

## **The role of renin – angiotensin system in prothrombotic state in essential hypertension**

Anna Remková<sup>1</sup> and Milan Remko<sup>2</sup>

<sup>1</sup> Department of Internal Medicine, Military Hospital, Bratislava, <sup>2</sup> Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic

### **Corresponding author:**

Prof. MUDr. Anna Remková, PhD, DrSc.

Nemocnica Ministerstva obrany SR, a.s.

Cesta na Červený most 1

833 31 Bratislava 37, Slovak Republic

[remkova@nmo.sk](mailto:remkova@nmo.sk), [remkova@gmail.com](mailto:remkova@gmail.com)

**Short title:** renin-angiotensin system and prothrombotic state

## **Summary**

Rheological, haemostatic, endothelial and platelet abnormalities appear to play a role in the thrombotic complications of hypertension. This prothrombotic/ hypercoagulable state in hypertension may contribute to the increased risk and severity of target organ damage. It can be induced by the activated renin - angiotensin system (RAS), with abnormalities in endothelial and platelet function, coagulation and fibrinolysis. Treatment of uncomplicated essential hypertension by RAS targeting antihypertensive therapy could result in a reversal of prothrombotic abnormalities, contributing to a reduction in thrombosis-related complications. Since angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) have two distinct mechanisms of RAS interruption it is hypothesized that each therapy might have different impact on the prothrombotic state in hypertensive patients. Some studies demonstrate a beneficial effect of both ACE inhibitors and ARBs on prothrombotic state, in addition to their efficacy to normalize elevated BP. The potentially antithrombotic effect of the RAS inhibiting agents may in turn support the preservation of cardiovascular function. Available data may offer an additional explanation for the efficacy of the RAS targeting agents in the prevention of cardiovascular events in patients with atherosclerotic vascular disease.

**Key words:** endothelium, platelet, coagulation, fibrinolysis, hypertension

## Introduction

Hypertension is a major risk factor for thrombotic events such as myocardial infarction and stroke, reflecting a prothrombotic state that is present in hypertensive patients. A number of rheological, haemostatic, endothelial and platelet abnormalities appear to play a role in the thrombotic complications of hypertension. This prothrombotic/ hypercoagulable state in hypertension may contribute to the increased risk and severity of target organ damage (Sechi et al. 2000, Nadar et al. 2004a, Spencer et al. 2004, Sechi et al. 2008, Tay and Lip 2008). Moreover, markers of a hypercoagulable state may predict subsequent cardiovascular events in hypertensives (Lip et al. 2002).

A growing body of evidence indicates that prothrombotic state can be induced by the activated renin - angiotensin system (RAS), which is more pronounced in hypertension. Numerous deleterious effects of angiotensin II, including vasoconstriction, sympathetic nervous activation, smooth muscle cell growth and proliferation, vascular inflammation, generation of reactive oxygen species and endothelial dysfunction are mediated through the angiotensin II type 1 (AT<sub>1</sub>) receptor (**Figure 1**). Angiotensin II opposes the effect of nitric oxide, stimulates the production of adhesion factors and plasminogen activator inhibitor-1 (PAI-1), promoting risk of thrombosis. It was demonstrated that angiotensin II and aldosterone interact synergistically to increase PAI-1 production in humans (Sawathiparnich et al. 2003). Previous studies have attempted to define the relative weights of renin and aldosterone in the induction of the prothrombotic state. In patients with essential hypertension, a relationship between plasma renin activity (PRA) and plasma aldosterone with markers of activated coagulation and decreased fibrinolytic activity was demonstrated. Elevated levels of PRA, aldosterone, and prothrombotic markers are associated with clinical and instrumental evidence of cardiac and renal hypertensive organ damage (Sechi et al. 2008).

The RAS plays an important role in the pathogenesis of atherosclerotic complications. It can influence not only vascular tone, but also disturb the balance of the haemostatic system, with abnormalities in endothelial and platelet function, coagulation and fibrinolysis (Sakata et al. 2002, Dielis et al. 2007). Therefore, it is of importance to regulate not only blood pressure (BP), but also the haemostatic system in the long-term antihypertensive therapy. From this point of view, treatment of uncomplicated essential hypertension by RAS inhibition-based antihypertensive therapy could result in a reversal of prothrombotic abnormalities, contributing to a reduction in thrombosis-related complications.

Evidence for the protective role of some RAS targeting agents against atherothrombotic cardiovascular disease is accumulating. A precise mechanism of their ability to prevent thrombotic events is of particular interest. Both angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 (AT<sub>1</sub>) receptor blockers (ARB) effectively decrease blood pressure, but it seems that there could be some differences in clinical outcome, which may be partly related to their effect on haemostatic abnormalities. Since ACE inhibitors and ARBs have two distinct mechanisms of RAS interruption, it is hypothesized that each therapy might have different impact on the prothrombotic state in hypertensive patients.

### **Laboratory markers of prothrombotic state in hypertension**

Laboratory markers of endothelial and platelet function, as well as markers of hypercoagulation and fibrinolytic activity can be used to document a prothrombotic state in hypertension. For example, plasma von Willebrand factor (vWF) and thrombomodulin (TM) are used as indicators of endothelial dysfunction and/or damage. Plasma  $\beta$ -thromboglobulin ( $\beta$ TG), platelet factor 4 (PF4), soluble P-selectin (sPsel), soluble glycoprotein V (sGpV) can be measured as indicators of *in vivo* platelet activation. Plasminogen activator inhibitor type 1 (PAI-1) and tissue type plasminogen activator (tPA) antigens are used mainly as indicators of

fibrinolytic activity, but partly also as markers of endothelial function. Soluble endothelial protein C receptor (sEPCR) is a new marker of hypercoagulation. Fibrinogen as a crucial component of coagulation system can be investigated as a risk factor for vascular changes. In our previous studies, a statistically significant increase of vWF, TM,  $\beta$ TG, sPsel, sGpV, platelet aggregation, PAI-1 antigen, tPA antigen, sEPCR and fibrinogen level in untreated patients with the early stages of mild-to-moderate essential hypertension compared to healthy subjects was found (Remkova and Kratochvilova 2002, Remková et al. 2008).

It is known that vWF mediates platelet adhesion to the vascular wall, platelet aggregation and serves as a plasma carrier for factor VIII. Most circulating vWF is derived from the endothelium. *Blann et al.* were the first to describe raised vWF in essential hypertension (Blann et al. 1993). Now, vWF is considered to be the gold standard plasma endothelial marker, indicating a state of endothelial dysfunction/ damage or activation. It has also attracted considerable interest as a predictor of cardiovascular disease (Spencer et al. 2004, Lee et al. 2005, Vischer 2006, Ceconi et al. 2007). Some findings in hypertension suggest that TM, another marker of endothelial cell injury, is released into the plasma only by true endothelial cell damage during development of vascular complications and probably a certain degree of endothelial injury is necessary for its plasma increase (Okrucká-Remková et al. 1998, Remkova et al. 2000, Remkova and Kratochvilova, 2002).

Plasma  $\beta$ TG and PF4, platelet-specific proteins released from the  $\alpha$ -granules, are established markers of platelet activity. Both sPsel and sGpV are considered to be another plasma markers of *in vivo* platelet activation. The adhesion molecule P-selectin has a role in modulating interactions between blood cells and the endothelium. It is a component of the membrane of the  $\alpha$  and dense granules of platelets, and also of the membrane of the Weibel-Palade bodies of endothelial cells. Although present on the external cell surface of both activated endothelium and activated platelets, it now seems clear that most, if not all of the

measured sPsel is of platelet origin (Blann et al. 2003). Increased levels of sPsel in the plasma have been demonstrated in a wide variety of acute and chronic cardiovascular and cerebrovascular disorders, including hypertension. The increased sPsel can be considered as a predictor of adverse cardiovascular events (Riondino et al. 1999, Blann et al. 2003, Nadar et al. 2004a, 2004b, 2004c, Spencer et al. 2004, Lee et al. 2005). Soluble GpV is cleaved from the platelet membrane surface by thrombin. While bound to the platelet membrane, GpV is associated with GPIb and GpIX to form the platelet GpIb-V-IX complex, which mediates platelet adhesion to the subendothelium by binding von Willebrand factor. Soluble GpV is significantly elevated in patients with atherothrombotic diseases (Blann et al. 2001, Wolff et al. 2005).

Fibrinolytic activity is determined by the balance between the levels of tPA and PAI-1, both of which are synthesized in the vascular endothelium, and endothelial injury induces an imbalance in fibrinolysis (Tomiyama et al. 1998). Besides endothelial cells, PAI-1 as a strong inhibitor of fibrinolysis, is produced mainly by adipose tissue (including the liver steatosis). Among haemostatic abnormalities, an increase of PAI-1 is considered as a core feature of metabolic syndrome, and obesity is a main determinant of plasma PAI-1 concentrations (Aso et al. 2005). Data from the Framingham Offspring Study showed that increased PAI-1 antigen and tPA antigen levels are independently associated with hypertension (Poli et al. 2000). High concentrations of tPA antigen also reflect impairment of the fibrinolytic system, because most of the tPA antigen measured is complexed with PAI-1 and is inactive. These data suggest that impaired fibrinolysis may play an important role in the pathogenesis of cardiovascular disease in hypertensive patients. The mechanism by which increasing BP may result in impaired fibrinolysis is unclear, but it may be related to endothelial dysfunction. It is known that angiotensin II (via AT<sub>1</sub> receptor) stimulates the expression of PAI-1, resulting in an increase of PAI-1 release from endothelial cells (Sakata et al. 2002, Koh et al. 2004, Dielis et al. 2007).

However, it was demonstrated that angiotensin IV, which is a metabolite of angiotensin II, can stimulate PAI-1 expression from endothelial cells (Ruiz-Ortega et al. 2007). Angiotensin IV has drawn a lot of attention since it exerts a wide range of distinct biological effects, including protection against cerebral ischaemia, activity at the vascular level and an involvement in atherogenesis. It is involved in the vascular inflammatory response since it regulates cell growth, activates the adhesion molecules and the cytokines. Some of these effects are AT<sub>1</sub> receptor dependent but others most likely result from binding of angiotensin IV to a specific AT<sub>4</sub> receptor, which has recently been identified as the membrane-bound insulin-regulated aminopeptidase (IRAP). This protein appears to have multiple physiological roles that are tissue-specific. The exact mechanisms of action that mediate the angiotensin IV-induced effects following this binding are until now not fully known (Ruiz-Ortega et al. 2007, Stragier et al. 2008).

Recently, the first evidence was offered that hypertension could contribute to the increased sEPCR levels (Remková et al. 2008). EPCR is a transmembrane glycoprotein restricted to the endothelium, which activates protein C via the thrombin-TM complex. Unlike TM, it is expressed mainly on the endothelium of large vessels. The previous studies confirm an *in vivo* function of sEPCR in the regulation of coagulation in humans (Saposnik et al. 2004). The release of sEPCR is inducible by inflammation and/ or thrombin generation. Increased sEPCR levels may be prothrombotic (Saposnik et al. 2004). Lower sEPCR levels reflect a decrease of thrombin generation. In patients free of evident inflammatory disease, plasma levels of sEPCR can be a potential marker for the prothrombotic state and a predictor of cardiovascular disease in hypertensives (Remková et al. 2008).

It is known that hypertensives demonstrate abnormalities of rheological function. Plasma fibrinogen and vWF are important determinants of blood viscosity and are involved in haemostasis. Elevated levels are found in patients with hypertension and an extensive body of

evidence indicates they are independent predictors of cardiovascular disease. High plasma levels of fibrinogen observed in hypertensives are in keeping with a prothrombotic or hypercoagulable state in hypertension (Spencer et al. 2004, Remková et al. 2008). The highest levels of fibrinogen are found in nondipper patients with concurrent high ambulatory pulse pressure (Lee et al. 2005). Plasma fibrinogen is significantly related to the presence and severity of target organ damage in patients with essential hypertension and may contribute to the development of atherosclerotic disease in these patients (Sechi et al. 2000, 2008).

### **Effect of RAS inhibition on prothrombotic state in hypertension**

Modern antihypertensive therapy has focused not only on BP control but also on the favorable modification of known prognostic indices, such as endothelial and platelet dysfunction or coagulation and fibrinolytic abnormalities.

In our previous study, besides a normalization of high BP in hypertensive patients, a significant decrease of plasma vWF antigen, sPsel, sGpV, PAI-1 antigen and tPA antigen level after one month of perindopril therapy, as well as a significant decrease of sEPCR and fibrinogen level after one month of telmisartan therapy was observed (Remková et al. 2008). This finding can document a beneficial effect of both treatment strategies on prothrombotic state in hypertensive patients.

A decrease of vWF after perindopril therapy can indicate the improvement of endothelial function in patients with hypertension. In contrary, no significant changes of vWF in hypertensives treated by telmisartan were found (Remková et al. 2008). This finding is supported by results of another study, in which only antihypertensive treatment by perindopril but not telmisartan was able to improve conduit artery endothelium-dependent vasodilatation (Ghiadoni et al. 2003). It could be hypothesized that this beneficial effect of perindopril may be related to bradykinin-dependent mechanisms (Taddei et al. 2002). Although many clinical



studies have found that ACE inhibitors improve endothelial function, it is not clear whether ARBs have beneficial effects comparable to those of ACE inhibitors (Matsumoto et al. 2003). A possible vascular and anti-atherosclerotic effect of long-lasting ACE inhibition by perindopril, as shown in different experimental and other studies, was the background hypothesis for *EUROPA* study (Ferrari et al. 2003). The *EUROPA* study demonstrated reduction in cardiovascular mortality and myocardial infarction with ACE inhibition by perindopril in patients with coronary artery disease, including even stable patients at lower risk. The positive outcome occurred also in patients with normal blood pressure and was not related to the degree of blood pressure reduction achieved by the drug (EUROPA Trial Investigators 2003). In order to verify the mechanism of action of perindopril in secondary prevention, *PERTINENT* substudy examined the effects of perindopril on endothelial function, thrombosis, and inflammation (Ceconi et al. 2007). Data from *PERTINENT* suggest that long-term (1 year) ACE inhibition with perindopril 8 mg/day exerts a direct positive effect on the vascular endothelium. At baseline, vWF was at the upper limit of the normal values and even more elevated in the diabetic patients, indicating endothelial damage. Furthermore, vWF levels were correlated with prognosis, and 1 year of treatment with perindopril significantly reduced them independently from other risk factors. These data in patients with coronary artery disease do not provide information on whether long-term administration of perindopril is needed or whether this can be the result of an acute effect. The results of a later study demonstrate that also a short-term, i.e. one-month administration of perindopril in a half of the dose used in *EUROPA* study, has a benefit on endothelial function, even in patients with lower risk, without manifest coronary artery disease (Remková et al. 2008).

In early-stage hypertensive patients without microalbuminuria, which is also a marker of widespread endothelial dysfunction, only borderline changes in hypertensives and no changes

in plasma TM can be found during short-term treatment by perindopril or telmisartan (Remková et al. 2008).

Antihypertensive treatment by perindopril but not by telmisartan was reported to reduce platelet activation, with a decrease of sPsel and sGpV (Remková et al. 2008). In the other study, normalization of BP in elderly hypertensives with an ACE-inhibitor and/or a calcium antagonist resulted in a reduction in plasma levels of sPsel (Riondino et al. 1999). The improvement of platelet function in hypertensives treated by perindopril is in accordance with previous findings of decreased platelet aggregation in a similar group of treated patients (Okrucká-Remková et al. 1998). Those results suggest that increased platelet activation need not be restored to normal after effective anti-hypertensive therapy alone (Okrucká-Remková et al. 1998). Indirectly, these findings can reflect a protective effect of perindopril on the vascular wall, with improvement of endothelial function. This hypothesis is likely supported by findings that perindoprilat enhances inhibition of platelet activation by human umbilical vein endothelial cells (HUVEC). Enhanced endothelial anti-platelet properties (greater amount of prostacyclin released) were able to stabilize platelets, making them less responsive to agonists in an *ex vivo* situation (Kishi et al. 2003). Thus, perindopril exhibited an ability to inhibit platelet aggregation by augmenting endothelial anti-platelet mechanisms. On the other hand, candesartan had no effects on inhibition of platelet aggregation by HUVEC (Kishi et al. 2003). Since human platelets have been demonstrated to express angiotensin II (AT<sub>1</sub> type) receptor, the action of ACE inhibitor on the platelets could be related to angiotensin II blockade. However, exogenous angiotensin II does not modify platelet activation (Montón et al. 2000). These results suggest that some RAS antagonists reduce platelet activation independently of angiotensin II involvement. Platelet activation may also be suppressed, in the presence of intact endothelial cells, through increased prostacyclin and nitric oxide (NO) release, induced by elevated bradykinin levels due to ACE inhibition. This last mechanism is

absent in patients treated by ARB. Actually, telmisartan therapy failed to show any changes in platelet markers of hypertensive patients (Remková et al. 2008). Drugs such as RAS targeting agents possess some sympathetic activity inhibitory effect. A subsequent reduction of catecholamine release could be a further factor for a favorable effect on platelet function in patients with essential hypertension.

The ARBs may impair the fibrinolytic system due to increased angiotensin IV, which results from an increase in angiotensin II (Sakata et al. 2002). The ACE inhibitors are thought to favourably affect the fibrinolytic balance by decreasing angiotensin II-mediated PAI-1 release and/or by increasing bradykinin-induced tPA release from endothelial cells (Matsumoto et al. 2003). Modification of the hypofibrinolytic state may have a beneficial effect on the increased cardiovascular risk in subjects with hypertension. It was reported, that perindopril increases the ability of bradykinin to stimulate the release of tPA in the human coronary circulation, which is not seen with losartan (Matsumoto et al. 2003). According to several comparative studies, using two therapeutic regimens, ACE inhibition may have greater beneficial effects than AT<sub>1</sub> receptor antagonism (Matsumoto et al. 2003). For instance, in hypertensive postmenopausal women, the PAI-1 antigen levels were significantly decreased by trandolapril, but not by losartan therapy (Fogari et al. 2001a).

To date, controversial results have been reported in both experimental and clinical studies about the effect of ARBs (with different pharmacokinetic and pharmacodynamic properties) on fibrinolysis, with some studies showing no influence and others a reduction or even an increase of PAI-1 levels (Fogari et al. 2001b, Erlinger et al. 2002, Koh et al. 2004, Dielis et al. 2007). The mechanism for these differences is not clear, but it might be related to the different inhibitory characteristic of the various ARBs. One possible explanation for the lack of effect on fibrinolysis by some ARBs might be that receptor subtypes other than AT<sub>1</sub> mediate the effect of angiotensin II on endothelial PAI-1 expression. In fact, there have been conflicting

findings also on the effects of ACE inhibitors on fibrinolysis. In general, they may result from the study population and design, such as drug dosage etc. (Sakata et al. 2002). It was shown that perindopril enhances fibrinolysis (with a decrease of PAI-1 as well as tPA antigens), but telmisartan does not attenuate it in essential hypertension (Remková et al. 2008). These different effects of each drug on fibrinolysis appear to be associated with the changes in angiotensin metabolites. The results are in accordance with findings of other authors, showing that levels of PAI-1 antigen are reduced after antihypertensive treatment with perindopril (Erdem et al. 1999, Fogari et al. 2002). On the other hand, it was reported that expression of PAI-1 was *in vitro* attenuated by telmisartan in cultured mesangial cells, possibly owing to its lipophilic and antioxidant properties (Shao et al. 2007).

According to a finding of decreased sEPCR due to telmisartan therapy, it is probably possible to improve the prothrombotic state in hypertensive patients (Remková et al. 2008). The mechanism of this effect is not clear; but because of the endothelial cell receptor expression, a speculation about the improvement of functional properties of large arteries can arise as explanation.

Previous studies examining the influence of therapeutic intervention on fibrinogen plasma levels have shown conflicting results. For example, treatment with either irbesartan or atenolol (Makris et al. 2000) and with telmisartan (Remková et al. 2008) in hypertensive patients was associated with a significant decrease in the levels of fibrinogen, but no changes of fibrinogen were observed in 20 subjects with insulin resistance after telmisartan administration (Nagel et al. 2006). In another studies in hypertensive patients, plasma fibrinogen levels were reduced by perindopril (Fogari et al. 1998) but not by losartan (Fogari et al. 1998, Li-Saw-Hee et al. 2001). On the other hand, no changes of fibrinogen level were found in another studies in hypertensive patients after treatment by enalapril (Li-Saw-Hee et al. 2001) or perindopril (Remková and Kratochvířová 2000, Remková et al. 2008). It has not

yet been determined whether the decrease in fibrinogen levels, whenever observed, is related directly to BP reduction or is associated with coexisting properties of the drugs used. It has been suggested that it may result from haemodilution caused by vasodilating agents or by a decrease in red cell rigidity (Makris et al. 2000).

The supposed mechanisms of the effect of therapy by RAS inhibiting agents on prothrombotic state are shown in **Figure 2**.

There is only scarce information about the effect of other RAS targeting agents, i.e. aldosterone receptor antagonists and renin inhibitor on haemostatic markers in hypertension.

Spirolactone through mineralocorticoid receptor antagonism prevents the effect of activation of the RAS on PAI-1 antigen in normotensive subjects and improves fibrinolytic balance in hypertensive subjects. In hypertensive patients, spironolactone significantly decreased PAI-1 antigen (Ma et al. 2005). In experimental chronic heart failure model, spironolactone added to an ACE inhibitor trandolapril beneficially modulates the balance of nitric oxide and superoxide anion formation (Bauersachs et al. 2002). In a similar experimental model, monotherapy with ACE inhibitor trandolapril or with selective aldosterone receptor antagonist eplerenone partially reduced the increased platelet activation, which was completely reduced to basal levels by combination therapy (Schäfer et al. 2003).

*In vitro* effects of a renin inhibitor aliskiren on a total of 33 biomarkers of platelet activity, coagulation and fibrinolysis were assessed in subjects with multiple risk factors for vascular disease. In the therapeutic concentration, aliskiren does not affect haemostatic markers *in vitro* (Serebruany et al. 2008). It is suggested that antithrombotic properties of aliskiren should be explored further in an *ex vivo* clinical setting.

## Conclusion

In conclusion, available data may offer an additional explanation for the efficacy of the RAS targeting agents in the prevention of cardiovascular events in patients with atherosclerotic vascular disease (**Table 1**). Some studies demonstrate a beneficial effect of both ACE inhibitors and ARBs on prothrombotic state, in addition to their efficacy to normalize elevated BP. Despite the results of these studies, the possible role of blood pressure lowering effect on prothrombotic state should be always kept in mind. The potentially antithrombotic effect of the RAS targeting agents may in turn support the preservation of cardiovascular function.

## Acknowledgement

This work was supported by grant from the Slovak Ministry of Education (VEGA grant No 1/4301/07).

## References

- ALEIL B, MEYER N, WOLFF V, KIENTZ D, WIESEL ML, GACHET C, CAZENAVE JP, LANZA F: Plasma glycoprotein V levels in the general population: normal distribution, associated parameters and implications for clinical studies. *Thromb Haemost* **96**: 505-511, 2006.
- ASO Y, WAKABAYASHI S, YAMAMOTO R, MATSUTOMO R, TAKEBAYASHI K, INUKAI T: Metabolic syndrome accompanied by hypercholesterolemia is strongly associated with proinflammatory state and impairment of fibrinolysis in patients with type 2 diabetes. *Diabetes Care* **28**: 2211-2216, 2005.
- ATALAR E, HAZNEDAROGLU IC, KILIC H, OZER N, COSKUN S, OZTURK E, AKSOYEK S, OVUNC K, KIRAZLI S, OZMEN F: Increased soluble glycoprotein V

concentration during the acute onset of unstable angina pectoris in association with chronic cigarette smoking. *Platelets* **16**: 329-333, 2005.

BAUERSACHS J, HECK M, FRACCAROLLO D, HILDEMAN SK, ERTL G, WEHLING M, CHRIST M: Addition of spironolactone to angiotensin-converting enzyme inhibition in heart failure improves endothelial vasomotor dysfunction: role of vascular superoxide anion formation and endothelial nitric oxide synthase suppression. *J Am Coll Cardiol* **39**: 351-358, 2002.

BLANN AD, NAQVI T, WAITE M, MCCOLLUM CN: Von Willebrand factor and endothelial damage in essential hypertension. *J Hum Hypertens* **7**: 107-111, 1993.

BLANN AD, LANZA F, GALAJDA P, GURNEY D, MOOG S, CAZENAVE JP, LIP GY: Increased platelet glycoprotein V levels in patients with coronary and peripheral atherosclerosis – the influence of aspirin and cigarette smoking. *Thromb Haemost* **86**: 777-783, 2001.

BLANN AD, NADAR SK, LIP GYH: The adhesion molecule P-selectin and cardiovascular disease. *Eur Heart J* **24**: 2166-2179, 2003.

CECONI C, FOX KM, REMME WJ, SIMOONS ML, BERTRAND M, PARRINELLO G, KLUFT C, BLANN A, COKKINOS D, FERRARI R on behalf of the EUROPA and the PERTINENT Investigators: ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. *Cardiovasc Res* **73**: 237-246, 2007.

DIELIS AWJH, SMID M, SPRONK HMH, HOUBEN AJHM, HAMULYÁK K, KROON AA, TEN CATE H, DE LEEUW PW: Changes in fibrinolytic activity after angiotensin II receptor blockade in therapy-resistant hypertensive patients. *J Thromb Haemost* **5**: 1509-1515, 2007.

ERDEM Y, USALAN C, HAZNEDAROGLU IC, ALTUN B, ARICI M, YASAVUL U, TURGAN C, CAGLAR S: Effects of angiotensin-converting enzyme and angiotensin II

receptor inhibition on impaired fibrinolysis in systemic hypertension. *Am J Hypertens* **12** (part 1): 1071-1076, 1999.

ERLINGER TP, CONLIN PR, MACKO RF, BOHANNON AD, MILLER III ER, MOORE TJ, SVETKEY LP, APPEL LJ: The impact of angiotensin II receptor blockade and the DASH diet on markers of endogenous fibrinolysis. *J Hum Hypertens* **16**: 391-397, 2002.

FERRARI R, ARBUSTINI E, BLANN A, COKKINOS D, KLUFT C, DE MAAT MPM, TAVAZZI L: PERTINENT – PERindopril - Thrombosis, InflammatioN, Endothelial dysfunction and Neurohormonal activation Trial: A sub-study of the EUROPA study. *Cardiovasc Drug Ther* **17**: 83-91, 2003.

FOGARI R, ZOPPI A, LAZZARI P, PRETI P, MUGELLINI A, CORRADI L, LUSARDI P: ACE inhibition but not angiotensin II antagonism reduces plasma fibrinogen and insulin resistance in overweight hypertensive patients. *J Cardiovasc Pharmacol* **32**: 616-620, 1998.

FOGARI R, ZOPPI A, PRETI P, FOGARI E, MALAMANI G, MUGELLINI A: Differential effects of ACE-inhibition and angiotensin II antagonism on fibrinolysis and insulin sensitivity in hypertensive postmenopausal women. *Am J Hypertens* **14**: 921-926, 2001a.

FOGARI R, ZOPPI A, MALAMANI G, MARASI G, PESCE RM, BANDERALI A, MUGELLINI A: Effects of four angiotensin II-receptor antagonists on fibrinolysis in postmenopausal women in hypertension. *Curr Ther Res Clin Exp* **62**: 68-78, 2001b.

FOGARI R, MUGELLINI A, ZOPPI, CORRADI L, PRETI P, LAZZARI P, DEROSA G: Losartan and perindopril effects on plasma plasminogen activator inhibitor-1 and fibrinogen in hypertensive type 2 diabetic patients. *Am J Hypertens* **15** (part 1): 316-320, 2002.

GHIADONI L, MAGAGNA A, VERSARI D, KARDASZ I, HUANG Y, TADDEI S, SALVETTI A: Different effect of antihypertensive drugs on conduit artery endothelial function. *Hypertension* **41**: 1281-1286, 2003.



HLUBOCKÁ Z, UMNEROVÁ V, HELLER S, PELEŠKA J, JINDRA A, JÁCHYMOVÁ M, KVASNIČKA J, HORKÝ K, ASCHERMANN M: Circulating intercellular cell adhesion molecule-1, endothelin-1 and von Willebrand factor-markers on endothelial dysfunction in uncomplicated essential hypertension: the effect of treatment with ACE inhibitors. *J Hum Hypertens* **16**: 557-562, 2002.

KISHI Y, OHTA S, KASUYA N, SAKITA S, ASHIKAGA T, ISOBE M: Perindopril augments ecto-ATP diphosphohydrolase activity and enhances endothelial anti-platelet function in human umbilical vein endothelial cells. *J Hypertens* **21**: 1347-1353, 2003.

KOH KK, CHUNG WJ, AHN JY, HAN SH, KANG WC, SEO YH, AHN TH, CHOI IS, SHIN EK: Angiotensin II type receptor blockers reduce tissue factor activity and plasminogen activator inhibitor type-1 antigen in hypertensive patients: a randomized, double-blind, placebo controlled study. *Atherosclerosis* **177**: 155-160, 2004.

LEE KW, BLANN AD, LIP GY: High pulse pressure and nondipping circadian blood pressure in patients with coronary artery disease: Relationship to thrombogenesis and endothelial damage/dysfunction. *Am J Hypertens* **18**: 104-115, 2005.

LIP GYH, BLANN AD, EDMUNDS E, BEEVERS DG: Baseline abnormalities of endothelial function and thrombogenesis in relation to prognosis in essential hypertension. *Blood Coag & Fibrinolys* **13**: 35-41, 2002.

LI-SAW-HEE FL, BEEVERS DG, LIP GYH: Effect of antihypertensive therapy using enalapril or losartan on haemostatic markers in essential hypertension: a pilot prospective randomised double-blind parallel group trial. *Internat J Cardiol* **78**: 241-246, 2001.

MA J, ALBORNOZ F, YU C, BYRNE DW, VAUGHAN DE, BROWN NJ: Differing effects of mineralocorticoid receptor-dependent and -independent potassium-sparing diuretics on fibrinolytic balance. *Hypertension* **46**: 310-320, 2005.

MAKRIS TK, STAVROULAKIS GA, KRESPI PG, HATZIZACHARIAS AN, TRIPOSKIADIS FK, TSOUKALA CG, VOTTEAS VV, KYRIAKIDIS MK: Fibrinolytic/hemostatic variables in arterial hypertension: response to treatment with irbesartan or atenolol. *Am J Hypertens* **13**: 783-788, 2000.

MATSUMOTO T, MINAI K, HORIE H, OHIRA N, TAKASHIMA H, TARUTANI Y, YASUDA Y, OZAWA T, MATSUO S, KINOSHITA M, HORIE M: Angiotensin-converting enzyme inhibition but not angiotensin II type 1 receptor antagonism augments coronary release of tissue plasminogen activator in hypertensive patients. *J Am Coll Cardiol* **41**: 1373-1379, 2003.

MONTÓN M, JIMÉNEZ A, NÚÑEZ A, LÓPEZ-BLAYA A, FARRÉ J, GÓMEZ J, ZALBA LR, SÁNCHEZ DE MIGUEL L, CASADO S, LÓPEZ-FARRÉ A: Comparative effects of angiotensin II AT-1-type receptor antagonists in vitro on human platelet activation. *J Cardiovasc Pharmacol* **35**: 906-913, 2000.

NADAR SK, BLANN AD, KAMATH S, BEEVERS DG, LIP GY: Platelet indexes in relation to target organ damage in high-risk hypertensive patients: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). *J Am Coll Cardiol* **44**: 415-422, 2004a.

NADAR SK, BLANN AD, LIP GY: Platelet morphology and plasma indices of platelet activation in essential hypertension: effects of amlodipine-based antihypertensive therapy. *Ann Med* **36**: 552-557, 2004b.

NADAR SK, BLANN AD, LIP GY: Platelet morphology, soluble P selectin and platelet P-selectin in acute ischaemic stroke. The West Birmingham Stroke Project. *Thromb Haemost* **92**: 1342-1348, 2004c.

NAGEL JM, TIETZ AB, GÖKE B, PARHOFER KG: The effect of telmisartan on glucose and lipid metabolism in nondiabetic, insulin-resistant subjects. *Metabolism* **55**: 1149-1154, 2006.

OKRUCKÁ - REMKOVÁ A, PECHÁŇ J, KRATOCHVÍLOVÁ H: Effects of the angiotensin-converting enzyme (ACE) inhibitor perindopril on endothelial and platelet functions in essential hypertension. *Platelets* **9**: 63-67, 1998.

POLI KA, TOFLER GH, LARSON MG, EVANS JC, SUTHERLAND PA, LIPINSKA I, MITTLEMAN MA, MULLER JE, D'AGOSTINO RB, WILSON PWF, LEVY D: Association of blood pressure with fibrinolytic potential in the Framingham Offspring Study. *Circulation* **101**: 264-269, 2000.

RIONDINO S, PIGNATELLI P, PULCINELLI FM, LENTI L, DI VEROLI C, MARIGLIANO V, GAZZANIGA PP: Platelet hyperactivity in hypertensive older patients is controlled by lowering blood pressure. *J Am Geriatr Soc* **47**: 943-947, 1999.

REMKOVA A, KOVACOVA E, PRIKAZSKA M, KRATOCHVILOVA H: Thrombomodulin as a marker of endothelium damage in some clinical conditions. *Eur J Int Med* **11**: 79-84, 2000.

REMKOVÁ A, KRATOCHVÍLOVÁ H: Effect of the angiotensin-converting enzyme inhibitor perindopril on haemostasis in essential hypertension. *Blood Coag & Fibrinolys* **7**: 641-644, 2000.

REMKOVA A, KRATOCHVILOVA H: Effect of the new centrally acting antihypertensive agent rilmenidine on endothelial and platelet function in essential hypertension. *J Hum Hypertens* **16**: 549-555, 2002.

REMKOVÁ A, KRATOCHVÍLOVÁ H, ĎURINA J: Impact of the therapy by renin-angiotensin system targeting antihypertensive agents perindopril versus telmisartan on prothrombotic state in essential hypertension. In press, accepted by *J Hum Hypertens*, **22**: 338-345, 2008.

RUIZ-ORTEGA M, ESTEBAN V, EGIDO J: The regulation of the inflammatory response through nuclear factor-kappaB pathway by angiotensin IV extends the role of the renin angiotensin system in cardiovascular diseases. *Trends Cardiovasc Med* **17**: 19-25, 2007.

SAKATA K, PAWLAK R, URANO T, TAKADA A: Effects of a long-term pharmacological interruption of the renin-angiotensin system on the fibrinolytic system in essential hypertension. *Pathophysiol Haemost Thromb* **32**: 67-75, 2002.

SAPOSNIK B, RENY JL, GAUSSEM P, EMMERICH J, AIACH M, GANDRILLE S: A haplotype of the EPCR gene is associated with increased plasma levels of sEPCR and is candidate risk factor for thrombosis. *Blood* **103**: 1311-1318, 2004.

SAWATHIPARNICH P, MURPHEY LJ, KUMAR S, VAUGHAN DE, BROWN NJ: Effect of combined AT1 RECEPTOR AND ALDOSTERONE RECEPTOR ANTAGONISM ON PLASMINOGEN ACTIVATOR INHIBITOR-1. *J Clin Endocrinol Metab* **88**: 3867-3873, 2003.

SECHI LA, ZINGARO L, CATENA C, CASACCIO D, DE MARCHI S: Relationship of fibrinogen levels and hemostatic abnormalities with organ damage in hypertension. *Hypertension* **36**: 978-985, 2000.

SECHI LA, NOVELLO M, COLUSSI G, DI FABIO A, CHIUCH A, NADALINI E, CASANOVA-BORCA A, UZZAU A, CATENA C: Relationship of plasma renin with a prothrombotic state in hypertension: relevance for organ damage. *Am J Hypertens* **21**: 1347-1353, 2008.

SEREBRUANY VL, MALININ A, BARSNESS G, VAHABI J, ATAR D: Effects of aliskiren, a renin inhibitor, on biomarkers of platelet activity, coagulation and fibrinolysis in subjects with multiple risk factors for vascular disease. *J Hum Hypertens* **22**: 303-310, 2008.

SHAO J, NANGAKU M, INAGI R, KATO H, MIYATA T, MATSUSAKA T, NOIRI E, FUJITA T: Receptor-independent intracellular radical scavenging activity of an angiotensin II receptor blocker. *J Hypertens* **25**: 1643-1649, 2007.

SCHÄFER A, FRACCAROLLO D, HILDEMAN S, CHRIST M, EIGENTHALER M, KOBAR A, WALTER U, BAUERSACHS J: Inhibition of platelet activation in congestive heart failure by aldosterone receptor antagonism and ACE inhibition. *Thromb Haemost* **89**: 1024-1030, 2003.

SPENCER CGC, MARTIN SC, FELMDEN DC, BLANN AD, BEEVERS GD, LIP GYH: Relationship of homocysteine to markers of platelet and endothelial activation in 'high risk' hypertensives: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial. *Internat J Cardiol* **94**: 293-300, 2004.

STRAGIER B, DE BUNDEL D, SARRE S, SMOLDERS I, VAUQUELIN G, DUPONT A, MICHOTTE Y, VANDERHEYDEN P: Involvement of insulin-regulated aminopeptidase in the effects of the renin-angiotensin fragment angiotensin IV: a review. *Heart Fail Rev* **13**: 321-327, 2008.

TADDEI S, VIRDIS A, GHIADONI L, SUDANO I, SALVETTI A: Effects of antihypertensive drugs on endothelial dysfunction – Clinical implications. *Drugs* **62**: 265-284, 2002.

TAY KH, LIP GYH: What “drives” the link between the renin-angiotensin-aldosterone system and the prothrombotic state in hypertension? *Am J Hypertens* **22**: 1278-1279, 2008.

THE EUROPEAN TRIAL ON REDUCTION OF CARDIAC EVENTS WITH PERINDOPRIL IN STABLE ARTERY DISEASE INVESTIGATORS: Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double - blind, placebo-controlled, multicentre trial (EUROPA). *Lancet* **362**: 782-788, 2003.

TOMIYAMA H, KIMURA Y, MITSUHASHI H, KINOUCHI T, YOSHIDA H, KUSHIRO T, DOBA N: Relationship between endothelial function and fibrinolysis in early hypertension. *Hypertension* **31** (part 2): 321-327, 1998.

TRIFILETTI A, BARBERA N, SCAMARDI R, BAGNATO L, PIZZOLEO MA, NEVOSO A, LASCO A, PEDULLA M, FRISINA N: Effects of medium-term antihypertensive therapy on haemostatic parameters in patients with essential hypertension. *Haemostasis***27**: 35-38, 1997.

VISCHER UM: Von Willebrand factor, endothelial dysfunction and cardiovascular disease. *J Thromb Haemost* **4**: 1186-1193, 2006.

WOLFF V, ALEIL B, GIROUD M, LORENZINI JL, MEYER N, WIESEL ML, CAZENAVE JP, LANZA F: Soluble platelet glycoprotein V is a marker of thrombosis in patients with ischemic stroke. *Stroke* **36**: e17-e19, 2005.

Table 1 The effect of renin-angiotensin system (RAS) targeting agents on prothrombotic state in hypertensive patients

<b>Markers</b>	<b>RAS inhibiting agents</b>	<b>Effect</b>	<b>Mechanism</b>
Endothelial function	ACE inhibitors	Beneficial (benazepril -Tomiyama et al. 1998, perindopril - Erdem et al. 1999, Ghiadoni et al. 2003, Ceconi et al. 2007, Remková et al. 2008, quinapril - Hlubocká et al. 2002) or neutral (cilazapril - Trifiletti et al. 1997)	Bradykinin dependent mechanism
	ARBs	Neutral (losartan - Matsumoto et al. 2003, telmisartan - Ghiadoni et al. 2003, Remková et al. 2008)	Bradykinin dependent mechanism is absent
Platelet function	ACE inhibitors	Beneficial (perindopril - Okrucká et al. 1998, Riondino et al. 1999, Kishi et al. 2003, Remková et al. 2008, trandolapril - Schäfer et al. 2003) or neutral (cilazapril - Trifiletti et al. 1997, quinapril - Hlubocká et al. 2002)	Enhanced endothelial antiplatelet properties (increased prostacyclin and nitric oxide release) induced by elevated bradykinin levels
	ARBs	Neutral (candesartan - Kishi et al. 2003, telmisartan - Remková et al.	This mechanism is absent

		2008)	
Fibrinolysis	ACE inhibitors	Beneficial (benazepril - Tomiyama et al. 1998, perindopril - Erdem et al. 1999, Fogari et al. 2002, Matsumoto et al. 2003, Remková et al. 2008, trandolapril - Fogari 2001a, quinapril - Sakata 2002) or neutral (cilazapril - Trifiletti et al. 1997)	Inhibition of angiotensin II-mediated PAI-1 release and/or stimulation of bradykinin induced tPA release from endothelial cells
	ARBs	Neutral (losartan - Fogari et al. 2001a, Fogari et al. 2001b, Matsumoto et al. 2003, valsartan, irbesartan - Fogari et al. 2001b, eprosartan - Dielis et al. 2007, telmisartan - Remková et al. 2008) or even harmful (candesartan - Fogari et al. 2001b, losartan - Sakata et al. 2002, Erlinger et al. 2002, Koh et al. 2004)	Increased angiotensin IV (due to angiotensin II increase) and its effect on endothelial PAI-1 expression and increase of PAI-1 levels
Fibrinogen	ACE inhibitors	Neutral (perindopril - Remková and Kratochvířová 2000, Remková et al. 2008, enalapril - Li-Saw-Hee et al. 2001) or even beneficial (perindopril - Fogari et al. 1998)	Unknown mechanism, probably haemodilution due to vasodilation or decrease in blood cell rigidity



	ARBs	Neutral (losartan - Fogari et al. 1998, Li-Saw-Hee et al. 2001, telmisartan - Nagel et al. 2006) or even beneficial (irbesartan - Makris et al. 2000, telmisartan - Remková et al. 2008)	
Other sEPCR	ACE inhibitors	Neutral (Remková et al. 2008 perindopril)	
	ARBs	Beneficial (Remková et al. 2008 telmisartan)	Unknown mechanism (improved function of large arteries?)

ACE – angiotensin converting enzyme, ARBs – angiotensin II receptor blockers, PAI-1 – plasminogen activator inhibitor type 1, tPA – tissue type of plasminogen activator, sEPCR – soluble endothelial protein C receptor

### **Figure 1. Pathophysiologic effects of angiotensin II**

Legend: VSMC – vascular smooth muscle cells, NO – nitric oxide, PGI<sub>2</sub> – prostacyclin, PAI-1 – plasminogen activator inhibitor type 1, tPA – tissue type plasminogen activator

### **Figure 2. The effect of ACE inhibitors and angiotensin II (AT<sub>1</sub>) receptor blockers (ARB) on haemostasis**

Legend: ACE – angiotensin converting enzyme, Ang – angiotensin, PAI-1 – plasminogen activator inhibitor type 1, tPA – tissue plasminogen activator, NO – nitric oxide, PGI<sub>2</sub> – prostacyclin.

The accumulation of bradykinin due to ACE inhibition induces NO and PGI<sub>2</sub> release with antiplatelet effect and tPA release with increase of fibrinolysis. This mechanism is absent in ARBs. Inhibition of Ang II formation due to ACE inhibition decreases PAI-1 release. In contrary, ARBs through the increase of Ang IV stimulate PAI-1 release, followed by a decrease of fibrinolysis.

Figure 1

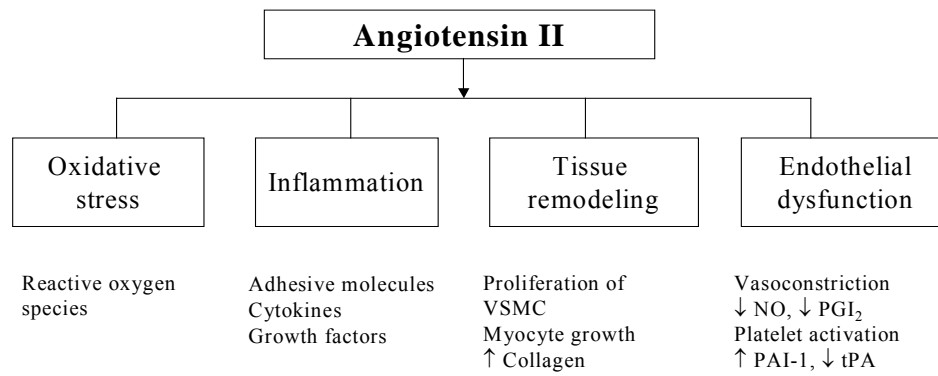


Figure 2.

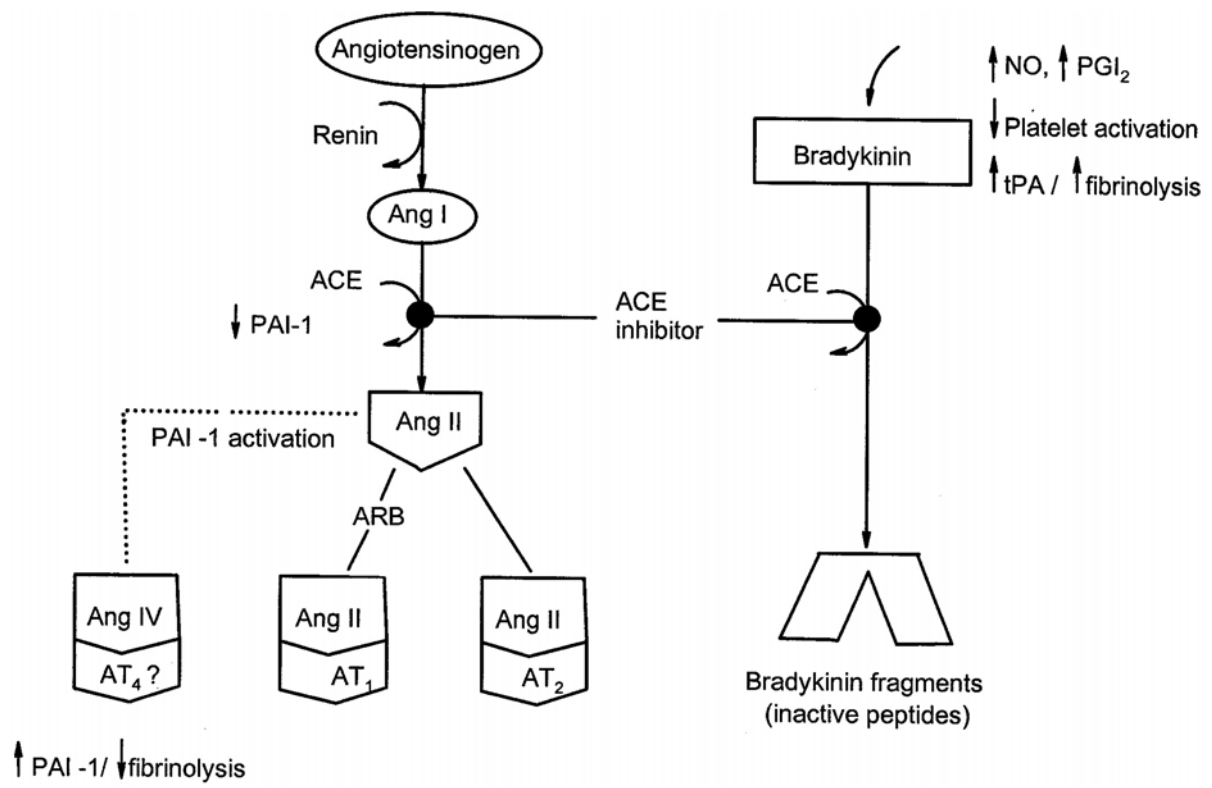


Fig. 2