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Protective effect of 2-aminoethyl diphenylborinate on acute ischemia–reperfusion injury in the rat kidney

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ABSTRACT

Background: To investigate the protective effect of 2-aminoethyl diphenylborinate (2-APB) against ischemia–reperfusion (I/R) injury in the rat kidney by an experimental study.
Materials and methods: Thirty male Sprague-Dawley rats were randomly divided into the following three groups: (1) sham group, (2) I/R group, and (3) I/R + 2-APB group. Renal I/R injury was induced by clamping the left renal pedicle for 45 min after right nephrectomy, followed by 3 h of reperfusion. The therapeutic agent 2-APB was administered intravenously at a dose of 2 mg/kg 10 min before renal ischemia. Glutathione, superoxide dismutase, total antioxidant capacity, malondialdehyde, tumor necrosis factor α, interleukin 6, aspartate aminotransferase, alanine aminotransferase, and creatinine levels were measured from blood samples, and the rats were sacrificed subsequently. Tissue samples were scored histopathologically. Visualization of apoptotic cells was performed using the terminal deoxynucleotidyl transferase dUTP nick end labeling staining method.
Results: 2-APB significantly reduced serum malondialdehyde, tumor necrosis factor α, interleukin 6, aspartate aminotransferase, alanine aminotransferase, and creatinine levels in the I/R injury group. However, glutathione, superoxide dismutase, and total antioxidant capacity levels increased significantly. Histopathologic scores were significantly better and the rate of apoptosis was lower in the 2-APB group.
Conclusions: 2-APB reduces oxidative stress and damage caused by renal I/R injury. The results of this study demonstrate that 2-APB can be used as an effective agent against I/R injury in the kidney.

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1. Introduction

Renal ischemia–reperfusion (I/R) injury is the primary cause of acute kidney damage [1]. This condition usually occurs during hemorrhagic shock, renal transplantation, and urological surgery procedures requiring clamping of the renal artery. Clinically, it can lead to prolonged hospitalization or even organ loss [1–3].

The pathogenesis of I/R injury is multifactorial [4]. Several mechanisms including disturbances of the cell Ca^{2+} metabolism, disruption of the generation of free radicals, activation of phospholipases by the production of toxic lipid metabolites, and loss of cell volume have been blamed [5]. The rise in intracellular and mitochondrial Ca^{2+} concentrations in association with a decrease in adenosine triphosphate during ischemia is also known to play an important role in cell damage, causing phospholipase, nuclease, and protease activation, and thus an increase in reactive oxygen species (ROS) occurs [4,6]. ROS, which are normally present in low-moderate concentrations in the body, are involved in physiological processes, such as functions in the cellular signaling pathway and defense against infectious agents [7]. For cases involving excessive intracellular ROS production, such as post-ischemic reperfusion, protein, DNA, lipid, and mitochondrial damage occurs in the cell [1,7]. The functional consequences of renal injury emerge because of this damage, which initiates the apoptotic process, affecting tubular cells in the kidney [3,8].

Store-operated calcium channels (SOCs) expressed in the membranes of most cells, including renal efferent arterioles, are the members of the ion channel family that permit the passage of Ca^{2+} into the intracellular space [6,9]. The chemical agent 2-aminoethyl diphenylborinate (2-APB) has an inhibitory effect on Ca^{2+} release from the extracellular space into the cell by blocking SOCs [6,10]. The inhibitor effect of 2-APB on mCa^{2+} (mitochondrial calcium) uptake by mCa^{2+} uniporter channels and the reduction of mitochondrial calcium deposition have been well demonstrated in a previous study of hepatic I/R injury [4]. Previous studies have also described the preventive effect of 2-APB on liver damage after hepatic I/R injury [4,6].

In this experimental study, we investigated the effect of 2-APB administration on acute kidney injury in a rat model. We hypothesized that 2-APB might have a protective effect on I/R injury in the kidney by blocking SOCs in the efferent arterioles or the mCA^{2+} uniporter channels in the proximal tubules and reducing oxidative stress. As a sign of this protective effect, we measured the serum sample levels of biochemical markers that revealed antioxidant activity and the histopathologic examination was performed.

2. Materials and methods

2.1. Chemicals

2-APB was obtained from Sigma-Aldrich (Sigma-Aldrich, Inc, Louis, MO), ketamine from Ketalar-Pfizer (Pfizer, Inc, New York), and xylazine (Alfazyne) from Ege Vet (Alfasan International, Woerden, Hollanda).

2.2. Animals and treatment protocol

Approval of Ethical Committee for Animal Experiments of Balıkesir University was obtained before the study. Animals were allowed *ad libitum* access to food and drink up to the time of the study. The animals were treated humanely throughout the protocol in line with national health institution guidelines and rules on guide for the care and use of laboratory animals.

Thirty male Sprague-Dawley rats weighing 250–300 g were randomly divided into the following three groups: (1) sham group ($n = 10$), (2) I/R group ($n = 10$), and (3) I/R + 2-APB group ($n = 10$). Rats were anesthetized under aseptic conditions with intramuscular injection of ketamine-xylazine mixture (ketamine, 90mg/kg; xylazine, 10 mg/kg). Intravenous (i.v.) injections were performed using the penile vein. The abdominal region was shaved and cleansed with povidone–iodine. Ten minutes after the i.v. injection of 1.0 mL/kg of 0.9% NaCl, laparotomy and right nephrectomy were performed on the sham group. The abdomen was closed 45 min after nephrectomy. In the I/R group, laparotomy and right nephrectomy were performed 10 min after the i.v. injection of 1.0 mL/kg of 0.9% NaCl. The left renal pedicle was occluded for 45 min using a vascular clamp. The clamp was then removed and reperfusion was established subsequently. Once the kidney was seen to be reperfused, the abdomen was closed. The I/R + 2-APB group was given 2 mg/kg 2-APB (i.v.) 10 min before laparotomy and the same procedures were performed as in the I/R group. Throughout laparotomy, 50 mL/kg of warm 0.9% NaCl was dripped into the abdominal cavity. Blood samples were subsequently collected by cardiac puncture 3 h after the experimental procedure, and all rats were sacrificed.

2.3. Antioxidant enzymes, malondialdehyde, proinflammatory cytokines, aspartate aminotransferase, alanine aminotransferase, and creatinine measurement

Blood samples were kept for 2 h at room temperature to ensure proper clotting. The samples were then centrifuged at 2500*g* at 4°C for 15 min and stored at −20°C until analysis.

Reduced glutathione (GSH) levels in whole blood were estimated with 5,5′-bis-dithionitrobenzoic acid reagent [11]. The samples were incubated with xanthine oxidase solution for 1 h at 37°C to measure superoxide dismutase (SOD) activity in serum. Absorbance was read at 490 nm to generate superoxide anions. SOD activity was determined as the inhibition of chromagen reduction. In the presence of SOD, superoxide anion concentration is reduced, yielding less colorimetric signal. SOD activity was expressed in percent. Lipid peroxidation was determined using the procedure described by Yoshioka *et al.* [12], in which malondialdehyde (MDA), an end product of fatty acid peroxidation, reacts with thiobarbituric acid to form a colored complex with a maximum absorbance at 532 nm. Total antioxidant capacity (TAS) of the serum was determined using an automated measurement method with a commercially available kit developed by Rel (Total Antioxidant Status Assay kit, Rel Assay Diagnostics, Turkey). The antioxidative effect of the sample against the potent-free radical reactions initiated by the reduced hydroxyl radical is measured using this method. The results were expressed as