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Short communication

Antithrombotic effect of captopril and enalapril in old rats

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Abstract:
In the present study, we have shown considerably accelerated thrombosis in old rats in comparison with adult rats, which may be related to the impaired hemostatic balance in these animals. In old rats, captopril and enalapril caused a marked reduction of venous thrombus weight. The mechanism of antithrombotic action of these drugs seems to be dependent on the suppression of coagulation cascade and the enhancement of the fibrinolytic processes.

Key words:
aging, angiotensin converting enzyme inhibitors, thrombosis, hemostasis, rat

Introduction

Clinical and experimental data point to the role of renin-angiotensin system in the development of thrombotic disease and suggest that angiotensin-converting enzyme inhibitors (ACE-Is) may prevent thrombus formation [6, 9]. In our previous study, we have demonstrated that captopril (CAP) and enalapril (ENA), when given at equipotent dose, exerted antithrombotic effect in a venous thrombosis model in adult rats and young rats [1, 7] *via* endothelium-dependent mechanism. What is more, the antithrombotic potency of ACE-Is was significantly stronger in young rats (2 months) than in adult rats.

It is generally accepted that the incidence of thrombotic complications increases with age. Aging is associated with endothelium dysfunction mainly represented by an increased production of reactive oxygen radicals [3]. An increase in superoxide O_2^- occurs concurrent to the decrease in nitric oxide (NO) bioavailability. Dysfunction of endothelium has been proved to strongly influence blood coagulation and fibrinolysis leading to hypercoagulation [5]. Since ACE-Is are widely used in elderly patients, we decided to examine if ACE-Is preserve their antithrombotic effect in old rats.

Materials and Methods

Animals

Male Wistar rats (670–950 g, 18–20 months old) were used in the study. The control values were also measured in adult rats (300–350 g, 5 months of age).

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 Use of Animals in Biomedical Research (Thromb Haemost, 1987, 58, 1078–1084).

Venous thrombosis model

Old rats received CAP (25 mg/kg twice daily; n = 10, RBI, USA), ENA (15 mg/kg, n = 11, KRKA, Slovenia,), VEH (distilled water, n = 10) or 18% ethyl alcohol (n = 10) in the same volume and route for 10 days, *per os*. Doses of CAP and ENA were equipotent in inhibiting angiotensin converting enzyme. On the 11th day venous thrombosis was induced by ligation of the vena cava [8] following pentobarbital anesthesia (45 mg/kg *ip*). After two hours, the animals were re-anesthetized, the abdomen was re-opened, the vena cava was carefully dissected and the thrombus was collected, dried at 37°C for 24 h and weighed.

Plasma coagulation and fibrinolytic activity

Plasma coagulation and fibrinolytic activity was assayed by the method of He et al. [4] with our modification [2]. Blood was obtained from the heart. The collection started 2 h after the venous thrombosis induction. Blood was collected to disposable syringe containing 3.13% sodium citrate. The ratio of blood to sodium citrate was 9:1 v/v. Blood was centrifuged in plastic test-tubes for 20 min at 2500 rpm, and at the temperature of 4°C to obtain the plasma. Briefly, two fibrin time curves, changing during clot formation, were made by the registration of optical density (OD) *via* microplate reader (Dynex Tech., USA). To make the first one, determining overall coagulation potential (OCP), $CaCl_2$ (36 mM) and thrombin (0.09 IU/ml) were added to the Tris buffer (66 mM Tris and 130 mM NaCl, pH = 7) and mixed with plasma sample. OCP was calculated by summation of the OD values recorded from the time of noticeable OD increase until 15 min. From the second fibrin time curve, created by adding t-PA (1500 ng/ml) to the above-described mixture, clot lysis time (CLT) was calculated. CLT was the time of the curve fall from the maximum to the minimum OD value passing the plateau.

Data analysis

The data were expressed as the mean ± SEM. To test whether the mean of the TW, OCP and CLT differs between two groups, two-tail, Mann-Whitney test was used. The p values less than 0.05 were considered significant.

Results and Disscusion

The value of overall coagulation potential was significantly higher in old rats in comparison to adult rats, which was accompanied by the tendency to prolongation of clot lysis time (Tab. 1). These animals were more liable to develop thrombosis to a significantly greater extent than adult animals what was demonstrated by 100% thrombus formation frequency and 3-fold increase in thrombus weight (Tab.1). Thus, we confirmed previous observations that thrombotic complications increased with age what might be related to impaired hemostatic balance in old individuals [5].

In old rats, CAP and ENA caused a marked reduction of thrombus weight (2.5-fold decrease in relation to its original weight) while the incidence of venous thrombosis was not affected by both drugs. The anti-

Tab. 1. Effects of ACE-Is on thrombus formation and hemostasis in old rats

	TW [mg]	TF [%]	OCP [%]	CLT [min]
		ADULT RATS		
VEH (dist. water)	1.20 ± 0.40	98	788 ± 34	5.0 ± 0.5
		OLD RATS		
VEH CAP (dist. water)	3.72 ± 0.31^	100	938 ± 58^	6.0 ± 0.3
CAP	1.47 ± 0.32*	100	390 ± 130**	3.8 ± 0.7*
VEH ENA (18% alcohol)	3.50 ± 0.50	100	920 ± 40	6.1 ± 0.4
ENA	1.40 ± 0.30*	100	645 ± 93*	3.8 ± 0.6*

The thrombus weight (TW), thrombosis frequency (TF), overall coagulation potential (OCP) and clot lysis time (CLT) in rats treated with captopril (CAP, 2 x 25 mg/kg *po*, n = 10), enalapril (ENA, 15 mg/kg *po*, n = 11) or their vehicle (VEH, the same volume and route, n = 10, distilled water for CAP and 18% ethyl alcohol for ENA). Data are expressed as the mean ± SEM. * $p < 0.05$, ** $p < 0.01$ *vs.* VEH; ^ $p < 0.05$ *vs.* adult rats

thrombotic effect of CAP and ENA groups was accompanied by the significant reduction of plasma coagulability and activation of fibrinolytic system (Tab. 1). It is interesting to note that the antithrombotic effect of ACE-Is in old rats was less pronounced than in young and adult rats in which 8–16-fold decrease in thrombus weight and 20–50% reduction of thrombotic frequency occured [1, 7]. The present data suggest that the mechanism of antithrombotic action of ACE-Is in old rats is likely to be complex and dependent on the suppression of coagulation cascade and the enhancement of the fibrinolytic processes.

In conclusion, we assume that the weaker antithrombotic effect of ACE-Is may be dependent first on hypercoagulability and second, on the impaired fibrinolytic activity in old rats. According to our previous data [1, 7], we do not also exclude the possibility that the age-related endothelium dysfunction is, at least partially, responsible for less pronounced antithrombotic effect of ACE-Is in old animals. Thus, our observations raise the question about the cardiovascular benefit of ACE-I in elderly patients. Further investigation is required to explain a precise mechanism of the antithrombotic effect of ACE-Is in old rats.

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