Biomedicine & Pharmacotherapy 85 (2017) 7–15

Available online at
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www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Protective effect of *Nigella sativa* oil on cisplatin induced nephrotoxicity and oxidative damage in rat kidney

Zeba Farooqui[a], Faizan Ahmed[a], Sana Rizwan[a], Faaiza Shahid[a], Aijaz Ahmed Khan[b], Farah Khan[a,*]

[a] Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, U.P., India
[b] Department of Anatomy, Faculty of Medicine, J. N. Medical College, Aligarh Muslim University, Aligarh 202002, U.P., India

ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form 11 November 2016
Accepted 27 November 2016

Keywords:
Cisplatin
Nephrotoxicity
Nigella sativa oil
Brush border membrane
Oxidative stress

ABSTRACT

Background: Nephrotoxicity is a severe complication in patients undergoing cisplatin (CP) chemotherapy. Previous studies in our lab have shown that administration of a single dose of CP results in decrease in the activities of brush border membrane (BBM) and free radical scavenging enzymes and induces oxidative stress in rat kidney. *Nigella sativa*, is one of the most revered medicinal plant known for its numerous health benefits. *Nigella sativa* seed/oil has been shown to improve kidney functions in animal models of acute kidney injury.
Objective: The present study was undertaken to investigate whether *Nigella sativa* oil (NSO) can prevent the CP-induced nephrotoxic effects.
Results: The effect of NSO was determined on CP induced alterations in various serum parameters and on enzymes of carbohydrate metabolism, BBM and antioxidant defense system in renal cortex and medulla. Administration of NSO (2 ml/kg bwt. orally), prior to and following a single dose CP treatment (6 mg/kg bwt. i.p), significantly attenuated the CP induced increase in serum creatinine (Scr) and blood urea nitrogen (BUN) and decrease in the activities of BBM enzymes in renal cortical and medullary homogenates as well as in isolated BBM vesicles (BBMV). NSO administration also precluded CP induced alterations in the activities of carbohydrate metabolism enzymes and in the enzymatic and non-enzymatic antioxidant parameters. Histopathological observations showed extensive kidney damage in CP treated animals and remarkably reduced renal injury in CP and NSO co-treated group.
Conclusion: The biochemical and histological data suggest a protective effect of NSO against CP-induced acute kidney injury.

1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum-II, CP) is an effective chemotherapeutic agent that has been largely used in the treatment of wide variety of solid tumors [1]. The kidney while playing its primary role as eliminator of numerous endogenous and exogenous substances including drugs, accumulate large amount of these substances particularly in the proximal tubules. The concentration of CP in the proximal tubular epithelial cells was found to be about 5 times higher than the serum concentration [2]. This disproportionate accumulation of CP and its metabolic activation to a more potent toxin in proximal tubular cells underlie, the susceptibility of proximal tubules to CP toxicity [3]. Several studies have demonstrated that acute and chronic CP treatments, cause onset of kidney injury and increase the risk of renal failure [4,5]. Approximately 25–35% of patients develop evidence of acute kidney injury (AKI) following a single dose of

Abbreviations: ACPase, Acid phosphatase; ALP, Alkaline phosphatise; ALT, Alanine aminotransferase; AKI, Acute kidney injury; AST, Aspartate aminotransferases; BUN, Blood urea nitrogen; BBM, Brush border membrane; CAT, Catalase; Scr, Serum creatinine; BBMV, BBM vesicles; CP, Cisplatin; FBPase, Fructose 1,6-bisphosphatase; G6Pase, Glucose 6-phosphatase; G6PDH, Glucose 6-phosphate dehydrogenase; GGTase, γ-Glutamyl transferase; Glc, Glucose; GR, Glutathione reductase; GSH, Glutathione; GSH-Px, Glutathione peroxidise; GST, Glutathione S-transferase; HK, Hexokinase; H2O2, Hydrogen peroxide; LAP, Leucine aminopeptidase; LDH, Lactate dehydrogenase; LPO, Lipid peroxidation; MDA, Malondialdehyde; MDH, Malate dehydrogenase; ME, Malic enzyme; μm, Micrometer; NADPH, Nicotinamide adenine dinucleotide phosphate reduced; NADP, Nicotinamide adenine dinucleotide phosphate; NSO, *Nigella sativa* oil; Pi, Inorganic phophate; PUFA, Polyunsaturated fatty acids; ROS, Reactive oxygen species; SH, Sulfhydryl; SOD, Superoxide dismutase; TCA, Tricarboxylic acid; TR, Thioredoxin reductase.

* Corresponding author.
E-mail address: dr.farahkhan23@gmail.com (F. Khan).

http://dx.doi.org/10.1016/j.biopha.2016.11.110
0753-3322/

cisplatin [6]. AKI remains a significant cause of increased morbidity and mortality among patients undergoing CP chemotherapy.

Morphologically, CP nephrotoxicity is characterized by the loss of microvilli, tubular cell vacuolization, tubular dilation and condensation of nuclear chromatin. CP induced injury and necrosis has been shown to be localized predominantly in S3 subsegment of proximal tubules. Tubular damage may range from a mere loss of brush border of epithelial cells to an overt tubular necrosis in severe cases. Decreased glomerular filtration rate and increased serum creatinine and blood urea nitrogen levels reflect CP-induced alterations in renal functions [6,7]. Increasing evidences indicate that multifactorial mechanisms are involved in cisplatin-induced AKI and some of the well known mechanisms are enhanced generation of free radicals such as hydroxyl and superoxide radicals, mitochondrial dysfunction, increased activity of calcium independent nitric oxide synthase and more recently apoptosis [8,9]. Mitochondrial dysfunction is considered as a key event in cisplatin induced renal damage. Recent reports signified that CP accumulates in the mitochondria and induces the generation of mitochondrial reactive oxygen species (ROS), which trigger oxidative stress and result in mitochondrial deterioration [8,10]. Despite side effects, CP remains the drug of preference in chemotherapy, mainly because of its efficacy and low cost. Therefore, it is important to develop effective strategies to prevent CP induced AKI. Natural antioxidants such as quercetin, rutin and hesperidin have been used as therapeutic agents against CP induced nephrotoxicity in experimental animals [11,12]. These studies indicate that naturally occurring dietary substances having free radical scavenging and/or antioxidant properties could have excellent scope in mitigation of CP induced toxicity.

Nigella sativa (NS) is an annual flowering plant that belongs to the family Ranunculaceae. Its seeds and other derivatives have been used in food for centuries in Middle East, Northern Africa and Southwest Asia. *Nigella sativa* is an enriched source of nutritionally essential constituents and its oil (NSO) is rich in polyunsaturated fatty acids (PUFA), phytosterols and several other phytochemicals including thymoquinone, carvacrol, t-anethole and 4 terpinol, that exhibit strong antioxidant properties. The synergistic antioxidant activities of these NSO constituents might contribute to the therapeutic effects attributed to NS seed and/or its oil [13]. Presence of omega (ω)-3 and ω-6 PUFA in the recommended optimal dietary intake ratio of 1:4, makes NSO an important source of PUFA and that might be an additional factor contributing to its healing effects. Several studies have demonstrated marked protective effect of NSO against toxicities induced by a variety of oxidative stress generating agents including gentamicin induced nephrotoxicity [14], propoxur-induced brain injury [15], alcohol induced gastric mucosal injury [16], cyclosporine induced cardiotoxicity [17] and more recently CP induced hepatotoxicity [18]. Preliminary reports have also shown partial protection by NSO against CP induced nephrotoxicity [19]. However the present study illustrates the effect of orally administered NSO on CP induced damage in renal cortex and medulla separately. We also report for the first time the protective effect of NSO on CP induced alterations in the enzymes of carbohydrate metabolism and renal brush border membrane (BBM). These studies are important since disruption of energy metabolism and alterations in the activities of BBM enzymes can affect the reabsorptive functions of the kidney and lead to AKI.

2. Material and methods

2.1. Chemicals and drugs

Nigella sativa oil was supplemented from Mohammedia Products, Red Hills, Nampally, Hyderabad, India. Cisplatin was obtained from Sigma–Aldrich Chemical Corp., St. Louis, MO, USA. All other chemicals used were of analytical grade and were purchased either from Sigma Chemical Corp. or SRL (Mumbai, India). CP solution (2 mg/ml) was freshly prepared in 0.9% normal saline by continuous stirring at room temperature for 10 min.

2.2. Diet

A nutritionally adequate laboratory pellet diet was obtained from Aashirwaad Industries, Chandigarh (1544, Sector 38-B, Chandigarh, India).

2.3. Ethical statement for animal experimentation

Animal experiments were permitted by Ministry of Environment, Forests and Climate Change, Government of India under registration no. 714/GO/Re/02/CPCSEA issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) dated 29th October 2014 and approved by the Institutional Animal Ethic Committee (IAEC) of Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, India.

2.4. Experimental design

Adult male Wistar rats (6 rats/group) weighing between 150–200 gm were purchased from Central Animal House Facility, Jamia Hamdard University, New Delhi, India. All animals were acclimatized for a week on standard rat laboratory pellet diet and allowed water ad libitum under controlled conditions of $25 \pm 2\,^{\circ}C$ temperature, $50 \pm 15\%$ relative humidity and normal photoperiod (12 h dark and light). Four groups of rats entered the study after acclimatization; Control (C), CP treated (CP), *Nigella sativa* oil treated (NSO) and *Nigella sativa* oil + CP treated (CPNSO). Rats in the group CPNSO were administered NSO (2 ml/kg bwt, orally) by gavage for 14 days prior to and 4 days following CP treatment. However, rats in NSO group were administered NSO alone without CP treatment. A single intraperitoneal injection of CP (6 mg/kg bwt.) in 0.9% normal saline was administered to the animals in CP and CPNSO groups, while animals in the control and NSO group received an equivalent amount of normal saline. Body weight of rats was recorded at the start and completion of the treatment protocol. Urine samples were collected for 4 h in standard metabolic cages a day before the sacrifice. Animals were sacrificed on the fifth day after CP injection under light ether anesthesia. Blood samples were collected from overnight fasted rats and the kidneys were removed and processed for the preparation of cortical and medullary homogenates and brush border membrane vesicles (BBMVs) as described below. Each preparation of BBMV was made by pooling the tissue (cortex) from 2 animals in each group. Thus, the number of animals mentioned above contributed to 3 different preparations for each group. Analyses of various parameters were performed simultaneously under similar experimental conditions to avoid any day to day variations.

2.5. Preparation of homogenates and brush border membranes

The decapsulated kidneys were kept in ice-cold 154 mM NaCl, 5 mM Tris–HEPES buffer, pH 7.5. The cortical and medullary region were carefully separated and homogenized separately by using glass-teflon homogenizer (Thomas PA, USA) by passing 5 pulses at $4\,^{\circ}C$. The homogenates were then subjected to high speed Ultra-Turrex Kunkel homogenizer (Type T-25, Janke & Kunkel GMBH & Co. KG. Staufen) for 3 pulses of 30 s each with an interval of 30 s between each stroke. One part of the homogenate was saved at $-20\,^{\circ}C$ for estimation of total sulfhydryl (-SH) groups, GSH and