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ORIGINAL ARTICLE

Investigation of possible prophylactic, renoprotective, and cardioprotective effects of thromboprophylactic drugs against ischemia–reperfusion injury

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Abstract The aim of this study was to investigate whether anticoagulant and antiaggregant agents have protective effects against oxidative damage induced by peripheral ischemia–reperfusion (I/R). Groups were created as follows: control group, I/R group (sham group), I/R plus acetylsalicylic acid (Group I), I/R+clopidogrel (Group II), I/R+rivaroxaban (Group III), I/R+bemiparin sodium (Group IV), and I/R+enoxaparin sodium (Group V). In Groups I, II, III, IV, and V, drugs were administered daily for 1 week before I/R creation. Peripheral I/R was induced in the I/R groups by clamping the right femoral artery. The rats were sacrificed 1 hour after reperfusion. Nitrogen oxide levels, malondialdehyde (MDA) levels, paraoxonase-1 (PON1) activity, and prolidase activity were evaluated in both cardiac and renal tissues. There was no significant difference in nitrogen oxide levels between the groups. However, cardiac and renal MDA were significantly higher and PON1 activity was markedly lower in the I/R groups compared with the control group ($p < 0.05$). Although elevated prolidase activity was detected in both the cardiac and renal tissue of the I/R groups, only the sham group and Group V had significantly higher renal prolidase activity ($p < 0.05$). Group V had significantly higher cardiac MDA, PON1, prolidase levels, and renal prolidase activity compared with the sham group ($p < 0.05$). Significant improvement in renal MDA levels was only observed in Group III, and marked improvement was observed in the cardiac MDA levels of Group II when compared with

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the sham group ($p < 0.05$). Thromboprophylactic agents appear to provide partial or prominent protection against I/R injury.

Introduction

Thrombosis can locally obstruct distal flow and cause distal ischemia or disrupt distant organ perfusion via emboli [1]. Therefore, thrombosis prophylaxis regimens for prevention of ischemic complications in high-risk populations are frequently investigated [2]. Thrombosis and embolic events can occur despite sufficient management and even when appropriate antithrombotic therapy is administered [3]. Treatment for both acute embolic and thrombotic occlusion focuses on reperfusion of the ischemic limb. Reducing the adverse outcomes of the ischemic injury is also a main target of treatment [1]. Furthermore, the duration of ischemic exposure is an important determinant of tissue recovery and of the undesired effects of reperfusion [4,5]. Therefore, receiving prophylaxis prior to thrombosis-induced ischemia is important.

Antiaggregant and anticoagulant agents can be used for thromboprophylaxis [6]. Previous investigations of antiaggregant agents have claimed that these agents modulate vascular smooth muscle and induce apoptosis, as well as inhibit platelet adhesion and aggregation [7]. Previous reports have also identified antioxidant effects of antiaggregant drugs [8]. Similarly, antithrombotic agents have cellular effects, on growth factor regulation and cell adhesion, in addition to preventing new thrombosis formation [9,10]. Previous studies have also mentioned possible antioxidant features of antithrombotic agents [10,11].

The purpose of the present study was to investigate and compare the possible cardiac and renal protective effects of thromboprophylactic agents against ischemia—reperfusion (I/R) injury in an experimental peripheral I/R model.

Materials and methods

All animal procedures were approved by the Animal Research Committee of Dicle University and were performed in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the ethics committee. The principal investigator, animal laboratory staff, and the primate handling staff were present for all procedures. Study design and protocol was created according to our previous experimental modeling [12].

Animals

Fifty-six male Sprague—Dawley rats (aged 8—12 weeks) weighing 230 ± 30 g (mean ± standard deviation) were used for this study. The rats were obtained from the Laboratory Animal Production Unit of the university. All rats were maintained in standard humidity (50 ± 5%) and temperature (22 ± 2°C) controlled cages with a 12-hour light/dark cycle for 1 week prior to the start of the study.

Study protocol

The rats were randomly divided into seven equal groups. All operations were performed simultaneously on sham and study groups for sample standardization. All rats were anesthetized with ketamine (Ketalar®; Pfizer, Täby, Sweden) at a dose of 130 mg/kg and xylazine (Rompun®; Bayer, Gothenburg, Sweden) at a dose of 20 mg/kg via an intraperitoneal line. Maintenance of anesthesia was provided with ketamine hydrochloride (50 mg/kg).

Experimental ischemia/reperfusion injury model preparation

The right femoral artery was clamped for 6 hours and then the clamp was removed to create reperfusion. Two milliliters of blood samples were obtained from inferior vena cava at the 1st hour of reperfusion. The rats were sacrificed 1 hour after reperfusion, and cardiac tissue samples and left kidneys were obtained.

Eight of the rats were placed in the control group at the beginning of the study, and 2 mL of blood samples from inferior vena cava, cardiac tissue samples, and left kidneys were obtained for determining the baseline nitrogen oxide (NOx) levels, malondialdehyde (MDA) levels, and paraoxonase-1 (PON1) and prolidase activity in blood, renal, and cardiac tissue without any intervention. The remaining rats were separated for experimental I/R injury induction.

The six peripheral I/R injury groups of eight animals each were as follows:

Sham group: I/R only.

Group I (n = 8): Acetylsalicylic acid (ASA, Coraspin; Bayer, Leverkusen, Germany) was administered orally via gavage at a dose of 30 mg/kg/day beginning 1 week prior to the start of study. I/R was induced after 1 week of drug administration.

Group II (n = 8): Clopidogrel bisulfate (Planor; Koçak Farma, Tekirdağ, Turkey) was administered orally via gavage at a dose of 1 mg/kg/day beginning 1 week prior to the start of the study. I/R was induced after 1 week of drug administration.

Group III (n = 8): Rivaroxaban (Xarelto; Bayer Schering Pharma AG, Wuppertal, Germany) was administered orally via gavage at a dose of 3 mg/kg/day beginning 1 week prior to the start of the study. I/R was induced after 1 week of drug administration.