Phytomedicine 20 (2013) 453–460

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage

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ARTICLE INFO

Keywords:
Hesperidin
Cisplatin
Nephrotoxicity
Inflammation
Apoptosis
Oxidative stress

ABSTRACT

Nephrotoxicity is an important complication in cancer patients undergoing cisplatin therapy. Oxidative stress, inflammation and apoptosis/necrosis are the major patho-mechanisms of cisplatin induced nephrotoxicity. In the present study, hesperidin, a naturally-occurring bioflavonoid has been demonstrated to have protective effect on cisplatin-induced renal injury in rats. Cisplatin intoxication resulted in structural and functional renal impairment which was revealed by massive histopathological changes and elevated blood urea nitrogen and serum creatinine levels, respectively. Renal injury was associated with oxidative stress/lipid peroxidation as evident by increased reactive oxygen species (ROS) and malondialdehyde (MDA) formation with decreased levels of antioxidants such as reduced glutathione, vitamin C, catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase and glutathione-*S*-transferase. Cisplatin administration also triggered inflammatory response in rat kidneys by inducing pro-inflammatory cytokine, TNF-α, with the increased expression of myeloperoxidase (MPO). Furthermore, cisplatin increased the activity of caspase-3 and DNA damage with decreased tissue nitric oxide levels. Hesperidin treatment significantly attenuated the cisplatin-induced oxidative stress/lipid peroxidation, inflammation (infiltration of leukocytes and pro-inflammatory cytokine), apoptosis/necrosis (caspase-3 activity with DNA damage) as well as increased expression of nitric oxide in the kidney and improved renal function. Thus, our results suggest that hesperidin co-administration may serve as a novel and promising preventive strategy against cisplatin-induced nephrotoxicity.

Introduction

The treatment decisions of a disease or disorder are often based on the side effects of drugs rather than their therapeutic efficacy. Platinum derivative compound like cisplatin (cis-diamine dichloro-platinum II, CDDP), is considered as highly effective anti-tumor agent against various types of solid tumors. Unfortunately its therapeutic efficacy is limited because of its dose and duration dependent nephrotoxicity which primarily affects the S3 segment of the proximal renal tubule (Sahu et al. 2011). Although several therapeutic strategies like intensive hydration or use of alternative chemotherapeutic agents have been suggested for prevention of cisplatin-induced renal injury, about 25–35% of the patients develop a reversible decline in renal function after the first course of therapy (Kang et al. 2009).

There are multiple lines of evidence suggesting the release of pro-inflammatory cytokines (TNF-α) (Ramesh and Reeves 2004), recruitment of inflammatory cells, such as macrophages and leukocytes (Faubel et al. 2007) and mitochondrial dysfunction (Rodrigues et al. 2009) in the pathogenesis of cisplatin-induced nephrotoxicity. Growing evidence also implicates that administration of nephrotoxic doses of cisplatin produces both necrosis and apoptosis in the renal tissues by activating caspase-3 which plays a central role in the execution-phase of cell apoptosis (Miller et al. 2010; Pabla and Dong 2008). Our previous study demonstrated the role of oxidative stress/lipid peroxidation and nitric oxide in cisplatin-induced acute kidney injury (Sahu et al. 2011). In this context, combinatorial strategies which target multiple mechanisms such as improvement in impaired antioxidant defense mechanisms or diminish free radical production with anti-inflammatory and cytoprotective properties in the kidney may offer best chance for clinically meaningful prevention of nephrotoxicity.

Abbreviations: Ac-DEVD-AMC, acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin; BUN, blood urea nitrogen; BW, body weight; CAT, catalase; DCFH-DA, 2,7-dichlorofluorescein diacetate; DTNB, 5,5-dithio-bis (2-nitrobenzoic acid); NED, *N*-(1-naphthyl)-ethylene-diamine dihydrochloride; GR, glutathione reductase; TNF-α, tumor necrosis factor-α; MPO, myeloperoxidase; GPx, glutathione peroxidase; GST, glutathione-*S*-transferase; DNPH, dinitrophenyl hydrazine; MDA, malondialdehyde; NADPH, β-nicotinamide adenine dinucleotide 3-phosphate; NO, nitric oxide; PAS, periodic acid Schiff; SOD, superoxide dismutase; tROS, total reactive oxygen species.

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http://dx.doi.org/10.1016/j.phymed.2012.12.001

Hesperidin (3,5,7-trihydroxy flavanone-7-rhamnoglucoside) is a pharmacologically active bioflavonoid found in citrus fruits, with good free radical scavenging as well as anti-lipid peroxidation properties in biological membranes (Suarez et al. 1998). Hesperidin possesses highest reducing power, chelating activity on Fe^{2+}, hydrogen radical scavenging and hydrogen peroxide scavenging activities when compared with natural and synthetic antioxidants such as α-tocopherol, ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and trolox (Hussein and Othman 2011). It was reported that hesperidin protects acetaminophen-induced hepatic stress (Ahmad et al. 2012) as well as isoproterenol-induced cardiotoxicity (Selvaraj and Pugalendi 2012). Clinical and experimental data further showed the antihypertensive, lipid-lowering, insulin-sensitizing, antioxidative and anti-inflammatory properties of hesperidin (Chanet et al. 2012). However, the protective role of hesperidin against cisplatin-induced renal injury has not been investigated. Hence we proposed to investigate whether administration of hesperidin offers protection against cisplatin-induced acute renal injury and also to study the mechanism of its protection.

Materials and methods

Drugs and chemicals

Hesperidin, cisplatin, proteinase K, agarose, NADPH (β-nicotinamide adenine dinucleotide 3-phosphate), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), glutathione reductase, 2-thiobarbituric acid (TBA), glutathione peroxidase, reduced glutathione (GSH), glutathione oxidized (GSSG), catalase, 2,7-dichlorofluorescein diacetate (DCFH-DA), caspase 3 assay kit, fluorimetric, superoxide dismutase assay kit, Bradford reagent, O-dianisidine, HTAB (hexadecyl trimethyl ammonium bromide), etc. were purchased from Sigma–Aldrich Co, St Louis, MO, USA. 1-Chloro-2,4-dinitrobenzene (CDNB) was obtained from Avra synthesis Pvt. Ltd., Hyderabad, India. Hydrogen peroxide was purchased from Fisher Scientific, Mumbai, India. All other chemicals unless until mentioned were of analytical grade.

Animals

The protocol of this study was approved by the Institutional Animal Ethical Committee (IAEC) of Indian Institute of Chemical Technology (IICT), Hyderabad, India in accordance with the CPCSEA guidelines for the safe use and care of experimental animals. Male wistar rats weighing between 200–220 g were obtained from National Institute of Nutrition (NIN), Hyderabad, India and were housed them in a controlled environment (temperature, 24 ± 3 °C and relative humidity, 55 ± 15%) with a 12-h light/dark cycle. During the experimental period animals were allowed free access to food and water.

Experimental design

The animals were randomly divided into five groups, containing six rats in each. Based on our previous study, nephrotoxicity was induced by intraperitoneal (i.p) administration of cisplatin dissolved in normal saline at the dose of 7.5 mg/kg body weight (Sahu et al. 2011). Experimental design was performed as follows:

(1) *Normal control (N)*. Gum acacia suspension (2%) was administered per oral for 10 days and a single intra peritoneal (i.p) injection of 0.5 ml isotonic saline on 5th day.
(2) *Hesperidin control (H)*. Rats were given a suspension of hesperidin (200 mg/kg/day) in gum acacia (2%) per oral for 10 days.
(3) *Cisplatin control (C)*. Gum acacia suspension (2%) was administered per oral for 10 days and a single intraperitoneal (i.p) injection of cisplatin (7.5 mg/kg, i.p) dissolved in normal saline was administered on 5th day.
(4) *Hesp (100 mg/kg) + Cis(CH-100)*. Rats were given a suspension of hesperidin (100 mg/kg/day) in gum acacia (2%) per oral for 10 days and a single dose of cisplatin (7.5 mg/kg, i.p) on 5th day, 1 hour prior to hesperidin dose.
(5) *Hesp (200 mg/kg) + Cis(CH-200)*. Rats were given a suspension of hesperidin (200 mg/kg/day) in gum acacia (2%) per oral for 10 days and a single dose of cisplatin (7.5 mg/kg, i.p) on 5th day, 1 hour prior to hesperidin dose.

Sampling and biochemical assays

After 5 days of cisplatin administration, body weights of rats were recorded and blood samples were collected from intra cardiac puncture. Serum was separated by centrifugation at 4000 rpm (4 °C) for 15 min and stored at −80 °C until analysis. Rats were euthanized in CO_2 chamber and kidney tissues were dissected, weighed, immediately frozen in liquid nitrogen and stored at −80 °C until analysis. A 10% homogenate of kidney tissue was prepared in ice cold phosphate buffer saline (PBS) (0.05 M, PH 7).

Assessment of renal functions

The development of nephrotoxicity was assessed in rats after 5 days of cisplatin administration by estimating creatinine (Cr) and blood urea nitrogen (BUN) in the serum samples using auto blood analyzer (Siemens, Dimension Xpand^{plus}). Furthermore, body weight reduction and kidney to body weight ratios were calculated as indices of kidney hypertrophy.

Assessment of renal oxidative stress

A part of the homogenate was mixed with equal volume of 10% trichloroacetic acid (TCA), centrifuged at 5000 rpm for 10 min and the supernatant was used for the estimation of reduced glutathione (GSH) (Ellman 1959), malondialdehyde (MDA) (Ohkawa et al. 1979) and vitamin C (Omaye et al. 1979). The pellet sediments were used for the estimation of protein carbonyl content (Dalle-Donne et al. 2003). The remaining part of homogenate was centrifuged at 17,000 × *g* for 60 min at 4 °C and the supernatant was used for the estimations of total protein (Bradford 1976), catalase (CAT) (Aebi 1974), glutathione peroxidase (GPx) (Paglia and Valentine 1967), glutathione reductase (GR) (Carlberg and Mannervik 1975), glutathione-*S*-transferase (GST) (Habig et al. 1974), superoxide dismutase (SOD) (SOD assay kit (Sigma–Aldrich Co, St Louis, MO, USA), reactive oxygen species (ROS) and tissue nitrite levels (Sahu et al. 2011). The kidney tissue samples of rats from different experimental groups were fixed in 10% neutral buffered formalin for histopathology.

Assessment of renal inflammation

Serum was collected by centrifuging the blood at 4000 rpm for 15 min at 4 °C and used for estimation of TNF-α using commercially available kit (rat TNF-α ELISA kit, eBioscience, USA). Myeloperoxidase (MPO) activity was assessed as a marker of neutrophil infiltration according to the method of Xia and Zweier (1997). Serum lactate dehydrogenase levels were estimated as a marker of tissue injury using LDH assay kit by auto blood analyzer (Siemens, dimension Xpand^{plus}).