JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1999, 50, 4, 499—507

Review article

W. BUCZKO, T. MATYS, I. KUCHAREWICZ, E. CHABIELSKA

THE ROLE OF ENDOTHELIUM IN ANTITHROMBOTIC EFFECT OF THE RENIN-ANGIOTENSIN SYSTEM BLOCKADE

Department of Pharmacodynamics, Medical University, 15-230 Białystok, Mickiewicza Str 2C, Poland

Drugs blocking the renin — angiotensin system, angiotensin converting enzyme inhibitors and AT_1 receptor antagonists, among many pharmacological effects may exert an antithrombotic action. The mechanisms, which mediate their antithrombotic activity are associated with enhanced nitric oxide and prostacyclin release or with attenuation of angiotensin II action (*Fig. 1, 2*). Nevertheless, endothelium plays an important role in this process linking the renin-angiotensin and fibrinolysis / coagulation systems.

Key words: *renin-angiotensin system, endothelium, thrombosis*

INTRODUCTION

Recently there has been a growing number of experimental and clinical studies pointing to the role of angiotensin II, the main substance of the renin — angiotensin system (RAS) in regulation of coagulation and fibrinolysis. It has been shown that an infusion of angiotensin II (Ang II) to healthy volunteers results in the increase of plasminogen activator inhibitor-1 (PAI-1) plasma level (1). It has also been demonstrated that Ang II regulates the expression of PAI-1 in cultured human endothelial cells (2). In other studies a significant increase in tissue factor activity following Ang II stimulation was observed (3), suggesting that not only fibrinolysis but also coagulation may be regulated by RAS. On the other hand, angiotensin converting enzyme inhibitors (ACE-Is) have been shown to limit infarct size and to prolong survival time after myocardial infarction in animals (4). These findings were confirmed in humans by the SAVE study, in which long — term administration of captopril to patients with left ventricular dysfunction after myocardial infarction reduced the incidence of recurrent coronary thrombosis (5).

All the above findings support the hypothesis that RAS blockade should be efficient in preventing thrombosis. Endothelium, being a site of synthesis of many active agents regulating fibrinolysis and coagulation, serves as a link between RAS and fibrinolytic / coagulation system.

RENIN — ANGIOTENSIN SYSTEM

RAS is one of the most important mechanisms regulating blood pressure and electrolyte / blood volume homeostasis (6). Renin, produced in the juxtaglomerular cells in the kidney, cleaves a decapeptide, angiotensin I from liver protein, angiotensinogen. The main active substance of this system is octapeptide Ang II, which is cleaved from angiotensin I by angiotensin — converting enzyme (ACE). Ang II acts on at least two subtypes of receptors called AT_1 and AT_2, exerting in many points opposing actions (7).

The breakdown of angiotensin II by angiotensinases leads to angiotensin III and IV production, which are still biologically active. The function and binding site of angiotensin III is not clearly defined, while angiotensin IV is known to act on AT_4 receptor, which is believed to be associated with PAI-1 release (8). Other fragment of Ang II, angiotensin — (1—7) causes vasodilatation and lowers blood pressure (9).

Nowadays it has been known that RAS is present not only in the circulation and that its components can be found at cellular level in kidneys, heart, brain or vessels. It is believed that local tissue systems are associated with long — term effects (e.g. hypertension development) while circulating system is related to short — term functions (e.g. water and sodium retention) (16). Although conversion of angiotensin I by ACE is the main way of Ang II synthesis, local tissue systems are able to generate Ang II from angiotensin I or directly from angiotensinogen via non — ACE — dependent pathways, i.e. by heart — specific chymase, CAGE (chymostatin — sensitive Ang II — generating enzyme), cathepsin G, tonin or tissue plasminogen activator (t-PA) (11).

ANTITHROMBOTIC EFFECT OF RAS BLOCKADE

Feasible mechanisms by which RAS blockade influences the process of thrombus formation are not fully defined, but interaction of NO and PGI_2 with fibrinolysis, platelet and leukocyte function as well as hypotensive effect of ACE-Is and AT_1 — receptor antagonists (AT_1-A) must be taken under consideration besides Ang II synthesis modification.

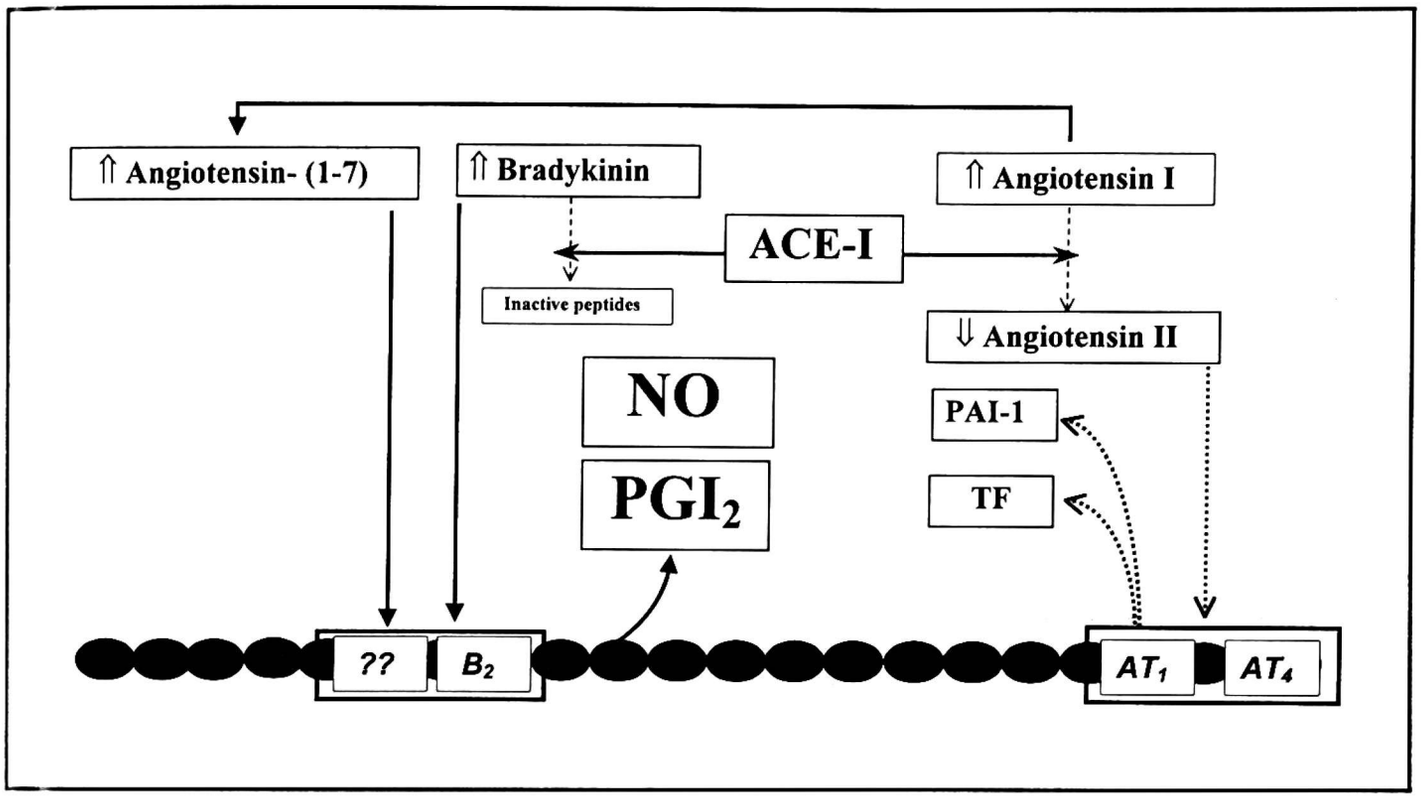

Fig. 1. Mechanism of antihrombotic action of ACE inhibitors

The role of nitric oxide and prostacyclin

Besides attenuating the formation of Ang II, ACE-Is inhibitors may exert part of their pharmacological effects by affecting the kallikrein — kinin system. It is widely known that ACE inhibition prevents the breakdown of bradykinin (12). In addition, ACE-Is probably interact with kinins at the receptor level, changing their affinity to B_2 receptor (13). Accumulation of bradykinin and related peptides results in increased nitric oxide (NO) and prostacyclin (PGI_2) formation mediated by B_2 receptor (14).

As far as AT_1- A are concerned, the contribution of PGI_2, NO and kinins to the pharmacological action of these drugs has been suggested (15). During AT_1 receptor blockade, Ang II binds solely to AT_2 receptors which results in enhanced NO synthesis and increased cGMP level (16). In addition, Ang (1—7), which level in conditions of AT_1 blockade is higher (17), exerts similar effect (9).

We have performed several studies to evaluate potential antithrombotic action of RAS blockade and the participation of NO and PGI_2 in this action. Both losartan and captopril administered chronically in arterial thrombosis prolonged the time of aortic loop occlusion (18), which evidences antithrombotic action of these drugs.

In venous thrombosis in normotensive rats, captopril administered in a chronic manner exerted a dose- dependent decrease in thrombus weight, while NO and/or PGI_2 synthesis blockade with L-NAME or indomethacin abolished the antithrombotic action of the drug (19). Similar effect was

observed in two kidney — one clip (2K1C) hypertensive rats [data not published].

In the same model losartan was ineffective in normotensive rats, however it decreased the thrombus weight in spontaneously hypertensive rats (20) and 2K1C hypertensive rats (21). In both studies the action of AT_1 receptor antagonist was attenuated after NO — synthase inhibition with L-NAME (20, 21) while PGI_2 synthesis blockade did not influence the antithrombotic action of losartan (20).

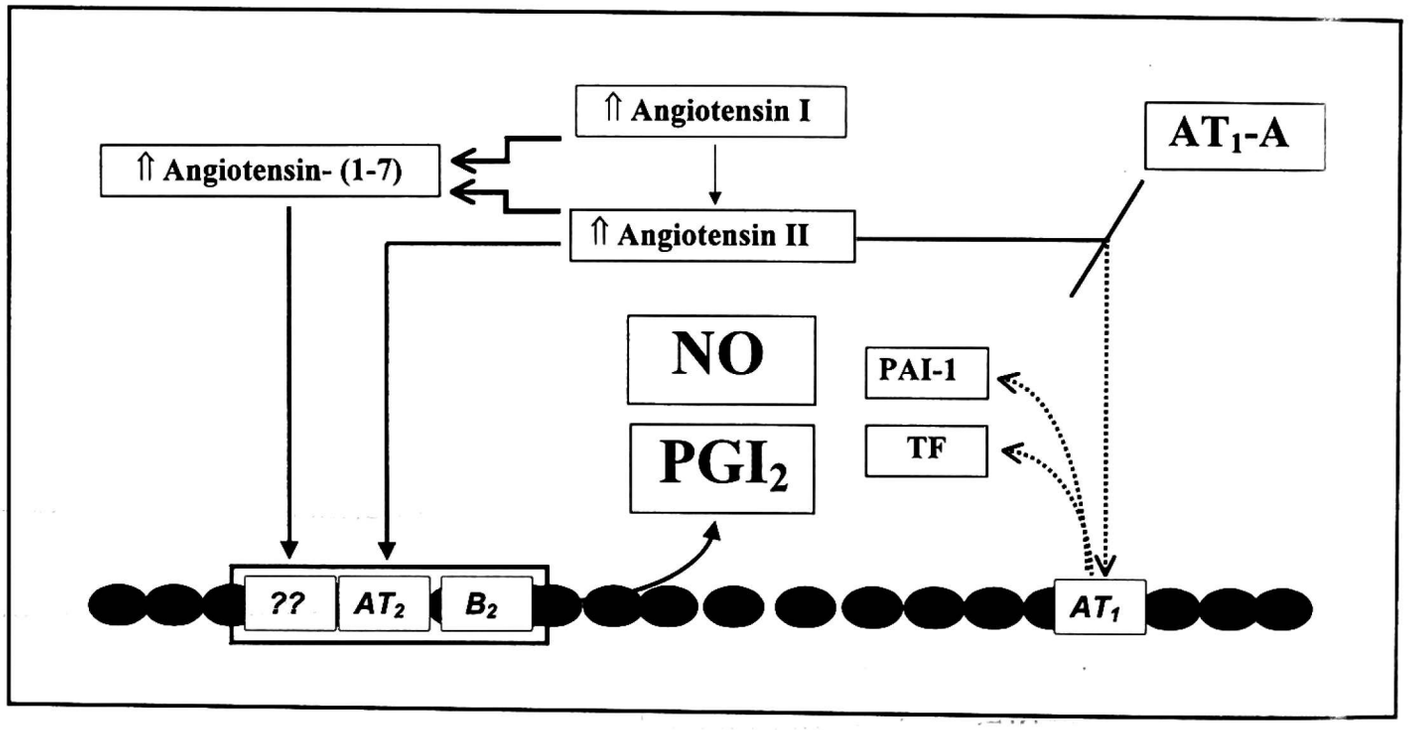

Fig. 2. Mechanism of antihrombotic action of AT_1 receptor antagonists

The role of fibrinolysis and coagulation

As mentioned above, Ang II stimulation results in PAI-1 (1, 2) and tissue factor release (3) from the endothelium, although some recent studies have shown paradoxically opposite effect (22). ACE inhibition was shown to be associated with lower PAI-1 and higher t-PA levels (23). It is unclear which angiotensin receptor is related to PAI-1 release. Some studies indicate that losartan decreases PAI-1 release, suggesting AT_1 receptor participation in this process (3). In other studies PAI-1 mRNA expression was attenuated by Ang IV receptor (AT_4) blockade, being influenced by neither AT_1 nor AT_2 receptor antagonists (8). Thus, AT_1 receptor antagonists treatment, condition known to increase Ang II and derivative peptides levels, may cause enhanced PAI-1 release.

Several studies show that t-PA release from the endothelium is associated with NO and PGI_2 production (24). Thus, it may be speculated that antithrombotic action of ACE inhibition may be in fact depending on NO, PGI_2 and t-PA release, which synergize in their profibrinolytic action (25).

In our studies with venous thrombosis either after treatment with captopril or losartan no significant changes in basic haemostatic parameters in the systemic blood were observed (20, 21, 26). In the blood collected from the site of thrombus formation we observed no changes in these parameters in rats treated with losartan (21). However, our recent studies have indicated that captopril administration to normotensive rats results in local activation of fibrinolysis and extrinsic coagulation pathway suppression in place of thrombus formation (27). Therefore, participation of fibrinolytic system in observed effects cannot be excluded.

The role of platelet and polymorphonuclear leukocyte function

ACE inhibition was found to be associated with inhibition of platelets aggregation (28), although some studies do not confirm these findings (29). Nevertheless, it is not fully understood whether this effect is related to enhanced endothelial mediators production or attenuated Ang II action.

As described previously, both ACE inhibition and AT_1 receptor antagonism lead to enhanced synthesis of PGI_2 and NO, which possess antiaggregatory properties and were found to act synergistically to inhibit platelet aggregation (30). NO inhibits platelet adhesion to collagen fibrills and endothelial cell matrix (31) while PGI_2 exerts only a weak inhibitory effect on platelet adhesion (32) and both mediators do not synergize in this action (30). NO inhibits the expression of platelet surface glycoproteins GPIIb/IIIa (33) which are crucial for platelet interactions with the vessel wall.

The involvement of Ang II in platelet function is not clearly determined. Angiotensin II does not influence platelet aggregation by itself but it was shown to enhance aggregation induced by other agents (34). AT_1 receptors are found on platelets and study with losartan indicates that it inhibits Ang II binding to its receptor on human platelets (35). It is worth mentioning that losartan was also found to be a weak antagonist of platelet thromboxane A_2 / prostaglandin H_2 receptors (36) and to inhibit thromboxane A_2 — induced platelet aggregation (37).

In our studies concerning venous thrombosis, captopril and enalapril administered in acute manner diminished platelet aggregation (38) while chronic treatment with ACE-Is did not change this parameter (19, 39). On the contrary, losartan inhibited platelet aggregation when given in chronic (39) but not acute manner (20, 21).

Acute losartan administration to 2K1C hypertensive rats resulted in 20% reduction of platelet adhesion to fibrillar collagen (21).

Surprisingly, antithrombotic effect of examined drugs occurred within groups in which platelet aggregation was unchanged. However, correlation of platelet function *in vitro* and thrombotic tendency *in vivo* remains controversial

(40). Besides, a growing body of evidence suggests important interactions of platelets with vessel wall and leukocytes adhering to endothelial cells, which relations cannot be examined in an aggregometer.

Polymorphonuclear leukocytes (PMNs) play a crucial role in destruction of endothelium and exposition of collagen fibres, being the first cells to adhere to endothelium in reduced flow conditions (41). The next step in thrombus formation is interaction of activated platelets with PMNs, vessel wall and other platelets, resulting in a formation of a haemostatic plug. It has been demonstrated that NO is able to modulate PMNs function and expression of adhesion molecules, mediating interaction of PMNs with endothelial cells (42). In addition, NO decreases the expression of platelet surface P-selectin which mediates adhesion of activated platelets to neutrophils and monocytes and supports neutrophils adhesion under shear stress conditions (43). Prostacyclin mimetics have also been demonstrated to inhibit neutrophil function (44). Therefore, feasible mechanisms of antithrombotic effect of RAS blockade may include also the above actions, however this hypothesis demands further investigation.

The role of sulfhydryl group in antithrombotic action of some ACE-Is

Several ACE-Is (captopril, alacepril, fentiapril, zofenopril) contain a sulfhydryl group (-SH), which seems to modulate their pharmacological action. It is known that substances possessing -SH group are able to bind NO and prolong its half-life in plasma (45). S-nitrosothiols, formed this way, possess antithrombotic activity related to the spontaneous release of NO from the moiety (46) and may act as free radical scavengers. These actions have been demonstrated also for captopril (47, 48). The importance of this fact is related to the finding, that reactive oxygen species, which can be scavenged by sulfhydryl agents, modulate PAI—1 release from endothelium (49) and may condition coagulation activation, thus promoting thrombosis (50).

Our previous studies (19, 38) have shown superiority of captopril over enalapril, ACE-I devoid of -SH group, in preventing venous thrombosis development. To evaluate the role of -SH group in this effect we have compared captopril with two other sulfhydryl — containing agents: epicaptopril (SQ14534), an isomer of captopril virtually devoid of ACE-inhibitory properties, and N-acetylcysteine (27). All three substances exerted similar antithrombotic effect accompanied by decreased euglobulin — clot lysis time and, with exception of N- acetylcysteine, by a prolongation of prothrombin time (PT) in the blood obtained from the site of thrombus formation. Thus, captopril seems to have a dual mechanism of antithrombotic action sharing the advantages of RAS blocker and -SH group containing agent.

The role of hypotension

One may suggest that the antithrombotic activity of ACE-Is and AT_1 -- A is a result of blood pressure reduction. Since L-NAME when administered alone enhanced blood pressure in our experiments but did not affect venous thrombus formation, the contribution of the hypotensive component should be excluded.

REFERENCES

1. Ridker PM, Gaboury CL, Conlin PR, Seely EW, Williams GH, Vaughan DE. Stimulation of plasminogen activator inhibitor *in vivo* by infusion of angiotensin II. Evidence of a potential interaction between the renin-angiotensin system and fibrinolytic function. *Circulation* 1993; 87: 1969—1973.
2. Vaughan DE, Lazos SA, Tong K. Angiotensin II regulates the expression of plasminogen activator inhibitor-1 in cultured endothelial cells. A potential link between the renin-angiotensin system and thrombosis. *J Clin Invest* 1995; 95: 995—1001.
3. Nishimura H, Tsuji H, Masuda H *et al.* Angiotensin II increases plasminogen activator inhibitor-1 and tissue factor mRNA expression without changing that of tissue type plasminogen activator or tissue factor pathway inhibitor in cultured rat aortic endothelial cells. *Thromb Haemost* 1997; 77: 1189—95.
4. Ertl G, Kloner RA, Alexander RW, Braunwald E. Limitation of experimental infarct size by an angiotensin-converting enzyme inhibitor. *Circulation* 1982; 65: 40—48.
5. Pfeffer MA, Braunwald E, Moýe LA *et al.*. on behalf of the SAVE Investigators. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the survival and ventricular enlargement trial. *N Engl J Med* 1992; 327: 669—677.
6. Griendling KK, Murphy TJ, Alexander WA. Mollecular biology of the renin — angiotensin system. *Circulation* 1993; 87: 1816—1828.
7. Timmermans PBMW, Wong PC, Chiu AT. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 1993; 45: 205—251.
8. Kerins DM, Hao Q, Vaughan DE. Angiotensin induction of PAI—1 expression in endothelial cells is mediated by the hexapeptide angiotensin IV. *J Clin Invest* 1995; 96: 2515—2520.
9. Brosnihan KB, Li P, Ferrario CM. Angiotensin — (1—7) dilates canine coronary arteries through kinins and nitric oxide. *Hypertension* 1996; 27: 523 — 528.
10. Huber K, Pachinger O, Pichler M, Klein W. Pathophysiologische Mechanismen des Renin — Angiotensin — Systems und deren pharmakologische Beeinflusung durch ACE — Hemmer oder Angiotensin — II — (Typ-1-) Rezeptorblocker bei kardiovaskulären Erkrankungen. *Z Kardiol* 1997; 86: 239—250.
11. Urata H, Nishimura H, Ganten D. Mechanisms of angiotensin II formation in humans. *Eur Heart J* 1995; 16: 79—85.
12. Kramer HJ, Glanzer K, Meyer-Lehnert H, Mohaupt M, Predel HG. Kinin- and non-kinin-mediated interactions of converting enzyme inhibitors with vasoactive hormones. *J Cardiovasc Pharmacol* 1990; 15: S91—S98.
13. Hecker M, Bara AT, Busse R. Potentiation of the biological efficacy of bradykinin by ACE inhibitors: a shift in the affinity of the B_2 receptor ? *Immunopharmacology* 1996; 33: 93—94.
14. Wiemer G, Schölkens BA, Becker RHA, Busse R. Ramiprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium — derived bradykinin. *Hypertension* 1991; 18: 558—563.

15. Cachofeiro V, Maeso R, Rodrigo E, Navarro J, Ruilope LM, Lahera V. Nitric oxide and prostaglandins in the prolonged effects of losartan and ramipril in hypertension. *Hypertension*, 1995; 26 : 236 — 243.
16. Gohlke P, Pees C, Unger TH. Stimulation of AT_2 receptors increases aortic cyclic GMP levels in SHRSP by kinin — dependent mechanism. *Hypertension* 1998; 31: 349—355.
17. Campbell DJ, Menard J, Azizi M, Gonzales MF. Synergistic interaction between angiotensin — converting enzyme inhibition and angiotensin II antagonism in spontaneously hypertensive rats. *J Hypertens* 1997; 15 (Suppl 4): S63.
18. Chabielska E, Pawlak R, Golatowski J, Buczko W. The antithrombotic effect of captopril and losartan on experimental arterial thrombosis in rats. *J Physiol Pharm* 1998; 49 : 251—260
19. Pawlak R, Chabielska E, Golatowski J, Azzadin A, Buczko W. Nitric oxide and prostacyclin are involved in antithrombotic activity of captopril in venous thrombosis in rats. *Thromb Haemost* 1998; 79: 1208—1212
20. Chabielska E, Pawlak R, Golatowski J, Ró łkowski R, Pawlak D, Buczko W. Losartan inhibits experimental venous thrombosis in spontaneously hypertensive rats. *Thromb Res* 1998; 90 : 217—278.
21. Chabielska E, Pawlak R, Wollny T, Ró łkowski R, Buczko W. Antithrombotic activity of losartan in two kidney, one clip hypertensive rats. A study on the mechanism of action. *J Physiol Pharm* 1999; 50: 99—109.
22. Larsson PT, Schwieler JH, Wallen NH, Hjemdahl P. Acute effects of angiotensin II on fibrinolysis in healthy volunteers. *Blood Coagul Fibrin* 1999; 10: 19—24.
23. Wright RA, Flapan AD, Alberti KGM, Ludlam CA, Fox KAA. Effects of captopril therapy on endogenous fibrinolysis in men with recent, uncomplicated myocardial infarction. *J Am Coll Cardiol* 1994; 24: 67—73.
24. Lidbury PS, Thiemermann Ch, Korbut R, Vane JR. Endothelins release tissue plasminogen activator and prostanoids. *Eur J Pharmacol* 1990; 186: 205—212.
25. Gryglewski RJ : Interactions between endothelial mediators. *Pharm Toxicol* 1995; 77: 1—9.
26. Pawlak R, Chabielska E, Ró łkowski R, Buczko W. Influence of captopril on some haemostatic parameters in rats with ongoing process of venous thrombosis. *Acta Physiol Hung* 1996; 84: 327—328.
27. Pawlak R, Chabielska E, Matys T, Kucharewicz I, Ró łkowski R, Buczko W. The role of thiol repletion for venous thrombosis: relation to the antithrombotic action of captopril [Second European Congress of Pharmacology, Budapest 1999].
28. Someya N, Morotomi Y, Kodama K *et al.* Suppresive effect of captopril on platelet aggregation in essential hypertension. *J Cardiovasc Pharmacol* 1984; 6: 840 — 843.
29. Gow IF, Flapan AD, Morris M *et al.* A lack of effect of captopril on platelet aggregation in patients with congestive heart failure. *Eur J Clin Pharmacol* 1991; 41: 47—49.
30. Radomski MW, Palmer RMJ, Mocada S. The anti-aggregating properties of vascular endothelium interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987; 92: 639 — 646.
31. Radomski MW, Palmer RMJ, Mocada S. The role of nitric oxide and cGMP in platelet adhesion to vascular endothelium. *Biochem Biophys Res Commun* 1987; 148: 1482—1489.
32. Higgs EA, Moncada S, Vane JR, Caen JP, Michel H, Tobelem G. Effect of prostacyclin (PGI_2) on platelet adhesion to rabbit arterial subendothelium. *Prostaglandins* 1978; 16: 17—22.
33. Michelson AD, Benoit SE, Furman MI *et al.* Effects of nitric oxide/EDRF on platelet surface glycoproteins. *Am J Physiol* 1996; 270: H1640 — H1648.
34. Ding YA, Mac Intyre DE, Kenyon CJ. Potentiation of adrenaline induced platelet aggregation by angiotensin II. *Thromb Haemost* 1985; 54: 717—720.

35. Burnier M, Centeno G, Grouzmann E, Walker P, Waeber B, Brunner R. *In vitro* effects of DuP 753, a nonpeptide angiotensin II receptor antagonist, on human platelets and rat vascular smooth muscle cells. *Am J Hypertens* 1991; 4: 438—443.
36. Liu EC, Hedberg A, Goldenberg HJ, Harris DN, Weed ML. DuP753, the selective angiotensin II receptor blocker, is a competitive antagonist to human platelet thromboxane A_2 prostaglandin H_2 (TP) receptors. *Prostaglandins* 1992; 44: 89—99.
37. Li P, Ferrario CM, Brosnihan KB. Losartan inhibits thromboxane A_2- induced platelet aggregation and vascular constriction in spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 1998; 32: 198—205.
38. Pawlak R, Chabielska E, Buczko W. Antithrombotic effect of enalapril, an angiotensin-converting enzyme inhibitor, on venous thrombosis in rats. *Pol J Pharmacol* 1996; 48: 117—120
39. Chabielska E, Pawlak R, Buczko W. Effects of drugs affecting the renin-angiotensin system on venous thrombosis in normotensive rats. *Pol J Pharmacol* 1996; 48: 89—91.
40. Hornstra G, Kalafusz R, Wierts JW., Kester ADM. Does platelet function *in vitro* reflect thrombosis tendency *in vivo ?Thromb Haemost* 1991; 65: 1085.
41. Schaub RG, Simmons CA, Koets MH, Romano PJ, Steward GJ. Early events in the formation of a venous thrombus following local trauma and stasis. *Lab Invest* 1984; 51: 218—224.
42. De Caterina R, Libby P, Peng HB *et al.* Nitric oxide decreases cytokine — induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995; 96: 60—68.
43. Yeo EL, Sheppard J-AI, Feuerstein IA. Role of P-selectin and leukocyte activation in polymorphonuclear cell adhesion to surface adherent activated platelets under physiological shear stress conditions (an injury vessel wall model). *Blood* 1996; 88: 146—157.
44. Wise H. The inhibitory effect of non-prostanoid prostacyclin mimetics on rat neutrophil function. *Prostaglandins Leukot Essent Fatty Acids* 1996; 54 : 351—360.
45. Ignarro LJ, Lippton H, Edwards JC et al. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitropruside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. *J Pharmacol Exp Therap* 1981; 218: 739—749.
46. Mendelsohn ME, O'Neill S, George D, Loscalzo J. Inhibition of fibrinogen binding to human platelets by S-nitroso-N-acetylcysteine. *J Biol Chem* 1990; 265: 19028—19034.
47. Goldschmidt JE, Tallarida RJ. Pharmacological evidence that captopril possesses an endothelium-mediated component of vasodilation: effect of sulfhydryl groups on endothelium — derived relaxing factor. *J Pharmacol Exp Therap* 1991; 257: 1136—1145.
48. Bagchi D, Prasad R, Das DK. Direct scavenging of free radicals by captopril, an angiotensin converting enzyme inhibitor. *Biochem Biophys Res Com* 1988; 158: 52 — 57.
49. Cheng JJ, Chao YJ, Wung BS, Wang DL. Cyclic strain-induced plasminogen activator inhibitor—1 (PAI—1) release from endothelial cells involves reactive oxygen species. *Biochem Biophys Res Commun* 1996; 225: 100—105.
50. Jennings PE, McLaren M, Scott NA, Saniabadi AR, Belch JJ. The relationship of oxidative stress to thrombotic tendency in type 1 diabetic patients with retinopathy. *Diabet Med* 1991; 8: 860—865.

Received: July 21, 1999
Accepted: September 21, 1999

Author's address: W. Buczko, Department of Pharmacodynamics Medical University 15-230 Białystok, Mickiewicza Str 2C, Poland