Life Sciences 147 (2016) 85–91

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Morphological and morphometric study of protective effect of green tea in gentamicin-induced nephrotoxicity in rats

Milica Veljković [a,*], Dragana R. Pavlović [b], Nenad Stojiljković [a], Sonja Ilić [a], Aleksandar Petrović [c], Ivan Jovanović [d], Mirjana Radenković [a]

[a] Department of Physiology, University of Niš, Faculty of Medicine, Bulevar Dr Zoran Djindjić 81, Niš, Serbia
[b] Department of Pharmacy, University of Niš, Faculty of Medicine, Bulevar Dr Zoran Djindjić 81, Niš, Serbia
[c] Department of Histology, University of Niš, Faculty of Medicine, Bulevar Dr Zoran Djindjić 81, Niš, Serbia
[d] Department of Anatomy, University of Niš, Faculty of Medicine, Bulevar Dr Zoran Djindjić 81, Niš, Serbia

ARTICLE INFO

Article history:
Received 25 October 2015
Received in revised form 17 January 2016
Accepted 22 January 2016
Available online 23 January 2016

Keywords:
Gentamicin
Nephrotoxicity
Green tea
Oxidative stress

ABSTRACT

Aims: One of the most popular beverages worldwide, green tea, was investigated for its potential protective effect in a rat model of gentamicin-induced nephrotoxicity by monitoring functional and morphological changes in kidneys.
Main methods: The study was conducted on four groups of rats: control group (C), treated with only gentamicin (GM), treated with only green tea (GT) and treated with both gentamicin and green tea (GT + GM). Kidney function, oxidant and antioxidant parameters of renal tissue, as well as histopathological studies were assessed. Morphometric analysis was used to quantify these histopathological changes.
Key findings: Gentamicin caused significant elevations in serum creatinine and urea and oxidative stress parameter (AOPP), while antioxidative enzyme catalase was significantly decreased. Histological sections of kidneys in GM group revealed necrosis of proximal tubules, vacuolation of cytoplasm and massive mononuclear inflammatory infiltrates in interstitium. Coadministration of green tea with gentamicin histologically showed renoprotective effect. Histological results were confirmed and quantified by morphometric analysis. Also in this group we measured ameliorated parameters of renal functions and antioxidative defense.
Significance: Regenerative potential of green tea after renal injury induced by gentamicin could be explained through the decrease of oxidative stress and lipid peroxidation. Green tea is a natural antioxidant, with many health promoting effects, widely available and in accordance to that affordable. Because of the established habits, people largely consume it as a beverage. It could be beneficial in the reduction of oxidative stress and changes caused by it primarily in renal tubules and interstitium.

1. Introduction

Gentamicin (GM) is an aminoglycoside antibiotic effectively used in the treatment of severe Gram negative infections of the abdomen and urinary tract [14]. Since 30% of patients treated with GM showed some signs of acute renal failure, its use has been limited [2]. GM-induced nephrotoxicity was used as an animal model for acute renal failure in experimental research [5,25]. The specificity of GM nephrotoxicity is possibly related to it being excreted predominantly by glomerular filtration, reabsorbed by proximal tubules and accumulated within the renal cortex. The very mechanism by which GM causes nephrotoxicity is through oxidative and nitrosative stress [1,9]. GM generates reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, hydrogen peroxide and reactive nitrogen species [25] associated with an increase in lipid peroxidation (LPO) and decrease in antioxidant enzymes in the kidney [6,14].

Green tea (*Camellia sinensis* (L.) Kuntze, Theaceae) contains a wide range of polyphenols with strong antioxidative, anticancer and antimutagenic activity [10], also known as green tea catechins. They include catechin, epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate and gallocatechingallate [2,4,7]. It is documented that green tea has preventive effects against many chronic diseases including obesity, atherosclerosis, neurodegenerative disease and cancer [7]. Tea drinking, through antioxidant intake, may become beneficial in oxidative stress conditions [8], such as the acute renal failure caused by GM. Among catechins, epigallocatechin-3-gallate is the

* Corresponding author at: Department of Physiology, University of Niš, Faculty of Medicine, Bulevar Dr Zoran Djindjić 81, 18 000 Niš, Serbia.
E-mail address: milica.veljkovic@gmail.com (M. Veljković).

http://dx.doi.org/10.1016/j.lfs.2016.01.035
0024-3205/

most abundant polyphenol in green tea and the most powerful antioxidant [8]. It also has anti-inflammatory and antiproliferative activity and is capable of inhibiting cell growth and inducing apoptosis of various human cancer cells without adversely affecting normal cells [23,27].

In our study we sought to investigate if green tea ethanolic extract (GTE) could be used as an inhibitory agent against GM-induced acute renal failure because of its antioxidative property [25].

2. Materials and methods

2.1. Green tea ethanolic extract (GTE) preparation

Green tea sample was purchased from commercial sources (Institute of Medicinal Plants Research "Dr Josif Pančić", Pančevo, Serbia). Dried leaves were reduced to a fine powder and extracted with ethanol (70% *v*/v) by percolation, as described in European Pharmacopeia 6.0 [11]. GTE was obtained after evaporation to the dryness in vacuo under 40 °C and extraction yield was 25%. Chemicals, reagents and solvents used in the experiment were of analytical grade.

2.2. Determination of GTE constituents

2.2.1. Determination of total polyphenols

The total phenolic content of extract was estimated using the Folin–Ciocalteu colorimetric procedure [18]. Quantification was done on the basis of a standard curve with (+)-catechin (concentration span 1–10 μg/ml) and result was expressed as catechin equivalents (mg CE/g extract) per g of sample (average value of 5 probes ± standard error).

2.2.2. Determination of total tannins

Total tannins content was determined by the same Folin–Ciocalteu procedure after addition of insoluble matrix — polyvinylpolypyrrolidone [18]. Tannins content was measured as the reduction in phenolics that occur when a binding agent (polyvinyl polypyrrolidone, PVPP) is added to the extract. The assay was carried out with clear supernatant and result was also expressed as mg CE/g of sample (average value of 5 probes ± standard error).

2.2.3. Determination of total flavonoids

The content of total flavonoids was determined spectrophotometrically in GTE according to aluminum chloride method described by Lamaison and Carnat [15]. Quantification was performed using a standard curve with rutin (concentration span 1–5 μg/ml). The result was expressed as mg rutin equivalents (mg Ru/g extract) per g of sample as average value of 5 probes ± standard error.

2.3. Animals

Wistar albino rats of either sex, weighting 200–250 g were selected for this study. The animals were obtained from vivarium of Medical faculty, University of Niš. They were kept inside a well-ventilated room under environmentally controlled conditions. Room temperature was 20 ± 2 °C and the light/dark cycle was 12/12 h. Rodent chew diet and water were available to them ad libitum. Experimental protocols were done in accordance with the declaration of Helsinki and European Community guidelines for the ethical handling of laboratory animals (EEC Directive of 1986; 86/609/EEC) and approved by the Animal Ethics Board of the Medical Faculty in Nis. It was documented under number 01-2625-8.

2.4. Experimental procedure

The animals were divided into four groups of 8 animals each. The first 8 animals served as control (C group) and received 1 ml of saline solution per day intraperitoneally for 8 days. The green tea (GT) group orally received 300 mg/kg/day of GTE for 15 days using an intragastric enteral feeding protocol [20]. Nephrotoxicity in GM group was induced by intraperitoneal administration of GM (Actavis company, Leskovac, Serbia) 100 mg/kg/day for the last 8 days of experiment. The green tea and gentamicin group (GT + GM) was treated orally with green tea 300 mg/kg/day for 15 days. For the last 8 days of experiment simultaneously with green tea, intraperitoneal injection of GM (100 mg/kg/day) was given. At the end of the experiment, all animals were anesthetized using 80 mg/kg ketamine (Ketamidor 10%, Richter Pharma AG, Wels, Austria) and sacrificed. Blood samples for biochemical analysis were taken from the aorta (2 ml) and analyzed as markers of the acute renal failure. The kidney was subsequently removed and separated into two parts for biochemical analysis and light microscopic examination.

2.5. Homogenates preparation

Removed kidneys were cut into small pieces and homogenized in ice cold water using homogenizer (IKA® Works do Brasil Ltda, Rio de Janeiro, Brasil). A 10% (*w*/*v*) homogenate was centrifuged at 1500 g for 10 min at 4 °C to remove cell debris. The supernatant was saved and stored at −20 °C for the biochemical analysis.

2.6. Histological analysis

Kidney tissue was fixed in 10% paraformaldehyde, dehydrated in ascending graded series of alcohol and embedded in paraffin. Tissue samples were cut into slices of 5 μm thickness using a Histo Range microtome (model: LKB 2218, LKB-Produkter AB, Bromma, Sweden) and stained with hematoxylin and eosin (HE) and Periodic Acid Schiff (PAS) for light microscopic examination. Histological sections were examined and photographed on a light microscope Leica DMR (Leica Microsystems AG, Wetzlar, Germany).

2.7. Determination of protein oxidation

The concentration of advanced oxidation protein products (AOPP), as a marker of oxidative modified proteins and also inflammation, was determined [28]. The concentration of proteins was measured according to Lowry's method [17], using bovine serum as standard. Spectrophotometric method based on the reaction of AOPP with potassium iodide in an acidic medium was used to measure the levels of AOPP. The intensity of color was recorded instantly at 340 nm. The values were expressed in μmol/mg of protein.

2.8. Determination of catalase activity

Tissue catalase (CAT) activity was determined according to Goth [12]. The method is based on the ability of catalase to dissolve the substrate (H_2O_2), whereby enzymatic reaction is stopped by the addition of ammonium molybdate. Yellow complex of molybdate and H_2O_2 was measured at 405 nm and enzyme activity was expressed in catalytic units per gram of protein (kU/g).

2.9. Morphometric analysis

After rat kidneys were histologically processed, digital pictures of them were taken by using a camera connected to a light microscope Leica DMR (Leica Microsystems AG, Wetzlar, Germany). Morphometric analysis was done by using computer program ImageJ (http://rsbweb.nih.gov/ij/). Before each analysis spatial calibration by object micrometer was performed. Measured morphometric parameters included: glomerular area (μm^2), area of proximal tubules (μm^2), interstitial nuclei area (μm^2) and circularity of interstitial nuclei. From each experimental group 4 animals were randomly chosen, and their tissue preparation stained by PAS was analyzed. In each animal at least 50 glomerules, proximal tubules and interstitial nuclei were analyzed. Morphometric