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THE ANTITHROMBOTIC EFFECT OF CAPTOPRIL AND LOSARTAN ON EXPERIMENTAL ARTERIAL THROMBOSIS IN RATS

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The mechanism by which ACE-Is (angiotensin converting enzyme inhibitors) reduces the rate of coronary thrombosis among patients with left ventricular dysfunction is not known. A potential interaction between the renin-angiotensin system (RAS) and the thrombotic process has been suggested. The goal of the present study was to evaluate the antithrombotic action of drugs which block the RAS by different mechanisms; captopril (50 mg/kg p.o.) — the angiotensin converting enzyme inhibitor and losartan (30 mg/kg p.o.) — the selective AT_1 receptor antagonist. The normotensive rats were treated in acute or chronic manner (7 days) and then the arterial thrombosis was induced by insertion of a loop-shaped cannula into the abdominal aorta. The occlusion time (the period during which the loop was totally occluded by thrombus) was significantly prolonged in comparison with the control groups after chronic treatment with captopril (by 46%; $p < 0.01$) and losartan (by 42%; $p < 0.05$). Our results provide experimental evidence that the drugs blocking RAS exert an antithrombotic effect in the arterial thrombosis model in rats. This effect was independent from changes in blood pressure and primary hemostasis.

K e y   w o r d s: *captopril, losartan, animal thrombosis model, primary hemostasis.*

INTRODUCTION

It was more than 10 years ago that the first data indicating the beneficial effect of captopril on the cardiac function in rats with acute myocardial infarction appeared (1). Recent clinical trials indicate that the long-term administration of ACE-Is (angiotensin converting enzyme inhibitors) reduces the incidence of recurrent infarct by over 20% in patients with left ventricular dysfunction after myocardial infarction (2). The mechanism of this newly recognised effect of ACE inhibition is not completely understood.

However, a potential link between RAS and thrombosis was suggested on the basis of the observation that an infusion of angiotensin II (Ang II) to normotensive and hypertensive volunteers results in an increase in

plasminogen activator inhibitor -1 (PAI-1) plasma level (3). It may be the result of Ang II-induced overproduction and the release of PAI-1 and PAI-2 from endothelial cells and smooth muscle cells (4). Recently, a significant increase in tissue factor activity after Ang II stimulation has also been demonstrated (5). These results suggest that Ang II reduces the antithrombotic properties of the endothelial cells, which increases the risk of thrombosis. Thus, inhibition of the renin-angiotensin system may be beneficial in preventing thrombus formation. Indeed, it was demonstrated that captopril promotes fibrinolysis, which can be detected as suppression of PAI-1 activity (6) or the decrease in tissue plasminogen activator (t-PA) antigen levels (7). It has also been reported that ACE-Is may elevate bradykinin concentration, with a subsequent increase in the production of prostacyclin, nitric oxide (NO), and t-PA, which can prevent thrombus formation (8).

Losartan is the first of a new class of selective and competitive AT_1 receptor antagonists (9) with established indications in hypertension (10) and is, at present, under evaluation for the treatment of heart failure (11). However, data concerning its influence on blood coagulation and fibrinolysis are very limited. Although the antihypertensive action of losartan is attributed mainly to AT_1 receptor blockade, the contribution of NO and prostaglandins in this action was also suggested (12), which may indicate the suppressive effect of the drug on the thrombotic process.

We have recently reported that captopril, enalapril and losartan exerted an antithrombotic effect in experimental venous thrombosis in rats (13, 14). Therefore, in the present study we have decided to evaluate the possible antithrombotic action of captopril and losartan in the „chronic" model of arterial thrombosis in rats.

MATERIAL AND METHODS

Animals

Experiments were conducted on male Wistar rats (270—300 g), maintained under controlled light and temperature conditions. The rats were fed a normal rat chow and had free access to tap water.

Drug dosing

In acute experiments, captopril (50 mg/kg) or losartan (30 mg/kg) were administered p.o. 2 hours before loop insertion. In chronic experiments, the drugs (the same dose regimen) were administered once daily p.o. for 7 days. On the 8 th day, the aortic loop was inserted 2 hours after the last drug administration. The treatment was continued throughout the following days until total loop occlusion in both acute and chronic treatment.

Arterial thrombosis

Arterial thrombosis was induced according to the Hornstra method (15). The rats were anaesthetised with pentobarbital (40 mg/kg, i.p.). Then, the abdomen was surgically opened on the median line. The section of the aorta between the renal arteries and the common iliac arteries was detached from the surrounding tissue. The section between the spermatic and iliolumbar arteries was also carefully cleaned. The aorta was clamped above the spermatic arteries and below the iliolumbar arteries. A loop-shaped polythene cannula (1 mm internal diameter, 2 mm external diameter, Portex, Hythe, Kent, England) was inserted into this section of the abdominal aorta. The aorta loop was fixed by two ligatures, the clamps were removed and the blood flow was restored *via* the loop. The muscles and the skin were sutured allowing the loop to protrude out of the abdominal wall. The authors believe that the loop insertion, by damaging the vascular wall and by changing the blood flow, triggered thrombus formation at the site of the vascular damage. When an occluding thrombus was formed, the colour of the loop changed from light-red *via* dark-red to blue or black. The time between insertion and complete obstruction of the loop, called „occlusion time" (OT), was a measure of arterial thrombosis development. The loop was inspected twice daily (every 12 ± 3 h).

Blood pressure measurement

Systolic blood pressure (SBP) was monitored indirectly in conscious rats before the operation, by the „tail cuff" method (16), using a Harvard Indirect Rat Tail Blood Pressure Monitor, and was calculated as the average of at least three consistent determinations of systolic pressure.

„Template" bleeding time

Bleeding time was measured before the operation, according to Dejana *et al.* (17). The rats were placed in a plastic cylinder and the standardised device was applied longitudinally on the dorsal part of the tail between 6 and 9 cm from the tip, taking care to avoid the large veins. Immediately after injury, the tail was placed into the cylinder with isotonic saline at 37° C. Bleeding time was measured from the moment the tail was surgically cut until bleeding stopped completely (no rebleeding within 30 sec). Bleeding time was expressed in seconds.

Platelet count

Platelets were counted by a phase contrast microscope after dilution of the blood by the Unopette system (Becton — Dickinson, New Jersey, USA).

Drugs

Captopril (RBI, USA), Losartan (DuP 753, kindly provided by DuPont Merck Pharmaceutical Co., USA) and pentobarbital (Vetbutal, Polfa, Poland) were used in the experiments.

Statistics

Multiple group comparisons were performed by one-way Analysis of Variance (ANOVA), and when significant intergroup differences were assessed by a Dunnet's test. A value of $p < 0.05$ was considered statistically significant. The data are shown as mean $\pm$ SEM.

RESULTS

Occlusion time of the aortic loop

Captopril treatment: The occlusion time (OT) of the loop in the control animals was 63.6 ± 4 h. Chronic administration of captopril induced a significant prolongation of the occlusion time to 92.0 ± 6.8 h, p < 0.01, while acute captopril administration did not significantly prolong OT (63.6 ± 4 h vs 74.9 ± 5.4 h) (*Table 1*). In the control rats, the occlusion time of the loop ranged between 2—4 days. (*Fig. 1*) shows the percentage of occluded loops in the course of the experiment, in the control animals and in those treated with captopril. In the control group, 100% of loop occlusion was reached after 4 days. On the 3rd day, 58.3% of the loops were occluded in the control animals, whereas only 40% of the loops were completely occluded after captopril acute treatment. All the loops in the chronically treated animals appeared to be free of occlusive thrombus at that time.

Table 1. Occlusion time in rats treated with 50 mg/kg of captopril in acute and chronic manner. Means $\pm$ SEM, ** p < 0.01.

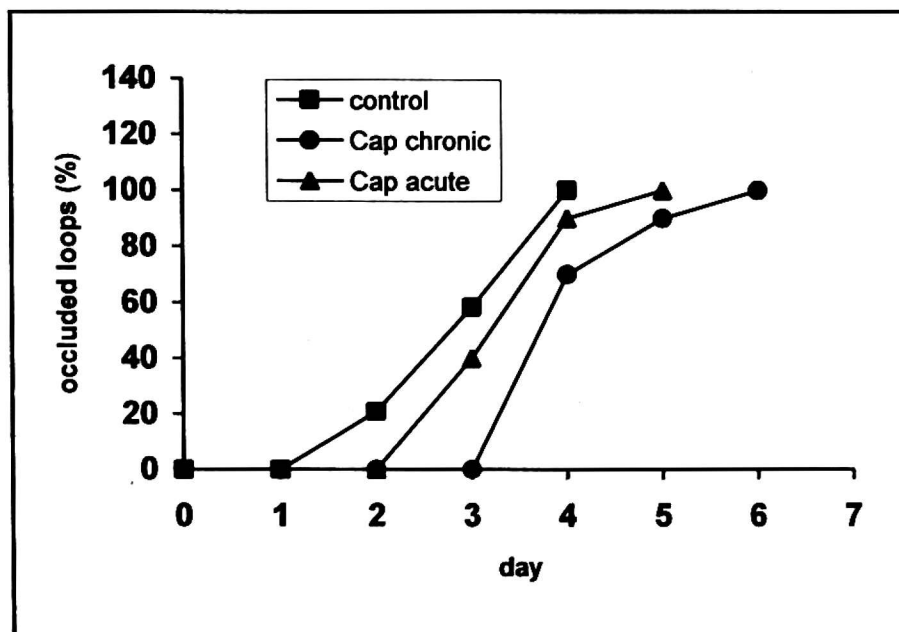| Group	n	OT (h)
Control	23	63.6 ± 4.0
Captopril acute	10	74.9 ± 5.4
Captopril chronic	10	92.0 ± 6.8 **

Fig. 1. Incidence of occluded loops after captopril treatment

Losartan treatment: The average occlusion time (OT) of the loops in the control animals was 56.7 ± 3.4 h. Acute losartan administration (30mg/kg) did not influence the time of thrombus formation (67.8 ± 6.1 h, not significant) (*Table 2*). Chronic administration of losartan induced a significant prolongation of occlusion time by 42% (to 80.6 ± 10.7 h; $p < 0.05$). (*Fig. 2*) shows the incidence of occluded loops presented as a percentage of occlusions in the controls and the animals treated with losartan. On the 2nd day, 42% of loops were occluded in the control group, but only 27% in the chronically treated animals. In the acute experiment, all the loops were free of occlusive thrombi at that time. In the control group, 100% of loop occlusion was reached after 4 days. At the same time, 87.5% and 72.7% of the loops were occluded after acute and chronic losartan administration, respectively.

Table 2. Occlusion time in rats treated with 30 mg/kg of losartan in acute and chronic manner. Means $\pm$ SEM, $^*p < 0.05$.

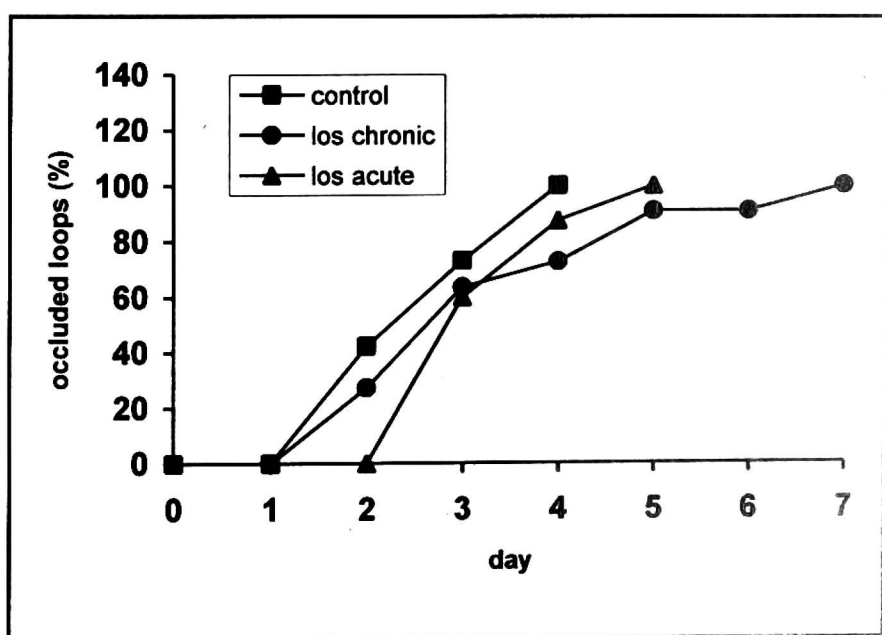| Group	n	OT (h)
Control	26	56.7 ± 3.4
Losartan acute	10	67.8 ± 6.1
Losartan chronic	11	$80.6 \pm 10.7\,^*$

Fig. 2. Incidence of occluded loops after losartan treatment

Blood pressure

Acute captopril administration caused a significant reduction in systolic blood pressure (101 ± 3 mmHg vs 124 ± 2 mm Hg in control; $p < 0.01$), while its chronic treatment did not change this parameter (*Table 3*). Acute losartan treatment did not reduce SBP, although such a tendency could be observed

after its chronic administration (108 ± 11 mmHg vs 124 ± 2 mmHg in control; not significant) (*Table 3*).

Table 3. The influence of acute and chronic treatment with captopril (50 mg/kg) and losartan (30 mg/kg) on systolic blood pressure, "template" bleeding time and platelet number. Mean ± SEM, *p < 0.05.

Group	SBP (mm Hg)	BT (sec)	Platelet number (10^9/l)
Control	124 ± 2	114 ± 12	940 ± 20
Captopril acute	101 ± 3 *	121 ± 13	842 ± 19
Captopril chronic	118 ± 4	120 ± 18	998 ± 17
Losartan acute	113 ± 9	111 ± 16	1001 ± 25
Losartan chronic	108 ± 11	120 ± 15	958 ± 12

Bleeding time

Bleeding time was not influenced by captopril and losartan in either the acute or the chronic experiments (*Table 3*).

Platelet number

Captopril and losartan in both schedules of treatment did not significantly change the platelet number (*Table 3*).

DISCUSSION

In the present study, we have demonstrated the antithrombotic properties of captopril and losartan in the model of arterial thrombosis in rats. Chronic treatment with these drugs was associated with a significant prolongation of the occlusion time of the aorta loops, indicating a tendency of decreased arterial thrombosis. We have failed to demonstrate a similar effect after acute administration of captopril or losartan, although the tendency to prolong the occlusion time (OT) was still observed.

In the present study, we used the well characterised model of arterial thrombosis in rats, which allowed observation of the thrombus formation during quite a long period (a few days), reflecting the process occurring in human arteries. Because of the endothelial damage at both ends of the aorta-loop and disturbed flow conditions the thrombi gradually increased in size. About 30% of animals exhibited an occluded thrombus on the second day after loop insertion and 100% of prosthesis were occluded on the 4th or 5th

day. As we have shown before (18), activated platelets spread on the internal surface of the cannula one minute after its insertion into the aorta, and 24 hours later numerous platelet aggregates supported by the fibrin network could be observed. The model is also substantially dependent on fibrinolytic potential, since the systemic inhibition of PAI-1 expression significantly prolonged the time of loop occlusion (19). Therefore, we can conclude that the platelet activation and aggregation as well as blood coagulation and fibrinolysis are critical steps in this model, as in human diseases.

In the present study, the changes in the platelet numbers were not registered. Our earlier studies demonstrated the lack of any influence of chronic captopril and losartan administration on platelet aggregation (13, 20). It may therefore be speculated that in the present experiments the inhibition of platelet aggregation is not responsible for the antithrombotic action of captopril and losartan. On the other hand, an aspirin with its well documented antiaggregating potential did not prolong the occlusion time in our model (21). It should be taken into consideration that the potential changes in the platelet function at the place of thrombus formation may be not reflected in the systemic circulation. Similarly, the previous report by Hornstra *et al.* (22) showed the lack of relationship between *in vivo* and *in vitro* measurements of platelet function in animal studies.

Since captopril and losartan are used as antihypertensive drugs, one might consider that their antithrombotic activity is a result of the reduction in blood pressure. However, at the time of arterial thrombosis induction, we did not observe any differences in SBP between the control animals and the rats treated chronically with captopril or losartan. Moreover, in the only group where captopril — administered acutely — significantly reduced SBP, it failed to significantly prolong the occlusion time. Therefore, the contribution of a hypotensive effect in the antithrombotic effect of the tested drugs should be excluded.

The antithrombotic treatment is often connected with the bleeding tendency that manifests itself by a prolonged bleeding time (23), suggesting an altered primary hemostasis. The drugs such as HMW heparin (24) and ticlopidine (25) which demonstrated an antithrombotic effect in the model used here also prolonged the bleeding time. The fact that the bleeding times were not prolonged in our study indicates that primary hemostasis was not involved in the antithrombotic mechanism of captopril and losartan.

In conclusion, we can say that captopril and losartan, after chronic administration, showed a significant antithrombotic effect on arterial thrombosis in rats. This effect is comparable to that shown by antiplatelet (25) and anticoagulant (26) drugs in the same model. The antithrombotic effect was connected neither with the hypotensive action of captopril and losartan nor with their influence on primary hemostasis. It is noteworthy that the

antithrombotic activity of both drugs is more prominent during the first two days after loop insertion and could involve an acute activity on the process of thrombus formation.

Taking these observations into account, it is difficult to suggest now the exact mechanism of the antithrombotic action of captopril and losartan. It is documented that captopril and losartan are able to stimulate NO synthesis (8, 27). It is also well known that vascular NO strongly inhibits platelet adhesion and therefore is thought to be an important determinant of the arterial thrombosis tendency *in vivo* (28). Thus, the antithrombotic action of these drugs can be NO-dependent, which was previously demonstrated for captopril (29), and losartan (30) in the venous thrombosis model. The fact that the delay in time of the thrombus formation was more pronounced after captopril administration (which contains the sulfhydryl group in the structure) may be connected with its property to potentiate NO effects by prolonging its half-life in plasma (31). The NO-donors are known to exhibit fibrinolytic activity through the inhibition of PAI-1 release from platelets (32). There is a growing amount of evidence proving the profibrinolytic properties of both studied drugs (6—8). Therefore, the role of fibrinolytic processes in the antithrombotic activity of captopril and losartan should also be taken into consideration. Finally, the local generation of prostacyclin may be amplified by captopril (33) and losartan (34) and may play a role in the defence against prothrombotic stimuli. The explanation of the precise antithrombotic mechanism(s) of the drugs blocking the renin-angiotensin system should be further investigated.

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