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Antithrombotic effect of eplerenone - selective mineralocorticoid receptor antagonist - in arterial thrombosis model in diabetic rats

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Introduction

There is mounting evidence for a link between aldosterone and hemostasis process. We have recently demonstrated prothrombotic effect of aldosterone in experimental venous thrombosis in rats. The EPHESUS trial has shown an effectiveness of eplerenone (EPL), a new selective mineralocorticoid receptor antagonist, in reduction of cardiovascular events (acute myocardial infarction, stroke) in patients with congestive heart failure following acute myocardial infarction, mostly pronounced in diabetic patients. Thus, we hypothesize that particular benefits observed after EPL treatment depend on its influence on acute intravascular thrombosis. So far there are no data confirming the effect of EPL on arterial thrombosis *in vivo*.

Aim

To study the antithrombotic effect of EPL in arterial thrombosis in streptozotocin-induced diabetic rats.

Materials and methods

Male Wistar rats (250-350 g; n= 10-17) were used. Diabetes was induced with a single injection of streptozotocin (STZ; 65 mg/kg; *i.p.*). STZ rats were treated with eplerenone (EPL, 100 mg/kg per day by gavage) or vehiculum (VEH) for 10 days. Arterial thrombosis was induced by electrical stimulation of the common carotid artery on the 11th day. One hour later, the presence of a thrombus and its weight was checked. Carotid blood flow was monitored continuously during the study using a Doppler flow probe (1 mm-diameter, Hugo Sachs Elektronik – Harvard Apparatus GmbH, Germany). The thrombus was collected together with a fragment of the vessel wall and stained with hematoxylin and eosin and next subjected to von Willebrand factor (vWF) immunohistochemistry. Washed platelet adhesion to fibrillar collagen ex vivo, levels of tissue factor (TF) and plasminogen activator inhibitor type-1 (PAI-1) as well as thrombin time (TT) were measured. Plasma aldosterone level was measured by radioimmunoassay.

Results

STZ rats showed amplified thrombus formation manifested by higher incidence of artery occlusion and increased thrombus mass. The thrombus showed highly organized fibrin structure with densely packed platelets and large numbers of leukocytes. Hypertrophy of the tunica media of the carotid artery, numerous vacuoles within the myocytes and lack of expression of vWF in the endothelium were observed. Hypercoagulable state was manifested by an increase in the platelet adhesion, TF and PAI-1 plasma levels as well as shortening of TT.

After EPL treatment the decrease in the incidence of occlusion in the artery and a marked decrease in the thrombus mass were observed. The thrombus in the EPL-treated rats showed weakly organized fibrin structure with low amount of leukocytes. Inhibition of hypertrophy of the tunica media and the moderate expression of vWF in the carotid artery wall were observed after EPL treatment. Inhibition of platelet adhesion, prolongation of TT and a decrease in TF and PAI-1 plasma levels were observed. EPL did not change the glucose and aldosterone levels.

Conclusion

Our study demonstrates a novel and important effect of EPL on the arterial thrombotic process in diabetic rats. Antithrombotic effect of EPL was associated with changing in the thrombus structure, improvement of the endothelial cells structure and function, inhibition of primary hemostasis, suppression of coagulation and enhancement of fibrinolysis. The potential importance of pleiotropic effects of EPL should be evaluate in further clinical studies.