Review article

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

THE ROLE OF ENDOTHELUM 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 AT₁ 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 decapetide, 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 AT₁ and AT₂, 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 AT₄ 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 PGI₂ with fibrinolysis, platelet and leukocyte function as well as hypotensive effect of ACE-Is and AT₁ — receptor antagonists (AT₁-A) must be taken under consideration besides Ang II synthesis modification.
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\textsubscript{2} receptor (13). Accumulation of bradykinin and related peptides results in increased nitric oxide (NO) and prostacyclin (PGI\textsubscript{2}) formation mediated by B\textsubscript{2} receptor (14).

As far as AT\textsubscript{1} - A are concerned, the contribution of PGI\textsubscript{2}, NO and kinins to the pharmacological action of these drugs has been suggested (15). During AT\textsubscript{1} receptor blockade, Ang II binds solely to AT\textsubscript{2} receptors which results in enhanced NO synthesis and increased cGMP level (16). In addition, Ang (1—7), which level in conditions of AT\textsubscript{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\textsubscript{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\textsubscript{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).

![Diagram of the mechanism of antithrombotic action of AT\(_1\) receptor antagonists](image)

**Fig. 2.** Mechanism of antithrombotic 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 GPIb/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
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 sulphydryl group in antithrombotic action of some ACE-Is

Several ACE-Is (captopril, alacepril, fentiapril, zofenopril) contain a sulphydryl 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 sulphydryl 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 sulphydryl — 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-I's and AT\textsubscript{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


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