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Type 2 Diabetes and Fibrinolysis

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1. Introduction

Type 2 diabetes (DM2) is associated with complex hemostasis disorders characterised with enhanced coagulation, platelet dysfunction and hypofibrinolysis. These pathological conditions predispose type 2 diabetics towards increased morbidity and mortality due to thrombotic complications. In fact, DM2 is considered the most frequent acquired thrombophilic state. Under physiological conditions, fibrinolytic system is responsible for the lysis of fibrin clot, thus maintaining the blood fluidity. It was first described by Astrup in 1956. The most important molecule in fibrinolytic system is plasmin, which cleaves fibrin clot. It is released in its inactive form called plasminogen from the liver to the blood stream and gets activated by tissue plasminogen activator (t-PA), urokinase plasminogen activator (u-PA), clotting factor XII (FXII) and kallikrein. Plasmin deactivation occurs via several molecules. The best known and most important is α2-plasmin inhibitor (α-2PI). Another one, which was described in early nineteen nineties, is called thrombin-activatable fibrinolysis inhibitor (TAFI). Plasminogen activation and thus plasmin formation is inhibited by plasminogen activator inhibitor type 1 (PAI-1), PAI-2 and PAI-3. Fibrinolysis disorder due to disequilibrium between plasminogen activators and its inhibitors is the most characteristic feature of prothrombotic state associated with diabetes. The fibrinolytic activity in DM2 is influenced by numerous factors, the most crucial ones are glycaemic control, insulinaemia, blood lipid profile, blood pressure, genetic factors and different sorts of medication (Brummel-Ziedins et al., 2009, Alzahrani & Ajjan, 2010a).

2. Fibrinolysis

2.1 Plasminogen and its activation

Under physiological conditions, fibrinolytic system is responsible for the lysis of fibrin clot, thus maintaining the blood fluidity. The central protein in whole fibrinolytic system is plasmin, a serin protease, which cleaves fibrin clot. Plasminogen, the single-chain proenzyme of plasmin, is a protein of 791 amino acids, m.w. 92 000 synthesized primarily in the liver (Raum et al., 1980). The lysine-binding domains of plasminogen mediate its specific interactions with fibrin, cell surface receptors and other proteins, including its circulating inhibitor α2-plasmin inhibitor (α2-PI), which is also known as α2-antiplasmin (Hajjar et al., 1986; Plow et al., 1991). Activation of plasminogen, by cleavage of a single Arg-Val peptide bond at position 560–561 (Holvoet et al., 1985), gives rise to the active serine protease, plasmin.

Plasminogen can be converted to plasmin by both t-PA and u-PA. t-PA is a molecule of m.w. 72 000 which consists of 527 amino acids (Pennica et al., 1983). Cleavage of the Arg275-Ile276 peptide bond by plasmin converts t-PA to a disulphide-linked, two-chain form (Pennica et al., 1983). While single-chain t-PA is less active than two-chain t-PA in the fluid phase, both forms demonstrate equivalent activity when fibrin bound. The t-PA is synthesized and secreted primarily by endothelial cells. u-PA is the second endogenous plasminogen activator. Single-chain u-PA or prourokinase, is a glycoprotein of m.w. 54 000, consisting of 411 amino acids. Cleavage of the Lys158-Ile159 peptide bond by plasmin or kallikrein converts single-chain u-PA to a disulphide-linked two-chain derivative. The u-PA has much lower affinity for fibrin than t-PA, and is an effective plasminogen activator in both the presence and the absence of fibrin (Gurewich et al., 1984). Fibrin, the major plasmin substrate, regulates its own degradation by binding both plasminogen and t-PA on its surface, thereby localizing and enhancing plasmin generation. The affinity between t-PA and plasminogen is low in the absence of fibrin, but increases significantly in its presence. Once formed, plasmin cleaves fibrin, generating soluble degradation products, and exposing carboxy-terminal lysine (Lys) residues. 'Kringles' 2 of t-PA, and 1 and 4 of plasminogen contain lysine-binding sites, which mediate further binding to fibrin, leading to enhanced plasmin generation and fibrin removal (Plow et al., 1991).

This binding can be blocked by the recently discovered thrombin-activatable fibrinolysis inhibitor (TAFI). When activated by thrombin-thrombomodulin complex, TAFI removes carboxy-terminal Lys residues, thereby attenuating plasmin generation, stabilizing fibrin thrombi, and establishing a regulatory connection between coagulation and fibrinolysis. Fibrin dissolution is also regulated by inhibitors of plasminogen activation, such as PAI-1, and by inhibitors of plasmin itself, such as α2-PI. In addition, plasmin bound to fibrin is protected from α2-PI, due to occupancy of its lysine-binding sites. TAFI, on the other hand, decreases this protection by deleting plasmin-binding Lys residues on fibrin. Moreover, diverse cell types promote plasmin generation through their expression of cell surface receptors (Cesarman-Maus & Hajjar, 2005). Endothelial cells, monocytes, macrophages, neutrophils and some tumour cells, all bind plasminogen, as well as t-PA and/or u-PA. Their receptors localize cell surface fibrinolytic activity, serve as cofactors in acute or ongoing plasmin generation, and provide specialized environments that are protected from circulating inhibitors. Under certain conditions, proteases that are traditionally classified within the intrinsic arm of the coagulation cascade have been shown to activate plasminogen directly. These include kallikrein, factor XIa and factor XIIa, but normally account for no more than 15% of total plasmin-generating activity in plasma (Cesarman-Maus & Hajjar, 2005). In addition, the membrane type 1 matrix metalloproteinase (MT1-MMP) appears to exert fibrinolytic activity in the absence of plasminogen. Factor VII-activating protease has also been reported to serve as an in vitro activator of single-chain PLG activators, but its role *in vivo* remains to be determined (Römisch et al., 1999). Plasminogen together with its activators t-PA and u-PA, urokinase receptor (uPAR), plasminogen activator inhibitors PAI-1, PAI-2, PAI-3 (which is identical to the protein C inhibitor) and protease nexin forms the plasminogen activator system (PAS). (Brummel-Ziedins et al., 2009; Schmitt et al., 1997).

2.2 Inhibition of fibrinolysis

The major plasmin inhibitor is α2-PI. It is a single-chain glycoprotein of m.w. 70 000. α2-PI is also a constituent of platelet α granules (Plow & Collen, 1981). Thus, plasmin released into

flowing blood or in the vicinity of a platelet-rich thrombus, is immediately neutralized by α2-PI. α2-PI is cleaved at its Arg364-Met365 peptide bond, and then forms a lysine-binding site-dependent α2-PI–plasmin complex, which is cleared in the liver (Plow et al., 1991).

The PAI-1 is the most important and most rapidly acting physiological inhibitor of both tPA and uPA. This single chain glycoprotein of m.w. 52 000 is released by endothelial cells, monocytes, macrophages, hepatocytes, adipocytes and platelets (Ny et al, 1986). Release of PAI-1 is stimulated by many cytokines, growth factors and lipoproteins common to the global inflammatory response. After the release, PAI-1 quickly converts to an inactive form and circulates in plasma. Activation occurs by conformational change through binging to negatively charged phospholipids. It builds up a 1:1 complex binding with t-PA, thus inactivating it. This process is catalyzed by fibrin, which binds PAI-1 and enhances the t-PA plasma clearance. The complex dissociates from fibrin and is removed from circulation by liver (Cesarman-Maus & Hajjar, 2005).

PAI-2, the less important inhibitor of plasmin activation, is a 393 amino acid member of the serpin family. It was originally purified from human placenta. Functionally, PAI-2 inhibits both two-chain t-PA and two-chain u-PA with comparable efficiency, but it is less effective towards single-chain t-PA, and does not inhibit prourokinase. Significant levels of PAI-2 are found in human plasma only during pregnancy (Ye et al, 1987).

2.3 Plasminogen activator inhibitor type 1
2.3.1 Regulation of plasminogen activator inhibitor type 1 levels

PAI-1 is synthesized after stimulation. Its gene is localized on chromosome 7. In the circulation PAI-1 is rapidly converted (half-life 5 min.) to a latent inactive form which represents the major form of PAI-1 in plasma. Vitronectin stabilizes the active form of PAI-1 in plasma and prolonges its half-life 2 to 4-fold. The vitronectin levels are increased in relation to the cytokine dependent synthesis in the liver and hence the PAI-1 active form circulates in plasma during inflammation at high concentration. PAI-1 is removed from the circulation in the liver, in particular through binding at low density lipoprotein receptor-related protein (LRP), with the half-life of 10 min. (Lijnen, 2004; Loskutoff & Mimuro, 1991; Schleef & Loskutoff, 1988; Wiman, 1995).

According to multicompartmental and multifactorial model, the regulation of the PAI-1 plasma levels is complex and depends on multiple sites of PAI-1 synthesis and various stimuli, including hormones involved in the lipid and glucose metabolism. PAI-1 is synthesized in vascular (endothelium, vascular smooth muscles, platelets storage pool) and extravascular, i.e. metabolic (adipocytes, hepatocytes) compartments. These two PAI-1 compartments are regulated in a different way with various effect of insulin on PAI-1 synthesis. Factors regulating the PAI-1 production include stimulating effects of insulin, proinsulin, very low density lipoproteins (VLDL), inflammatory cytokines and oxidative stress (Galajda et al., 1997b, 1998a, 1998e, 1999; Galajda & Mokáň, 2001).

2.3.1.1 PAI-1 metabolic compartment

PAI-1 metabolic compartment is formed by visceral adipocytes, stromal fat cells (in particular macrophages) and hepatocytes. These cells regulate the postprandial changes of fibrinolysis in splanchnic system. The principle site of PAI-1 production in mice is adipose tissue. The cultures of visceral adipocytes are more important source of PAI-1 *in vitro* than hepatocytes. Experimental studies of PAI-1 *in vivo* found that PAI-1 production in obese mice is 7-fold increased in adipocytes whilst it is only 2-fold higher in hepatocytes compared to lean mice. In

human adipose tissue, PAI-1 production is localized mainly in the stromal fat cells and macrophages. Adipose tissue can be the principal source of PAI-1. Visceral adipose tissue produces 5-fold higher amount of PAI-1 compared to subcutaneous adipose tissue and levels of PAI-1 in obese subjects are related rather to the amount of visceral than subcutaneous fat. Thus PAI-1 levels are better correlated with indices of the central obesity (waist-to-hip ratio - WHR). Insulin stimulates PAI-1 production in adipose tissue with synergistic effect of VLDL and free fat acids (FFAs). Therefore the adipose tissue is the most important source of PAI-1 related to insulin. Moreover, experimental studies in mice proved that the insulin application increases the expression of PAI-1 mRNA much more in adipose tissue than in the liver. Locally formed corticoids, inflammatory cytokines (TNF) and growth factors (TGF-β) can also stimulate the PAI-1 production in visceral adipose tissue. Under certain circumstances, hepatocytes can be an important source of PAI-1, especially in fatty liver disease. Insulin, proinsulin (PI) including des-31,32-split form and insulin like growth factor (IGF-1) stimulate production of PAI-1 in the liver. The most potent form among them is des-31,32-split PI, whilst PI and insulin stimulate the production of PAI-1 in liver with lower effect. The PAI-1 production is also stimulated by hyperlipidemia. VLDL act relatively rapidly and in synergy with insulin (Alessi et al., 1988, 1997, 2006; Hamsten & Eriksson, 1994; Juhan-Vague et al., 1991; Juhan-Vague & Alessi, 1997; Lijnen, 2004; Lijnen et al., 2005; Loskutoff & Mimuro, 1991; Loskutoff & Samad, 1998; Schneider & Sobel, 1996; Samad & Loskutoff, 1997).

2.3.1.2 PAI-1 vascular compartment

PAI-1 vascular compartment includes endothelium, subendothelial pool (vascular smooth muscles, fibroblasts, macrophages) and a rapid release pool (platelets). PAI-1 is released to circulation mainly from the endothelium. Vascular smooth muscles can participate in regulation of PAI-1 levels under certain conditions. The platelets are the only cells in human body which store PAI-1. The total amount of PAI-1 is 4-fold higher in platelets compared to plasma, but they do not significantly impact on the PAI-1 plasma levels. The platelet PAI-1 plays a role in the local accumulation and action of the inhibitor. In our DM2 patients, hyperinsulinemic (HI) non-diabetics and healthy controls the PAI-1 levels did not correlate with platelet factor 4 (PF_4) - the marker of platelet activation. This finding supports the hypothesis about more significant effect of the endothelium on PAI-1 plasma levels than the release of PAI-1 from platelet α granules (Galajda et al., 1997a; Galajda & Mokáň, 2001). Regulation of PAI-1 production in the endothelium has some specifics and differs from its regulation in metabolic compartment. The inflammatory cytokines as tumor necrosis factor (TNF), interleukin 1 (IL-1), transforming growth factor β (TGF-β), thrombin and angiotensin II and metabolic factors as proinsulin, hyperglycaemia, oxidative stress and lipids including VLDL and FFAs represent important stimulators of PAI-1 synthesis in the endothelium. On the contrary insulin inhibits synthesis of PAI-1 in the endothelium induced by TGF-β, probably due to activated cGMP and cAMP systems. Insulin along with leptin also stimulates the PAI-1 production in vascular smooth muscles (Eriksson et al., 1998; Hamsten & Eriksson, 1994; Kawai et al., 1996; Kim et al., 1997; Li et al., 1997; Peiretti et al., 1997; Vaughan, 1997, 2005; Yamauchi et al., 1997).

2.3.2 The control of PAI-1 levels in circulation

PAI-1 levels depend on the PAI-1 expression and release from various tissues which are induced by the stimulatory effects of different factors. Adipose tissue, liver and vascular endothelium are considered to be the most important sources of PAI-1 production.

2.3.2.1 Genetic factors

Up to 100-fold individual variability of PAI-1 levels has been described in the general population. Genetic factors take part in this variability in only as much as 3-5%. Increased PAI-1 levels are associated with several known PAI-1 gene polymorphisms. One of them is the PAI-1 promotor region gene polymorphism **-844** G/A (Xho I) which represents a potential binding site for Ets transcription factor. Another one is the polymorphism **-675 4G/5G** which affects the binding of nuclear proteins involved in the regulation of PAI-1 gene transcription. The PAI-1 5G allele leads to binding of a repressor protein thereby decreasing gene transcription. Consequently, higher plasma PAI-1 concentrations have been found among homozygotes for the 4G- allele compared to heterozygotes and homozygotes for the 5G-allele (Burzotta et al, 2003; Dawson et al., 1993; Eriksson et al, 1995). Majority of the europoid population are heterozygous carriers of the 4G/5G allele with a frequency of approximately 50%. Total frequency of 4G allele associated with a higher PAI-1 production is 52-54% in europoid, 30-46% in mongoloid and 24% in negroid population. Another polymorphism **Hind III** in the 3´-end of PAI-1 gene is associated with different regulation of PAI-1 by metabolic factors. Allele 2/2 is linked with more potent stimulatory effect of VLDL on PAI-1 production in the endothelial cell culture while a less frequent allele 1/1 (present in 20% of general population), is characterized by the stimulatory effect of insulin on PAI-1 production in endothelial cells *in vitro*. Recently identified **G12078A dimorphism** in 3´-end of PAI-1 can influence the PAI-1 levels by modifying of post-transcription mechanisms (Green, 1996; Lijnen, 2004; Mansfield et al., 1997; McCormack et al., 1996).

2.3.2.2 Insulin, proinsulin and insulin resistance

The grade of insulin resistance (IR) is responsible for 49% interindividual variability of the PAI-1 levels in europoid men and 29% variability in women. It is a principle factor associated with PAI-1 levels but it includes various stimulatory mechanisms. Insulin is considered to be one of the main physiologic regulators of PAI-1 levels, it is responsible for a linkage of PLG system with human metabolic activity. The effect of insulin is different in various compartments of the PAI-1 production. Adipose tissue is the most important source of circulating PAI-1, its *in vivo* production is insulin dependent. Insulin stimulates the PAI-1 synthesis in synergy with VLDL and FFAs. Insulin also enhances the PAI-1 production in the liver. The effect of insulin on PAI-1 production in the endothelium is rather more complex. Insulin impacts neither PAI-1 basal production in the culture of human endothelial cells *in vitro* nor PAI-1 production in the vessels of mice splanchnic system after insulin application *in vivo*. On the contrary, insulin can inhibit PAI-1 production in the culture of endothelial cells induced by TGFβ *in vitro*. The inhibitory effect of insulin on the PAI-1 endothelial production can be mediated by cGMP and cAMP. Insulin increases the activity of cGMP by stimulation of NO production, it also increases the amount of cAMP by subsequent cGMP dependent inhibition of specific phosphodiesterase (PDE$_3$). The decrease of PAI-1 during postprandial HI can be explained by inhibitory effect of insulin on the PAI-1 production in endothelium *in vivo*. Similarly, we confirmed the significantly decreased PAI-1 levels which were independent from other metabolic parameters in our DM2 patients with endothelial dysfunction on long-term insulin treatment compared to those treated with oral antidiabetics **(Table 1 and 2) (**Galajda & Mokáň, 2001). These findings verify the inhibitory effect of insulin on PAI-1 production in the endothelial cells. On the other hand some studies found locally increased PAI-1 levels in human antebrachial vessels which were

induced by the insulin application. This finding can be explained either by the release of PAI-1 from the vascular smooth muscles, as insulin stimulates PAI-1 release from the muscles *in vitro* or by heterogeneity of insulin effect on different types of endothelium. Another explanation can be found in the interindividual differences in human endothelial response to insulin as it was proved in Hind III polymorphism of PAI-1 gene. In this polymorphism a less frequent allele 1/1 is associated with stimulatory effect of insulin on PAI-1 production in the culture of human umbilical vein endothelial cells (HUVEC). **Proinsulin (PI)** including its incomplete split forms (des-31,32; des-61,62) stimulates the PAI-1 synthesis in liver and endothelial cells. The des-31,32 split PI form has the most potent stimulatory effect on PAI-1 production in hepatocytes whereas PI stimulates PAI-1 production with the same effect as insulin. **Insulin-like growth factor 1 (IGF-1)** stimulates, similarly to insulin, the PAI-1 production in the liver but not in the endothelium. **C-peptide,** which possesses certain hormonal activity of insulin, does not influence the PAI-1 production (Alessi et al., 1988, 1997, 2007; Alessi & Juhan-Vague, 2006; Grant et al., 1990; Gray et al., 1997; Juhan-Vague et al., 1991; Lijnen 2004; Nagi et al., 1990; Nordt et al., 1994; Panahloo et al., 1996; Pandolfi et al., 1996; Yamauchi, et al., 1997).

2.3.2.3 Free fat acids and lipoproteins with very low density

VLDL are important molecules stimulating PAI-1 synthesis. From among them, the bigger $VLDL_1$ stimulate PAI-1 production more than the smaller $VLDL_2$. Unsaturated FFAs are proper stimulators of PAI-1 released from the VLDL, which intracellularly activate specific VLDL/FA - inducible transcription factor. This factor binds at -672 to -657 site of the PAI-1 gene promotor region marked as VLDL/FA-RE. This site has 67% homology with binding sites of so called peroxisome proliferator-activated receptors (PPARs) and is situated next to -675G promotor which is responsible for the binding of PAI-1 transcription repressor (factor B). Binding of VLDL/FA - inducible transcription factor at VLDL/FA-RE decreases the binding of transcription repressor (factor B) resulting in the increased PAI-1 production. VLDL increase synthesis of PAI-1 in the endothelium by this mechanism whilst in hepatocytes VLDL stimulate the PAI-1 synthesis synergistically with insulin by stabilization of mRNA. The monounsaturated, ω-3 and ω-6 polyunsaturated FFAs have a stimulatory effect on PAI-1 production in hepatocytes and endothelium *in vitro* while saturated FFAs do not influence PAI-1 production. Regulation of PAI-1 by PPARs has different mechanisms. Binding of PPARγ to corresponding bond sites (PPRE) on PAI-1 gene promotor can be associated with the increased basal mRNA expression for PAI-1 but the main effect is inhibition of PAI-1 production induced by inflammatory cytokines TNF and IL-1. Also the application of glitazones for patients with DM2 is associated with decreased PAI-1 independently on metabolic control and in correlation with decreased insulin and FFAs. Activation of PPARα by fibrates do not influence basal production of PAI-1 in the endothelium *in vitro*, but significantly decreases PAI-1 production mediated by TGFβ. The fibrates decrease PAI-1 production stimulated by insulin in hepatocytes *in vitro*. (Alessi & Juhan-Vague, 2006; Alessi et al., 2007; Asplund-Carlson et al., 1993; Eriksson et al., 1998; Li et al., 1997; Nilsson et al., 1998; Schneider & Sobel, 1996; Stiko-Rahm et al., 1990).

2.3.2.4 Inflammatory cytokines, growth factors, leptin

Inflammatory cytokines TNF, IL-1, IL-6 stimulate very effectively PAI-1 synthesis. TNF induces PAI-1 production in endothelium and adipose tissue more than in hepatocytes. IL-1

is an important stimulator of PAI-1 production in endothelium. Inflammatory cytokines have an increased effect on the 4G/5G promotor region PAI-1 gene polymorphism. The 5G allele has a bond site for inhibitory transcription factor and therefore the 4G insertion causes dramatic response to IL-1 with the increased PAI-1 levels. IL-6 also increases PAI-1 production in hepatocytes. Leptin, hormon of adipocytes, stimulates PAI-1 production in vascular smooth muscles. Adiponectin inhibits PAI-1 production in adipose tissue. TGFβ stimulates the endothelial PAI-1 production while growth factors related to the receptor protein tyrosine kinase (PTK) like insulin and IGF-1, do not act on PAI-1 synthesis in the endothelium but stimulate PAI-1 production in hepatocytes. This regulation is connected with indirect inhibitory effect of PAI-1 on the endothelial proliferation that needs proteolytic phenotype mediated by plasmin. Mentioned growth factors related to the receptor PTK induce endothelial proliferation. On the contrary, TGFß inhibits their growth effect, directly blocks the endothelial proliferation and increases PAI-1 endothelial production with an indirect antiproliferative effect. TGFß increases PAI-1 production in adipocytes and its effect is several times higher compared to insulin (Alessi & Juhan-Vague, 2006; Kawai et al., 1996; Loskutoff & Samad, 1998; Peiretti et al., 1997; Samad & Loskutoff, 1997; Vaughan, 2005).

2.4 Tissue plasminogen activator

t-PA is a serine protease which cleaves plasminogen to plasmin. t-PA has a structure of glycoprotein with two polypeptide chains (m.w.70 kDa) and disulphide-linked bond. Fibrin acts as a main cofactor of t-PA activity in the activation of fibrinolysis. tPA is synthesized particulary in the endothelium. After its synthesis by gene localized at 8th chromosome the t-PA is either stored in reserved compartment of the endothelium which is different from Weibel-Palade bodies or released to the circulation. t-PA is bound in circulation on the surface proteoglycan layer of endothelium or minority of t-PA circulates in plasma bound in complexes with its inhibitor PAI-1. Release of t-PA from the endothelium differs according to the types of vessels and takes part on heterogeneity of the fibrinolytic potential of vessels. The most of t-PA is localized on the surface of veins, especially in the upper part of body (release of t-PA in the veins of arms is 10 times higher than in the veins of legs), less of t-PA is produced in microvascular circulation and in aorta, and at least is localized in the endothelium of arteries. The highest release of t-PA was found in splanchnic system and coronary arteries. Liver is the site of t-PA uptake with a half-life of 5 min. (Hinsgbergh, 1988; Jern et al., 1997b).

2.4.1 The effects on t-PA levels

The t-PA antigen and activity levels depend on various factors.

2.4.1.1 Release of t-PA from stories in granules

The t-PA is stored in reserved granules of the endothelium which are different from Weibel-Palade bodies for vWF and small vesicules for protein S. Rapid degranulation and the t-PA release to plasma appears after the Ca_i-dependent stimulus. Factors activating receptors of phosphoinositol system by G-protein signaling pathway such as thrombin, acetylcholine, bradykinin, histamin, platelet activating factor (PAF) and endothelin stimulate the t-PA release by this way. Hormones like catecholamines activating cAMP system via β-receptors and vasopressin via V_2-receptor also increase the t-PA release to plasma. Physical or mental

stress is associated with the activation of fibrinolysis and increased t-PA but without changes in PAI-1 levels. The t-PA released by stress is dependent on the intensity and duration of the stressor action. Strong or long-acting stressor against decreases the release of t-PA and increases PAI-1 production (Chandler et al., 1995; Kooistra et al., 1994; Teger-Nilsson et al., 1991).

2.4.1.2 Formation of complexes with PAI-1

The most of circulating t-PA antigen is coupled in complex with PAI-1 and only 5% of total t-PA circulates in free form. Measurement of the total t-PA by enzyme immunosorbent assay (EIA) does not distinguish the amount of free and bound t-PA. Close correlation between t-PA and PAI-1 can be explained especially by the increased level of t-PA/PAI-1 complexes because the PAI-1 level is fundamental for t-PA level. But only a small amount of circulating t-PA is in active form and therefore the t-PA activity does not correlate with t-PA antigen (Loo et al., 1995; Wiman, 1991).

2.4.1.3 Endothelial damage

t-PA is bound on the surface proteoglycan layer of endothelium and can be released to plasma after the endothelial damage. The increased t-PA levels correlate with vWF a TM in patients with coronary or peripheral atherosclerosis supporting the role of t-PA as a marker of endothelial damage. The t-PA levels are also an independent predictive marker for the risk of acute coronary events. So, the increased t-PA levels reflect severity of the endothelial dysfunction and are associated with a deterioration of fibrinolytic activity in plasma (Blann et al., 1995; Galajda et al., 1998d; Galajda & Mokáň, 2001).

2.5 Thrombin activatable fibrinolysis inhibitor

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a procarboxypeptidase and member of the family called metallocarboxypeptidases. Activation of TAFI occurs by trypsin, plasmin, thrombin and meizothrombin. The catalytic efficiency of thrombin to activate TAFI is increased about three orders of magnitude in the presence of thrombomodulin. Thus, the thrombin-thrombomodulin complex is most likely the physiologic activator of TAFI. TAFI removes C-terminal lysine residues from partially degraded fibrin, thus inhibiting further plasminogen activation. It could be stated that TAFI acts as an important link between coagulation and fibrinolysis (Bajzar et al., 1996; Staško et al., 2004).

3. Fibrinolysis in diabetes mellitus type 2

Fibrinolysis disorder due to excessive PAI-1 production is the most characteristic feature of the prothrombotic state associated with DM2. Of all the various haemostatic measures, PAI-1 has been most consistently associated with insulin resistance (IR) and is generally accepted as being an important part of the risk cluster.

3.1 PAI-1 in patients with diabetes mellitus type 2

PAI-1 levels are strongly correlated with components of the IR syndrome such as body mass index (BMI), blood pressure, plasma triglycerides (TG) and insulin in healthy subjects with IR, DM2 patients, and patients with known coronary heart disease (CHD). Elevated PAI-1 levels were an independent risk factor for the development of DM2 in healthy subjects in the Insulin Resistance Atherosclerosis Study (IRAS), suggesting that

they may be a very early risk marker for the development of the metabolic syndrome and DM2. This view was supported by subsequent serial analysis of PAI-1 in the IRAS in which baseline and follow-up PAI-1 levels were higher in subjects who subsequently converted to DM2. This link is strengthened by interventional studies, in which PAI-1 levels fell following improved insulin sensitivity (weight loss, exercise, metformin therapy). Festa et al. suggested that a fall in PAI-1 may in itself be associated with a reduction in conversion to DM2. However, the observation that elevated PAI-1 precedes the development of DM2 and is, in the presence of IR, independent of glycaemia (Natali et al., 2006) provides compelling evidence that abnormalities in fibrinolysis occur very early in the course of these disorders. (Juhan-Vague et al., 1987, 1989, 1993; Mansfield et al., 1997; Grant, 2007; Sakkinen et al., 2000; Vague et al., 1986; Landin et al., 1990a, 1990b; Hamsten et al., 1987; Sundell et al., 1989; Asplund-Carlson et al., 1993; Meigs et al., 2000; Festa et al., 1999, 2002, 2006; Natali et al., 2006). Our work also proved PAI-1 to be increased in early stages of diabetes (Kubisz et al., 2010).

3.1.1 PAI-1 as a predictive factor of cardiovascular risk

The role of PAI-1 as a predictive factor of cardiovascular risk is different compared to parameters of subclinical inflammation (CRP, fibrinogen) and endothelial dysfunction (von Willebrand factor - vWF) that are the independent cardiovascular risk factors but their predictive role for the risk of DM2 development is dependent on presence of obesity. PAI-1 was supposed as a risk factor for development of coronary heart disease (CHD) due to the results of Northwick Park Heart Study that found an independent relation of decreased fibrinolytic activity to the risk of future CHD. However, metaanalysis of the studies showed that PAI-1 levels have not independent effect as a predictor of the CHD risk. Paradoxically the increased levels of t-PA can be predictive for development of CHD. The IRAS study confirmed that increased PAI-1 levels have strong effect as a predictor of DM2 risk that is independent from obesity (Alessi & Juhan-Vague, 2006; Festa et al., 2002; Lowe et al., 2004; Lowe, 2005; Meade et al. 1993).

3.1.2 Heterogeneity of the insulin effects on PAI-1 levels in diabetes mellitus type 2

It was considered in previous model that the overproduction of PAI in IR states is a result of direct stimulatory effect of the portal hyperinsulinaemia (HI). This was confirmed by a stimulatory effect of insulin in the culture of hepatocytes *in vitro*, by the increased PAI-1 levels during oral glucose tolerance test (OGTT) and clinically proved by the correlation of PAI-1 levels to insulinaemia. The endothelial production of PAI-1 was considered as independent on insulin. This model was modified by a discovery that the principal source of PAI-1 which is dependent on insulin is adipose tissue but there are some controversies. The effect of insulin is different in adipose tissue, liver and vascular system, dependent on duration and character of concurrent factors and influenced by clearence of PAI-1 in liver and endothelial cells (Jokl et al., 1994; Juhan-Vague et al., 1991; Potter van Loon et al., 1993; Galajda & Mokáň, 2001).

3.1.2.1 Dependence on compartment

Insulin stimulates production of PAI-1 in visceral adipose tissue and liver. On the contrary insulin in the endothelial cells inhibits production of PAI-1 induced by cytokines. However, insulin has a stimulatory effect on PAI-1 synthesis in the subendothelial compartment,

which is probably responsible for a local release of PAI-1 in the vascular stream of forearm muscles during infusion of insulin. The increased PAI-1 levels in subjects with IR can be a result of either the stimulatory effect of HI on adipose tissue mass in obesity or the missing inhibitory effect of insulin on endothelium in IR (Jern et al., 1997a; Juhan-Vague et al., 1991; Negri et al., 1997; Yamauchi et al., 1997).

3.1.2.2 Dependence on duration of insulin action

Decreased PAI-1 levels were found after a short-term insulin infusion or HI clamp. The same PAI-1 decrease was showed also in the control group with an administration of physiological solution without HI induction. These results confirmed that short-term application of insulin does not influence the PAI-1 levels and that PAI-1 decrease is a result of the PAI-1 diurnal rhytm variability. The PAI-1 levels after a long-term application of insulin in DM2 subjects were found decreased independently on other metabolic parameters. This effect can be caused by the stimulatory action of proinsulin during insulin treatment. We proved the inhibitory effect of insulin especially in DM2 subjects with endothelial dysfunction. This result supports, in accordance with an evidence of the inhibitory effect of PAI-1 on endothelium *in vitro,* the possibility of a direct inhibitory action of insulin during its long-term application (Galajda et al., 1998a, 1998b; Galajda & Mokáň, 2001; Nordt et al., 1993; Panahloo & Yudkin, 1996).

3.1.2.3 Dependence on concurrent factors

Short-term infusion of insulin, lipids or glucose does not influence PAI-1 levels but their mixed application leads to the increased PAI-1 levels. Synergistical effect of insulin and lipids on PAI-1 levels was confirmed also in the culture of hepatocytes. Insulin supports the stimulatory effect of glucose on PAI-1 synthesis in the endothelial cells but it inhibits the PAI-1 production in the endothelium mediated by cytokines (Calles-Escandon et al., 1998; Panahloo & Yudkin, 1996; Schneider & Sobel, 1996; Yamauchi et al., 1997).

3.1.2.4 Dependence on PAI-1 clearence.

PAI-1 synthesized by a visceral adipose tissue under the stimulation by insulin forms complexes with the increased t-PA produced in splanchnic system. Liver is a site of the rapid and effective uptake of these complexes. Rapid uptake of PAI-1/t-PA complexes can be responsible for the postprandial PAI-1 decrease. Intravenous application of glucose leads to the rapid PAI-1 decrease accelerated by sulodexid. This result supports the endothelial uptake of PAI-1. The similar finding was confirmed in subjects with diabetes mellitus type 1 (DM1) and it supposes the idea that postprandial PAI-1 levels variability can be independent on insulin (Ceriello et al., 1993; Jern et al., 1997b).

3.1.3 The effect of insulin treatment on PAI-1 levels in subjects with DM2

Insulin treatment in DM2 is associated with a decrease of PAI-1 levels. This is opposite to the confirmed stimulatory effect of insulin on PAI-1 levels in adipose tissue and liver. Although insulin has not the effect on basal PAI-1 production in endothelial culture *in vitro*, it inhibits the PAI-1 production induced by cytokines. This effect can exist also *in vivo*. In our clinical study we examined two groups of subjects with DM2 treated by sulphonylurea derivates (DM2-SU) and those treated 2-3 months by insulin (DM2-INS) (**table 1, table 2**).

	DM2 - SU (n=17)	**DM2 - INS** (n=11)	**p**
PAI-1 (ng/ml)	48,1 (14-108)	27,9 (2-53)	<0,05
t-PA (ng/ml)	8,1 (4,4-11,8)	8,8 (4,6-14,0)	n.s.
vWF (IU/ml)	1,0 (0,7-1,4)	1,0 (0,8-1,3)	n.s.
TFPI(ng/ml)	60,1 (33,2-84,3)	69,1 (29.1-86,7)	n.s.
TM (ng/ml)	27,8 (10-45)	31,0 (11-50)	n.s.
PF$_4$ (ng/ml)	52,1 (1-172)	50,8 (6-106)	n.s.
CP (ng/ml)	1,5 (0,5-5,9)	1,7 (0,4-3,8)	n.s.
TG (mM/l)	2,7 (0,9-6,8)	2,9 (0,4-7,4)	n.s.

DM2-SU (diabetics treated by sulphonylurea derivates), DM2-INS (diabetics treated by insulin), PAI-1 (plasminogen activator inhibitor type 1), t-PA (tissue plasminogen activator), vWF (von Willebrand factor), TFPI (tissue factor pathway inhibitor), TM (thrombomodulin), PF$_4$ (platelet factor 4), CP (C-peptid), TG (triglycerids)

Table 1. Subjects with diabetes mellitus type 2 without endothelial dysfunction

	DM2 - SU (n=17)	**DM2 - INS** (n=13)	**p**
PAI-1 (ng/ml)	80,7 (43-217)	16,7 (7-34)	<0,0001
t-PA (ng/ml)	13,7 (5,6-25,3)	7,1 (3,4-18,9)	<0,01
vWF (IU/ml)	1,9 (1,3-2,4)	1,8 (1,0-2,3)	n.s.
TFPI(ng/ml)	81,4 (50,2-107,1)	89,0 (47,3-112,8)	n.s.
TM (ng/ml)	54,7 (2-104)	46,9 (13-179)	n.s.
PF$_4$ (ng/ml)	70,1 (3-126)	68,3 (6-146)	n.s.
CP (ng/ml)	1,8 (0,6-7,9)	1,7 (0,9-5,6)	n.s.
TG (mM/l)	2,3 (0,9-8,2)	2,3 (0,9-6,1)	n.s.

DM2-SU (diabetics treated by sulphonylurea derivates), DM2-INS (diabetics treated by insulin), PAI-1 (plasminogen activator inhibitor type 1), t-PA (tissue plasminogen activator), vWF (von Willebrand factor), TFPI (tissue factor pathway inhibitor), TM (thrombomodulin), PF$_4$ (platelet factor 4), CP (C-peptid), TG (triglycerids)

Table 2. Subjects with diabetes mellitus type 2 with endothelial dysfunction

These two groups did not differ by metabolic parameters (glycaemia, glycosylated HbA1c, triglycerides, BMI), endothelial and platelet parameters (vWF, thrombomodulin-TM, PF$_4$). We found the significantly decreased PAI-1 levels in the insulin treated subjects compared to those with sulphonylurea derivates. These results can admit the inhibitory effect of insulin on PAI-1 levels independently to metabolic parameters. We evaluated also the influence of endothelial dysfunction measured by the endothelial markers. We found the significantly increased PAI-1 levels in subjects treated with sulphonylurea in subgroup with the increased vWF levels. Our results supported the idea that presence of the endothelial dysfunction was associated with the increased PAI-1 levels and this relation was not influenced by treatment with sulphonylurea derivates. There were confirmed decreased PAI-1 levels in subjects treated with insulin especially in those with the endothelial dysfunction when compared groups of subjects treated

with insulin and sulphonylurea derivates. In accordance with the confirmed inhibitory insulin effect on PAI-1 production mediated by cytokines in the endothelium *in vitro* our results suppose the possibility of direct inhibitory action of insulin *in vivo* (Galajda et al., 1998b, 1998c; Galajda & Mokáň, 2001).

3.1.4 The effect of microalbuminuria on PAI-1 levels

Several studies found high PAI-1 levels in DM2 subjects with normo- and microalbuminuria. These results are in agreement with our results. We suggest decreased fibrinolysis in DM2 subjects presenting with increased levels of PAI-1 in both normoalbuminuric (NAU) and microalbuminuric (MAU) diabetics. We found a positive correlation between PAI-1 levels and BMI in the MAU group and between PAI-1 and triglycerids in our NAU subjects, which proves that obesity, dyslipidemia and hypofibrinolysis are closely linked together (Chudý et al., 2011; Soares et al., 2007; Umpaichitra et al., 2005; Sobel et al., 2005).

3.2 t-PA in subjects with diabetes mellitus type 2

The t-PA levels were described to be increased in DM2 in an early phase of disease while t-PA levels in DM1 are increased later in presence of vascular complications. There is a correlation between t-PA and PAI-1 levels in subjects with DM2 and the principle cause of the t-PA increase is formation of the t-PA/PAI-1 complexes. Therefore it seems that the relation between t-PA and some parameters of IR as HI, BMI and arterial hypertension (AH) is mediated by correlation of IR parameters with PAI-1. The t-PA/PAI-1 complexes are increased in diabetics of both types with AH, microangiopathy (nephropathy including microalbuminuria) and macroangiopathy, however t-PA levels are increased in the early phase in DM2 subjects and also dependence between t-PA levels and PAI-1 and IR is higher in the phase of complications than dependence between t-PA and presence of complications in DM2. (Cho et al., 1994; Collier et al., 1992; Kvasnička et al., 1997).

t-PA was considered to be a marker of endothelial dysfunction because the circulating t-PA is of endothelial origin. However, EIA does not distinguish between a free t-PA and circulating t-PA bound in t-PA/PAI-1 complexes. Due to this problem with interpretation of t-PA results it is necessary to evaluate t-PA levels together with PAI-1 levels in relation to the type of disease. In our study we verified the relation between t-PA and PAI-1 levels in subjects with DM1 and DM2, treated by insulin (DM2-INS) or oral antidiabetics (DM2-AD), subjects with IR and HI and healthy controls (**table 3**). Since the PAI-1 levels are increased due to the extravascular production in adipose tissue it is evident that t-PA levels in complex with PAI-1 could not reflect a severity of the endothelial dysfunction in DM2 subjects. In accordance with other studies we confirmed the correlation between t-PA and PAI-1 in DM2 subjects treated with oral antidiabetics (DM2-AD) and subjects with IR. This correlation was even more significant in control healthy group (**table 3**). In subjects with DM2 and IR there is the increase of PAI-1 levels with the determinant effect on t-PA antigen levels. It was proved by the increased t-PA/PAI-1 complexes levels and inverse correlation between t-PA antigen and t-PA activity. Since the circulating PAI-1 is of combined vascular and extravascular origin with a close relation to parameters of IR there is not possible to consider the t-PA levels as a marker of endothelial dysfunction in DM2 and IR. But it can be of benefit to measure t-PA levels as a marker of endothelial dysfunction e.g. in subjects with DM1 and normal PAI-1 levels. The t-PA plasma levels increase in the period of vascular

complications and correlate with endothelial markers in subjects with DM1. Predictive value of the increased t-PA levels for worse disease prognosis was confirmed in subjects with atherosclerosis. The levels of t-PA and PAI-1 antigen in our subjects with DM1 did not correlate and the PAI-1 levels were in most of them normal. Thus the t-PA increase can reflect its release from the endothelium and measurement of t-PA in this case can be considered as a marker of endothelial dysfunction. We did not prove the correlation between t-PA and PAI-1 levels in DM2 subjects treated with insulin (DM2-INS). Insulin can inhibit the endothelial production of PAI-1 with the following decrease of tPA/PAI-1 complexes levels but insulin does not influence the t-PA release from endothelium and t-PA plasma levels. This can be explanation of the absent correlation between t-PA and PAI-1 levels in our DM2 subjects treated with insulin (Galajda & Mokáň, 2001).

	t-PA (ng/ml)	PAI-1 (ng/ml)	Correlation t-PA/PAI-1 p
DM1 (n=19)	4 (1-13)	7 (4-99)	n.s.
DM2-INS (n=27)	8 (1-17)	28 (2-270)	n.s.
DM2-AD (n=39)	11 (2-26)	58 (14-217)	p<0,05
NDIAB (n=22)	7 (2-15)	35 (6-217)	p<0,01
NORMAL (n=22)	3 (2-13)	9 (16-50)	p<0,01

DM1 (subjects with diabetes mellitus type 1), DM2 (subjects with diabetes mellitus type 2, treated by insulin - INS, oral antidiabetics - AD), NDIAB (hyperinsulinemic nondiabeticics), NORMAL (healthy controls), t-PA (tissue plasminogen activator) and plasminogen activator inhibitor type 1 (PAI-1)

Table 3. Correlations between tPA and PAI-1 levels

3.3 Thrombin activatable fibrinolysis inhibitor in subjects with diabetes mellitus type 2

Hori et al. found TAFI antigen levels and activity to be significantly higher in plasma of DM2 subjects compared to healthy controls. The plasma levels of TAFI antigen and D-dimers were inversely and significantly correlated in all DM2 subjects. These observations support the role of TAFI in the mechanism of diabetes-associated hypofibrinolysis (Hori et al., 2002).

Yano at al. found that the plasma levels of TAFI were significantly increased in DM2 subjects with microalbuminuria (MAU) compared to those in DM2 subjects with normoalbuminuria (NAU) and in normal subjects (Yano et al., 2003). Yener et al. observed in normotensive DM2 subjects without diabetes-related complications that PAI-1 level was significantly elevated, but level of TAFI antigen did not differ from healthy controls (Yener et al., 2009).

Our results in DM2 subjects (Chudý et al., 2011) showed that TAFI was significantly increased only in the MAU group compared to the controls (**table 4**). Neither the difference in TAFI levels between NAU and controls, nor the difference between diabetic subgroups MAU and NAU were statistically significant. These results indicate that the disease progression in DM2 subjects leads to more profound TAFI mediated inhibition of fibrinolysis (Chudý et al., 2011).

	NAU (n=42)	**MAU** (n=42)	**controls** (n=42)
TAFI (µg/mL)	63.5 (48-88)	72 (59-122)**	47 (36-55)
PAI-1 (ng/mL)	71.95 (57-90.9)**	102 (63.4-124.4)**	27.9 (10.2-47.7)
t-PA (ng/mL)	10.6 (8.7-12.9)	11.4 (7.9-15.6)*	8.15 (4.9-11.7)
F1+2 (nmol/L)	2.05 (1.7-5.3)**	2.9 (1.5-9.2)**	0.8 (0.5-1.4)**

* $0.05 < p < 0.1$, ** $p < 0.01$ compared to the control group.

TAFI: thrombin-activable fibrinolysis inhibitor, PAI-1: plasminogen activator inibitor type 1, t-PA: tissue plasminogen activator, F1+2: prothrombin fragments 1+2, NAU: normoalbuminuric group, MAU: microalbuminuric group

Table 4. Levels of TAFI, PAI-1, t-PA and F1+2 in subjects with DM2 based on albuminuria and in controls

Recent results are concordant with those of previous studies (Hori et al., 2002; Yano et al., 2003; Yener et al., 2009). We found no significant correlation between TAFI and PAI-1 in either group of subjects. This suggests that TAFI mediated inhibition of fibrinolysis in DM2 might be regulated independently from PAI-1. While PAI-1 is up-regulated mainly in obesity and in endothelial dysfunction, TAFI is activated in conditions of enhanced coagulation activation, which was also proved by positive correlation between TAFI and F1+2 in our MAU group as well as TAFI and fibrinogen in the NAU group. TAFI may be up-regulated by arterial hypertension, which is supported by the significantly positive correlation found between TAFI and systolic blood pressure in our MAU subjects, while dyslipidemia defined by elevated total cholesterol seems to down-regulate TAFI, which is supported by significant inverse correlation between TAFI and total cholesterol in our MAU subjects (Chudý et al., 2011). This finding is in agreement with previous study in subjects with essential hypertension where diastolic blood pressure significantly correlated with TAFI concentrations in untreated subjects, and in beta-blocker-treated subjects (Malyszko & Tymcio, 2008).

Our work also suggests fibrinolysis inhibition mediated by TAFI in early stages of diabetes defined by microalbuminuria in type 2 diabetic subjects without macrovascular complications (Chudý et al., 2011).

3.4 Modulation of hypofibrinolysis in subjects with diabetes mellitus type 2

In subject with DM2 there is hemostatic balance tipped towards a hypofibrinolytic phenotype which, coupled with atheromatous vascular changes and platelet hyperreactivity, predisposes to increased cardiovascular ischemic events (Alzahrani & Ajjan, 2010a). In the next section various agents used in diabetes are discussed.

3.4.1 Hypoglycaemic agents

3.4.1.1 Metformin

This agent is usually used in DM2 subjects. Metformin lowers PAI-1 levels *in vivo*. This beneficial effect of metformin on fibrinolysis (and on clot structure) may, in part, explain findings from the UK prospective Diabetes Study (UKPDS), which demonstrated reduced CHD risk in metformin users compared with other hypoglycaemic agents (UKPDS Group, 1998). Metformin therapy was associated with enhanced fibrinolytic potential of the clot in 850 DM2 subjects, further providing mechanistic explanation for the cardioprotective properties of this agent (Alzahrani et al., 2010b).

3.4.1.2 Sulphonylureas

Gliclazide has been shown to reduce clot permeability, creating a prothrombotic clot structure that is resistant to fibrinolysis (Dhall & Nair, 1994). Improving glycemic control with glipizide is associated with a fall in PAI-1 levels thereby enhancing fibrinolysis (Cefalu et al, 2002).

3.4.1.3 Thiazolidinediones

Thiazolidinediones are peroxisome proliferator-activated receptor (PPAR) - γ stimulators and possess antithrombotic properties. These agents lower PAI-1 levels which improves fibrinolysis (Haffner et al., 2002; Buckingham, 2005; Chen et al., 2010; Perriello et al., 2007). Clinical studies have shown that glitazones can delay progression of atherothrombotic lessions although the pioglitazone as well as rosiglitazone have failed to show a reduction in cardiovascular events in DM2 subjects (Dormandy et al., 2005; Nissen & Wolski, 2007; Home et al., 2009).

3.4.1.4 Gliptins and GPL-1 agonists

Number of clinical outcome studies are currently underway to clarify the role of these relatively new agents used in DM2 subjects in prevention of cardiovascular complications (De Caterina et al., 2010). Both native GLP-1 and the GLP-1 analogue liraglutide attenuate glucose-stimulated PAI-1 expression in human vascular endothelial cells (Liu et al., 2009).

3.4.1.5 Insulin

Insulin-treated DM2 subjects are at greater risk of cardiovascular events compared with non-insulin-treated subjects, which may be a reflection of longer disease duration increasing the risk of complications (Margolis et al., 2008). Hyperinsulinemia increases PAI-1 levels, which explains elevated levels of this protein in insulin-resistant states (Alessi & Juhan-Vague, 2008). In healthy individuals, insulin has antithrombotic effects. But according to the results of some recent studies the insulin treatment is associated with prothrombotic changes in the presence of IR and diabetes, as it increases fibrinogen and PAI-1 levels (Alzahrani & Ajjan, 2010a).

3.4.2 Anti-platelet agents

3.4.2.1 Aspirin

Aspirin has been shown to acetylate fibrinogen, resulting in a less compact clot structure that is easier to lyse (Ajjan et al., 2009b). Ajjan et al. have shown in DM1 subjects that addition of aspirin to plasma samples *ex vivo* resulted in either no effect or a paradoxical increase in clot lysis time in the presence of poor glycemic control, and this was reversed once glycemic control improved (Ajjan et al., 2009a).

3.4.2.2 Thienopyridines

Ticlopidine, clopidogrel´s precursor, has been associated with a reduction in plasma fibrinogen levels in a meta-analysis (Mazoyer et al., 1994). However, this was not confirmed in a randomized multi-centre study, which showed no difference in fibrinogen levels comparing subjects treated with cloppidogrel or aspirin following acute myocardial infarction (Woodward et al., 2004).

3.4.3 Lipid-lowering agents

3.4.3.1 Statins

Statins affect the fibrinolytic system through upregulation of thrombomodulin expression and reduction in plasma PAI-1 levels. Indirect effects on the coagulation system are related to lowering cholesterol levels which may be associated with reduced PAI and improved tPA release (Tekin et al., 2004; Masamura et al., 2003; Ludwig et al., 2005).

3.4.3.2 Fibrates

Fibrates are PPARα activators and are mainly used in subjects with raised triglyceride levels or in those intolerant to a statin. Fibrinogen and PAI-I levels are reduced in hyperlipidemic subjects following fibrate treatment (Okopien et al., 2001). Clinical study failed to show a reduction in cardiovascular events in diabetes subjects on fibrate treatment, but this may be related to a study design that has been repeatedly criticised (Keech et al., 2005).

3.4.3.3 Ezetimibe

Recent animal work has shown that PAI-1 expression in aortic and adipose tissue is reduced following ezetimibe administration (Yamamoto et al., 2009). The exact mechanism is unknown but may be related to decreased levels of oxidised low-density lipoprotein.

3.4.4 Angiotensin-converting enzyme inhibitor and angiotensin receptor blocker

The renin–angiotensin system has a role in hypofibrinolysis by stimulating PAI-1 synthesis. Both angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) reduce fibrinogen levels (Makris et al., 2004) thereby reducing the risk of cardiovascular events. ACEI can also modulate PAI-1 levels in diabetes subjects, whereas ARB has a less consistent effect (Fogari et al., 2002; Sola et al, 2005).

4. Conclusion

Diabetes mellitus is a metabolic disorder associated with increased vascular risk. Fibrinolysis disorders characterized with increased PAI-1 and t-PA antigens are considered a risk factor for future development of diabetes. This prothrombotic state is in diabetics further enhanced by decrease in t-PA activity and increase in TAFI antigen and activity. Consistant antidiabetics therapy may improve diabetes related hypofibrinolytic condition.

5. Acknowledgement

6. References

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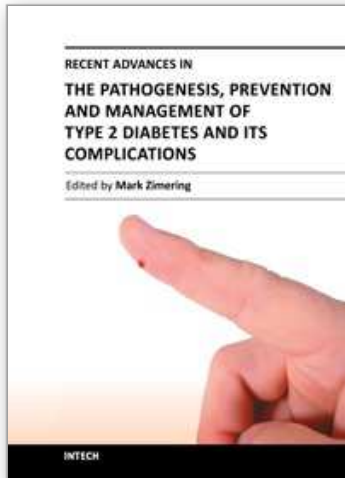
Recent Advances in the Pathogenesis, Prevention and Management of Type 2 Diabetes and its Complications
Edited by Prof. Mark Zimering

Type 2 diabetes â€œmellitusâ€ affects nearly 120 million persons worldwide- and according to the World Health Organization this number is expected to double by the year 2030. Owing to a rapidly increasing disease prevalence, the medical, social and economic burdens associated with the microvascular and macrovascular complications of type 2 diabetes are likely to increase dramatically in the coming decades. In this volume, leading contributors to the field review the pathogenesis, treatment and management of type 2 diabetes and its complications. They provide invaluable insight and share their discoveries about potentially important new techniques for the diagnosis, treatment and prevention of diabetic complications.

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