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Impact of aliskiren on some hemostatic parameters in experimental arterial thrombosis in rats

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ABSTRACT

Background: Aliskiren is the first orally active inhibitor of renin to be approved for clinical use as an antihypertensive agent. A number of studies show a link between aliskiren and intravascular thrombosis.
Materials and methods: The goal of the present study was to investigate the impact of aliskiren on arterial thrombosis in normotensive and renovascular hypertensive rats. The contribution of each coagulation and fibrinolytic parameters in the mode of aliskiren action was determined. Six weeks after clipping of the left renal artery rats developed hypertension which was confirmed by the "tail cuff" method. Animals were treated with aliskiren (10, 30 and 100 mg/kg/day) *per os* for 10 days. Arterial thrombosis was induced by electrical stimulation of the common carotid artery.
Results: It was found that aliskiren in a dose-dependent manner decreased weight of the arterial thrombus in normotensive and hypertensive rats. It has been shown that this result was not associated with the effects on blood pressure, TF, PT, APTT, fibrinogen and hematological parameters. It was found that aliskiren caused increase of t-PA activity and decrease of its inhibitor activity.
Conclusions: The presented results indicate that aliskiren inhibits hemostasis in the arterial thrombosis in rats. The antithrombotic effect is related with improvement of the fibrinolytic balance, and also depends on antiplatelet action.

Introduction

Myocardial infarction and ischemic stroke belong to the most serious cardiovascular diseases. The main pathogenic factor responsible for those urgent clinical events is intravascular arterial thrombosis. The most effective drugs which reduce the risk of death with the cardiovascular causes are angiotensin converting enzyme inhibitors (ACE-Is) and AT_1 receptor antagonists (ARBs) [1–3]. Our previous experiments provided evidence for the antithrombotic effect of ACE-Is and ARBs in arterial thrombosis

Abbreviations: 2K-1C, two-kidney one-clip; ACE-Is, angiotensin converting enzyme inhibitors; AL, aliskiren; Ang II, angiotensin II; ANP, atrial natriuretic peptide; APTT, activated partial thromboplastin time; ARBs, AT_1 receptor antagonists; ASA, aspirin; Fg, fibrinogen; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; HCT, hematocrit; HGB, hemoglobin; ICAM-1, intercellular adhesion molecules-1; L-NAME, NG-nitro-L-arginine methyl ester; MAP, mean arterial pressure; MCV, mean corpuscular volume; NADPH, oxidase nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PAI-1, plasminogen activator inhibitor; PRA, plasma renin activity; PGI_2, prostacyclin; PLT, platelet; PT, prothrombin time; RAS, renin–angiotensin system; RBC, red blood cell count; SBP, systolic blood pressure; TF, tissue factor; TNF (alpha), tumor necrosis factor alpha; t-PA, tissue plasminogen activator; TT, thrombin time; WBC, white blood cell count; VCAM-1, vascular cell adhesion molecule-1; VEH, vehicle; vWF, Von Willebrand factor.

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model [4]. Their high therapeutic efficacy is associated with a number of pleiotropic effects, such as anticoagulant activity, fibrinolytic, antiplatelet action and endothelial functions [1–3]. The discovery of aliskiren (AL), which is the first renin inhibitor active after oral administration, made it possible to more optimally blockade of the renin–angiotensin system (RAS) and a more effectively inhibit the production of angiotensin II (Ang II) [5,6]. Treatment with aliskiren decreases plasma renin activity (PRA). It is known that high PRA level is associated with the risk of cardiovascular events in patients with hypertension, as well as in those with normal blood pressure [7–9]. AL lowers blood pressure, decreases albuminuria, cardiac hypertrophy, significantly reduces the thickness of the left ventricular wall and increases the survival of treated rats as compared to untreated [10]. It is also responsible for reduction in biomarkers of heart failure (ANP) [11] and improvement of post-MI systolic and diastolic function of the left ventricle [12]. Additionally it inhibits ischemia-induced signaling pathways associated with inflammation and reduces the number of apoptosis biomarkers [13]. An indication that they studied aliskiren action independent of renin inhibition since this drug showed about 130-fold lower specificity for rat renin in comparison with human. AL reduces cardiac hypertrophy, cardiac remodeling and prevents the extension of the left ventricle, at a dose not affecting the blood pressure [12].

Recent reports indicate that aliskiren may inhibit thrombosis, a major complication caused by multiple vascular pathologies [14–17]. Indeed, it decreases the level of Ang II suggesting that this drug may inhibit the arterial thrombotic process. It has been also shown that AL causes a significant reduction of cardiac fibrosis, macrophage infiltration, remodeling and improves coronary endothelial function [13]. Moreover aliskiren enhances nitric oxide (NO) bioavailability [17] among other, due to increase cardiac bradykinin level. Those factors play a key role in endothelial thromboresistance. It has been also documented that AL down-regulates tissue factor expression in HUVECs and decreases human plasminogen activator inhibitor (PAI-1) antigen and activity [16].

The aim of the present work was to estimate the antithrombotic effects of aliskiren on arterial thrombus formation and on hemostatic parameters such as platelet activation, coagulation and fibrinolysis. Hypotensive response to aliskiren and its effect on blood cell count were also examined.

Materials and methods

Animals

Animals were purchased from and housed in the Centre of Experimental Medicine of Medical University of Bialystok according to Good Laboratory Practice rules. Male Wistar-Crl:WI(Han) rats were used in the experiment. Animals were housed in a room and maintained on a 12 h light/dark cycle, in group cages as appropriate and allowed to have access to tap water and a standard rat chow.

Ethics

All the procedures involving animals and their care were approved by a local bioethics committee and conducted in accordance with the institutional guidelines that are in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research [18].

Chemicals and drugs

Aliskiren tablets were kindly provided by Novartis (Warszawa, Poland). Routine laboratory reagents to determine prothrombin time, activated partial thromboplastin time, and Fg levels *in vitro* in rat plasma were purchased from HemosIL, Instrumentation Laboratory (Lexington, MA, USA). Rat tissue factor kit was bought from MyBioSource (USA), rat plasminogen activator inhibitor-1 activity kit was bought from Hyphen, BioMed (France), whereas rat tissue plasminogen activator activity kit was bought from Innovative Research Inc. (USA). Reagents for prothrombin time, activated partial thromboplastin time and fibrinogen level measurement were bought from HemosIL, Instrumentation Laboratory (USA). Collagen (Chrono-log, USA), Tris buffer [Tris(hydroxymethyl)-aminomethane] (Merck, Germany), pentobarbital (Vetbutal, Biovet, Poland) trisodium citrate (Polish Chemicals, Poland) and ready-to-use kits for blood cell count were also used in the study.

Induction of renovascular hypertension

Rats (80–120 g) were anesthetized with pentobarbital (45 mg/kg, *ip*). Two-kidney one-clip (2K-1C) renovascular hypertension was induced by partial, standardized clipping of the left renal artery [19]. After 6 weeks most of the animals (250–300 g) developed hypertension which was confirmed by the blood pressure measurement. Sham-operated rats (SO) served as a control to 2K-1C rats. They received the same surgical intervention without the clipping of the renal artery.

Experimental protocol

To study the effect of AL on arterial thrombosis, the animals received aliskiren at doses 10, 30 and 100 mg/kg/day, once daily for 10 days, *po* or vehicle (VEH; 5% aqueous gummi arabici solution). Last dose was administered 20 min before the induction of carotid artery injury. Doses of aliskiren were chosen on the basis of previously published data [20,21]. Vehicle (VEH; 0.9% NaCl) served as a control to aliskiren-treated rats.

Blood pressure measurement

Systolic blood pressure (SBP) was monitored in conscious rats before and after 10 days of AL administration with use of the "tail cuff" method (Student Oscillograph, Harvard Rat Tail Blood Pressure Monitor, UK) [22]. To verify hypertension development we used the same method. Each value was the average of three consecutive readings. Hypertensive (SBP higher than 140 mmHg) and normotensive rats were used in the further experiments.

Induction of arterial thrombosis

Male renovascular hypertensive and normotensive Wistar Crl:WI(Han) rats were anesthetized by intraperitoneal injection of pentobarbital (40 mg/kg) and then placed in the supine position on a heated (37 °C) operation table. We induced arterial thrombosis by electrical stimulation of the right common carotid artery as previously described [23,24]. Briefly, the anode, a stainless steel L-shaped wire, was inserted under the artery and connected with constant current generator. The cathode was attached subcutaneously to the hind limb. The artery stimulation (1 mA) took 10 min. One hour after the commencement of the stimulation the segment of the common carotid artery with the formed thrombus was dissected than opened lengthwise and the thrombus was completely removed, air-dried in 37 °C and weighed 24 h after the end of experiment. The blood was collected before thrombus removal by heart puncture and transferred to a tube containing 3.13% sodium citrate (citrate/blood = 1:9). Platelet poor plasma was obtained by centrifugation of blood at 3500 rpm for 20 min (temp. 4 °C). Samples were stored at −80 °C until assayed.