Toxicology and Applied Pharmacology 277 (2014) 8–20

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Naringin ameliorates gentamicin-induced nephrotoxicity and associated mitochondrial dysfunction, apoptosis and inflammation in rats: Possible mechanism of nephroprotection

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ARTICLE INFO

Article history:
Received 2 December 2013
Revised 24 February 2014
Accepted 28 February 2014
Available online 14 March 2014

Keywords:
Gentamicin-induced nephrotoxicity
Naringin
Apoptosis
Inflammation
Mitochondrial dysfunction

ABSTRACT

Gentamicin-induced nephrotoxicity has been well documented, although its underlying mechanisms and preventive strategies remain to be investigated. The present study was designed to investigate the protective effect of naringin, a bioflavonoid, on gentamicin-induced nephrotoxicity and to elucidate the potential mechanism. Serum specific renal function parameters (blood urea nitrogen and creatinine) and histopathology of kidney tissues were evaluated to assess the gentamicin-induced nephrotoxicity. Renal oxidative stress (lipid peroxidation, protein carbonylation, enzymatic and non-enzymatic antioxidants), inflammatory (NF-kB [p65], TNF-α, IL-6 and MPO) and apoptotic (caspase 3, caspase 9, Bax, Bcl-2, p53 and DNA fragmentation) markers were also evaluated. Significant decrease in mitochondrial NADH dehydrogenase, succinate dehydrogenase, cytochrome c oxidase and mitochondrial redox activity indicated the gentamicin-induced mitochondrial dysfunction. Naringin (100 mg/kg) treatment along with gentamicin restored the mitochondrial function and increased the renal endogenous antioxidant status. Gentamicin induced increased renal inflammatory cytokines (TNF-α and IL-6), nuclear protein expression of NF-κB (p65) and NF-κB-DNA binding activity and myeloperoxidase (MPO) activity were significantly decreased upon naringin treatment. In addition, naringin treatment significantly decreased the amount of cleaved caspase 3, Bax, and p53 protein expression and increased the Bcl-2 protein expression. Naringin treatment also ameliorated the extent of histologic injury and reduced inflammatory infiltration in renal tubules. U-HPLS-MS data revealed that naringin co-administration along with gentamicin did not alter the renal uptake and/or accumulation of gentamicin in kidney tissues. These findings suggest that naringin treatment attenuates renal dysfunction and structural damage through the reduction of oxidative stress, mitochondrial dysfunction, inflammation and apoptosis in the kidney.

Introduction

Gentamicin (GM) is probably one of the most commonly used aminoglycoside antibiotics for the treatment of serious and life-threatening infections caused by Gram-negative aerobes (Negrette-Guzman et al., 2013). Despite its beneficial effects, low cost and low levels of resistance, serious complications like nephrotoxicity and ototoxicity are dose-limiting factors in the use of aminoglycosides (Ali et al., 2011; Sun et al., 2013). It has been reported that aminoglycoside antibiotics induce a dose-dependent nephrotoxicity in 10–25% of therapeutic courses; despite rigorous monitoring of serum drug concentrations and adequate fluid volume control (Martínez-Salgado et al., 2007). Although several studies have been undertaken to investigate the mechanisms underlying these unwanted side effects, the mechanism of nephrotoxicity induced by gentamicin is not completely known and remains to be studied further. Experimental evidence suggests the role of reactive oxygen/nitrogen species, in association with increased lipid peroxide formation and decreased activity of antioxidant enzymes in gentamicin-induced nephrotoxicity (Balakumar et al., 2010; Lee et al., 2012). Recent studies have also postulated that renal inflammation, which is characterized by infiltration of inflammatory cells such as monocytes/macrophages and subsequent release of pro-inflammatory cytokines and activation of NF-κB in

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http://dx.doi.org/10.1016/j.taap.2014.02.022
0041-008X/

response to oxidative stress, is involved in this process (Bae et al., 2013; Kalayarasan et al., 2009). Furthermore, apoptosis/necrosis of renal tubular epithelial cells (Juan et al., 2007; Lee et al., 2012; Sue et al., 2009), mitochondrial dysfunction (Morales et al., 2010; Negrette-Guzman et al., 2013; Servais et al., 2005) and activation of renal matrix metalloproteinases (Romero et al., 2009) are also involved in gentamicin-induced nephrotoxicity.

Flavonoids occur ubiquitously in the plant kingdom and are common components of the human diet. In recent years, the use of naringin had received considerable attention as dietary antioxidant. Naringin (4′, 5, 7-trihydroxy flavanone 7-rhamnoglucoside) is a major and active flavanone glycoside found in grape fruits and other related citrus species (Singh et al., 2004). Numerous reports have documented the protective actions of naringin in various models of oxidative stress due to its direct free radical scavenger activity and its indirect antioxidant properties (Amudha and Pari, 2011; Badary et al., 2005; Singh et al., 2004). It is also reported that naringin up-regulates the gene expression of superoxide dismutase, catalase and glutathione peroxidase (Jeon et al., 2001). Experimental data further showed the hypolipidemic (Jeon et al., 2004), anticancer (Marchand et al., 2000; So et al., 1996), anti-inflammatory (Gopinath and Sudhandiran, 2012; Nie et al., 2012), cardioprotective (Rajadurai and Prince, 2009), antimutagenic (Higashimoto et al., 1998) and antimicrobial (Kim et al., 1998) properties of naringin. To the best of our knowledge, the effect of naringin against gentamicin-induced nephrotoxicity has not been studied. Based on these finding, we hypothesized that combining naringin with gentamicin would be a novel strategy to protect the kidney from gentamicin induced renal side effects. Therefore, the present study is initiated with an aim to evaluate the protective effect and the potential mechanisms of naringin against nephrotoxicity following gentamicin administration in rats.

Materials and methods

Drugs and chemicals

Gentamicin sulfate was obtained from Abbott Healthcare Pvt Ltd, India. Naringin (purity: ≥90% from citrus fruit), reduced glutathione (GSH), glutathione oxidized (GSSG), catalase, glutathione reductase, glutathione peroxidase, 2-thiobarbituric acid (TBA), superoxide dismutase assay kit, caspase 3 fluorimetric assay kit, caspase 9 substrate colorimetric, cytochrome c oxidase assay kit, o-dianisidine, Bradford reagent, 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) were obtained from Sigma-Aldrich Co., St Louis, MO, USA. Antibodies against NF-kB (p65), cleaved caspase-3, Bax, Bcl-2, p53, lamin B1, β-actin and HRP-conjugated secondary antibody were purchased from Cell Signaling Technology (Boston, MA). NE-PER nuclear and cytoplasmic extraction kit was obtained from Pierce Biotechnology, Rockford, IL, USA. NF-κB (p65) transcription factor assay kit was obtained from Cayman Chemical Company, Ann Arbor, MI. Rat TNF-α and IL-6 ELISA (Ready-SET-Go) kits were obtained from eBioscience, USA. All other chemicals were of analytical grade.

Experimental animals

Male Sprague–Dawley rats weighing between 180 and 200 g were obtained from National Institute of Nutrition (NIN), Hyderabad, India. Animals were housed in a central facility under controlled conditions (12 h light schedule, temperature at 22 ± 2 °C). Food and water were provided ad libitum. The experiments involved with animals were conducted according to the ethical norms of the CPCSEA, Government of India and after obtaining approval from the Institutional Animal Ethics Committee (IAEC) of the institute.

Experimental design

Rats were randomly divided into five groups consisting of eight rats in each and were treated as follows:

Group I: Vehicle control (Control); rats were orally treated with gum acacia (2%) and an intraperitoneal (i.p.) injection of normal saline daily for a period of 7 consecutive days.
Group II: Naringin control (Nar); rats were orally treated with naringin (100 mg/kg body weight) and an intraperitoneal (i.p.) injection of normal saline daily for a period of 7 consecutive days.
Group III: Gentamicin control (GM); rats were orally treated with gum acacia (2%) and an intraperitoneal (i.p.) injection of gentamicin (120 mg/kg body weight) daily for a period of 7 consecutive days.
Group IV: GN50; rats were treated daily with both naringin (50 mg/kg body weight, orally) and gentamicin (120 mg/kg body weight, intraperitoneally) at an interval of 1 h for a period of 7 consecutive days;
Group V: GN100; rats were treated daily with both naringin (100 mg/kg body weight, orally) and gentamicin (120 mg/kg body weight, intraperitoneally) at an interval of 1 h for a period of 7 consecutive days.

Because of very low aqueous solubility, naringin (purity ≥ 90% from citrus fruit, Sigma-Aldrich Co., St Louis, MO, USA), was suspended in 2% gum acacia. The dose of gentamicin (120 mg/kg, i.p.) was selected based on our pilot study (data not shown) and the doses of naringin (50 mg/kg and 100 mg/kg) were selected based on reported literature, in which naringin did not produce any detectable toxicity to experimental animals (Li et al., 2013). At the end of the study, whole blood samples were collected through retro-orbital plexus to obtain serum for the quantification of serum specific renal function parameters (blood urea nitrogen and creatinine). Body weights of all animals were recorded and the animals were humanely euthanized using carbon dioxide gas in an air-tight chamber. Kidney tissues were harvested, fatty and conjunctive tissue layer were removed, rinsed in normal saline and stored in −80 °C freezer for further biochemical and immunoblot studies.

Preparation of kidney tissues

A known weight of the kidney tissue (right side) was homogenized in phosphate buffer saline (PBS, 50 mM, pH 7.4) containing 1% protease inhibitor cocktail (Sigma-Aldrich) to give a 10% homogenate suspension. A part of the homogenate was centrifuged at 14,000 rpm for 1 h, at 4 °C and the supernatant obtained was used for the estimation of various biochemical parameters. The protein contents were measured by using Bradford reagent (Sigma-Aldrich) against bovine serum albumin (BSA) as standard. To another part of the homogenate, an equal quantity of 10% trichloroacetic acid (TCA) was added, mixed properly and centrifuged at 5000 rpm for 15 min at 4 °C. The supernatant obtained was used for estimation of thiobarbituric acid reactive substance (TBARS) and vitamin C and the pellet obtained was used for estimation of protein carbonyl content.

Biochemical analysis

Non-enzymatic antioxidants

Reduced glutathione was measured using DTNB [5, 5′ dithiobis-(2-nitrobenzoic acid)]. This reagent reacts with the –SH groups to produce a yellow colored complex which has peak absorbance at 412 nm (Ellman, 1959). GSH was determined from a standard curve produced using commercially available standard GSH (Sigma-Aldrich). Levels of GSH were expressed as μg/g tissue. Kidney tissue vitamin C level from different experimental groups was estimated by using 2, 4-dinitrophenyl