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ANTITHROMBOTIC EFFECT OF *TISSUE* AND *PLASMA* TYPE ANGIOTENSIN CONVERTING ENZYME INHIBITORS IN EXPERIMENTAL THROMBOSIS IN RATS.

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This study compared the antithrombotic effect of *plasma* angiotensin converting enzyme inhibitors (ACE-Is): captopril (CAP), enalapril (ENA) and *tissue* ACE-Is: perindopril (PER), quinapril (QUIN) in experimental venous and arterial thrombosis. Normotensive Wistar rats were treated p.o. with CAP (75 mg/kg), ENA (20 mg/kg), PER (2 mg/kg) and QUIN (3 mg/kg) for 10 days. The influence of ACE-Is on coagulation and fibrinolytic systems as well as platelet function was evaluated. The hypotensive effect of ACE-Is was equal in all groups. QUIN maintained the final carotid blood flow at the highest value in comparison to PER and *plasma* ACE-Is. The arterial thrombus weight was reduced in PER and QUIN groups while venous thrombus weight was also reduced after CAP. *Tissue* and *plasma* ACE-Is caused the inhibition of platelet adhesion and aggregation. A reduction of fibrin generation, prolongation of prothrombin time (PT), activated partial thromboplastin time (APTT) and shortening of euglobulin clot lysis time (ECLT) were observed after PER and QUIN treatment. In conclusion, given in equipotent hypotensive doses, *tissue* ACE-Is exerted more pronounced antithrombotic effect than *plasma* ACE-Is in experimental thrombosis. The differences between *tissue* and *plasma* ACE-Is in terms of their more pronounced inhibition of experimental thrombosis may be related to the intensified activation of fibrinolysis and inhibition of coagulation.

Key words: *tissue ACE-Is, plasma ACE-Is, thrombosis, haemostasis, rat.*

INTRODUCTION

Randomized trials have shown that some of the ACE-Is when given in specific doses, increase survival in specific populations of patients with heart disease (1-4). In 2004 Pilote and coworkers (5) published the results of the retrospective head - to - head studies in which they showed that among 7 ACE-Is used, only ramipril and perindopril reduced 1-year mortality in patients after acute myocardial infarction. It is generally accepted, that inhibiting of *tissue* angiotensin converting enzyme (ACE) are an effective target for preventing premature death, myocardial infarction and stroke. Thus beneficial cardiovascular effects of some ACE-Is may result from inhibition of *tissue* ACE caused by their unique pharmacokinetic properties. It may be related to their chemical structure and bioavailability (functional group, dissociation constants from the enzyme, lipophilicity) (6-10). Therefore ACE-Is are now categorized into 2 groups depending on their relative affinity to *tissue* ACE (11). Higher - affinity ACE-I (*tissue* ACE-Is) consisted of quinapril, perindopril, benazepril, ramipril and lower - affinity ACE-Is (*non-tissue, plasma* ACE-Is) consisted of lisinopril, enalapril and captopril.

Since 90% of the enzyme is found locally as tissue-bound ACE, presumably *tissue* ACE-Is could improve more effectively the endothelium function (8, 9). Vascular endothelial cells constitute an important source of ACE where circulating or locally produced Ang I serves as available substrate for local production of Ang II (12). *Tissue* ACE-Is through their high affinity to endothelium significantly prevent the local synthesis of Ang II. Inhibition of ACE, being the kininase II, cause the subsequent increase of bradykinin level and mediated by BK₂ receptor release of nitric oxide (NO), prostacycline (PGI₂) and tissue plasminogen activator (t-PA) (13, 14).

In our previous study we observed NO and PGI₂ dependent antithrombotic effect of *plasma* ACE-Is: captopril and enalapril in thrombosis models in rats (15). Since *tissue* ACE-Is have higher affinity to endothelium we assumed that *tissue* ACE-Is could exert stronger antithrombotic effect than *plasma* ACE-Is. No clinical and experimental studies comparing the antithrombotic effect of *tissue* and *plasma* ACE-Is have been carried so far. Therefore the aim of our study has been to compare the antithrombotic effect of the *tissue* and *plasma* ACE-Is and the influence on haemostasis in the same experimental conditions.

MATERIAL AND METHODS

Chemicals

The following drugs were used in the study: captopril (Research Biochemicals International, USA), enalapril (KRKA, Slovenia), perindopril (Servier, Poland), quinapril (Pfizer, Poland), collagen (Chronolog, USA), trisodium citrate, calcium chloride, Tris Buffer (Polish Chemical

Reagents, Poland), Thrombin (Polfa, Poland) and gummi arabici (Polish Chemical Reagents, Poland).

Animals

Male, normotensive Wistar rats, weighing 300-400 g were used in our study. They were housed ten per cage in air-conditioned colony room at the natural light-dark cycle, with food and water continuously available. Procedures involving the animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research (16).

Experimental protocol

Animals received *per os* the following drugs for ten days. Group I - captopril: 75 mg/kg/day (twice daily) (CAP), Group II - enalapril: 20 mg/kg/day (ENA), Group III - perindopril: 2 mg/kg/day (PER), Group IV- quinapril: 3 mg/kg/day (QUIN). Group V - control (CON) rats received 5% aqueous gummi arabici solution (VEH). The last dose of drugs was given 12 hours (CAP) and 24 hours (ENA, PER and QUIN) before the experiments. The dosage of drugs and sampling time were chosen basing on the range of doses used in other (17, 18, 19) and our previous studies (15, 20, 21) as well as on the results of SBP measurement, in which ACE-Is exerted similar hypotensive effect. Experiments were done on the 11th day after starting this treatment. Twelve hours before experiments rats were deprived of food but had free access to water.

Blood pressure measurement

The systolic blood pressure (SBP) was measured in conscious rats, by the "tail cuff" method (Harvard Indirect Rat Tail Blood Pressure Monitor) before and after 10 days of treatment (22). Each value was the average of three consecutive readings.

Venous thrombosis induction

The venous thrombosis was induced as previously described by Reyers et al. (23). The rat abdomen was opened under pentobarbital anesthesia (40 mg/ kg, *i.p.*) and the vena cava was carefully separated from the surrounding tissues and then ligated tightly with a cotton thread just below the left renal vein. Subsequently, the abdomen was closed with a double layer of sutures (peritoneum with muscles and the skin separately). After 2 hours the animals were reanaesthetized, the abdomen was reopened; the vena cava was carefully dissected and inspected for the presence of a thrombus. The thrombus was air-dried at 37° C and after 24 hours its weight was measured.

Arterial thrombosis induction

Thrombosis was induced by an electric stimulation according to Schumacher, et al. as follows (24). Rats were anesthetized (pentobarbital, 40 mg/kg *i.p.*), fixed on an operation table and kept warm by radiant heat from a light bulb. The left common carotid artery was exposed at the minimum length of 15 mm and carefully freed from the surrounding tissue. A piece of parafilm "M" was placed under the exposed vessel for electrical isolation. A stainless steel L-shaped wire was placed on the dorsal surface of the artery (anode). The cathode was a subcutaneous metal clip attached to the hindlimb. The electrodes were connected into a circuit with a miliamperometer and potentiometer. A Doppler flow probe (1 mm-diameter) was connected to the exposed artery upstream from the electrode and connected to a blood flowmeter (Transonic System Inc.). After 5

min stabilization, the baseline blood flow was determined. Electrical current of 1 mA was delivered by a constant current stimulator for 10 minutes. To demonstrate evidence of thrombus formation, carotid blood flow (CBF) was continuously measured before, during and 45 minutes after electrical stimulation (*Fig. 1*). At the end of the experiment a segment of common carotid artery was removed, the thrombus was isolated, dried at 37° C for 24 hours and weighted. Immediately after removal of the thrombus the blood was collected from the heart for the measurement of haemostatic parameters.

Fibrin generation

For fibrin generation assay we used previously described method (25) modified and adapted to the use of laboratory animals (26). Blood samples were drawn immediately after arterial thrombus removal from carotid artery of animals treated for 10 days with ACE-Is or VEH. Blood was added to 3.13% trisodium citrate (9:1), then centrifuged at 2000 g in 4°C for 20 minutes and plasma was stored deep-frozen in aliquots of 1 ml at -70°C until further assays were performed. Fibrin time curve was made by adding CaCl₂ (36 mM) and thrombin (0.09 IU/ml) to the Tris buffer (66 mM Tris and 130 mM NaCl, pH=7.4) and mixing this with rat plasma. Optical density (OD) was measured via microplate reader (Dynex Tech., USA) at 1 minute intervals for 10 minutes. Based on the principle of integrals the area under the curve expressed by summation of OD values reflected the intensity of fibrin generation.

Haemostatic parameters

Prothrombin time (PT), and activated partial thromboplastin time (APTT) were automatically determined by optical method (Coag-A-mate XM; Organon Teknika, Belgium) adding routine laboratory reagents (Organon Teknika) to collected rat plasma. Euglobulin clot lysis time (ECLT) was evaluated according to Lidbury et al. (27).

Preparation of washed platelets

Blood samples were taken from the heart on anticoagulant (170mM trisodium citrate, 130mM citric acid and 101mM glucose) in volume ratio 9:1. Platelets washing was carried out as described previously (28). In brief, platelet rich plasma (PRP) was obtained by centrifugation of blood at 180g for 20 minutes at room temperature. PRP was then centrifuged at 400g for 15 minutes and obtained platelets were washed with calcium-free Tyrode's buffer (137mM NaCl, 2.6mM KCl, 12mM NaHCO₃, 0.9mM MgCl₂, 5.5mM glucose, 0.35% albumin, apyrase 0.5U/ml, pH 6.5) by a centrifugation at 400 g for 15 min. The washed platelets were finally suspended in a calcium - free Tyrod - Hepes buffer (137mM NaCl, 2.6mM KCl, 12mM NaHCO₃, 0.9mM MgCl₂, 5.5mM glucose, 0.35% albumin, pH 7,4) The final concentration of platelets was 3×10^5 platelets/ μ l.

Platelet adhesion to fibrillar collagen

Platelet adhesion was assayed according to Mant (29). The 250 μ l of washed platelet samples were incubated in an Elvi 840 aggregometer at 37°C and stirred at 900 rpm with EDTA (5mM) to prevent platelet aggregation. After 5 minutes preincubation, collagen (50 μ l/ml) was added and platelets were further incubated for 10 minutes. Samples of the suspension were obtained before and 15 minutes after adding the collagen, then platelets were counted in a haemocytometer after dilution with Unopette system. Index of adhering platelets was calculated using a formula [(platelet count

before adding the collagen - platelet count after adding the collagen)/platelet count before adding the collagen] \times 100%.

Platelet aggregation in the whole blood

Platelet aggregation in the whole blood was monitored by measuring electric impedance using a Chronolog aggregometer (Chrono Log, Havertown, PA) according to Cardinal et al. (30). 1 ml samples (0.5 ml of citrated blood and 0.5 ml of 0.9% NaCl) were left to equilibrate at 37°C for 5 minutes before the agonist (collagen 5 mg/ml) addition. The platelet aggregation was evaluated by measuring of the electric impedance (Ω) 5 minutes after the agonist addition.

Statistical analysis

The data are shown as mean \pm SEM. While calculating thrombus weight, the lack of the thrombus was marked as 0 mg (23). Incidence of occlusion in carotid artery was calculated by Fisher's exact test. Multiple group comparisons were performed by Kruskal-Wallis nonparametric ANOVA, followed by Dunn's multiple comparisons test. Values of $p < 0.05$ were considered significant.

RESULTS

Blood pressure

Plasma and *tissue* ACE-Is in the applied doses had similar hypotensive effect after 10 days of treatment. SBP decreased to 113 \pm 3 mmHg, 111 \pm 3 mmHg, 113 \pm 2 mmHg and 112 \pm 3 mmHg vs 127 \pm 2 mmHg in CON (n=25), for CAP (n=15), ENA (n=15), PER (n=15) and QUIN (n=15), respectively ($p < 0.001$).

Carotid Blood Flow

The mean Initial Carotid Blood Flow (I-CBF) was similar in CON and ACE-Is-treated groups (*Fig. 1, Tab. 1*). Electrical stimulation led to gradual flow fall caused by the increasing arterial thrombus formation in the lumen of carotid artery. Whereas CBF decreased to zero in most of the VEH treated rats, vascular occlusion was prevented by ACE-Is. Mean CBF over the duration of experiment was significantly higher in rats treated with QUIN in comparison to other groups of animals (*Fig. 1*). As shown in *Fig. 1* and *Tab. 1* the final flow was significantly higher in OUIN, PER and CAP treated groups than in CON. The incidence of occlusion was reduced only in OUIN treated group ($p < 0.05$), (*Tab. 1*)

Arterial and Venous thrombus weight

Administration of PER and QUIN caused marked decrease in arterial thrombus weight 0.17 \pm 0.05 mg; ($p < 0.01$) and 0.32 \pm 0.1 mg; ($p < 0.001$) vs CON (1.06 \pm 0.16 mg) respectively (*Fig. 2A*). CAP and ENA did not influence arterial thrombus weight.

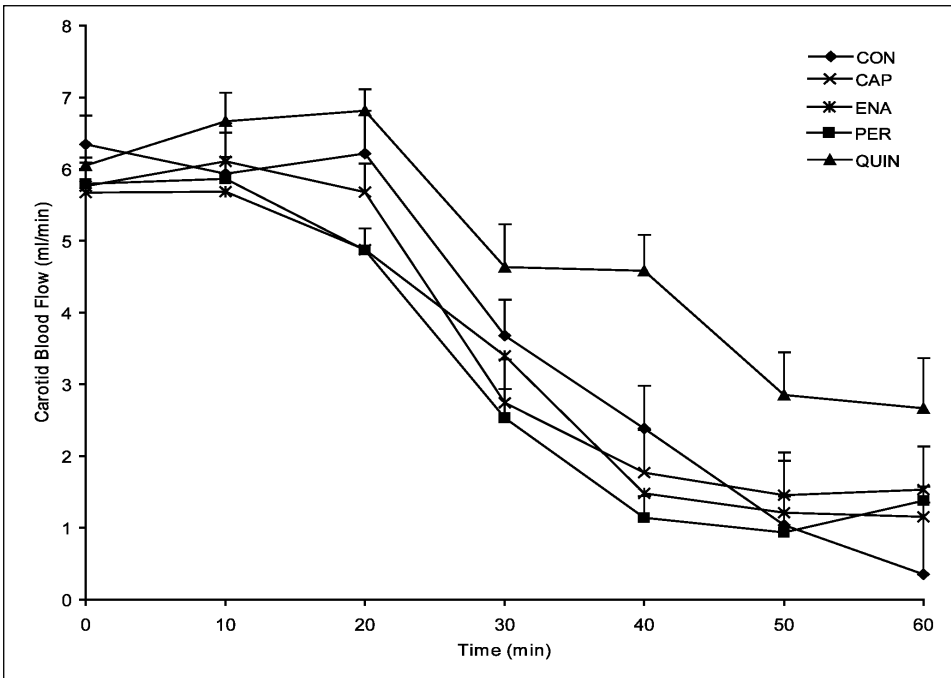


Fig. 1. Carotid blood flow (CBF) during the arterial thrombus formation.

CON (control, n=25) CAP (captopril, n=15), ENA (enalapril, n=15), PER (perindopril, n=15), QUIN (quinapril, n=15).

Tab. 1. Flow parameters in the carotid artery of rats during arterial thrombus formation

Groups	n	Initial blood flow (I-CBF) (ml/min)	Final blood flow (F-CBF) (ml/min)	Incidence of occlusion
CON	25	6.3±0.4	0.4±0.2***	21/ 25
CAP	15	5.8±0.6	1.6±0.8***##	12/ 15
ENA	15	5.6±0.7	1.1±1.0**	13/ 15
PER	15	5.7±0.3	1.3±0.5***##	12/ 15
QUIN	15	6.7±0.3	2.5±1.2**###	7 /15#

** p<0.01, *** p<0.001 vs initial flow,

#p<0.05, ##p<0.01, ###p<0.001 vs control.

In venous thrombosis model PER, QUIN and CAP significantly reduced the thrombus weight vs CON ($0.90\pm 0.2\text{mg}$, $0.54\pm 0.06\text{mg}$ and $1.20\pm 0.3\text{ mg}$ vs $2.25\pm 0.16\text{mg}$; $p<0.01$, $p<0.01$, $p<0.05$; respectively) (Fig. 2B).

When comparing the differences between *tissue* and *plasma* ACE-Is, in arterial thrombosis PER was more effective in reducing thrombus weight vs CAP ($p<0.05$) and ENA ($p<0.001$) while QUIN only vs ENA ($p<0.01$). Both PER and

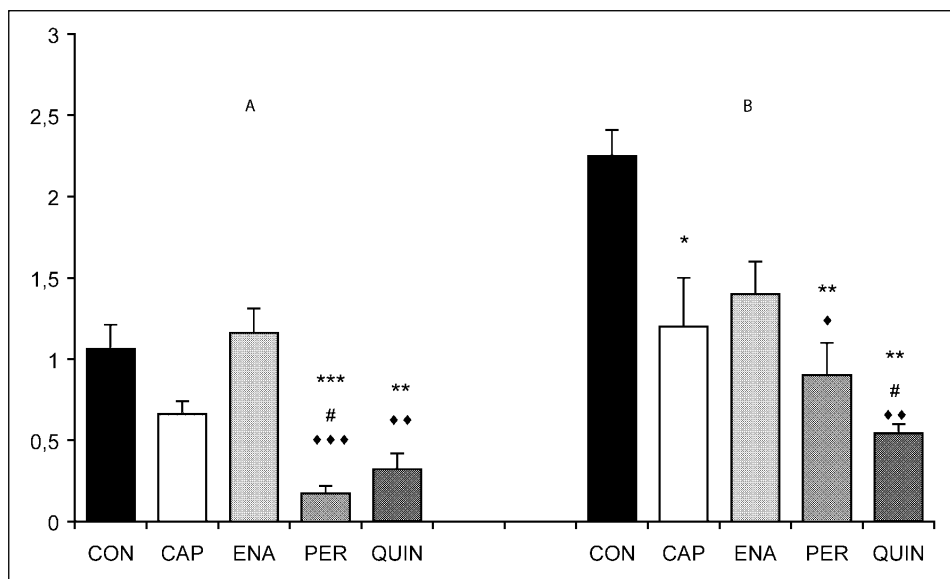


Fig. 2. The columns represent the thrombus weight in arterial thrombosis (A) and venous thrombosis (B) in rats treated with ACE-Is.

CON (control, n=25), CAP (captopril, n=15), ENA (enalapril, n=15), PER (perindopril, n=15), QUIN (quinapril, n=15). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs CON, # $p < 0.05$ vs CAP, ♦ $p < 0.05$, ♦♦ $p < 0.01$, ♦♦♦ $p < 0.001$ vs ENA.

QUIN reduced the thrombus weight stronger in comparison to ENA both in arterial ($p < 0.01$; $p < 0.01$) as well as venous thrombosis ($p < 0.01$; $p < 0.001$).

Fibrin Generation

ENA, PER and QUIN decreased the fibrin generation (Fig. 3) and the optical density value in the 10th minute was significantly lower in comparison to VEH treated animals (79 ± 5 , 63 ± 9 and 54 ± 5 vs 89 ± 4 in CON group; (ns, $p < 0.05$, $p < 0.001$) respectively. ENA failed to influence fibrin generation.

Haemostatic parameters

Neither CAP nor ENA changed PT and APTT (Tab. 2). QUIN and PER significantly prolonged PT ($p < 0.05$, $p < 0.01$) as well as APTT ($p < 0.01$) vs CON. CAP slightly shortened ECLT (Tab. 2). ENA had no influence on this parameter. QUIN ($p < 0.01$) and PER ($p < 0.01$) shortened ECLT in comparison to CON.

Platelet adhesion to fibrillar collagen

When the platelets were incubated with collagen, $34.7 \pm 0.8\%$ of the platelets adhered. Pretreatment with CAP, ENA, PER and QUIN resulted in a significant reduction of platelet adhesion (Tab. 2).

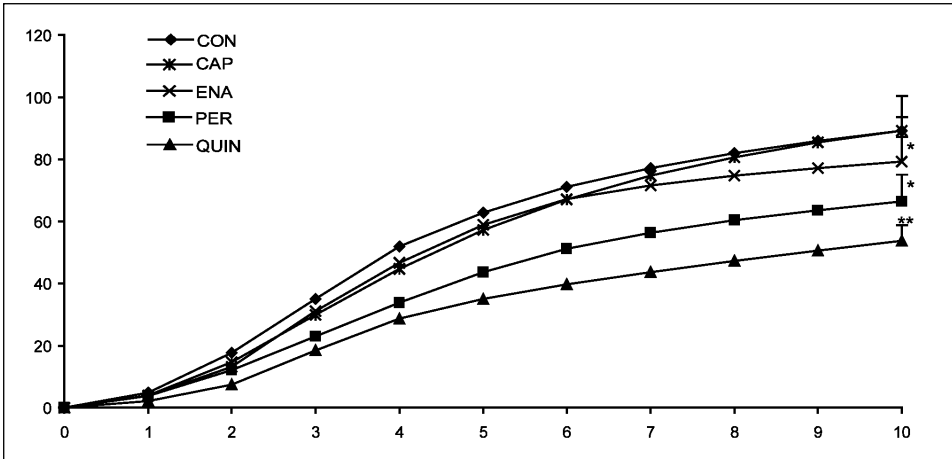


Fig. 3. The curves represent optical density (OD) changes during fibrin generation in plasma of rats treated with ACE-Is.

CON (control, n=15), CAP (captopril, n=8), ENA (enalapril, n=8), PER (perindopril, n=8), QUIN (quinapril, n=8). *p<0.05, **p<0.01 vs CON in 10th minute.

Tab. 2. Coagulation parameters, platelet adhesion to fibrillar collagen and whole blood platelet aggregation in rats with arterial thrombosis.

Groups	n	PT (s)	APTT (s)	ECLT (min)	Platelet aggregation (Ω)	Platelet adhesion (%)
CON	15	28.7±0.8	22.7±1.2	370±60	6.1± 1.0	34.7±0.8
CAP	8	29.8±1.5	21.7±0.6	250±30	5.2± 0.9	27.9±1.1**
ENA	8	27.9±1.2	23.8±0.9	320±25	4.7±0.7 *	27.2±0.5 **
PER	8	33.5±1.3**	29.8±1.3**	147±22**	4.3 ±0.8 *	29.3±1.9*
QUIN	8	31.8±1.1*	30.7±0.3**	190±14**	4.1 ±0.9 *	31.2±1.3*

*p<0.05, ** p<0.01 vs CON

Whole blood platelet aggregation

The collagen induced platelet aggregation was inhibited significantly after ENA (p<0.05), PER (p<0.05) and QUIN (p<0.05) treatment (Tab. 2), while CAP did not significantly affect the whole blood platelet aggregation.

DISCUSSION

In the present study we have presented that given in equipotent hypotensive doses, *tissue* ACE-Is (quinapril and perindopril) exerted more pronounced antithrombotic effect than *plasma* ACE-Is (captopril and enalapril) in

experimental thrombosis. This effect was related to the more distinct activation of fibrinolysis and, inhibition of coagulation by *tissue* ACE-Is.

We have applied here experimental model of stasis induced venous thrombosis that not only resembles pathological conditions of thrombus formation in human, but also separates the hemodynamic and haemostatic effects of the studied substances, due to the minor relationship between arterial and venous blood pressure. Moreover, in this model further activation of renin-angiotensin-system (RAS) occurs due to false information about hypovolemia as a result of the vena cava ligation (23), which enables studying the effects of various compounds affecting RAS. Using this model we have previously demonstrated that captopril and AT₁-antagonist (losartan) or Ang-(1-7) exert strong antithrombotic effect (15, 20, 21). The drugs preventing venous thrombosis interfere with coagulation and act on endothelium to increase naturally occurring fibrinolytic activity.

We found that QUIN and PER reduced venous thrombus weight to a greater extent than CAP and ENA. CAP showed also stronger antithrombotic effect than ENA on venous thrombus formation. We have previously shown that more distinct antithrombotic effect of CAP was related to the presence of sulfhydryl group in the molecule (31). Thus it should be emphasized the role of thiol group which is responsible for the stabilization and prolongation of NO half-life potentiating its profibrinolytic and antithrombotic effect. This observation is in good agreement with a study which demonstrated that pre-treatment of endothelial cells with N-acetylcysteine abolished the strain-induced PAI-1 release and activated fibrinolysis (32).

We also used platelet dependent arterial thrombosis model in which electric current is a factor, causing damage of endothelium and deeper layers of vessel wall. Changes in blood flow are a reliable marker of platelet-rich thrombus formation (33). Although platelets initiate arterial thrombosis the activation of blood coagulation and fibrinolysis is the next critical step (34). We observed significant reduction of arterial thrombus weight in rats treated with PER and QUIN. Others have also shown inhibition of arterial thrombotic process by quinapril, imidapril and captopril in rats (18, 19, 35, 36, 37).

In our study we have observed more pronounced effect of QUIN in maintenance of the carotid blood flow over the period of experiment in comparison to PER, even though similar effect of both drugs on thrombus weight occurred. It could be related to the differences between QUIN and PER in the affinity with ACE. In *in vitro* and experimental animals studies it was shown, that the potency against *plasma* as well as *tissue* ACE was greater for QUIN than for PER and *plasma* ACE-Is (6-8). Moreover, on the basis of the earlier data related to the dynamics of arterial thrombus formation we could suggest, that only drugs with relatively strong antithrombotic potency (thrombin inhibitors, aspirin) maintain CBF on the initial level and markedly reduce the thrombus weight (24). For drugs with weaker antithrombotic potency the strong stimulus such as

electrical current causing severe endothelial damages, leads finally to closing the lumen of the vessel by growing thrombotic material.

It is possible, that differences between *tissue* and *plasma* ACE-Is in terms of their more pronounced inhibition of tissue RAS and in consequence in stronger impact on thrombosis occurred. Since the positive correlation between arterial thrombus weight reduction and inhibition of ACE activity has already been shown (36) and QUIN was also more effective than ENA in ACE inhibition and reducing *tissue* Ang II in rat (38, 39), in the present study we have not measured the *tissue* ACE activity. We thought that at least two mechanisms may account for stronger antithrombotic effect of *tissue* ACE-Is. First, *tissue* ACE-Is through their high affinity to endothelium considerably prevent the local synthesis of Ang II. This may result in attenuation of prothrombotic action of this peptide. Ang II is well known to contribute to endothelial dysfunction by inducing oxidative stress (40), inhibiting NO synthesis (41) and enhancing leukocytes infiltration and adhesion to vascular wall (42). Few observations indicate that Ang II may activate the coagulation cascade by increasing tissue factor (TF) expression (43). Moreover, Ang II inhibits fibrinolysis by increasing plasminogen activator inhibitor type 1 (PAI-1) expression (44). What's more, we proved prothrombotic effect of this peptide in *in vivo* model of venous and arterial thrombosis in hypertensive rats (45, 46). Secondly, ACE-Is by increasing bradykinin concentration enhances the release of NO, PGI₂ and t-PA-strong antithrombotic agents (47, 48). The fact that the reduction of thrombus weight was more pronounced after QUIN and PER administration may be linked to their greater ability to release these autacoids from endothelium. It has recently been shown that *tissue* ACE-Is (quinapril and perindopril) caused experimental thrombolysis in rats to a greater extent than captopril by the mechanism which involved bradykinin release from endothelium (49).

ACE-Is are known to affect haemostasis at different levels. As was previously shown antithrombotic effect of ACE-Is may depend on the inhibition of platelet and erythrocyte aggregation (35, 37), elevation of NO release (50), reduction of serum and aortic ACE activity, reduction of aortic PAI-1 protein level (36), down regulation of glycoprotein IIb/IIIa complex on platelet surface (37) and attenuation of tissue factor expression (47). This clearly suggests that various mechanisms are involved in the antithrombotic effect of ACE-Is.

In the second part of our study we compared the impact of studied drugs on haemostasis with respect to their antithrombotic activity. Since the studies evaluating the effect of ACE-Is on clinical outcomes were done in patients with artery disease (1-4), we compared the influence of *tissue* and *plasma* ACE-Is on haemostasis in rats developing arterial thrombosis. We found superiority of PER and QUIN over CAP and ENA in fibrinolytic system activation in rats. Fibrinolysis is largely regulated by endothelial expression of t-PA and PAI-1 and ACE is the crucial mediator of the interaction between PAI-1 and t-PA (48). Strong profibrinolytic effect of ACE-Is - as a result of bradykinin-depend release

of PGI₂ and t-PA from endothelium in rats was also shown by others (49,51,52). Clinical studies have also shown profibrinolytic effect of *tissue* ACE-Is, but large comparative studies have not been done yet (53,54,55).

We have also showed that *tissue* ACE-Is inhibited coagulation cascade. Both QUIN and PER and to a lesser extent ENA inhibited fibrin generation, prolonged PT and APTT pointing to the impact on extrinsic and intrinsic pathways of coagulation cascade. It has been previously shown, that fibrin generation assay depends on plasma levels of various coagulation factors, like V, VII, VIII, IX, X or endogenous thrombin. Moreover, fibrin generation increases after adding exogenous TF or von Willebrand factor but decreases when exogenous t-PA is added, indicating that the method is also sensitive to agents affecting vascular wall (25). The expression of TF on endothelial cell membrane is critical for the initiation of extrinsic coagulation cascade and platelet activation after vessel injury. Therefore a possibility that ACE-Is could prevent thrombus formation through reduction of TF expression in endothelium cannot be excluded. It was shown that ENA reduced plasma level of TF in patients after myocardial infarction (56). We assume that stronger inhibitory effect of QUIN and PER on coagulation cascade may depend on NO and PGI₂ release from endothelium. These mediators markedly decreased expression of TF (47). On the other hand it was demonstrated that *tissue* ACE-I - ramipril did not change the coagulation parameters in rats, rabbits or dogs but reduced the level of thrombin - antithrombin (TAT) complexes in patients with arterial hypertension (57, 58).

The first step of a haemostatic plug formation is adhesion of circulating thrombocytes to the subendothelial matrix of damaged vessel wall. Adhered platelets recruit additional platelets within developing aggregate (59). Among the components of the wall collagen is considered to be the most important element involved in that process since it is a unique ligand for platelet adhesion causing also platelet activation (60). ACE-Is are believed to inhibit platelet activity (61), thus a question arises whether this action could contribute to the effective prevention of arterial thrombosis. Our results show similar antiadhesive and antiaggregative effects of all studied ACE-Is. This suggest that platelets inhibition is not responsible for the stronger antithrombotic effect of *tissue* ACE-Is in our experimental condition.

We have recently showed that *plasma* and *tissue* ACE-Is have comparable effects on coagulation and fibrinolysis in nonthrombogenic rats (62). In the present study, when endothelium denudation is followed by thrombin generation, platelet activation and impairment of fibrinolysis, the differences in the effect of *tissue* ACE-Is are clearly demonstrated. Also in clinical studies cardioprotective benefits of ACE-Is was particularly apparent in high-risk patients (63, 64) Thus our observation may have clinical significance since thrombotic disorders often accompany cardiovascular disease.

CONCLUSION

In conclusion, we have demonstrated the more pronounced antithrombotic effect of *tissue* ACE-Is (quinapril and perindopril) in comparison to *plasma* ACE-Is (captopril and enalapril) in normotensive rats both in venous and arterial thrombosis models. The stronger antithrombotic effect of *tissue* ACE-Is seems to be complex and involves the activation of fibrinolytic pathway and inhibition of coagulation system. The demonstration that ACE-Is do not influence haemostasis in the same way could have an important clinical implication.

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