活性的临床研究甚少。最近关于 AST的动物模型研究提出, DES与 BMS置入后前 24小时 TF水平没有改变,但 TF是 AST的预测因素[22]。

TF作为外源性凝血过程的启动因子,在动脉粥样硬化斑块、冠状动脉疾病的发生和治疗后血管再狭窄以及在支架内血栓的形成过程中扮演了重要的角色。冠心病 (CHD)患者本身已经存在血小板及凝血功能亢进,纤溶活性受损和功能失调。有资料表明动脉粥样硬化斑块中富含大量 TF[23],包括动脉硬化损害的所有阶段都有 TF表达[24,25]。尽管本研究入选的所有稳定型心绞痛患者并且 PCI术前均给予预防性双联抗血小板治疗,但 PCI术前 TF的基线水平比正常参考值仍然高于 3倍。这个结果提示 CHD患者冠状动脉循环血内存在大量的 TF,预防性阿司匹林和氯吡格雷两种药物抗血小板治疗似乎不能完全抑制冠状动脉循环局部的 TF活性, TF水平对测定 CHD患者凝血前状态以及评估预防抗栓治疗的有效性是一个非常敏感的指标。 TF活性在两种支架之间比较没有统计学差异,表明 DES的药物涂层及支架本身并不引起凝血活性的早期改变。

支架置入后这种止血活性无即刻升高,说明支架置入可能具有一定的稳定血管内皮和止血活性的作用。具体原理还需要进一步阐明。

本研究主要对 DES置入后的冠状动脉循环内生物学指标的早期改变做初步探讨。样本量通过统计学方法得出,有一定的可靠性。由于每个患者作为自身对照,没能设立未用抗栓药物的正常对照组来进一步比较抗栓药物的有效性。我们的研究结果不适用于急性冠状动脉综合症、亚急性及晚期支架内血栓患者。置入 DES后冠状动脉循环内 TF活性是否会晚些升高(例如 30 min、1 h、3 h、24 h后等),是否手术前预防性使用抗凝药物可以有效降低循环血液中的 TF水平,以及 TF与炎性指标的相关性有待进一步临床研究。

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·文献摘译·

可卡因可增加经皮冠状动脉介入术后支架内血栓的风险

美国学者 McKee等对 71例可卡因成瘾患者行经皮冠状动脉介入术(PCI)后 9个月的心血管事件进行观察,发现支架内血栓发生率为 7.6%,而正常对照组为 0.6%($P < 0.001$),心肌梗死、猝死或再次血管血运重建差别无统计学意义。因此作者认为,可卡因增加了支架内血栓形成的几率。

(方玉强、杨成明摘译自 Am Heart J, 2007, 154: 144-150.)

II.3 Discussion on hemostasis activation and PCI

II.3.1 Pretreatment and local hemostasis activation

Anti-thrombotic and anticoagulant therapies minimize thrombotic complications after PCI. The baseline platelet characteristics are predictive of future platelet activity and thus may identify patients who would be most likely to benefit from more aggressive APA and anticoagulant regimens (Gurbel PA et al., 2000). In our study, the three-drug anti-thrombotic regimen maintained the hemostasis activation at the low state achieved before the procedure. Our results demonstrated that levels of CD62P and sGPV levels in the coronary ostium and in the coronary artery beyond lesion were similar before PCI procedure and they were within normal reference range. This finding suggests the baseline platelet characteristics are predictive of a low risk of stent thrombosis in patients with stable angina. Aspirin is a widely used and very well documented APA drug that lowers cardiovascular morbidity and mortality (Antithrombotic Trialists´ Collaboration., 2002 and Patrono C et al., 2001). The APA effects of aspirin are related to irreversible inhibition of platelet cyclooxygenase (COX), and reduced production of the potent vasoconstrictor and platelet activator thromboxane A2 (TxA2) (Patrono C et al., 2001). Recent placebo-controlled trials have shown excellent protection with 75 mg aspirin daily in patients with SA (Juul-Moller S et al., 1992) or UA (The RISC Group., 1990) pectoris. More recently, the "Clopidogrel in Unstable angina to prevent Recurrent Events" (CURE) Study demonstrated that the addition of clopidogrel to aspirin was associated with a further 20% reduction in the risk of death, myocardial infarction or stroke in patients with acute coronary syndromes (Yusuf S et al., 2001). Similarly, some recent studies have shown that clopidogrel, particularly as a loading dose of 300 mg, can rapidly and significantly inhibit platelet aggregation in both healthy subjects and patients with atherosclerotic disease, even after just 2 h (Savcic M et al., 1999). Enoxaparin is used more frequently in patients undergoing PCI. Our study shows that during PCI, no changes occurred in levels of platelet activation markers in patients under dual APA pretreatment. This finding suggests the rapid effect of the pretreatment in attenuating

intra-coronary platelet activity. Our results also suggest that dual APA pretreatment sufficiently inhibited platelet activation in patients who underwent DES or BMS. Corroborating our results, Mizuno et al. (Mizuno O et al., 2000) reported no changes in platelet activation in the "balloon angioplasty only" and "stent implantation" groups when aspirin and ticlopidine were administered; in contrast, the levels of platelet activation markers significantly increased immediately after percutaneous transluminal rotational coronary atherectomy in patients without using antiplatelet therapies. Similarly, Gregorini et al. (Gregorini L et al., 1997) reported that the combined use of ticlopidine, aspirin, and heparin effectively inhibited platelet activation in patients with angina pectoris who underwent percutaneous transluminal coronary angioplasty (PTCA). In addition, in our study, widespread use of statins, angiotensin converting enzyme inhibitors and angiotensin receptor blockers may also have played important roles in the inhibition of platelet activation. Our patients were also treated with calcium channel antagonists, which, in experimental animals, reduce platelet deposition and thrombus formation and prolong bleeding time (Foley JA et al., 1996, Rao GH et al., 1992 and Stone PH., 1987). Nitrous vasodilators also inhibit platelet activation through complex mechanisms that may involve increased production of endogenous nitric oxide and of cyclic guanosine monophosphate (GMP) while lowering intracellular cytosolic calcium (Adrie C., 1996). These treatments may thus have also contributed to the control of hemostasis activation in our patients.

II.3.2 Fibrinolytic gene polymorphism and hemostasis activation

Several observations suggest that the fibrinolytic system plays an important role in hemostatic response to artery wall injury. Family studies suggest that plasma PAI-1 and t-PA levels are influenced by genetic factors (Pankow et al., 1998). Previous studies usually investigated the effects of non-genetic and genetic factors individually on plasma levels of t-PA or PAI-1 in relatively small populations at high-risk for thromboembolic events (Mansfield MW et al., 1995, Juhan-Vague I et al., 1993 and Toft I et al., 1997). In our study we analysed t-PA and PAI-1 gene polymorphism; however, t-PA gene polymorphism influence on hemostasis following PCI is still

being analysed and thus is not reported in this thesis. The 4G/5G genotype has been related to the risk of MI (Boekholdt SM et al., 2001), sudden cardiac death (Anvari A et al., 2001), transplant CAD (He JQ et al., 2002), and in-stent restenosis after coronary stent implantation (Ortlepp JR et al., 2001). In our study, balloon angioplasty easily induced vessel shrinkage and arterial wall injury and transient local haemostatic activation. This response was more obvious in patients with 4G/5G polymorphism of the PAI-1 gene. Apparently, 4G/5G polymorphism did not influence the rapid return of all hemostasis and fibrinolysis markers at the baseline after stent implantation. Specific influence of t-PA polymorphism and possible interaction/synergy with PAI-1 polymorphism may perhaps modify our conclusions regarding the influence of genetic predisposition towards hemostasis activation after angioplasty. In addition, a long-term evaluation of the patients could make clear the possible influence of the transient activation of hemostasis/fibrinolysis activation after angioplasty, favored by PAI-1 gene polymorphism, on long-term thrombosis and/or restenosis after stenting. Such an evaluation would give some rationale to possible different attitudes towards pre-stenting anticoagulant and APA regimens in patients that are 4G/5G heterozygotes.

II.3.3 Methodology of the study

The sample size in our study is relatively small. This may have led to an underestimation of possible differences in platelet activation after angioplasty and/or possible differences between DES and BMS. We examined only biological criteria, without any follow-up of clinical events. Thus, our results need to be confirmed in a larger and long-term study. We selected low risk patients with stable angina and thus the results may not be applicable to different subsets of patients such as unstable angina and ACS. Furthermore, we concentrated on acute local hemostasis activation, and we can draw no conclusions about the possible risk of AST, SST or LST. A long-term clinical follow-up should give more insight in the actual consequences of hemostasis activation levels after angioplasty and/or genetic background of the patients on further stenosis. Our patients were pretreated with enoxaparin and it is

difficult to know if our results might be extrapolated to patients pretreated with unfractionated heparin. Pretreatment or other successful interventions initiated before the coronary intervention may have altered the plasma levels of the hemostasis markers and we did not assess CRP to study the relationship between early local inflammatory response after PCI and hemostasis activation. Finally there was no control group (i.e. SA patients with single vessel disease and who did not require PCI). However, our study was performed in patients pre-treated and managed according the state of the art, regarding anticoagulants and APA, and therefore the results might reasonably extrapolated to other patients with SA undergoing angioplasty and stenting.

The patients served as their own controls and in this context, we found that baseline levels of TF were actually above normal values, which may be explained by the fact that all patients had documented CAD. Measurement of these hemostatic markers in coronary artery circulation has been shown to be much more sensitive in detecting hemostasis activation than in venous blood (Eichinger S et al., 1994 and Weltermann A et al., 1999). Previous studies have shown that the catheter itself does not cause artificial increases in levels of hemostasis markers, and we may thus consider our data to be reliable (Marmur JD et al., 1994). This allowed us to demonstrate the transient activation of hemostatis/fibrinolysis after angioplasty and the return to baseline after DES stent implantation, whatever its type, which is an original finding.

II.4 Conclusion

Hemostatis activation occurs after balloon-induced vessel injury; however neither DES nor BMS further increases markers of platelet activation, coagulation or fibrinolysis in SA patients under dual APA and anticoagulant pretreatment. The transient local haemostatic activation is likely due to vessel shrinkage and arterial wall injury. This response is more obvious in patients with 4G/5G polymorphism of the PAI-1 gene. Thus, pretreatment with anti-thrombotic medications in patients with stable angina effectively prevents or limits further consequences of the initial injury caused by angioplasty following coronary DES or BMS implantation.

B. Prevention and Treatment of Thrombosis in Venous Thrombo-embolic Disease

I. Introduction: Venous Thrombo-Embolic Disease (VTE)

Deep vein thrombosis (DVT) and pulmonary embolism (PE) represent different manifestations of the same clinical entity, which is referred to as VTE. In patients with this condition, venous thrombosis occurs when red blood cells, fibrin and, to a lesser extent, platelets and leukocytes, form a mass within an intact cardiovascular system.

The mechanisms which support thrombus formation, i.e. platelet activation, hemostasis/coagulation, and eventually fibrinolysis, are quite similar to those involved in artery thrombosis, which have been extensively presented in Section 1 of this thesis.

A proximal DVT in the leg is one that is located within the popliteal, femoral (including the superficial femoral), or iliac veins. Acute pulmonary embolism (APE) occurs when a segment of a thrombus within the deep venous system detaches from the vessel, travels to the lungs, and lodges within the pulmonary arteries. The pelvic and deep veins of the lower extremities are the source of more than 70% of all pulmonary emboli (Browse NL et al., 1974). The superior vena cava, upper extremity veins, and right chambers of the heart are less common sources.

I.1 Incidence

It is difficult to determine the incidence of VTD. Clinical signs and symptoms are nonspecific, and screening tests are not always sensitive enough to detect disease in asymptomatic patients. According to population studies, the overall age- and sex-adjusted annual incidence of VTD is 1 to 2 per 1,000 people (Anderson FA Jr et al., 1991 and Silverstein MD et al., 1998). More than one-third of these cases represent recurrent disease (Silverstein MD et al., 1998). Extrapolation of these data suggests that more than 250,000 cases of VTE are diagnosed annually in the United States. At least 50,000 of these cases are fatal, although available autopsy data suggest that this figure is probably a significant underestimation of actual mortality. It has been estimated that PE occurs in approximately 600,000 patients annually in the

United States and causes or contributes to 50,000 to 200,000 deaths (Lilienfeld, DE et al., 1990). Similarly, VTD is a frequent pathological event in France, with an incidence in France, in the general population, of 180/100 000 people per year. This incidence increases with the age to attain 1000/100 000 after 75 years. Incidence of VTD in the United States is of 100-to 500/100 000 at 80 yr of age and 81.1/100 000 after 66 yr age in China (Lindblad B et al., 1991). PE seemed to be, until recently, a rare pathological event in China. However, the recent article by Tan et al (Tan XY et al., 2006) shows an increase of PE in hospitalized patients from 0.27% during the early 1970s to 0.94% in the XXIst century, and concludes that PE is “an increasingly significant disease” in modern China.

I.2 Pathophysiology

DVT: Venous thrombi typically develop within a deep vein at a site of vascular trauma and in areas of sluggish blood flow (eg, in the venous sinuses of the calf and within a valve cusp). An accumulation of fibrin and platelets causes rapid growth in the direction of the blood flow, potentially reducing venous return. Endogenous fibrinolysis results in a partial or complete resolution of the thrombus. Residual thrombus will organize and the vein may incompletely recanalize, which often results in narrowing of the lumen and valvular incompetency. An extensive collateral network can develop.

PE: PE is a relatively common cardiovascular emergency. Thrombi that embolize to the lungs will lodge within either the lobar arteries or the distal main pulmonary artery; occasionally they will straddle the pulmonary artery bifurcation (saddle embolus). Smaller thrombi can travel more distally. A PE causes several physiologic changes. Stimulation of irritant receptors causes alveolar hyperventilation, which increases the respiratory rate. Gas exchange becomes impaired because the affected lung tissue is ventilated but not perfused. Initially, this alveolar "dead space," and later the development of intrapulmonary shunting, cause bronchoconstriction and hypoxemia. Atelectasis and edema caused by the loss of alveolar surfactant can develop within hours. A decrease in the cross-sectional area of the pulmonary arterial

bed, hypoxia, and the release of humoral factors by activated platelets (eg, serotonin and thromboxane) increase pulmonary vascular resistance. Even so, an acute embolic event in a healthy individual will not generate a mean pulmonary artery pressure greater than 40 mm Hg (Miller GA et al., 1970). Pulmonary hypertension can result in right ventricular failure and, infrequently, decrease cardiac output. The severity of hemodynamic compromise, and hence symptoms, is dependent on the extent of arterial obstruction and the presence or absence of pre-existing cardiopulmonary disease.

I.3 Prevention and treatment of PE

The therapeutic goals for PE are to relieve symptoms, prevent the development of pulmonary hypertension and right ventricular failure, and ultimately, diminish the risk of death. Long-term prevention of recurrence should also be considered in all patients. To achieve these therapeutic objectives, anticoagulation has been widely used, where unfractionated heparin (UFH) or low-molecular- weight heparin (LMWH) is to be used for about a week, followed by oral administration of warfarin for at least 3-6 months. Heparin provides immediate anticoagulation and severs as a bridge until warfarin is fully effective.

I.3.1 Heparin

Heparin is an animal-derived large polysaccharide that binds to endogenous antithrombin. This heparin-antithrombin complex catalyzes the inactivation of several activated coagulation factors, including factor Xa and IIa (thrombin). Heparin can be administered by either intravenous infusion or subcutaneous injection. Its plasma half-life is generally 1 to 2 hours, although the duration lengthens with higher doses. Heparin is primarily cleared from the circulation by the reticuloendothelial system. A baseline complete blood count with platelets and an activated partial thromboplastin time (aPTT) should be documented prior to initiating therapy. Weight-based dosing (an 80 U/kg bolus followed by 18 U/kg/h) is associated with a lower risk of recurrent thrombo-embolism (Raschke RA et al., 1993). The appropriate dose is determined by

the aPTT value. The target value is 1.5 to 2.5 times the mean control value (which corresponds to 0.3 to 0.6 U/mL on the amidolytic anti-factor Xa assay) and should be checked 6 hours after a dose adjustment. Adverse drug reactions include bleeding, heparin-induced thrombocytopenia, and osteoporosis with prolonged use.

I.3.2 Low-Molecular-Weight Heparin (LMWH)

A LMWH is a small heparin fragment whose mechanism of action is similar to that of UFH. However, LMWH has less nonspecific binding to proteins, which results in a longer plasma half-life (4 h) and a more predictable dose-response relationship. Laboratory monitoring is usually not necessary, but it must be performed with a chromogenic anti-Xa assay (rather than the aPTT measurement) 4 hours after a dose has been given (therapeutic range: 0.6 to 1.0 IU/mL in most laboratories). LMWH is as effective as UFH for the treatment of DVT, and it might be associated with a lower risk of bleeding (Hull RD et al., 1992). Studies have demonstrated that LMWH is similarly efficacious and safe in treating PE (The Columbus Investigators., 1997 and Simonneau G et al., 1997). In appropriate patients, LMWH can facilitate the outpatient treatment of VTD because it is administered subcutaneously (Levine M et al., 1996 and Koopman MM et al., 1996). Although the incidence of heparin-induced thrombocytopenia and osteoporosis is lower with LMWH than with UFH, caution should be used in obese patients and in those with renal insufficiency because of the renal clearance of LMWH. In addition, the potential adverse effects of heparin are haemorrhage and thrombocytopenia (Quinlan DJ et al., 2004 and Levine MN et al., 2004). The risk of major bleeding is 1.0-5.0%, which increases with age (>70 years) and the dose of heparin (Levine MN et al., 2004). LMWH is associated with less major bleeding, such as intracranial or retroperitoneal haemorrhage, compared with unfractionated heparin in acute VTE (Levine MN et al., 2004).

Fondaparinux is a synthetic and selective inhibitor of factor Xa that has proven its efficacy and safety at different dose regimens as a preventive and curative treatment of thromboembolic disease when compared with heparins (Buller HR et al., 2004, Buller HR et al., 2003 and Turpie AG et al., 2002). Its pharmacokinetic properties

allow a simple, fixed-dose, once-daily regimen of subcutaneous injection without the need for monitoring. The use of pharmacological prophylaxis in patients undergoing major orthopedic surgery was suspected to entail potential increased risks of major bleeding, although this trend was not confirmed when higher curative dosing regimens were used in the treatment of DVT or low risk PE ((Buller HR et al., 2004, Buller HR et al., 2003 and Turpie AG et al., 2002).

I.3.3 Thrombolytic therapy

Thrombolytic drugs such as urokinase, streptokinase or recombinant tissue plasminogen activator (tPA) act by converting plasminogen to plasmin, which dissolves the thrombus. Especially, streptokinase and tPA are the two FDA approved thrombolytic agents for VTD. Streptokinase is administered as an intravenous 250,000 IU bolus over 30 minutes followed by 100,000 IU/h over 24 hours for a PE or up to 72 hours for a DVT. The recommended dose of tPA for thrombolysis of a PE is 100 mg over 2 hours also given intravenously. The main reason for thrombolytic therapy is that, in conjunction with anticoagulation, it may reduce the risk of pulmonary hypertension, right ventricular dysfunction or rate of death (Konstantinides S et al., 1997). The evaluation of thrombolytic therapy for PE began in the early 1970s. The first prospective randomised trial was the Urokinase Pulmonary Embolism Trial (UPET), where the effect of urokinase and heparin was compared in patients with APE (Urokinase pulmonary embolism trial., 1970). Within 24 hours of the drug administration, improvement in lung scans, pulmonary angiograms and right ventricular pressure was found only in the urokinase group (Urokinase pulmonary embolism trial., 1970). However, no significant differences in clinical outcomes were apparent between the two groups 24 hours after the drug administration (Urokinase pulmonary embolism trial., 1970).

The effect of tPA on PE was initially investigated in a small group of patients. Compared with heparin only therapy, a tPA, alteplase, led to a greater reduction in pulmonary pressure and better improvement in pulmonary angiography (Dalla-Volta S et al., 1992). However, even there were significant improvements in the objective

tests, no additional clinical benefits were observed in the thrombolytic therapy group (Dalla-Volta S et al., 1992). Further studies were conducted by Goldhaber and associates (Goldhaber SZ et al., 1988) and the European Cooperative Study investigators (Meyer G et al., 1992) to compare the effect of tPA and urokinase. These studies demonstrated that, although tPA initially produced a faster resolution of the pulmonary thrombus, clinical outcomes were the same 24 hours after the drug administration.

The impact of thrombolysis on mortality in patients with PE has been a subject of debate for many years. A recent meta-analysis on some major clinical trials on thrombolytic therapy has found that, compared with heparin, thrombolytic therapy is not associated with a statistically significant reduction in recurrent PE or death (Wan S et al., 2004). However, in trials that enrolled patients with massive PE and hypotension or cardiogenic shock, thrombolytic therapy is associated with a significant reduction in recurrent PE or death (Wan S et al., 2004).

Thrombolysis in patients with PE is associated with a significant risk of major bleeding. The bleeding rates from thrombolysis are higher than that from heparin. Pooled data from recent trials on PE have demonstrated that, major bleeding rate in thrombolysis and heparin group is 9.1% and 6.1%, respectively (Wan S et al., 2004). The rate of nonmajor bleeding in the thrombolysis group was also higher than that in the heparin group (22.7% vs 10.0%) (Wan S et al., 2004).

Due to the perceived lack of mortality benefit and long-term efficacy, thrombolysis has been mainly used for the management of massive PE, which is often accompanied by hypotension or cardiogenic shock (Goldhaber SZ et al., 2004). The role of thrombolytic therapy in patients with submassive PE and stable haemodynamics remains uncertain. A recent trial, the Management Strategies and Determinants of Outcome in APET (Kasper W et al., 1997), one of the largest trials in the field involving 256 patients, shows that, compared with heparin only treatment, alteplase (100 mg over 2 hours) plus heparin substantially reduces the need for intensive therapeutic measures such as mechanical ventilation, pressor agents or secondary thrombolysis during hospitalisation (Konstantinides S et al., 2002). However, a

reduction in death has not been demonstrated in the heparin-plus-alteplase group (Konstantinides S et al., 2002).

Thrombolytic agents convert plasminogen into plasmin. Plasmin degrades fibrin and causes thrombi to rapidly dissolve. However, a complete resolution of a thrombus is rare in the venous circulation. The indications for thrombolytic therapy are a massive iliofemoral DVT (especially in the case of phlegmacia cerulea dolens) and PE that is accompanied by hemodynamic instability. Nevertheless, thrombolytics have not been shown to decrease mortality in these patients (Hyers TM et al., 2001). Konstantinides et al reported that thrombolytics did lower 30-day mortality in clinically stable patients with right ventricular dysfunction (Konstantinides S et al., 1997). However, because enrollment in this study was not randomized, these results should be interpreted with prudence. Bleeding is a serious complication of thrombolytic therapy, and it can occur at vascular puncture sites, within the gastrointestinal tract, or in the retroperitoneum. Thrombolytics also carry a 1% to 2% risk of intracranial bleeding, so their potential benefits should be weighed against these bleeding risks (Hyers TM et al., 2001).

II. Safety and efficacy of fondaparinux as an adjunctive treatment to thrombolysis in patients with high and intermediate risk PE

II.1 Objectives of the study

Intravenous, UFH is still considered as the standard of anticoagulant therapy for patients with PE (Hyers TM et al., 2001). In recent years several studies evaluated LMWH as alternatives to UFH for this indication. However, due to the small number of PE patients in most of these trials, many clinicians have been reluctant to adopt LMWH for treatment of acute symptomatic PE. A recent meta-analysis combined data from 12 studies that included 1,951 patients with PE. It demonstrated that LMWH are as safe and as effective as intravenous UFH in the treatment of non-massive PE (Qunilan DJ et al., 2004).

In recent years, various novel anticoagulants have emerged that may offer benefit over traditional anticoagulants in the treatment of patients with venous thrombosis. Fondaparinux is an inhibitor of factor Xa. Although it is not a LMWH, it is similar in that it is given by subcutaneous injection and does not require monitoring of anticoagulation. Alternatives to unfractionated heparin are widely used for treating DVT, but they are less widely used for PE because of concerns about efficacy. A recent randomized, open-label study evaluated the efficacy of fondaparinux compared to intravenous UFH in 2213 patients with acute symptomatic PE (The Matisse Investigators. 2003). This is the largest randomized study published to date that evaluated a novel anticoagulant "specifically" in the treatment of patients with acute symptomatic PE. The study demonstrated that once daily, subcutaneous fondaparinux is at least as effective and as safe as UFH in treatment of patients with hemodynamically stable PE. There was an absolute difference in favor of fondaparinux of -1.2% compared to UFH with regards to the primary outcome of recurrent VTE, however this did not reach statistical significance. Safety and mortality were similar in both groups. At this time there are no direct head to head trials that compare LMWH and fondaparinux in the treatment of PE. Overall, the efficacy, safety, and mortality results are comparable for fondaparinux, LMWH, and

UFH. Therefore, both LMWH and fondaparinux seem to be reasonable alternatives to UFH in the treatment of patients with PE. Hawever, to date, the safety and efficacy of fondaparinux as adjunctive therapy to thrombolysis has never been assessed in the setting of high and intermediate risk acute PE, where UFH is currently the only recommended molecule (Torbicki A et al., 2008). In addition, data from studies of OASIS-6 conducted in ST elevation myocardial infarction (STEMI) patients receiving thrombolytic therapy demonstrated that 2.5 mg of fondaparinux given once daily significantly reduces mortality and reinfarction without increasing bleeding and strokes, when compared with UFH (Yusuf S et al., 2006). Whether these results can be extrapolated to patients suffering from high and intermediate risk pulmonary embolism is unknown, since the use of higher doses of fondaparinux as an adjunctive therapy to thrombolysis might be associated with an increased bleeding rate. Thus, in this study, the aim of our study was to evaluate the safety and efficacy of a combination of fondaparinux and thrombolysis in the setting of acute high and intermediate risk PE.

II.2 Results and personal publication

The results obtained in this prospective study is given in the publication "***Safety and efficacy of fondaparinux as an adjunctive treatment to thrombolysis in patients with high and intermediate risk pulmonary embolism***" (*J Thromb Thrombolysis*, DOI 10.1007/s11239-008-0288-9).

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Safety and efficacy of fondaparinux as an adjunctive treatment to thrombolysis in patients with high and intermediate risk pulmonary embolism

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Abstract No data are available on the efficacy and safety of a combination of fondaparinux and thrombolysis in the setting of high to intermediate risk pulmonary embolism (PE). Patients submitted to thrombolysis and fondaparinux, presenting with $\geq$1 of the following criteria were included: (1) cardiogenic shock, (2) syncope, (3) $\geq$1 proximal thrombo-embolus at CT scan, (4) positive troponin test, (5) echocardiographic findings indicating right ventricular (RV) dysfunction. In-hospital results included death, recurrent PE, persistent RV dysfunction at 48 h echocardiography, bleeding complications. Twenty seven patients were included; 22 received a 2 h infusion of rt-PA and 5 received a 2 h infusion of streptokinase. Ten patients presented with cardiogenic shock (37%), 8 with syncope (30%), all had RV dysfunction. 82% of patients had an uneventful in-hospital course. One patient died during hospital stay from refractory shock. Thrombolysis failed in 2 patients (7%), requiring successful rescue surgical embolectomy. Bleeding events occurred in 2 patients (7%), of whom 1 required blood transfusion. Despite the small sample size, our data suggest that fondaparinux procures adequate tolerability compared to standard current therapy in combination with thrombolysis in high to intermediate risk PE.

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Keywords Fondaparinux · Thrombolysis · Pulmonary embolism

Background

Fondaparinux is a synthetic and selective inhibitor of factor Xa that has proven its efficacy and safety at different dose regimens as a preventive and curative treatment of thromboembolic disease when compared with heparins [1–3]. Its pharmacokinetic properties allow a simple, fixed-dose, once-daily regimen of subcutaneous injection without the need for monitoring. The use of pharmacological prophylaxis in patients undergoing major orthopedic surgery was suspected to entail potential increased risks of major bleeding, although this trend was not confirmed when higher curative dosing regimens were used in the treatment of DVT or low risk PE [1–3].

To date, the safety and efficacy of fondaparinux as adjunctive therapy to thrombolysis have never been assessed in the setting of high and intermediate risk acute PE, where UFH is currently the only recommended molecule [4]. However, data from studies conducted in ST elevation myocardial infarction (STEMI) patients receiving thrombolytic therapy demonstrated that 2.5 mg of fondaparinux given once daily significantly reduces mortality and reinfarction without increasing bleeding and strokes, when compared with UFH [5]. Whether these results can be extrapolated to patients suffering from high and intermediate risk pulmonary embolism is unknown, since the use of higher doses of fondaparinux as an adjunctive therapy to thrombolysis might be associated with an increased bleeding rate.

In this context, the aim of our study was to evaluate the safety and efficacy of a combination of fondaparinux and

thrombolysis in the setting of acute high and intermediate risk PE.

Materials and methods

Selection of patients

The study population was derived from a prospective, single-center registry of patients with confirmed pulmonary embolism.

Twenty seven patients with proven recent high and intermediate risk pulmonary embolism (symptom onset <15 days) were included in the registry between October 2006 and January 2008 if they met at least one of the following criteria: (1) cardiogenic shock defined as systolic blood pressure ≤90 mmHg, or a pressure drop of ≥40 mmHg, associated with clinical signs of organ hypoperfusion and hypoxia; (2) syncope; (3) one or more proximal thrombo-embolus at CT scan; (4) positive troponin test; or (4) at least one echocardiographic finding(s) indicating right ventricular dysfunction (RV/left ventricular end-diastolic diameter ratio ≥1 in the 4-chamber view, paradoxical septal systolic motion or pulmonary hypertension defined as a RV/atrial gradient >30 mmHg).

Patients with the following criteria were excluded: (1) contraindication to thrombolytic therapy; or (2) renal failure on admission, defined as creatinine clearance <30 ml/min.

The study protocol was approved by the local Ethics Committee and informed consent was obtained for all patients enrolled in the study.

Medication

Streptokinase or rt-PA could be used, according to the following regimens: (1) streptokinase was administered as a continuous intravenous infusion of 1.5 million IU over 2 h (started after an infusion of 40 mg of methylprednisolone); and (2) rt-PA was infused at a dose of 70 mg or 100 mg over 2 h, according to body weight (if body weight >70 kg, bolus of 10 mg followed by 90 mg over 2 h; if body weight <70 kg, bolus of 7 mg followed by 63 mg over 2 h). Both thrombolytics were only administered once fibrinogen level rose above 1 g/l.

Fondaparinux was given immediately at the beginning of thrombolytic infusion or before starting thrombolysis while waiting for the confirmation of the diagnosis. In heparin-pretreated patients referred for thrombolysis from other departments, fondaparinux was administered 12 h after the last injection of low molecular weight heparin. All patients received a single daily subcutaneous injection of 5.0 mg fondaparinux (if their body weight was <50 kg), 7.5 mg (if their body weight was 50 to 100 kg), or 10.0 mg (if their body weight was >100 kg).

Vitamin K antagonists (fluindione) were initiated when the patients were hemodynamically stable (48 to 72 h after thrombolytic infusion). Doses were adjusted to obtain an INR (International Normalised Ratio) target of 2.5 (range 2.0–3.0), for at least 6 months. Fondaparinux was stopped when the INR stayed between 2.0 and 3.0 for at least 2 consecutive days.

Clinical end points and in-hospital follow-up

Efficacy end point

The efficacy endpoint of in-hospital course was a combined endpoint including persistent clinical instability and residual echocardiographic right ventricular dysfunction within the first 36 h. Persistent clinical instability was prospectively defined as the presence of at least two of the following criteria: refractory cardiogenic shock; systemic arterial hypotension (defined as systolic blood pressure of ≤90 mmHg or a pressure drop of ≥40 mmHg for >15 min if not caused by new-onset arrhythmia, hypovolemia, or sepsis); severe hypoxemia (ie, room-air pulse oximetry of ≤90% or PaO_2 without oxygen therapy of ≤55 mmHg); or tachycardia (heart rate, ≥110 beats/min). Residual echocardiographic right ventricular dysfunction was defined as the persistence of at least two initial right ventricular dysfunction criteria.

Adverse events such as death, recurrent PE, repeat thrombolysis, surgical embolectomy and bleeding complications were noted throughout the hospital stay. Perfusion lung scans were performed within 6–8 days after onset of treatment. Perfusion impairment was graded as to the proportion of lung not perfused [6]. Patients with symptoms suggesting PE and with new perfusion defects on the lung scan or pulmonary angiogram were interpreted as having recurrent PE.

Safety end point

The safety end point included major and important bleeding complications.

Major bleeding complications were prospectively defined as any bleeding event that required blood transfusion, surgical control, discontinuation of thrombolytic or anticoagulant treatment; hemorrhagic stroke confirmed by computed tomography or autopsy; or any bleeding causing death or defined as a fall of 15% in hematocrit. Important bleedings, defined as a fall of 10% in hematocrit were also recorded [7]. Other bleeding events were considered as minor bleedings, and were not included in the safety end point.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation; categorical variables are expressed as percentage.

Results

Clinical presentation

The clinical characteristics of patients at hospital admission are reported in Table 1.

The study population comprised 11 men (40.7%) and 16 women (59.3%), mean age 68 ± 11 years (range 42–86). Twenty five proximal pulmonary embolisms were diagnosed with CT scan and one with ventilation-perfusion lung scintigraphy. One patient who presented in cardiogenic shock and with echocardiographic findings of severe RV overload received thrombolytic therapy before spiral CT scan examination.

Initial severity of PE

Ten patients (37.0%) presented with initial shock, 8 (29.6%) with syncope. The remaining 9 patients (33.3%) were hemodynamically stable, but presented initially with high troponin I (i.e., TnI >0.15 ng/ml) and BNP (i.e., BNP >200 pg/ml) levels.

All patients had echographic criteria of right ventricular dysfunction. Mean systolic pulmonary artery pressure was 56 ± 15 mmHg and all patients had a RV/atrial gradient >30 mmHg.

Table 1 Patient characteristics at diagnosis

	$N = 27$
Sex	
Male	11 (40.7%)
Female	16 (59.3%)
Age (years)	68 ± 11
History of thromboembolic disease	9 (33.3%)
Cardiopulmonary disease	3 (11.1%)
Hypertension	15 (55.5%)
Cancer	5 (18.5%)
Onset of symptoms	
≤5 days	12 (44.4%)
>5 days	15 (55.5%)
Syncope	8 (29.6%)
Heart rate ≥100 beats/min	14 (51.8%)
Cardiogenic shock	10 (37.0%)
ECG with RV overload	20 (74.1%)
DVT	17 (63.0%)
TnI >0.15 ng/ml	12 (44.4%)
BNP >200 pg/ml	17 (63.0%)
Thrombolytic agent	
Streptokinase	5 (18.5%)
rt-PA	22 (81.5%)

ECG: Electrocardiogram; RV: right ventricular; DVT: deep vein thrombosis; TnI: Troponin I; BNP: Brain Natriuretic Peptide

Table 2 In-hospital clinical outcome

	$N = 27$
Efficacy endpoint	3 (11.1%)
Death (recurrent PE)	1 (3.7%)
Persistent clinical instability	2 (7.4%)
Non-fatal recurrent PE	0
Bleeding endpoint	2 (7.4%)
Major bleeding	1 (3.7%)
Important bleeding	1 (3.7%)
Uneventful in-hospital course	22 (81.5%)

Treatment and in-hospital course

Twenty two patients (81.5%) received rt-PA and 5 (18.5%) streptokinase. Mean duration of fondaparinux administration was 8.6 ± 4.0 days. Table 2 describes in-hospital clinical outcome. Mean heart rate decreased by 21% (from 100 ± 22 to 79 ± 18 beats/min), while mean systolic blood pressure remained stable. Overall, the in-hospital clinical course was uneventful in 22 patients (81.5%).

Efficacy outcomes

Three patients (11.1%) met the clinical efficacy endpoint. An 86-year-old woman died from recurrent pulmonary embolism and refractory cardiogenic shock 3 h after admission. Two patients (7.4%) had hemodynamic instability associated with persistent echocardiographic findings of severe right ventricular dysfunction 24 h after fibrinolysis. Both patients underwent successful surgical embolectomy.

Among the 24 remaining clinically stable patients, 3 (11.1%) had residual echocardiographic right ventricular dysfunction as previously defined (persistence of at least two initial right ventricular dysfunction criteria) (Table 3).

Safety outcomes

There was 1 major bleeding, and 1 important bleeding (total 2 events, 7.4%). The major bleeding complication occurred at the surgical site of a 71-year-old man who had undergone elective hip replacement two weeks previously. Management of bleeding in this patient required blood transfusion. Fondaparinux was replaced by UFH in

Table 3 In-hospital echocardiographic outcome

Echocardiographic findings	Pre-thrombolysis ($N = 27$)	Post-thrombolysis ($N = 26$)
RVEDD/LVEDD ratio ≥ 1	18 (66.7%)	4 (15.4%)
Spap >30 mmHg	27 (100%)	20 (76.9%)
Intracardiac thrombus	3 (11.1%)	0
Paradoxical septal motion	17 (63.0%)	4 (15.4%)
At least 2 of the above	22 (81.5%)	5 (19.2%)

RVEDD = Right ventricular end-diastolic diameter; LVEDD = Left ventricular end-diastolic diameter; sPAP = systolic pulmonary artery pressure

order that anticoagulant treatment could be neutralized if recurrent bleeding occurred. Infusion of recombinant coagulation factor VIIa and surgery were not required. A 57-year-old man with cirrhosis experienced an important bleeding complication without any identifiable bleeding site. Fondaparinux was not stopped. Clinical evolution of these 2 patients was favorable.

Discussion

Fondaparinux is a synthetic and selective inhibitor of factor Xa that has proven its efficacy and safety as a preventive and curative treatment of thromboembolic disease. The predictable and sustained anticoagulant effect of this drug for 24 h allows once-daily injection, and since it does not cross-react with heparin-induced antibodies, platelet count monitoring is no longer needed.

Our study is the first study to report the immediate clinical course of acute pulmonary embolism patients who received fondaparinux as adjunctive therapy to thrombolysis in the setting of acute high and intermediate risk pulmonary embolism.

Efficacy end point

Three patients (11.1%) met the clinical efficacy endpoint criteria, of whom one patient died from refractory shock and two patients required successful surgical embolectomy for persistent clinical instability. This confirms our previous findings reporting 8.2% of failed thrombolysis in UFH treated patients [8]. Moreover, the mortality rate was 4.3% in PE patients treated with thrombolysis and UFH in a recently published meta-analysis [9]. Fondaparinux thus seems to have similar in-hospital efficacy to UFH in the setting of PE submitted to thrombolysis, confirming results observed in the setting of stable thrombo-embolic disease [1, 2]. Additionally, this corroborates data on the efficacy of a fondaparinux-thombolysis association observed in the initial treatment of STEMI [5].

Safety end point

Two bleeding complications (7.4%) occurred during in-hospital course, including one important bleeding (3.7%) without any identifiable bleeding site and one major bleeding (3.7%) at the surgical site of an elective hip replacement. In a previous meta-analysis, fondaparinux 2.5 mg once daily was associated with a trend towards an increase in the incidence of major bleeding complications in patients undergoing orthopedic surgery [3]. However, in the initial treatment of hemodynamically stable patients with DVT and PE, fondaparinux 5–10 mg was associated with low rates of major bleedings (1.1% and 1.3%, respectively) [1, 2]. In the meta-analysis by Wan [9], major bleedings occurred in 9.1% of patients treated with thrombolysis and UFH, whereas in MAPPET-3, major bleedings occurred in 0.8% of patients submitted to thrombolysis [10]. Although the small sample size of this study does not make it possible to draw any definitive conclusions, the combination of fondaparinux and thrombolysis does not seem to be associated with an increase in bleeding events compared with UFH.

Limitations

This study is not a prospective randomized controlled trial and should be considered as pilot study derived from a single-center registry. As in most registries, neither diagnostic work-ups nor therapy were controlled. Moreover, our study is clearly under-powered, and no definitive conclusions can be drawn on the basis of these results.

Conclusion

Although the sample size of this pilot study is too small to firmly establish the safety and efficacy of fondaparinux as an adjunctive therapy to thrombolysis in the setting of massive and submassive acute PE, our results indicate that fondaparinux procures adequate tolerability compared to standard current therapy in this indication.

A randomized trial is warranted before administration of a combination of thrombolysis and fondaparinux in routine clinical practice can be advocated in this setting.

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II.3 Discussion

Fondaparinux is a synthetic and selective inhibitor of factor Xa that has proven its efficacy and safety as a preventive and curative treatment of thromboembolic disease. The predictable and sustained anticoagulant effect of this drug for 24 hours allows once-daily injection, and since it does not cross-react with heparin-induced antibodies, platelet count monitoring is no longer needed. Our study is the first study to report the immediate clinical course of APE patients who received fondaparinux as adjunctive therapy to thrombolysis in the setting of acute high and intermediate risk PE. In our study, three patients (11.1%) met the clinical efficacy endpoint criteria, of whom one patient died from refractory shock and two patients required successful surgical embolectomy for persistent clinical instability. This confirms our previous findings reporting 8.2% of failed thrombolysis in UFH treated patients (Meneveau N et al., 2006). Moreover, the mortality rate was 4.3% in PE patients treated with thrombolysis and UFH in a recently published meta-analysis (Wan S et al., 2004). Fondaparinux thus seems to have similar in-hospital efficacy to UFH in the setting of PE submitted to thrombolysis, confirming results observed in the setting of stable thrombo-embolic disease (Buller HR et al., 2004 and Buller HR et al., 2003). Additionally, this corroborates data on the efficacy of a fondaparinux -thombolysis association observed in the initial treatment of STEMI (Yusuf S et al., 2006).

In our study, two bleeding complications (7.4%) occurred during in-hospital course, including one important bleeding (3.7%) without any identifiable bleeding site and one major bleeding (3.7%) at the surgical site of an elective hip replacement. In a previous meta-analysis, fondaparinux 2.5 mg once daily was associated with a trend towards an increase in the incidence of major bleeding complications in patients undergoing orthopedic surgery (Turpie AG et al., 2002). However, in the initial treatment of hemodynamically stable patients with DVT and PE, fondaparinux 5-10 mg was associated with low rates of major bleedings (1.1% and 1.3%, respectively) (Buller HR et al., 2004 and Buller HR et al., 2003). In the meta-analysis by Wan, major bleedings occurred in 9.1% of patients treated with thrombolysis and UFH (Wan S et al., 2004), whereas in MAPPET-3, major bleedings occurred in 0.8% of

patients submitted to thrombolysis (Konstantinides S et al., 2002). Although the small sample size of this study does not make it possible to draw any definitive conclusions, the combination of fondaparinux and thrombolysis does not seem to be associated with an increase in bleeding events compared with UFH.

II.4 Conclusion

Our study suggests that the combination of fondaparinux and thrombolysis in acute massive and submassive PE is safe and effective. However, a pilot study, even though it may be convincing, is not enough to support formal recommendation. A controlled study including a higher number of patients with massive and submassive PE is needed to formally recommend fondaparinux use in this situation.

C. GENERAL DISCUSSION AND CONCLUSION

Thromboembolic events are serious and common diseases, affecting persons of all ages. Their etiology encompasses a wide variety of physiopathological causes and they frequently lead to hospitalization with serious consequences requiring prompt therapeutic intervention. AMI and stroke caused by compromised arterial flow resulting in ischemia make up the majority of these thromboembolic events. However, VTE continue to increase in incidence and have posed formidable treatment issues for clinicians involved in their management.

Venous and arterial thromboembolic disorders are usually considered as two distinct disease entities. At first this belief appears to be indisputable. Arterial thrombi consist mainly of platelets and are induced by arterial plaque ruptures which tend to occur at sites where shear rates are high. Conversely, venous thrombi mainly consist of red blood cells and fibrin and tend to occur at sites where the vein wall is often normal, but blood flow and shear rates are low (Agnelli G, et al., 2006). Furthermore, major, known risk factors for arterial thrombosis (e.g. tobacco smoking, arterial hypertension, diabetes, and dyslipidemia) are completely different from risk factors that are known to provoke venous thrombosis, such as trauma, surgery and cancer. However, mechanisms beyond the development of venous and arterial thrombi are far more complex and certainly have more links, such as endothelial dysfunction, inflammation and coagulation/fibrinolysis. Moreover, there also remains much to be understood about risk factors of venous thrombosis, given that currently as many as 26% to 47% of all VTE events remain classified as “unprovoked” or “idiopathic” (White RH., 2003). Our interest in the hemostasis/coagulation system, as a background for thromboembolic disorders, and the different situations of France and China regarding the epidemiology, diagnosis, and management of both coronary diseases (and their thrombobotic complications) and VTE, led us to work on both types of events related to thrombosis.

In our study, firstly, we investigated the early changes in local intracoronary hemostasis following DES and BMS implantation in patients with SA or silent ischemia under dual antiplatelet and anticoagulant pretreatment. In addition, the

present analysis was also performed to test for possible associations between the PAI-14G/5G gene polymorphism and early change in local intracoronary hemostasis following PCI procedures. Local levels of hemostasis markers were assayed at various time points before and after stent implantation. In addition, early local hemostasis response was compared before and after DES and BMS implantation to assess whether DES would induce earlier thrombogenicity than BMS. Our results demonstrated that the hemostatic activation occurs after balloon-induced vessel injury; however neither DES nor BMS further increases markers of platelet activation, coagulation or fibrinolysis in SA patients under dual antiplatelet and anticoagulant pretreatment. Balloon angioplasty induces a transient local haemostatic activation likely due to vessel shrinkage and arterial wall injury. This response is more obvious in patients with 4G/5G polymorphism of the PAI-1 gene. We conclused that pretreatment with anti-thrombotic medications in patients with SA effectively prevents or limits further consequences of the initial injury caused by angioplasty following coronary DES or BMS implantation.

PE is a common disorder and an important cause of morbidity and mortality. Anticoagulation is an effective treatment for PE. Anticoagulation, by preventing clot propagation, allows endogenous fibrinolytic activity to dissolve existing thromboemboli. Thrombolytic therapy, by actually dissolving thromboemboli, has several potential advantages over anticoagulation in the treatment of patients with PE. First, it should produce more rapid clot lysis and result in faster improvement in pulmonary perfusion, hemodynamic alterations, and gas exchange. Second, thrombolysis should eliminate venous thrombi and thereby reduce the incidence of recurrent PE. Third, rapid and complete clot resolution should prevent the development of chronic vascular obstruction and reduce the incidence of pulmonary hypertension. Finally, through all of these mechanisms, thrombolytic therapy should reduce morbidity and mortality from PE. Thus, the second aim of our study was to evaluate the safety and efficacy of a combination of fondaparinux and thrombolysis in the setting of acute high and intermediate risk PE. Our data suggest that the combination of fondaparinux and thrombolysis in acute massive and submassive PE is

safe and effective.

Xinjiang is a cold region of North-Western China. It is also a multi-ethnic residential area, shared by Uygur, Kazakh, Tajik, Hui, Uzbek, Kyrgyz, Man, Mongolia, Tatar, Darur, Xibo, and Russian “minorities” as well as by the ethnic group of Hans, which composes the majority of Chinese people. Because of the climate and original and diverse cultural habits, in Xinjiang, a high-calories and high-fat diet is common. Populations in Xinjiang may thus be more exposed to thrombo-embolic diseases than other parts of China, where the diet is rich in vegetables and poor in fat and in food of animal origin. Simple clinical observation, however, seems to show that incidence of coronary diseases is increasing, as part of the state of “epidemiological transition phenomenon”, as defined by the World Health Organisation, which accompanies “Western-style” economical development in China. Conversely, VTE seems to be still rather rare. Development of sophisticated diagnosis procedures, which are now available in our University Hospital in Xinjiang, should allow us to distinguish between an actual low incidence level of VTE and a lack of proper diagnosis of VTE conditions, espcecially PE. Based on our knowledge of thrombo-embolic events and of epidemiological methodologies gained from our personal training in France, we would like, first, to scientifically assess the epidemiology of thromboembolic events, especially VTE, in our hospital and more globally in Xinjiang, a region which gives us the opportunity to distinguish between populations with different genetic backgrounds and different diets, occupations and behaviors. We should also participate in the application of international recommendations for the treatment and follow-up of these diseases in our hospital. On the other hand, we would like to work on the clinical follow-up of patients after PCI to answer some of the questions which were not completely elucidated by our thesis work. We intend to develop protocols to further study the links between inflammation and hemostasis/coagulation in situ, during PCI, and the possible influence of genetic polymorphisms, that are likely different in the different ethnic groups. Ideally, such a work should associate France and China to allow us interesting comparisons.

Summary in French

Résumé

Dans les pays occidentaux, les manifestations thromboemboliques induisent une morbidité et une mortalité majeure. De part leur diversité d'expression clinique, les thromboses concernent en effet de nombreuses disciplines cliniques : angiologie, anesthésiologie, cardiologie, gynécologie obstétrique, hématologie, médecine interne, neurologie... Donc, la maladie thrombotique atteint le système veineux, où le débit et la pression sanguine sont faibles mais aussi le système artériel, où ces deux paramètres sont élevés. La composition du thrombus (riche en plaquettes dans les artères et riche en fibrine dans la veine) et la présence d'une lésion de la paroi vasculaire (athérome dans la thrombose artérielle), différencient la thrombose veineuse de la thrombose artérielle. Toutefois, les distinctions ne sont pas absolues et il existe des mécanismes communs. L'activation anormale de l'hémostase est au cœur de l'initiation de toutes les thromboses. Cette activation anormale peut être favorisée par une prédisposition génétique, présente durant toute la vie. Elle peut, par ailleurs, être favorisée par les effets transitoires ou prolongés, immédiats ou retardés, de facteurs environnementaux capables de moduler le risque artériel ou veineux.

Les techniques d'imagerie de plus en plus précises constituent un support indispensable à l'approche clinique des maladies vasculaires. En parallèle la biologie a apporté aux cliniciens de nouveaux outils permettant de mieux comprendre comment se développe la thrombose et pourquoi certains sujets sont plus exposés au risque de thrombose, artérielle ou veineuse. Cette définition des profils de risque thrombotique doit permettre à terme une meilleure prise en charge thérapeutique, reposant sur des consensus issus d'études cliniques rigoureuses.

A. Prévention et Traitement de la Thrombose en Cardiologie Interventionnelle

I. Introduction:

Pour le traitement de la maladie coronarienne, la mise en place d’un stent complète de

nos jours la plupart des angioplasties coronaires percutanées. Elle permet de traiter les complications locales dues à l'angioplastie par ballon et à plus long terme de diminuer le taux de resténose intra coronaire (Fischman DL et al., 1994 et Serruys PW et al., 1994).

Il existe deux problèmes secondaires à l'implantation du stent :

- Le taux de resténose à 6 mois. Il reste élevé, de 15 à 40% (Savage MP et al., 1994). Il s'agit d'une resténose intra-stent due à une hyperplasie intimale (Phillips DF et al., 1996 et Schwartz RS et al., 1998).
- Le taux de thrombose de stent. Il varie selon les études de 0,5% à 1,9% (Cutlip DE et al., 2001 et Orford JL et al., 2002).

La traduction clinique de la resténose et de la thrombose est très différente. La resténose s'exprime par un angor d'effort ou un syndrome coronarien aigu sans sus-décalage du segment ST. Il n'y a pas d'augmentation de la mortalité dans ce cas. La thrombose de stent est révélée par un syndrome coronarien aigu avec sus-décalage du segment ST. Elle est donc associée à un haut taux de morbidité et de mortalité (45 %) (Orford JL et al., 2002).

L'emploi de stents recouverts de sirolimus ou de paclitaxel, des drogues immuno-suppressives antiprolifératives a permis de réduire le taux de resténose à 6 mois de 70 % environ (Sousa JE et al., 2001, Liistro F et al., 2002, Morice MC et al., 2002, Arampatzis CA et al., 2003, Degertekin M et al., 2003, Park SJ et al., 2003, Schofer J et al., 2003, Babapulle MN et al., 2004, Colombo A et al., 2004, Holmes DR Jr et al., 2004, Schampaert E et al., 2004 et Serruys PW et al., 2004), au prix d'un possible risque accru de thrombose. Il n'y avait pas de différence significative dans les essais randomisés initiaux, qui avaient été réalisées avec un petit nombre de patients très sélectionnés. En revanche, 4 études prospectives de cohortes sur plusieurs milliers de patients ont fait état d'un risque accru de thrombose après stent actif (Drug eluting stent, DES) [1,3 % versus 0,6 % après stent conventionnel, (Bara-metal stent, BMS)] (Stabile E et al., 2004, Bavry AA et al., 2005, Derghazarian S et al,. 2005).

On distingue les thromboses de stent précoces des thromboses tardives. Un retard à

l'endothélialisation du stent expliquerait les thromboses tardives et la nécessité d'un traitement antiagrégant prolongé. Cette hypothèse physiopathologique est étayée par différentes études qui ont mis en évidence des facteurs de risque de thrombose compatibles avec ce retard à l'endothélialisation : arrêt prématuré du traitement antiagrégant, utilisation de DES long, lésions de bifurcation, sous-déploiement du stent (Kastrati A et al., 2005, Katritsis DG et al., 2005, Karvouni E et al., 2005, Kittleson MM et al., 2005, Mauri L et al., 2005, Moreno R et al., 2005, Ong AT et al., 2005)….

Un risque accru de thrombose précoce de DES a également été décrit [TAXUS V ISR randomized trial] (Stone GW et al., 2006). La thrombose précoce de DES aurait une autre explication physiopathologique. Il pourrait s'agir 1) d'une activation locale de la coagulation, de l'agrégation plaquettaire, et du système fibrinolytique du fait du traumatisme de la paroi artérielle par l'inflation du ballon ; et/ou 2) activation locale de la coagulation, de l'agrégation plaquettaire et du système fibrinolytique du fait de la présence du DES (métal + polymère + substance active).

Le diabète et l'insuffisance rénale, deux situations pour lesquelles on décrit une activation de l'hémostase à l'état basal, sont d'ailleurs des facteurs de risque prédictifs d'une thrombose de DES précoce (Hermiller JB et al., 2005, Iakovou I et al., 2005).

II. Objectifs de l'étude « Modifications précoces de l'activation de l'hémostase après intervention coronarienne percutanée chez des patients avec angor stable : comparaison entre stents actifs et stents conventionnels »

L'hypothèse d'une activation locale de l'hémostase après implantation d'un DES sous traitement anticoagulant et antiagrégant plaquettaire n'a jamais reçu de confirmation formelle.

Objectif principal :

Il était d'évaluer l'activation locale de la coagulation, des plaquettes et de la

fibrinolyse, à l'état basal, après angioplastie par ballonnet et après pose d'un DES, sous traitement antiagrégant et anticoagulant.

Objectif Secondaires :

Il était d'évaluer l'activation de l'hémostase locale après pose d'un BMS.

III. Critères de jugement

Le critère principal de jugement a été la mesure de l'activité du facteur tissulaire (TF), avec une augmentation attendue de 50% après pose du DES.

Les critères secondaires étaient l'étude de l'évolution entre l'état basal et la pose du DES pour les autres facteurs de l'activation de l'hémostase.

Une étude parallèle a été conduite dans les mêmes conditions avec le groupe de patients ayant bénéficié d'un BMS sachant que l'augmentation attendue pour l'activité du TF est de 30% après pose du BMS.

Critères d'inclusion

Les critères d'inclusion étaient les suivants :

1. Homme ou femme,
2. De 18 à 80 ans,
3. Lésion coronaire justifiant d'une angioplastie avec pose d'un BMS ou d'un DES,
4. Après signature d'un consentement éclairé.

Critère d'exclusion :

Les critères d'exclusion étaient les suivants :

1. Syndrome coronarien aigu avec sus-décalage du segment ST,
2. Lésions de bifurcations,
3. Présence de thrombus au sein de la lésion,
4. Patient sous anti-GIIbIIIa,
5. Patient avec une néoplasie active,
6. Patients insuffisants rénaux (clairance de la créatinine inférieure à 30 mL /min),
7. Patient ne pouvant donner leur consentement,
8. Femme enceinte.

IV. Méthodes

Les prélèvements ont été réalisés dans l'artère coronaire, au niveau de la lésion, avant l'angioplastie, 15 minutes après l'angioplastie et 15 minutes après la pose de stent. Ils ont été effectués à travers un cathéter de thrombo-aspiration. Il a été démontré dans plusieurs études que le prélèvement à travers un cathéter n'augmentait pas artificiellement les marqueurs de l'hémostase (Inoue T et al., 1996, Watkins MW et al., 1998).

Complément d'étude

Le même protocole a été réalisé chez des patients recevant un BMS. Les patients avaient des caractéristiques différentes dans les deux groupes, empêchant une comparaison de type « cas-témoin » mais donnant des informations sur la responsabilité du DES en lui-même.

Pour prendre en compte les caractéristiques génétiques des patients nous avons aussi inclus dans notre étude l'analyse du polymorphisme 4G/5G du PAI-1.

Marqueurs de l'hémostase étudiés

Pour essayer de mettre en évidence cette activation in situ de l'hémostase en dépit du traitement anticoagulant par enoxaparine et antiagrégant plaquettaire par aspirine et clopidogrel, nous avons choisi des marqueurs d'activation plaquettaire, de la coagulation et de la fibrinolyse:

- **L'activation plaquettaire**

Les patients sont sous traitement antiagrégant par aspirine en association avec du clopidogrel. Il y a donc peu d'activation plaquettaire attendue au cours de l'angioplastie. Pour essayer de mettre en évidence une activation plaquettaire malgré le traitement, nous avons choisi des marqueurs d'activation plaquettaire « sensibles » et reproductibles (Tomer A., 2004):

  - dosage de l'anticorps CD62(CD62P),
  - dosage de la glycoprotéine V (sGPV).

- **La coagulation et la fibrinolyse**

Les marqueurs de l'activation de la coagulation et de la fibrinolyse retenus ont montré leur pertinence dans différentes situations cliniques associées à un haut risque de thrombose ou à une activation locale de l'hémostase (Meade TW et al., 1996, Miller GJ et al., 1996, Zito F et al., 2000, Kalweit G et al., 2005).

Pour la coagulation nous avons réalisé

- la mesure de l'activité du facteur VII (FVIIa),
- la mesure de l'activité du facteur tissulaire (TF),
- le dosage des fragments 1 et 2 de la prothrombine (F1+2).

Pour la fibrinolyse nous avons réalisé

- le dosage des D-Dimères (DD),
- le dosage du fibrinogène (FIB),
- le dosage du *tissue plasminogen activator* (t-PA),
- le dosage des *plasminogen activator inhibitor type-1 complexes* (PAI-1).

Nombre de patients :

Il a été calculé selon les hypothèses de l'objectif principal : augmentation attendue de l'activité du TF de 50% après pose du DES et de 30% avec un BMS et pour une valeur moyenne du dosage de l'activité du TF à l'état basal de 9.5 pg/ml (Sd = 13.5), pour un risque $\alpha = 0.05$, un risque $\beta = 0.05$ (puissance statistique du test de 80%), (stent actif: $\mu o = 9.5$; $\mu a = 14.25$; $\Delta = 4.75$, stent conventionnel : $\mu o = 9.5$; $\mu a = 2.85$; $\Delta = 6.65$). Il a semblé raisonnable de prévoir l'inclusion de 20 sujets.

Analyse statistique :

- ***Description des patients inclus dans les deux groupes :*** la comparabilité des groupes a été vérifiée par une analyse bivariée. Les variables suivantes ont été considérées : variables démographiques (age, sexe, etc.) ; marqueurs de l'hémostase ; variables descriptives de la maladie coronaire (site d'angioplastie, taille du vaisseau, nombre de vaisseaux malades, fraction d'éjection du ventricule gauche, etc.) et variables descriptives du type d'angioplastie (taille et pression du ballon, type de prothèse).

- ***Pour chaque groupe :*** une analyse de variance a été réalisée sur mesures répétées en tenant compte du type de prothèse et de la taille de l'artère.

- La comparaison des variables quantitatives et qualitatives entre les deux groupes a été effectuée à titre exploratoire.

L'analyse statistique a été effectuée a l'aide des logiciels SAS.

V. Résultat

Nous n'avons pas observé de modification significative des marqueurs d'activation plaquettaire après la procédure d'angioplastie coronaire percutanée (Percutaneous Coronary Intervention, PCI). Les marqueurs de la coagulation augmentaient significativement 15 minutes après l'angioplastie par ballon (14% pour TF, p = 0.004 ; 40% pour F1+2, p = 0.001 ; et 31% pour FVIIa, p = 0.007)), de même que les marqueurs de la fibrinolyse (43% pour DD, 46% pour FIB, 60% pour t-PA et 70% pour PAI-1 ; p < 0.0001). Tous les marqueurs de l'hémostase sont revenus à l'état basal après la pose du stent. Il n y avait pas de différence significative entre DES et BMS quelque soit le marqueur d'hémostase.

S'agissant du polymorphisme 4G/5G du PAI-1, la distribution du génotype chez nos patients français avec une maladie coronaire stable était de 30% (6 patients) pour 4G/4G, 60% (12 patients) pour 4G/5G et 10% (2 patients) pour 5G/5G. L'importance de l'augmentation de PAI-1, DD et FVIIa après angioplastie était d'autant plus manifeste que les patients étaient hétérozygotes 4G/5G, comparés aux homozygotes 4G/4G et 5G/5G (*P* = 0.01, respectivement).

VI. Discussion

Prétraitement et activation de l'hémostase locale

Anti-agrégants plaquettaires et anticoagulants minimisent les complications de thrombose après PCI. Les caractéristiques basales des plaquettes de sont prédictives de l'activation ultérieure des plaquettes et peuvent identifier les malades qui pouraient le plus probablement profiter d'anti-agrégants plaquettaires et d'anticoagulants plus agressifs (Gurbel PA et al., 2000). Dans cette étude, la combinaison utilisée de trois

médicaments (aspirine, clopidogrel et enoxaparine) s'est révélée limiter l'activation de l'hémostase de façon satisfaisante avant la procédure. Nos résultats ont montré que les niveaux de CD62P et sGPV dans l'ostium coronaire et dans l'artère coronaire au-delà de la lésion étaient similaires avant la procédure de PCI et qu'ils étaient dans la gamme de référence normale. Ce résultat suggère que les caractéristiques basales des plaquettes sont prédictives d'un risque peu élevé de thrombose du stent chez les malades avec angor stable.

L'aspirine est un médicament anti-agrégant plaquettaire largement utilisé et très bien documenté pour réduire la morbidité et la mortalité cardio-vasculaires (Antithrombotic Trialists´ Collaboration. 2002). Les effets anti-agrégants plaquettaires de l'aspirine sont liés à l'inhibition irréversible de la cyclooxygénase (COX) des plaquettes, et à la diminution de la production du thromboxane A2, vasoconstricteur puissant et'activateur des plaquettes (TxA2). Les essais thérapeutiques récents contre placébo ont montré une excellente protection avec 75 mg d'aspirine quotidiennement chez les malades avec angine de poitrine stable (Juul-Moller S et al., 1992) ou instable (The RISC Group. 1990). Plus récemment, l'étude « CURE » a démontré que l'addition de clopidogrel à l'aspirine était associée à une réduction de 20% de la mortalité par infarctus du myocarde chez les malades avec syndromes coronariens aigus (Yusuf S et al., 2001). De même, quelques études récentes ont montré que le clopidogrel, notamment avec une dose de charge de 300 mg, supprimait rapidement l'agrégation des plaquette chez les sujets sains et les malades atteint d'athérosclérose, dès 2 h après la prise (Savcic M et al., 1999).

L'enoxaparine est utilisée fréquemment chez les malades qui doivent bénéficier d'une PCI. Les avantages théoriques des héparines de bas poids moléculaire (HBPM) par rapport à l'héparine non fractionnée comprennent 1) une activité anti-Xa supérieure à l'activité anti-IIa (3:1 pour l'enoxaparine), 2) un effet dose-réponse plus prévisible, qui réduit la nécessité d'un suivi biologique fréquent, 3) la facilité de l'administration sous-cutanée et 4) la diminution voire l'absence de risque de thrombopénie.

Plusieurs études rétrospectives ont suggéré que le prétraitement avec des statines

pourrait réduire l'incidence de l'infarctus du myocarde après l'intervention sur les coronaires (Pasceri V et al., 2004). Par ailleurs, une étude précédente a montré que les inhibiteurs des récepteurs de l'angiotensine II avaient aussi des propriétés anti-inflammatoire et diminuaient l'agrégation plaquettaire (Krämer C et al., 2002).

Notre étude montre qu'en cours de PCI, il n'y a aucune modification significative des marqueurs d'activation des plaquettes chez les malades sous traitement anti-agrégant plaquettaire combiné. Cette observation suggère un effet rapide du prétraitement pour supprimer l'activitation des plaquettes dans les coronaires. Nos résultats suggèrent aussi que ce prétraitement combiné inhibe suffisamment l'activation des plaquettes chez les malades avec angine de poitrine stable qui bénéficient d'une pose de stent. Ils confirment les observations de Mizuno et collaborateurs (Mizuno O et al., 2000) qui n'ont pas rapporté de modification dans l'activation des plaquettes après angioplastie par ballonnet ou après 'implantation de stent sous aspirine et ticlopidine ; en revanche, dans leur étude, les marqueurs d'activation des plaquette augmentent significativement tout de suite après une athérectomie rotative transluminale des coronaires chez les malades qui ne reçoivent pas de traitement anti-agrégant plaquettaire. De même, Grégorini et collaborateurs (Gregorini L et al., 1997) ont rapporté que l'usage combiné de ticlopidine, d'aspirine, et d'héparine prévenait efficacement l'activation des plaquettes chez les malades atteints d'angine de poitrine traités par athérectomie transluminale.

On ne peut exclure que, dans notre étude, l'utilisation fréquente des statines, des inhibiteurs de l'enzyme de conversion de l'angiotensine ou des inhibiteurs des récepteurs de l'angiotensine ait peut-être contribué à l'inhibition observée de l'activation des plaquettes.

L'activation locale des plaquettes après PCI

L'activation des plaquettes joue un rôle clé dans le développement de la thrombose précoce des stents. L'implantation de stents coronaires induit des altérations de la paroi vasculaire et une modification du flux sanguin local et active donc la voie intrinsèque du système de coagulation. Cette activation participe de façon importante

à la physiopathologie de la thrombose artérielle aiguë et subaiguë qui limitent le succès immédiat et à court terme des PCI. Dans la littérature, il n'y a pas de preuve d'une quelconque corrélation entre les complications qui accompagnent la pose de stent actif et des paramètres de l'hémostase, sauf pour l'activité du TF (Steffel J et al., 2005, Stahli BE et al., 2006 and Lüscher TF et al., 2007). A notre connaissance, notre étude est la première investigation de la réponse plaquettaire locale avant l'implantation de DES chez des malades avec angor stable prétraités par une HBPM (l'enoxaparine) et un traitement anti-agrégant plaquettaire associant aspirine et clopidogrel. L'observation intéressante apportée par notre étude est que les taux de CD62P et de sGPV ne changent pas au décours immédiat de la pose du DES chez les malades avec angine de poitrine stable. Cette absence de modification est également observée chez les malades traités avec un BMS et DES. On peut donc conclure que les médicaments immunosuppresseurs associés au stent « actif » ou le polymère qui permet d'en recouvrir le stent n'influencent pas la réponse plaquettaire locale, au moins à très court terme après la pose du stent. Ces résultats suggèrent que le risque de thrombose précoce sur stent est bas, et qu'il est similaire après implantation de DES et de BMS chez les malades avec angor stable et pré-traitement anti-agrégant plaquettaire combiné. Nos résultats, pourraient s'expliquer par le court délai entre les prélèvements et la pose du stent. Cependant, dans un modèle expérimental chez le porc, il a pu être démontré que l'implantation de DES ne s'accompagnait pas de plus de thrombose que celle de BMS dans les 24 premières heures après l'intervention (Gyöngyösi M et al., 2006). Ces observations expérimentales semblent confirmer nos propres résultats chez l'homme.

L'activation locale de la coagulation et de la fibrinolyse après PCI

L'autre résultat intéressant de notre étude est l'augmentation significative, dans la circulation intracoronaire, de la coagulation et de la fibrinolyse locales suite aux lésions pariétales induites par le ballonnet d'angioplastie. Ceci confirme des études précédentes montrant que la blessure de la paroi artérielle causée par la PCI peut entrainer l'activation de l'hémostase et conduire à une thrombose localisée et à une

embolisation distale ; les données des différentes études sont cependant discordantes sur ce point (Inoue T et al., 1996, Korovesis S et al., 2000, Borries M et al., 1999, Shammas NW et al., 1994). Il existe une activation importante et précoce du système de la coagulation, probablement liée à l'altération de l'endothelium et à la rupture de la plaque d'athérome lors de l'inflation du ballonnet qui représente un traumatisme pour la paroi artérielle. Ce phénomène active les facteurs prothrombiniques menant à l'activation de l'hémostase locale (Gyöngyösi M et al., 2006, Korovesis S et al., 2000 et Philipp R et al., 2003). Nous n'avons pas mesuré en parallèle les paramètres de coagulation et de fibrinolyse dans le sang périphérique. Cependant il est très vraisemblable que les modifications des marqueurs que nous avons observées dans la circulation coronaire ne font pas intervenir une réponse systémique, qui exige un temps plus long. D'ailleurs, il est important de constater que tous les marqueurs de coagulation et de fibrinolyse locale sont revenus aux valeurs de base après l'implantation du stent. Ces résultats sont conformes à une étude précédente qui montrait que l'activité du TF des monocytes n'était pas augmentée après l'implantation de stent chez des malades avec angor stable (Agraou B et al., 1997).

Plusieurs observations suggèrent que le système de fibrinolyse joue un rôle important dans la réponse hémostatique à la blessure de la paroi de l'artère. Les études cliniques qui ont étudié les taux de PAI-1 avant et/ou après PCI ont montré des résultats opposés. Plusieurs études ont suggéré l'existence d'une augmentation des taux de PAI-1 immédiatement après PCI (Ishiwata S et al., 1997 et Muldowney JA 3rd et al., 2007), bien que d'autres études aient rapporté une diminution de PAI-1 dans ces mêmes circonstances (Huber K et al., 1992 et Prisco D et al., 2001). Le FIB a été proposé comme marqueur prédictif des événements cliniques défavorables après PCI (Maresca G et al, 1999). Contrairement aux autres marqueurs d'hémostase, les D-dimères seraient plus stables et utiles pour déterminer non seulement l'activation de la fibrinolyse mais aussi la sévérité de l'état hypercoagulabilité. Nos résultats confirment que les quatre marqueurs sont sensibles au traumatisme de la paroi vasculaire puisque leurs taux sont augmentés après pré-dilatation par le ballonnet. Cependant, cette augmentation n'est que passagère et l'implantation d'un stent a pour

résultat un retour à la base de toutes ces valeurs, ce qui vraisemblablement leur enlève toute valeur prédictive. On peut supposer que l'implantation du stent qui suit la dilatation par inflation du ballonet lors de l'angioplastie est capable, en stabilisant la plaque d'athérome, de limiter les stimulus hémodynamiques et chimiques de l'agrégation plaquettaire et de réduire l'activation de la coagulation.

Comparaison entre DES et BMS pour leurs effects sur l'activation locale de l'hémostase intracoronarienne :

Malgré la réduction du taux de re-sténose, la fréquence des thromboses du stent n'a pas diminué avec les DES comparés aux BMS (Moreno R et al., 2005, Bavry AA et al., 2005 et Bavry AA et al., 2005). Bien que plusieurs études expérimentales aient étudié la pharmacocinetique locale de libération des médicaments associés au stent (Sakharov DV et al., 2002), l'influence des DES sur l'hémostase in situ, dans la circulation coronaire, n'est pas entièrement comprise.

A notre connaissance, notre étude est la première investigation de l'hémostase locale, mesurée in situ, en réponse à l'implantation de BMS et de DES. Nos résultats cependant, ne montrent pas de différence dans la réponse hémostatique locale en fonction du type de stent. L'activation de l'hémostase intracoronaire observée après pré-dilatation par ballonnet est réduite de façon similaire par DES et par BMS. Ces résultats ne donnent pas d'argument pour confirmer l'hypothèse qui avait été émise à la suite des études cliniques (Nordmann AJ et al., 2006, Daemen J et al., 2007, Moreno R et al., 2005 et Stahli BE et al., 2006) qui montraient un plus haut risque de trombose aiguë du stent lorsqu'un DES était implanté chez les malades et anticipaient que des altérations des parois vasculaires au contact de la surface du stent « actif » puisse représenter des stimulus précoces d'activation de l'hémostase intracoronaire. De plus, il n'y a pas, dans nos résultats, d'élément en faveur du rôle potentiel du polymère utilisé pour l'adsorption des médicaments sur le stent sur la réponse hémostatique locale immédiate chez les malades sous traitement combiné anti-agrégant plaquettaire et anticoagulant. Nos résultats, étant donné la méthodologie que nous avons suivie (étude in situ, dans les coronaires, avant et au décours immédiat de la poste des stents) ne peuvent exclure la possibilité d'un plus haut risque de

thrombose secondaire du stent après DES en relation avec un retard de l'endothélisation, un déploiement incomplet du stent ou des anomalies de l'agrégation plaquettaire après l'interruption du clopidogrel (Park DW et al., 2006, Cutlip DE et al., 2001, Honda Y et al., 2003, Iakovou I et al., 2005, Jeremias A et al., 2004). Ils tendent cependant à montrer que les thromboses observées à moyen ou long terme après DES ne sont vraisemblablement pas liées à des anomalies de l'hémostase qui seraient survenues immédiatement avant, pendant ou après la pose du stent.

Le polymorphisme génique dans le système PAI-1 de la fibrinolyse et l'activation de l'hémostase après PCI

Plusieurs observations suggèrent que le système de la fibrinolyse joue un rôle important dans la réponse hémostatique après lésions de la paroi de l'artère. Les études familiales suggèrent que les taux et l'activité de PAI-1 sont influencés par des facteurs génétiques (Pankow JS, et al., 1998). Les patients de génotype 4G/5G et 4G/4G avaient par ailleurs, des taux plasmatiques de PAI-1, DD, FVII et CD62P sensiblement plus élevés que ceux de génotype 5G/5G.

Les études précédentes ont habituellement analysé les effets de facteurs individuels non génétiques et génétiques sur les taux plasmatiques de PAI-1 dans de relativement petites populations à haut risque pour les événements thromboemboliques (Mansfield MW et al., 1995, Juhan-Vague I et al., 1993 et Toft I et al., 1997).

Le génotype 4G/5G de PAI-1 a été mis en relation avec le risque d'infarctus du myocarde (Boekholdt SM et al., 2001), la mort subite par arrêt cardiaque (Anvari A et al., 2001), les maladies coronariennes après transplantation (He JQ et al., 2002) , et la re-sténose après implantation de stent (Ortlepp JR et al., 2001). Il faut souligner également que le genotype 4G/5G designe des patients qui ont tendance à exprimer une pathologie trombo-embolique plus fréquemment que les autres. Des facteurs génétiques héréditaires jouent donc un rôle dans le niveau de l'activité plasmatique de facteurs importants pour la coagulation/fibrinolyse, donc dans la prédisposition à une bonne activité de coagulation en cas d'hémorragie, mais en contre-partie, dans la prédisposition à la thrombose pathologique.

L'étude du polymorphisme génétique de nos 20 cas de patients français atteints de maladie coronaire stable nous a permis de constater que le génotype PAI-1 4G/5G était le plus fréquent, suivi du génotype 4G/4G et du génotype 5G/5G (60% [12 patients] pour 4G/5G, 30% [6 patients] pour 4G/4G, et 10% [2 patients] pour 5G/5G). Cette répartition correspond à une frequence de 60 % de l'allèle 4G et de 40% de l'allèle 5G allele. Ils confirment d'autres observations (Eriksson P et al., 1995, Johan-vague I et al., 2003, ten Boeked E et al., 2003) qui suggèrent que la fréquence de la maladie coronarienne chez les hétérozygotes 4G/5G est plus élevée que chez les homozygotes 4G/4G et 5G/5G.

De plus, dans notre étude, l'importance de l'augmentation de PAI-1, DD et FVIIa après le geste d'angioplastie était d'autant plus manifeste que les patients étaient hétérozygotes 4G/5G, comparés aux homozygotes 4G/4G et 5G/5G (P = 0.01, respectivement). Les porteurs du génotype 4G/5G apparaissent donc plus sensible à la lésion de la paroi artérielle et aux modifications de l'endothélium vasculaire provoquées par le ballonnet lors de la dilatation. Cela montre que le facteur génétique est susceptible de jouer aussi un rôle important dans les phénomènes thrombotiques qui accompagnent la PCI. Maladie coronarienne et maladie thrombo-embolique sont des maladies multi-géniques. Seule une étude plus poussée sur un plus grand nombre de patients permettrait de savoir si le polymorphisme 4G/5G de PAI-1 est un facteur de risque indépendant de risque thrombotique après un geste interventionnel chez les patients coronariens.

Discussion sur la méthodologie de l'étude

La taille d'échantillon de malades étudiés est relativement petite ; de plus nous avons seulement examiné des critères biologiques, mesurés in situ, dans la circulation sanguine coronarienne, sans recueillir la suite des événements cliniques survenus après la pose du stent. Nos résultats ont donc besoin d'être confirmés par une étude de plus grande envergure. Nous avons volontairement choisi les malades d'un niveau de risque peu élevé avec angor stable ; aussi les résultats ne peuvent-ils pas être applicables à d'autres sous-ensembles différents de malades comme ceux atteints

d'angor instable et/ou de pathologies aiguës. De plus, nous nous somme concentrés sur l'activation de hémostase locale précoce, et nous ne pouvons pas en tirer de conclusion sur un risque possible de thrombose subaiguë et de thrombose tardive du stent. Tous les malades ont été prétraités par enoxaparine et il est difficile de savoir si nos résultats pourraient être extrapolés aux malades prétraités par héparine non fractionnée. Il est également possible que le prétraitement par statines, inhibiteurs de l'enzyme de conversion de l'angiotensine ou inhibiteurs des récepteurs de l'angiotensine et/ou d'autres facteurs préinterventionnels puissent avoir changé les niveaux des marqueurs de l'hémostase. Enfin, les malades sont leurs propres témoins ; il n'y avait pas, dans notre étude, de groupe contrôle (par exemple : des patients atteints d'angor stable sans indication de PCI). Dans ce contexte, nous avons trouvé des niveaux de base de TF au-dessus des valeurs normales, ce qui est compatible avec le fait que tous les malades avaient une CAD documentée. Il aurait certainement été utile d'évaluer en parallèle l'activation des facteurs d'inflammation, comme la CRP, pour étudier la relation entre la réponse inflammatoire précoce locale et l'activation de l'hémostase après PCI. Une étude que nous avons réalisée dans le Premier Hôpital Affilié de l'Université de Médecine du Xinjiang, chez des patients atteints de maladies coronariennes, nous a permis de confirmer l'élévation des marqueurs d'inflammation, plus importante chez les patients atteints des pathologies les plus graves. Ces résultats sont globalement en accord avec d'autres études faites dans d'autres pays sur des séries de patients plus importantes, mais elles ne peuvent bien évidemment pas s'appliquer aux gestes interventionnels où des études spécifiques devraient être menées.

VII. Conclusion

L'activation locale de l'hémostase est précoce et importante après la dilatation coronaire par ballonnet. La pose du stent fait rapidement revenir les paramètres d'hémostase et de fibrinolyse à la normale. Il n'y a cependant pas de différence significative entre DES et BMS, quelque soit le marqueur d'activation plaquettaire,

d’activation de la coagulation et d’activation de fibrinolyse, sous pré-traitement combiné antiagrégant et anticoagulant.

L’importance de l’augmentation des marqueurs de fibrinolyse après angioplastie est d’autant plus manifeste que les patients sont hétérozygotes 4G/5G pour le gène du plasminogen activator inhibitor type-1 complexes (PAI-1).

B. Prévention et traitement de la maladie thromboembolique Veineuse : efficacité et sécurite d'emploi

I. Introduction : la maladie thromboembolique veineuse

Incidence

La thrombose veineuse profonde (TVP) et l'embolie pulmonaire (EP) font partie d'une même entité pathologique, la maladie thrombo-embolique veineuse (MTEV). Plus de 70 % des EP sont dues à une TVP des membres inférieurs. La MTEV présente un risque immédiat potentiellement vital l'EP, alors qu'à distance de l'épisode aigu le risque est lié au développement d'une maladie post-thrombotique et plusrarement à l'évolution vers une pathologie pulmonaire chronique. Les estimations concernant l'incidence de la MTEV sont très imprécises faute d'éléments diagnostiques fiables puisque même le diagnostique post-mortem est soumis à discussion. On estime à 600 000 le nombre de cas annuels de MTEV aux Etats-Unis, dont 30% entraînent un décès, et 250 000 TVP. En France l'incidence annuelle de la MTEV est de l'ordre de 50 à 100 000 cas responsables de 5 à 10 000 décès. Cette incidence augmente avec l'âge pour atteindre 1% après 75 ans. Il s'agit d'une pathologie grave et encore très sous-estimée, puisque l'EP est responsable de plus de 10 000 décès annuels en France alors que les séries autopsiques font état d'EP ou de thrombose veineuse dans plus de 30% des cas sans que cette fréquence ne varie au cours des 30 dernières années. Ces chiffres suffisent à justifier la mise en œuvre de programmes de recherche destinés à améliorer l'efficacité des mesures préventives, diagnostiques et thérapeutiques dans la prise en charge de la MTEV.

Etiologie et physiopathologie

L'EP est l'obstruction de l'artère pulmonaire par la migration de thrombus provenant d'une thrombophlébite distale. Une fois libérés dans la circulation veineuse, les emboles gagnent les 2 poumons dans environ 65 % des cas, le poumon droit dans 25 % des cas, et le poumon gauche dans 10 % des cas. Les lobes inférieurs sont atteints 4 fois plus souvent que les lobes supérieurs. La plupart des embolies se fixent

dans les artères pulmonaires de gros ou de moyen calibre (élastiques ou musculaires) ; 35 % ou moins atteignent les artères de petit calibre.

L'EP aiguë est un processus dynamique. Les thrombus commencent à se lyser immédiatement après avoir atteint les poumons. Habituellement, la lyse complète survient en plusieurs semaines en l'absence de cardiopneumopathie préexistante ; dans certains cas, des caillots même volumineux peuvent se lyser en quelques jours. Les troubles fonctionnels s'atténuent au cours des heures et des jours à mesure que la circulation pulmonaire s'améliore. Cependant, une embolie massive peut provoquer la mort en quelques minutes ou heures sans que l'infarctus n'ait le temps de se constituer. Parfois, les embolies se répètent pendant des mois ou des années, provoquant une obstruction artérielle évolutive avec hypertension artérielle pulmonaire chronique, dyspnée croissante, et coeur pulmonaire chronique.

Les phénomènes physiopathologiques induits par l'EP comprennent des troubles de l'hémodynamique pulmonaire, des échanges gazeux, et de la mécanique ventilatoire. Le degré de perturbation de la fonction cardiopulmonaire dépend de l'étendue de l'occlusion, qui varie selon la taille et le nombre de thrombus embolisant et obstruant les artères pulmonaires, et l'état cardiopulmonaire du patient avant l'embolie. Les modifications physiopathologiques qui en résultent peuvent comporter une hypertension artérielle pulmonaire avec insuffisance ventriculaire droite et choc, une dyspnée avec tachypnée et une hyperventilation, une hypoxémie et un infarctus pulmonaire. La sérotonine plaquettaire et/ou l'augmentation locale des liquides interstitiels stimulent les récepteurs pulmonaires des neurones médullaires inspiratoires et expiratoires entraînant la polypnée. L'hypoxie artérielle est presque constante. Elle est due en partie au défaut de perfusion et surtout à une réduction de la ventilation par rapport à la perfusion au niveau de tout le poumon embolisé. L’évolution se fait vers un infarctus pulmonaire. L'obstacle à l'éjection du ventricule droit détermine en partie une augmentation de la pression systolique dans le ventricule droit et dans l'artère pulmonaire. Une hypertension artérielle pulmonaire (HTAP) se développe. Elle est proportionnelle au degré d'obstruction vasculaire. Elle est accentuée par la vasoconstriction réflexe secondaire à l'hypoxémie, et par la

sérotonine plaquettaire.

II. Prévention et traitement de l'EP

Les objectifs thérapeutiques des traitements anticoagulants et thrombolytiques, agents thérapeutiques majeurs dans l'EP, sont très différents ; leurs indications respectives établies sur des critères de sévérité cliniques restent sujet à controverse (Goldhaber SZ et al., 1999). Les héparines préviennent les récidives emboliques et l'extension des thrombus veineux et pulmonaire, alors que la thrombolyse a une action directe qui permet de lever l'obstruction vasculaire pulmonaire.

On ne dispose aujourd'hui d'aucune recommandation internationale précise sur les indications de la thrombolyse médicamenteuse dans l'EP et les positions exprimées à cet égard dans la littérature sont parfois très discordantes. Schématiquement, ces indications se discutent en fonction du retentissement clinique et hémodynamique de l'obstruction vasculaire pilmonaire, dans trois circonstances particuliès : les EP massives avec obstruction de plus de 50% du lit vasculaire pulmonaire associée à des signes de mauvaise tolérance clinique et hémodynamique, les EP massives avec bonne tolérance hémodynamique et signes échocardiographiques de surcharge ventriculaire droite et les EP sub-massives (<50% d'amputation vasculaire) mal tolérées en raison d'une pathologie cardiaque ou pulmonaire associée.

L'intérêt porté à la simplification des modalités d'administration du traitement thrombolytique avec adoption de protocoles courts, a imposél'activateur tissulaire du plaminogène comme agent thrombolytique de référence. Si la thrombolyse garde aujourd'hui des indications dans la prise en charge de l'EP, c'est en grande partie parce qu'elle s'est montrée supérieure à l'héparine dans la rapidité à améliorer la perfusion pulmonaire et l'hémodynamique droite (Petitpretz P et al., 1984). Cependant, le risque hémorragique induit par la thrombolyse demeure la préoccupation majeure de ce type de traitement. Ce risque est présent quels que soient le thrombolytique utilisé et la manière dont il est administré. La réduction du taux de

complications hémorragiques passe par un respect scrupuleux des contre-indications, et la prise en compte des facteurs prédisposants aux accidents hémorragiques.

Trois agents thrombolytiques ont à ce jour fait l'objet d'une autorisation de mise sur le marché dans le cadre du traitement de l'EP : la streptokinase (SK), l'urokinase (UK) et l'activateur tissulaire du plasminogène (t-PA).

La streptokinase (SK) est le thrombolytique le plus ancien. Elle est extraite à partir de filtrats de culture de streptocoques béta-hémolytiques et possède un fort pouvoir antigénique. Elle active indirectement le plasminogène lié à la fibrine et le plasminogène circulant par le biais d'un complexe streptokinase-plasmine.

L'urokinase (UK) isolée à partir de l'urine humaine ou de cultures de cellules rénales embryonnaires est une glycoprotéine non antigénique. Son mode d'action repose sur l'activation directe du plasminogène et comme pour la streptokinase s'accompagne d'une fibrinolyse, d'une fibrinogénolyse et d'effets sur la coagulation.

L'activateur tissulaire du plasminogène (t-PA) obtenu initialement à partir de cultures de cellules humaines de mélanome est aujourd'hui produit par génie génétique (recombinant t-PA ou rt-PA). Il s'agit de l'agent thrombolytique le plus évalué dans l'EP depuis vingtaine d'années. L'altéplase est un t-PA, sérine protéase naturelle produite par recombinaison génétique (rt-PA). Il se fixe à la fibrine qui potentialise alors son activité. Aux doses thérapeutiques, la fibrinogénolyse est moindre que celle de la streptokinase ou de l'urokinase.

Parallèlement à la thrombolyse, le traitement médicamentteux de l'EP repose dans la très grande majorité des cas sur le traitement anticoagulant. Il est aujourd'hui bien établi que ce traitement est le plus souvent efficase et bien toléré, et que relayées par les anticoagulants oraux, l'héparine non fractionnée (HNF) ou les héparines de bas poids moléculaire (HBPM) préviennent l'extention de la thrombose veineuse et les récidives emboliques, au prix d'un taux de complications hémorragiques réduit (The Colombus Investigators. 1997, Charbonnier B et al., 1998, Leizorovicz A et al., 1994). L'apparition des HBPM a fait beaucoup évoluer le traitement de la maladie thrombo-embolique ces dernières années. Les HBPM ont une meilleure bio-disponibilité, une plus forte efficacité anti-thrombine, un risque hémorragique ou

de thrombopénie induite moindre que l'HNF. Les études Colombus et Thésée ont montré que l'administration d'une HBPM adaptée au poids du patients est aussi efficace et bien tolérée que l'HNF dans le cadre du traitement de l'EP symptomatique, ne relevant pas de la thrombolyse ou de l'embolectomie chirurgicale (The Colombus Investigators., 1997 et Simonneau G et al., 1997). L' études Thésée en particulier fait état d'un taux de récidives emboliques de 1.6%, de saignements majeurs de 1%, et de décès de 3.9%, et conduit à envisager à court terme un remplacement de l'HNF par les HBPM dès lors qu'elles auront obtenu l'AMM dans cette indication (Simonneau G et al., 1997).

Le fondaparinux (Arixtra®) est un pentasaccharide, dérivé de la portion de l'héparine qui se lie à l'antithrombine ; inhibiteur synthétique sélectif du facteur Xa. Il est indiqué pour :

- la prophylaxie des troubles thromboemboliques veineux (TEV) consécutifs à une chirurgie orthopédique des membres inférieurs, par exemple, en cas de fracture de la hanche, de chirurgie du genou ou d'arthroplastie de la hanche, et ce pour une période allant jusqu'à un mois.
- le traitement de la TVP aiguë et de l'EP aiguë.

L'absence d'induction d'anticorps anti-plaquettes et de réaction croisée avec les anticorps induits par l'héparine dispense le clinicien de la surveillance plaquettaire imposée par l'utilisation de l'héparine et représente un avantage majeur. En prévention, il est prescrit à la dose de 2,5 mg à la sixième heure en post-opératoire des chirurgies de hanche et de genou. Dans cette indication, il est plus efficace que l'enoxaparine, au prix d'une augmentation significative des saignements majeurs (Turpie AG et al., 2002). Pour le traitement curatif, la dose est adaptée au poids du patient (5 mg pour un poids inférieur à 50 kg, 7,5 mg entre 50 et 100 kg, 10 mg au delà de 100 kg). Il est au moins aussi efficace que l'enoxaparine et l'HNF (TVP et EP respectivement)(Buller HR et al., 2003 et Buller HR et al., 2004) sans différence significative de tolérance.

Par ailleurs, le fondaparinux a été évalué comme traitement adjuvant à la

thrombolyse pour le traitement du syndrome coronarien aiguë (SCA). Ces deux études OASIS cumulant plus de 32 000 patients en SCA. L'étude OASIS-5 (Yusuf S et al., 2006), avait déjà montré l'année dernière chez les patients avec SCA sans sus-décalage persistant du segment ST, qu'une stratégie basée sur le fondaparinux à dose relativement modérée faisait jeu égal avec l'HBPM de référence à sa forte dose classique, mais avec moins de complications hémorragiques. On attendait donc la suite logique, l'étude OASIS-6 (Michelangelo-Organization to Assess Strategies for Ischaemic Syndromes) testant une stratégie utilisant la même dose modérée de fondaparinux (2,5 mg/j) dans le cadre des SCA avec sus-décalage persistant de ST. Dans OASIS-6 (Yusuf S et al., 2006), le fondaparinux (2,5 mg SC/jour) était administré en double aveugle précocement, dès la randomisation (< 24 heures, délai qui a été raccourci à < 12 heures en cours d'étude), et pour une durée (relativement longue) de 8 jours. Le design de l'étude en deux stratifications est assez complexe. En effet, le traitement de référence dépend de l'indication d'une héparinothérapie jugée par le clinicien référent. S'il pense que l'héparine n'était pas indiquée (stratum 1), le comparateur sera le placebo pendant 8 jours. S'il pense que l'administration d'héparine était indiquée (stratum 2), le comparateur était l'héparine administrée pendant 48 heures (bolus de 60 UI/kg puis 12 UI/kg/heure), avec un relais par placebo pendant 8 jours. Concernant la prise en charge des patients, 31 % ont bénéficié d'une angioplastie primaire (0,2 % du stratum 1 et 53 % du stratum 2) et 45% ont bénéficié d'une thrombolyse (78% du stratum 1 et 16 % du stratum 2). Les thrombolytiques étaient très majoritairement utilisés (la streptokinase dans 78 % des cas). Donc 24 % des sujets n'ont pas bénéficié d'une thérapeutique de reperfusion. OASIS-6 a recruté 2 092 patients dans 41 pays. Les résultats globaux de cette étude ont démontré que le fondaparinux était supérieur au traitement standard (HNF ou placebo) pour réduire le risque de décès et de récidive de crise cardiaque (réduction du risque de 14 % au jour 30, p = 0,008), une réduction significative ayant été observée dès le jour 9 (réduction du risque de 17 %, p = 0,003). Le fondaparinux a également produit une baisse significative de la mortalité toutes causes confondues (paramètre d'évaluation secondaire) au jour 9 (réduction du risque de 13 %, p = 0,043), baisse qui s'est

maintenue jusqu'à la fin de l'étude (réduction du risque de 12 %, p = 0,029). Le bénéfice a aussi été constaté chez les patients traités par thrombolyse et chez ceux qui n'ont pu bénéficier d'aucune procédure de reperfusion. Parallèlement, dans l'étude OASIS 6, la fréquence des hémorragies graves jusqu'au jour 9 a été semblable chez les patients traités par le fondaparinux et ceux recevant le traitement standard. En outre, l'étude OASIS 6 a démontré que le fondaparinux était associé à un rapport avantages/risques net significatif d'après l'ensemble des paramètres d'évaluation de l'efficacité et de l'innocuité (décès, IM et hémorragies graves), et ce, à tous les points d'évaluation (au jour 30, la réduction du risque était de 14 %, p = 0,005). La posologie est de 2,5 mg par jour dans cette indication, quel que soit le poids du patient.

III. Objectif de l'étude « Efficacité et sécurité d'emploi du fondaparinux comme traitement adjuvant à la thrombolyse chez les patients avec embolie pulmonaire massive ou submassive »

A l'heure actuelle, l'HNF reste le seul traitement adjuvant à la thrombolyse validé et recommandé pour la prise en charge des EP massives et submassives. Le fondaparinux n'avait pas encore été évalué dans cette indication. Il semblait a priori comporter un intérêt potentiel en termes d'efficacité; mais cet intérêt devait être validés par une étude spécifiquement dédiée. L'objectif de notre travail a donc été d'évaluer l'efficacité, et surtout la sécurité d'emploi, du fondaparinux à dose curative comme traitement adjuvant à la thrombolyse pour le traitement des EP massives et submassives.

IV. Critères de jugement

Critères d'efficacité de la thrombolyse

Le critère de jugement était un critère combiné incluant la persistance d'une instabilité

hémodynamique et/ou d'une dysfonction ventriculaire droite échographique. Sur le plan clinique, la persistance d'une instabilité hémodynamique était définie par la présence d'au moins deux des critères suivants : choc cardiogénique réfractaire, tension artérielle systolique inférieure à 90 mmHg ou chute de tension artérielle systolique d'au moins 40 mmHg (en dehors d'un sepsis, d'une hypovolémie ou d'une arythmie rapide), hypoxémie (définie par une PaO2 ≤ 55 mmHg sans oxygène ou SaO2 ≤ 90 %) ou tachycardie (≥ 110 battements / minute).

Sur le plan échographique, la dysfonction ventriculaire droite était définie par la persistance d'au moins deux des critères définis précédemment (dilatation ventriculaire droite, rapport des diamètres télédiastoliques ventriculaires droit et gauche supérieur à un en coupe apicale quatre cavités, mouvement systolique septal paradoxal, hypertension artérielle pulmonaire systolique définie par un gradient ventricule droit - oreillette droite supérieur à 30 mmHg).

La présence de thrombu proximaux obstructifs à l'angioscanner et la constatation d'une amputation vasculaire importante sur les clichés de scintigraphie pulmonaire de ventilation – perfusion pouvaient également appuyer la conviction de l'inefficacité du traitement administré.

Critères de sécurité d'emploi

Le critère évaluant la sécurité d'emploi était la survenue d'accidents hémorragiques graves incluant :

- les hémorragies cérébrales confirmées par un scanner ou une IRM cérébrale ;
- les hémorragies nécessitant une transfusion sanguine ou entraînant une chute de l'hématocrite supérieure à 10 points ;
- les hémorragies nécessitant une chirurgie d'hémostase ;
- les hémorragies entraînant le décès du patient.

La survenue des complications hémorragiques était recherchée par des examens cliniques et biologiques quotidiens. Devant une telle complication, un scanner thoracique, abdominal ou cérébral, une échographie abdominale ou des parties molles (pour les hématomes sous-cutanés ou musculaires) ou une endoscopie digestive

pouvaient être réalisés avant une prise en charge adaptée.

V. Méthodes

Population

Elle était issue du registre monocentrique prospectif d'EP mis en place au CHU de Besançon depuis 1993. 27 patients victimes d'EP massives ou submassives ont bénéficié d'un traitement fibrinolytique associé au fondaparinux en 2006 et 2007.

Critères d'inclusion

Les critères d'inclusion étaient les suivants :

- EP documentée par une scintigraphie de ventilation - perfusion ou un scanner thoracique spiralé ;
- début de la symptomatologie inférieur à 15 jours ;
- avoir au moins l'un des critères suivants :
    - une instabilité hémodynamique avec état de choc cardiogénique, définie par une tension artérielle systolique inférieure à 90 mmHg ou une chute de tension artérielle systolique d'au moins 40 mmHg ou des signes cliniques de choc ;
    - une syncope ;
    - un thrombus proximal documenté par un scanner thoracique spiralé ;
    - une troponine positive ;
    - au moins deux critères échocardiographiques de dysfonction ventriculaire droite (dilatation ventriculaire droite, rapport des diamètres télédiastoliques ventriculaires droit et gauche supérieur à un en coupe apicale quatre cavités, mouvement systolique septal paradoxal, hypertension artérielle pulmonaire systolique définie par un gradient ventricule droit - oreillette droite supérieur à 30 mmHg).

Critères d'exclusion

Les critères d'exclusion étaient :

- présence de contre-indications au traitement thrombolytique ;
- présence à l'admission d'une insuffisance rénale définie par une clairance de la créatinine inférieure à 30 ml / min.

Traitement

Thrombolytique

Le choix du traitement thrombolytique était laissé à la discrétion du médecin prenant en charge le patient. Les molécules disponibles étaient la streptokinase et l'altéplase, administrées selon des protocoles stricts :

- Thrombolyse par streptokinase : injection de 40 mg de méthylprednisolone par voie intraveineuse immédiatement avant le début de la thrombolyse ; 1 500 000 UI de streptokinase administrées en perfusion continue par voie intraveineuse sur deux heures dans 60 ml de sérum glucosé à 5 % ;
- Thrombolyse par altéplase : pour un poids supérieur à 70 kg, administration d'un bolus intraveineux de 10 mg suivi d'une perfusion continue de 90 mg sur deux heures ; pour un poids inférieur à 70 kg, administration d'un bolus intraveineux de 7 mg suivi d'une perfusion continue de 63 mg sur deux heures.

Anticoagulant

Le fondaparinux était débuté dès la confirmation du diagnostic, par voie sous-cutanée à dose curative : 5 mg / jour pour un poids inférieur à 50 kg, 7.5 mg / jour entre 50 et 100 kg, 10 mg / jour au delà de 100 kg.

Le relais par traitement anti-vitamine K (fluindione) était entrepris après la restauration d'une hémodynamique normale (en moyenne 72 h après l'administration de la thrombolyse), avec un objectif d'INR (International Normalized Ratio) compris entre 2 et 3 pendant au moins six mois (délai adapté en fonction de la situation clinique et des antécédents thrombo-emboliques). Le traitement par fondaparinux était arrêté après obtention de deux INR successifs supérieurs à 2.

Analyse statistique

Les variables quantitatives ont été exprimées en moyenne plus ou moins une déviation

standard. Les variables qualitatives ont été exprimées en pourcentage. Les tests de Student et du Chi^2 ont été employés pour comparer respectivement les variables quantitatives et qualitatives. Une valeur de *p* inférieure à 0,05 a été considérée comme statistiquement significative. Les analyses ont été effectuées avec le logiciel SAS.

VI. Résultats

Les patients étaient âgés de 68 ± 11 ans (42 à 86 ans) ; il s'agissait de 11 hommes (40,7 %) et 16 femmes (59,3 %). Vingt deux patients (81,5 %) ont reçu un traitement fibrinolytique par perfusion d'altéplase sur 2h et 5 patients (18,5 %) par perfusion de streptokinase sur 2h. Le traitement a été inefficace chez 3 patients (11,1 %), dont un est décédé d'un choc cardiogénique réfractaire suite à une récidive embolique. Les deux autres (7,4 %) avaient une dysfonction ventriculaire droite persistante au contrôle échographique et une obstruction artérielle proximale objectivée par l'angioscanner, justifiant une embolectomie chirurgicale de sauvetage, qui s'est avérée efficace. La seule récidive embolique a concerné le patient décédé. Vingt six patients ont eu un contrôle échocardiographique post-fibrinolyse, qui a montré une diminution du rapport VD/VG de 23 % (de 1,02 ± 0,17 à 0,78 ± 0,17) et de la pression artérielle pulmonaire systolique de 21 % (de 56 ± 15 à 44 ± 13 mmHg). Parmi les 19 angioscanners de contrôle (70,0 %), 12 ont objectivé la persistance de thrombus proximaux. Vingt patients (74 %) ont eu une scintigraphie pulmonaire de ventilation – perfusion : l'amputation moyenne était de 26 ± 13 % ; 8 patients avaient une amputation supérieure ou égale à 30 %. Deux complications hémorragiques (7,4 %) ont émaillé l'évolution hospitalière, dont 1 avec une chute d'hématocrite supérieure à 10 % ; une seule a nécessité un traitement par transfusion sanguine dans un contexte post-opératoire de chirurgie d'arthroplastie de hanche.

VII. Discussion

Efficacité

Trois des 27 patients (11,1 %) n'ont pas répondu au traitement. Ce résultat est comparable aux données de notre registre d'EP thrombolysées entre 1995 et 2005 : parmi les 488 patients inclus, le taux d'échec était de 8,2 % (Meneveau N et al., 2006). Par ailleurs dans une recente publication meta-analyse le taux de mortalité est de 4.3% chez les patients traités par thrombolyse et UFH (Wan S et al., 2004). Le fondaparinux semble avoir la même efficacité en milieu hospitalier que l'UFH dans le cadre de PE thrombolysée, confirmant les résultats observés dans le cadre de la stabilité de la maladie thrombo-embolique (Buller H et al., 2003 et 2004). En outre, cela corrobore les données sur l'efficacité d'une association thrombolyse-fondaparinux observée dans le traitement initial de STEMI (Yusuf S et al., 2006).

Complications hémorragiques

Deux patients (7,4%) ont eu une complication hémorragique au cours de l'hôpitalisation, dont un important saignement (3,7%), sans site de saignement identifiable et un grand saignement (3,7%) au site chirurgical pendant une arthroplastie de la hanche. Dans un précédent méta-analysis, le fondaparinux 2,5 mg une fois par jour a été responsable d'une augmentation de l'incidence des principales complications de saignement chez les patients subissant une chirurgie orthopédique (Turpie AG et al., 2002). Parcontre, dans le traitement initial des patients hemodynamiquement stable avec DVT et PE, 5-10 mg de fondaparinux était associé à de faibles taux d'hémorragies majeures (1,1% et 1,3%, respectivement) (Buller HR et al., 2003 et 2004). Dans la méta-analyse par Wan (Wan S et al., 2004), des hémorragies majeures se sont produits chez 9,1% des patients traités avec la thrombolyse et l'UFH, alors que, dans MAPPET-3, des hémorragies majeures se sont produite chez 0,8% des patients soumis à la thrombolyse (Konstantinides S et al., 2002). Bien que la petite taille de l'échantillon de cette étude ne permette pas de tirer des conclusions définitives, la combinaison du fondaparinux et de la thrombolyse ne semble pas être associée à une augmentation des hémorragies événements par rapport à l'UFH.

VIII. Conclusion

Cette étude pilote, issue d'une registre monocentrique, suggère que le fondaparinux pourrait être aussi efficace que l'héparine non fractionnée dans le traitement adjuvant à la thrombolyse des embolies pulmonaires massives et sub-massives, sans risque hémorragique accru. Une étude randomisée de grande ampleur est toutefois nécessaire avant de parvenir à recommander cette association dans cette indication spécifique.

Summary in Chinese

中文摘要

血栓栓塞性疾病（thromboembolic disease）主要包括动脉粥样血栓形成、静脉血栓栓塞症、 缺血性脑卒中、以及外周动脉闭塞性疾病等,在心血管科、呼吸科、血管外科、骨科、肾脏科等学科广泛存在,是目前多个学科领域面临的严重保健问题。随着社会的发展和人口老龄化的进程，血栓栓塞性疾病的发病率逐年增加。据世界卫生组织统计，全球每年有 1500 万人死于血栓栓塞性疾病。据统计我国每年血栓栓塞性疾病的发病人数有 1000 万，病死人数有 100 万，致残率也很高。因此提高血栓栓塞性疾病的防治水平，减少其发病率、 降低致残率、死亡率，已成为当务之急。

血栓形成（thrombosis）是人体组织血管损伤时形成止血性血凝块的过程。栓塞（embolism）是血管局部形成的血凝块顺血流嵌顿到其他部位血管，导致相应组织、器官缺血、坏死或者严重生理紊乱的过程。血栓的主要成分为白细胞、红细胞、血小板和纤维蛋白。动脉血栓形成一般发生在动脉粥样硬化基础上主要由血小板和少量纤维蛋白组成，形成上具有不可预知性，治疗主要以抗血小板治疗的同时辅以抗凝治疗。静脉血栓主要由纤维蛋白与红细胞组成，早期相对不稳定，可脱落引发肺栓塞（PE），治疗上以抗凝和抗栓药物为主。

本课题主要以冠状动脉循环内血栓形成及 VTE 为研究基础，主要探讨稳定性冠心病患者经皮冠状动脉介入（PCI）术对止血机制的影响及急性和亚急性大面积肺栓塞（PE） 的发病机制及治疗，试图揭示在双重抗血小板及抗凝治疗前提下，PCI 术对血小板、凝血及纤溶三大止血活性的早期影响以及评价新型抗凝

药物 Fondaparinux 在 PE 治疗方面的有效性和安全性。

一、 冠心病患者 PCI 术治疗前后止血活性的早期变化

药物涂层支架内血栓形成是目前全球范围内的热门话题。尽管双重抗血小板及低分子肝素抗凝治疗，但（Drug-eluting stent, DES）所致的急性支架内血栓（Acut stent thrombosis, AST）没有比普通裸支架（Bare metal stent, BMS）减少。AST 的发生率在 0.1–3%，发病机理尚未完全阐明，有学者提出由于 PCI 术导致的冠状动脉血管内膜急性损伤反应 ,可以引起血小板聚集及凝血活性的增高，从而进一步加重局部乃至全身凝血-纤溶系统平衡，形成局部急性或亚急性支架内血栓。最新研究表明 ,植入 DES 后 ,雷帕霉素(Rapamycin)和(Paclitaxel) 均可以使血液中组织因子（TF）表达增高而导致血栓前状态，因而产生了药物涂层支架是否增加早期止血活性的问题。本研究主要围绕此问题，通过在 PCI 术中在冠状动脉不同部位及支架置入前后不同时间采样，比较 DES 与 BMS 植入前后稳定性心绞痛患者早期冠状动脉循环内局部血小板、凝血以及纤溶活性的变化。此外研究中还纳入了有关纤溶活性的基因指标（PAI-1 4G/5G 基因多态性），通过比较 PCI 术、止血活性以及基因多态性的相关性，试图揭示 DES 及抗栓治疗对血小板、凝血以及纤溶三大止血系统水平的早期影响，对 AST 早期形成的生物学和分子生物学机制作初步探讨。

我们的研究结果显示冠心病稳定性心绞痛患者 PCI 术期间，球囊扩张可以引起冠状动脉循环内凝血活性 [组织因子（TF），凝血因子 VII（FVIIa）以及凝血酶原片段 1 和 2 （F1+2）]和冠状动脉循环内纤溶活性 [纤溶酶原激活剂抑制物-1（PAI-1），组织型纤溶酶原激活物（t-PA），D-二聚体（DD）以及纤维蛋白

原(FIB)] 的一过性增高。支架植入后 15 分钟这些指标又回落为术前的基础值，且这些改变在药物涂层支架与裸支架中无显著性差异。凝血指标 TF 术前基础值较正常参考值高，考虑与入选病人均为冠心病患者，这些患者本身已经存在血小板及凝血功能亢进，纤溶活性受损和功能失调有关。当我们测定冠状动脉循环内血小板活性[可溶性 P 选择素（CD62P）和可溶性血小板膜糖蛋白 V（sGPV）]时发现，球囊扩张和支架植入均没有改变这两种反映血小板活性的指标。此外这些血小板、凝血以及纤溶指标在冠状动脉入口处（Ostium）与病灶下方（Distal to the lesion）PCI 手术前后比较均无显著性差异。我们还测定了法国患者冠状动脉循环内纤溶酶原激活剂抑制物 1（PAI-1） 4G/5G 基因多态性。 其结果为（PAI-1） 4G/5G 基因多态性在法国人中分布为 4G/5G 型最多 12 （60%），4G/4G 型其次 6（30%），5G/5G 型最少 2（10%）。4G 和 5G 等位基因频率分别为 60%和 40%。具有 PAI-1 4G/5G 基因型患者冠状动脉循环中血浆 PAI-1、DD 以及 FVII 活性在球囊扩张后较球囊扩张前明显升高且有显著性差异。然而这些在指标球囊扩张前与支架植入后比较无显著性差异。

从以上研究结果得出结论：（1）球囊扩张较支架植入更易损伤血管内皮并导致冠状动脉内局部、早期止血活性的一过性增高。具有 PAI-1 4G/5G 基因型的法国患者对这种反应较为敏感，这也许是造成一些患者发生急性与亚急性支架内血栓形成的原因之一。直接冠状动脉支架植入术可能会减少这种一系列止血活性改变，同时可以减少球囊扩张后引起内膜撕裂的发生率及预扩张时心肌缺血事件的发生率，但有待进一步大样本临床研究。（2）药物涂层支架和裸支架植入均不引起冠状动脉循环内血小板、凝血以及纤溶活性的早期改变。（3）PCI 术前双联抗血小板药物及低分子肝素抗凝治疗可以有效抑制血小板活性。

二、 抗凝加抗栓治疗在急性和亚急性大面积肺栓塞的有效性和安全性

肺血栓栓塞症 （pulmonary thromboembolism， PE） 与深静脉血栓形成（deep venous thrombosis，DVT）合称为静脉血栓栓塞症(venous thromboembolism， VTE)。目前 VTE 作为一个独立的疾病提出，已经成为全球性的重大健康问题，引起了国际学术界和社会的广泛关注。西方 VTE 导致的死亡排在心脑血管疾病和恶性肿瘤之后的第三位。美国致死性和非致死症状性 VTE 发生例数每年超过 90 万，其中有 29.64 万例死亡，23.60 万例 PTE 和 37.64 万例症状性 DVT。在致死性病例中，有 60%的患者被漏诊，只有 7%的患者得到及时与正确的诊断和治疗，其中早期诊断、规范治疗是降低 VTE 相关的致残率和病死率的关键。随着诊断意识和诊断水平的提高，我国诊断的 PE 病例数越来越多。

PE 多发生在 DVT 形成的基础之上，尤其近端（腘静脉以上包括腘静脉）深静脉血栓形成,如不能及时诊断和有效治疗，死亡率可以高达 30%，多数死亡发生在 2 小时以内，1 小时内死亡 11%。因此 VTE 的预后不但取决于急性期的可靠诊断和有效治疗，长期治疗防止复发、防止发生血栓后综合征是提高远期生存率和改善远期预后非常重要的措施。

VTE 的预防和药物治疗措施主要包括抗凝和抗栓治疗，只有少数急危而不适合药物治疗的患者采用介入和手术治疗。溶栓治疗的根本任务是溶解血栓中的纤维蛋白，是由被激活的纤溶酶原形成的纤溶酶将纤维蛋白溶解成小碎片，使血栓溶解。溶栓的药物，一类是作用于纤溶酶原的激酶，如尿激酶、链激酶；另一类是作用于纤维蛋白的蛋白水解酶，如纤溶酶、胰蛋白酶、曲酶蛋白酶等。抗凝

治疗主要通过药物降低凝血因子浓度或阻止其激活,从而降低血液凝固性或高凝状态,预防血栓形成或阻止血栓发展。抗凝药物主要包括直接凝血酶抑制剂(重组水蛭素、阿加曲斑、Hirudins 等)、间接凝血酶抑制剂(普通肝素、低分子肝素)、维生素 K 依赖性抗凝剂(香豆素类、华法令、新抗凝等)、戊聚糖钠等。目前临床上应用最多的是肝素、低分子肝素和华法林。

近来,在 VTE 治疗中,低分子量肝素(LMWH)由于出血并发症发生率低,无需检测凝血功能等优点而逐渐取代普通肝素。新型抗凝药物合成戊糖-戊聚糖钠(Fondaparinux,璜达肝癸钠)是一种人工合成的戊糖化合物,是新型的选择性Xa 抑制剂,仅作用于Xa,不引起凝血酶的激活,其抗凝活性是 ATIII介导的选择性抑制Xa 的结果。Fondaparinux 选择性与抗凝血酶III(ATIII)上的戊聚糖结合位点结合,使 ATIII 发生不可逆的构象变化,增强了对Xa 因子的灭活,从而作用于内源性和外源性凝血途径的最终环节,阻断了凝血酶以及纤维蛋白的形成。

Fondaparinux 作为一种新型人工合成类 Xa 因子抑制剂,经多项大规模临床试验证实,在急性冠脉综合征(ACS)、ST 段抬高的急性心肌梗死(STEMI)及 VTE 的治疗中具有抗凝作用稳定可靠,出血反应少等特点,部分适应症中有效性及安全性较传统药物更具优势。OASIS 6 研究 (Organization to Assess Strategies for Ischaemic Syndrome 6) 是 Yusuf.S 等为进一步评价 Fondaparinux 在 STEMI 中的安全性和有效性进行的国际大型、随机双盲研究。OASIS-6 实验中,有一个亚组包括无肝素指征的患者(例如使用链激酶溶栓者),比较 7-8 天的 Fondaparinux 和安慰剂,在这些患者中 Fondaparinux 能使 30 天死亡率和心肌梗死降低 21%,而不增加出血危险(抗凝时间延长到 7~8 天),

说明 Fondaparinux 除了降低死亡率和心肌梗死发生率外，还有降低出血的趋势，并证实 Fondaparinux 在链激酶溶栓患者中的益处。此外 CREATE 试验也证实了链激酶溶栓后延长抗凝时间的益处，然而，尽管低分子肝素 reviparin 能降低 30 天死亡、心肌梗死或卒中的危险，但能增加 7 天时致命性出血。因此和其他低分子肝素相比， Fondaparinux 并不增加出血风险，更为安全和易于使用。

近来的 9 项急性 PE 试验荟萃分析提示，与抗凝治疗比较，溶栓治疗能更加迅速地改善影像学和血流动力学异常，但这些获益是短暂的，溶栓和抗凝治疗患者的临床预后如死亡率或症状缓解没有差异。因此，对急性 PE 究竟哪些选择溶栓哪些选择抗凝治疗，一直存在争议。PE 治疗的首要目的之一即降低死亡率，当然还包括限制血栓进展、预防慢性血栓栓塞性肺动脉高压的发生等。临床上评价抗凝治疗和溶栓治疗对 PE 患者的获益时，在考虑上述终点的同时应兼顾治疗带来的风险，如出血等并发症。综上所述，目前 Fondaparinux 与溶栓治疗在急性和亚急性 PE 疗效及安全性方面的资料尚欠缺，因此这也是为什么我们要进行此研究的主要原因。

本研究选择 2006-2007 年期间，在法国 Jean-Minjoz 医院住院治疗的 27 例急性和亚急性大面积 PE 患者。溶栓药物包括链激酶（streptokinase）和纤溶酶（altéplase）。用药方法为：1 500 000 UI 的 streptokinase 用 5%的葡萄糖 60ml 稀释后静脉点滴 2 小时。Altéplase(体重超过 70 kg 先静脉给予 10mg 后续以 90mg 静脉持续点滴 2 小时，如果体重低于 70kg, 静脉先给 7mg 随后再给 63mg 静脉持续点滴 2 小时）。溶栓前立即给予或确诊前先给予 Fondaparinux 皮下注射治疗。Fondaparinux 给药剂量为：体重小于 50 kg 为 5 mg/日，体重

在 50-100 kg 之间为 7,5 mg/日，体重超过 100 kg 为 10 mg/日。当 48-72 小时后患者血液动力学稳定，检测国际化标准率在平均 2.5 (2-3) 时，停用 Fondaparinux，开始给予维生素 K 依赖性抗凝剂（fluindione）口服至 6 月。

研究结果显示：

入选的 27 例患者临床特征为：平均年龄为 68 ± 11（42-86 岁），平均心率为 100 ± 22 次/分。其中男性患者 11，占全部患者 40.7 %。女性患者 16，占全部患者 59.3 %。25 例患者经肺动脉断层 CT 确诊为近端的肺栓塞。10 例患者（37%）临床表现为心源性休克，8 例患者（29.6%）表现为晕厥。其中 9 例患者（33.3%）血液动力学稳定，但临床化验肌钙蛋白（TnI 大于 0.15 ng/ml）和脑钠素（BNP 大于 200 pg/ml）偏高。全部 27 例患者心脏超声检查均证实合并右心室功能不全，肺动脉收缩压的平均值为 56 ± 15 mmHg。 其中 22 例患者（81.5%）接受 rt-PA（altéplase），5 例患者（18.5%）接受 streptokinase 溶栓治疗。这些患者使用 Fondaparinux 平均时间为 8.6±4.0 天。患者一般情况治疗前后比较，平均心率较前降低 21%（从 100±22 次/分降为 79± 18 次/分），但动脉收缩压无明显改变。 临床指标治疗前后比较显示：26 例患者治疗后复查心脏超声结果，RVEDD/LVEDD （Right ventricular end-diastolic diameter/Left ventricular end-diastolic diameter） 比值降低 23 % (治疗前 1,02 ± 0,17，治疗后为 0,78 ± 0,17)，肺动脉收缩压降低 21%(治疗前 56 ± 15 mmHg，治疗后为 44 ± 13 mmHg)。19 例患者复查肺动脉 CT 检查，其中 12 例仍见近端血栓。 1 例患者（3.7%）由于肺栓塞再复发而死亡。2 例患者（7.4%）由于血液动力学情况持续不稳定，肺动脉 CT 证实合并近端血栓而做外科取栓术有效。 2 例（7.4%）患者出现出血并发症，其中 1 例患者（3.7%）由于大出血而给予输血

治疗。22 例患者（81.5%）病情稳定治疗有效。

本研究首次报道急性和亚急性大面积 PE 患者在 Fondaparinux 抗凝治疗基础上合并溶栓治疗的有效性和安全性。研究结果显示有 3 例患者（11.1%）治疗无效，其中 1 例因肺栓塞再复发且合并心源性休克死亡，另 2 例由于血液动力学情况持续不稳定而行外科取栓术。此结果与我们曾经在 1995-2005 年做过的一项研究，488 例患者用普通肝素抗凝加溶栓加治疗失败率达 8.2%比较，具有可比性。此外最近的一个 Meta 分析结果显示用普通肝素抗凝加溶栓的治疗死亡率为 4.3%。说明 Fondaparinux 抗凝加溶栓有一定的疗效。此外我们的研究中 27 例患者 Fondaparinux 抗凝加溶栓治疗后有 2 例出现出血并发症，这与其他 ICOPER 及一些 Meta 分析结果比较，并发症的发生率明显低，提示抗凝治疗基础上加用溶栓治疗，Fondaparinux 具有明显疗效和出血并发症低等优越性。由于此研究为预实验，研究结果还需要进一步随机大样本来证实。

三、 对未来展望

新疆是一个严寒且多民族聚集地区，由于各民族独特的高脂高热量饮食习惯，血栓栓塞性疾病的发生率很高。在新疆，随着医疗水平和医生诊断意识的不断提高，PE的诊断率也逐步提高，但比较内地，尤其国外还差甚远，漏诊、误诊率情况还是严重。是否我国PE的本身的发病率较欧美国家低还是我们的诊治水平低仍需要进一步深入研究。因此目前收集PE-DVT的流行病学基线资料，探讨国人VTE发生相关的遗传机制，进行凝血、纤溶和血管内皮相关的基础研究，探索出适宜于国人的诊断和治疗方案至关重要。我们关于冠心病患者PCI术与止血活性相关性研究存在一些遗憾和不足之处，由于严格的病例入选条件和在冠状

动脉病灶处取血样本操作难度大等原因造成了样本量少，由于耗时耗资太大等原因使我们不得不放弃CRP等有关炎性指标和血小板聚集测定等有较大临床意义的科研计划内容。因此回国后利用新疆具有的独特优势，主要以血栓栓塞性疾病为研究方向，延续且填补以上课题不足之处外，在这方面做深入研究也是我今后的进一步打算。

List of personal publications on the subject of the thesis

List of publications

Liste des publications

1. Mahemuti A, Meneveau N, Seronde MF, Schiele F, Descotes-Genon V, Ecarnot F, Blonde MC, Mercier M, Racadot E, Bassand JP. Early changes in local hemostasis activation following percutaneous coronary intervention in stable angina patients: a comparison between drug-eluting and bare metal stents. *J Thromb Thrombolysis,* 2008 Sep 3. [Epub ahead of print] PMID: 18766300.

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DE L'UNIVERSITE DE FRANCHE-COMTE

EN SCIENCES DE LA VIE ET DE LA SANTE

Présentée par Madame, Monsieur Ailiman MAHEMUTI

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