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ORIGINAL ARTICLE

The protective effects of tadalafil on renal damage following ischemia reperfusion injury in rats

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Abstract Ischemia-reperfusion injury can cause renal damage, and phosphodiesterase inhibitors are reported to regulate antioxidant activity. We investigated the prevention of renal damage using tadalafil after renal ischemia reperfusion (I/R) injury in rats. A total of 21 adult male Wistar albino rats were randomly divided into three groups of seven, including Group 1-control, Group 2-I/R, and Group 3-tadalafil + I/R group (I/R-T group) received tadalafil intraperitoneally at 30 minutes before ischemia. Inducible nitric oxide synthase, endothelial nitric oxide synthase, malondialdehyde, and total antioxidant capacity levels were evaluated, and histopathological changes and apoptosis in the groups were examined. Tadalafil decreased malondialdehyde levels in the I/R group and increased the total antioxidant capacity level. Histopathological and immunohistochemical findings revealed that tadalafil decreased renal injury scores and the ratios of injured cells, as measured through apoptotic protease activating factor 1, inducible nitric oxide synthase, and endothelial nitric oxide synthase levels. We suggest that tadalafil has protective effects against I/R-related renal tissue injury.

Conflicts of interest: All authors declare no conflicts of interest.

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Introduction

Renal ischemia leads to the depletion of cellular energy, accumulation of intracellular sodium, calcium, reactive oxygen species (ROS), and activation of multiple enzyme systems, including proteases, nitric oxide synthases (NOSs), phospholipases, and endonucleases; these result in cell damage and death [1]. Following reinstitution of blood flow, the final stage of injury occurs during reperfusion, called reperfusion injury (I/R injury). I/R injury is an immediate nonspecific inflammatory response [2].

Nitrogen oxide (NO) is an important mediator of the physiological and pathological processes during renal I/R injury [1,2]. Renal reperfusion following ischemia activates NOS and increases its expression [3]. Phosphodiesterase type 5 (PDE5) inhibitors, such as sildenafil, tadalafil, and vardenafil, comprise the first-line therapy for treating erectile dysfunction in males. Inhibiting phosphodiesterase also decreases renal injury [4–7].

In this study, we investigated the effect of tadalafil on renal I/R injury.

Methods

This study was approved by the Ethics Council of Bülent Ecevit University Medical Faculty, Zonguldak, Turkey.

Twenty-one Wistar albino rats (weight, 400–450 g) were divided into three groups. General anesthesia was administered with 40 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) with intraperitoneal injection. An approximate 3 cm skin incision was made in the abdominal region. The groups were divided as the following. Group 1, Control group: No treatment was given. The abdominal median laparotomy was conducted, blood was taken, and a bilateral nephrectomy was performed. Group 2, I/R group: The right kidney pedicle was clamped for 1 hour with a nontraumatic microvascular clamp (bulldog clamp), and the kidney was subjected to ischemia. No clamp was put on the left kidney pedicle. Following ischemia, the right kidney pedicle clamp was removed, and the kidney was perfused for 1 hour. Blood was then taken, and a bilateral nephrectomy was performed. Group 3, Tadalafil + I/R group (I/R-T group): Before subjecting the rat to ischemia, a 1 mL suspension of 10 mg/kg tadalafil was injected intraperitoneally. Then, the right kidney pedicle was clamped for 1 hour, as described for the I/R group. Following ischemia, the right kidney was subjected to 1-hour reperfusion by removing the clamp from the right kidney pedicle. Blood was then collected, and a bilateral nephrectomy was performed.

Tissue sampling

At the end of the experiment, the blood samples were centrifuged at 500*g* for 3 minutes and stored at −80°C for biochemical evaluation of malondialdehyde (MDA) and total antioxidant capacity (TAC).

After cutting each kidney into two parts, one part was stored at −80°C for biochemical evaluations, and the other was fixed in 10% formalin for histological studies.

Biochemical analyses

Preparation of tissue samples

Tissues were homogenized on ice with an homogenizer (Ultra Turrax Type T25-B, IKA Labortechnic, Germany) by adding 0.1M Tris-hydrochloride buffer solution including 0.1% Tween 80 [8]. Then, the homogenates were centrifuged at 800*g* and 4°C. Protein concentrations of supernatants were assessed using the Lowry et al [9] method.

MDA working procedure

Serum and tissue MDA levels were evaluated with high-performance liquid chromatography (HPLC) using an Agilent 1200 HPLC (Agilent Technologies, Munchen, Germany). MDA was measured using commercial immunodiagnostic kits (Merck, Bensheim, Germany). Solutions of 1,1,3,3-tetraethoxypropane (TEP), trichloroacetic acid, 2-thiobarbituric acid, and dinitrophenylhydrazine (DNPH), sodium hydroxide, perchloric acid, hydrochloric acid, sulfuric acid, methanol, and ethanol were obtained from Merck (Frankfurter, StraBe 250, Germany). Acetonitrile was obtained from Sigma-Aldrich (Frankfurter, StraBe 250, Germany). Fifty μL of 6M sodium hydroxide was added to 0.250 mL serum and then incubated in a 60°C water bath for an hour. The hydrolyzed sample (0.125 mL) was acidified with 0.125 mL of 35% (v/v) perchloric acid. After centrifugation (500*g*) for 10 minutes, 0.250 mL supernatant was mixed with 25 μL of 5mM DNPH solution and incubated in dark for half an hour. One hundred μL of the reaction mixture was directly injected into HPLC system [10]. MDA standard was prepared by dissolving 25 μL TEP in 100 mL of water to give a 1mM stock solution. Working standard was prepared by dilution of 1 mL stock solution in 50 mL of 1% sulfuric acid and incubation for 2 hours at room temperature. The resulting MDA standard of 20 μmol/L was further diluted with 1% sulfuric acid to yield different concentration. The 0.250 mL of standard was mixed with 25 μL DNPH solution and incubated in dark for 30 minutes. One hundred microlitres of the reaction mixture was directly injected into HPLC system. The analytical column was 125 mm × 4 mm ODS-2 C18 reserve phase column with particle size of 5 μm (Thermo, Theale/Berkshire, UK). The mobile phase was acetonitrile-distilled water (34:66, V/V) containing 0.2% (V/V) acetic acid. All separations were performed at isocratic conditions with a flow rate of 1 mL/min. MDA peaks were determined according to its retention time and confirmed by spiking with added exogenous standard. Concentrations of MDA were calculated from standard curve prepared from TEP.

The detection limit of this procedure was 0.15μM and linearity was 100μM.

TAC working procedure

The TAC measurement principle is based on the reaction of antioxidants with hydrogen peroxide in the sample. The antioxidants sample was taken, with the same amount of hydrogen peroxide. Antioxidants in the sample bind to the hydrogen peroxide, and the increased hydrogen peroxide converts TMB (3,3′,5,5′-tetramethylbenzidine) to a colored