Free Radical Research, February 2011; 45(2): 177–187

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healthcare

Amelioration of cisplatin-induced nephrotoxicity in mice by oral administration of diphenylmethyl selenocyanate

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(Received date: 25 March 2010; In revised form date: 31 August 2010)

Abstract

Cisplatin is one of the most potent and active cytotoxic drug in the treatment of cancer. However, side-effects in normal tissues and organs, notably nephrotoxicity in the kidneys, limit the promising efficacy of cisplatin. The present study was designed to ascertain the possible *in vivo* protective potential of a synthetic organoselenium compound diphenylmethyl selenocyanate (3 mg/kg.b.w.) against the nephrotoxic damage induced by cisplatin (5 mg/kg.b.w. for 5 days) in Swiss albino mice. Treatment with diphenylmethyl selenocyanate markedly reduced cisplatin-induced lipid peroxidation, serum creatinine and blood urea nitrogen levels. Renal antioxidant defense systems, such as glutathione-S-transferase, glutathione peroxidase, superoxide dismutase, catalase, activities and reduced glutathione level, depleted by cisplatin therapy, were restored to normal by the selenium compound. The selenium compound also reduced renal tubular epithelial cell damage, nitric oxide levels and expression of COX-2, and iNOS in kidneys injured by cisplatin. These results demonstrate the protective effect of diphenylmethyl selenocyanate against cisplatin-induced nephrotoxicity in mice.

Keywords: *Cisplatin, diphenylmethyl selenocyanate, oxidative stress, nephrotoxicity*

Introduction

Cisplatin, a platinum co-ordinated complex, is currently one of the most important cytostatic agents, against a variety of neoplasms [1]. The ability of cisplatin to react with nucleophilic bases in DNA and form intra- and inter-strand cross-links has been suggested to be the main mechanism behind its anticancer activity [2,3]. The major adverse effect of cisplatin use is nephrotoxicity, in which kidney proximal tubule cells are especially sensitive [4]. As a principal site for drug filtration, concentration and excretion renal tissues and cells are exposed to toxic concentration of cisplatin during cancer therapy, as a result nephrotoxicity is the major factor during cisplatin treatment. Cisplatin generates active oxygen species such as superoxide anion and hydroxyl radical [5–7] and inhibit the activity of antioxidant enzymes and enhanced lipid peroxidation in renal tissue [8]. The nephrotoxic injury typically affects the S3 segment of the proximal tubule in the outer stripe of the outer medulla including a thinning or local loss of brush border, cellular swelling and condensation of the nuclear chromatin and focal areas of necrosis [9].

Continued aggressive high-dose cisplatin chemotherapy necessitates the investigation of way for prevention of the dose-limiting side-effects that inhibits the cisplatin administration at tumoricidal doses. It has been reported that dietary antioxidants may detoxify ROS/RNS and also enhance the anti-cancer efficacy of chemotherapeutic drugs and reduce the side-effects [10]. Selenium is an essential dietary trace

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ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.3109/10715762.2010.521155

element, it acts as a potent antioxidant against oxidative stress induced by xenobiotic compounds of diverse nature [11]. Inorganic selenium, as well as some of the organoselenium compounds, has been found to be an effective chemoprotective agent in animal assays [12]. However, the clinical use of inorganic selenium compounds might be limited due to their potential toxicity. Organoselenium compounds are usually found to be less toxic than inorganic forms of the element [13,14] and the organoselenium compound exhibits better antioxidant activity in different experimental models [15,16]. Studies in our laboratory have established that diphenylmethyl selenocyanate has the ability to counter oxidative stress induced by xenobiotics of diverse nature *in vivo* [17,18]. The compound also protects against chemotherapy cytotoxicity *in vivo*. We found that chemoprotection can be obtained without blocking the anti-tumour effects of chemotherapy [19,20]. The objective of the present study is to obtain information about the possible protective effects of diphenylmethyl selenocyanate against cisplatin-induced nephrotoxicity in Swiss albino mice.

Materials and methods

Experimental animals

Adult (5–6 weeks) Swiss albino male mice (23 ± 2 g), bred in the animal colony of Chittaranjan National Cancer Institute (CNCI) (Kolkata, India) and used for this study, were maintained at controlled temperature under alternating light and dark conditions. Standard food pellets (Lipton India Ltd., Calcutta, West Bengal, India) and drinking water were provided *ad libitum*. The experiments were carried out following strictly the Institute's guideline for the Care and Use of Laboratory Animals.

Chemicals

Cisplatin from Cadila Pharmaceuticals (Bhat, Ahmedabad, India), diphenylmethyl bromide, potassium selenocyanate (KSeCN), 1-Chloro-2, 4-dinitrobenzene (CDNB), ethylene diaminetetra acetic acid (EDTA), reduced glutathione (GSH), pyrogallol, 5,5'-dithio-bis(2-nitro benzoic acid) (DTNB), sodium dodecyl sulphate (SDS), bovine serum albumin (BSA), thioglycolate broth, acrylamide, bis-acrylamide, ammonium persulphate, polyvinylidene difluride membrane (PVDF), TEMED, *β*-nicotinamide adenine dinucleotide phosphate (reduced) (*β*-NADPH), glutathione reductase, RPMI 1640, N-(1-napthyl) ethylenediamine dihydrochloride and sodium azide (NaN_3) were obtained from Sigma-Aldrich Chemicals Private Limited (Bangalore, India). Hydrogen peroxide 30% (H_2O_2), thioberbituric acid (TBA), propylene glycol, hexane, orthophosphoric acid and sodium nitrite were obtained from Merck Specialities Limited (Worli, Mumbai, India). Sulphanilamide, Tris-HCl and acetone were obtained from Sisco Research Laboratories Private Limited (Mumbai, Maharashtra, India). Diethyl ether, dipotassium hydrogenphosphate (K_2HPO_4) and potassium dihydrogenphosphate (KH_2PO_4) were obtained from Spectrochem Private Limited (Mumbai, Maharashtra, India). Foetal calf serum (FCS) was obtained from Hyclone Laboratories (Logan, UT). Serum Urea and Creatinine assay kits were obtained from Span Diagnostics Limited (Udhna, Surat, India). Rabbit polyclonal COX-2, iNOS, β-actin primary antibody, Anti-rabbit IgG-HRP (secondary antibody) and luminol reagent were obtained from Santa Cruz Biotechnology, Inc (California, USA).

Synthesis of diphenylmethyl selenocyanate

Diphenylmethyl selenocyanate was prepared following a literature procedure [21]. Briefly, diphenylmethyl bromide was treated with potassium selenocyanate (KSeCN) in acetone at 60–70°C for 5 h. Acetone was removed under reduced pressure and the resulting solid was extracted with diethyl ether. Usual work up then afforded the desired compound (Figure 1), which was crystallized from hexane to get a colourless crystalline solid m.p. 68–69°C. The compound was 99.9% pure. The purity was determined by using HPLC: waters (μ-Bondapak C-18 steel column; 30 cm × 3.9 mm; isocratic mobile phase acetonitrile water (50:50) at a flow rate of 1.0 ml/min at ambient temperature; UV detection at 220 nm; retention time 9.11 min). Purity of compound was checked at a regular interval during the experiment.

Drug preparation

Synthetic organoselenium compound diphenylmethyl selenocyanate was used as a suspension in 5.5% propylene glycol in water. It was prepared each day of experiment just before treatment.

Experimental groups

Animals were divided into five groups containing six animals ($n = 6$) in each group (Figure 2).

- *Control group* (*Gr. I*): Animals of this group were given 5.5% propylene glycol in water by oral gavage from day 1 to day 9 and kept as control.
- *Diphenylmethyl selenocyanate-treated group* (*Gr. II*): Animals were treated with diphenylmethyl selenocyanate only at a dose of 3 mg/kg. b.w. throughout the experimental period.
- *Cisplatin-treated group* (*Gr. III*): Each animal was injected with cisplatin intraperitoneally at a dose of 5 mg/kg b.w. from day 1 to day 5.

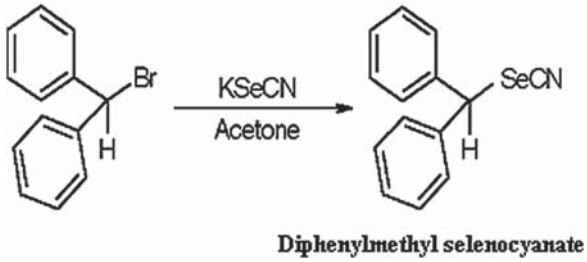

Figure 1. Synthesis of diphenylmethyl selenocyanate.

- *Pre-treatment group* (*Gr. IV*): Diphenylmethyl selenocyanate was administered orally at a dose of 3 mg/kg b.w., 7 days prior to cisplatin and then continued up to day 9 and cisplatin was given from day 1 to day 5.
- *Concomitant treatment group* (*Gr. V*): Diphenylmethyl selenocyanate was administered orally at a dose of 3 mg/kg b.w. from day 1 to day 9 and cisplatin was given from day 1 to day 5.

The animals were sacrificed on day 10 and the parameters described below were studied.

Biochemical estimation

Quantitative estimation of lipid peroxidation (LPO). LPO was estimated in kidney microsomal fraction. The level of lipid peroxides formed was measured using thiobarbituric acid and expressed as nmol of thiobarbituric acid reactive substances (TBARS) formed per mg of protein using the extinction co-efficient of 1.56×10^5 M^{-1} cm^{-1} [22].

Estimation of reduced glutathione (GSH) level. GSH level was estimated in kidney cytosol spectrophotometrically by determination of dithiobis (2-nitro)-benzoic acid (DTNB) reduced by –SH groups by measuring the absorbance at 412 nm. The level of GSH was expressed as nmol/mg of protein [23].

Estimation of glutathione-S-transferase (GST) activity. GST activity was measured in the kidney cytosol. The enzyme activity was determined from the increase in absorbance at 340 nm with 1-chloro-2-4-dinitrobenzene (CDNB) as the substrate and specific activity of the enzyme was expressed as formation of 1-chloro-2-4-dinitrobenzene (CDNB)-GSH conjugate per minute per mg of protein [24].

Estimation of glutathione peroxidase (GPx) activity. GPx activity was measured by NADPH oxidation using a coupled reaction system consisting of reduced glutathione, glutathione reductase and hydrogen peroxide [25]. Briefly, 100 µl of enzyme sample was incubated for 10 min with 800 µl reaction mixture (0.25 M potassium phosphate buffer containing 2.5 mM EDTA and 2.5 mM sodium azide, 10 mM reduced glutathione, 2.5 mM NADPH and 2.4 units of glutathione reductase). The reactions started by adding 100 µl H_2O_2 and follow the decrease in NADPH absorbance at 340 nm for 3 min. The enzyme activity was expressed as micromol NADPH utilized/min/mg of protein, using an extinction coefficient of NADPH at 340 nm as 6200 M^{-1} cm^{-1}.

Estimation of catalase (CAT) activity. CAT in kidney cytosol was determined spectrophotometrically at 250 nm and expressed as unit/mg of protein where the unit is the amount of enzyme that liberates half the peroxide oxygen from H_2O_2 in seconds at 25° C [26].

Estimation of superoxide dismutase (SOD) activity. SOD activity was determined by quantification of pyrogallol auto-oxidation inhibition and the amount of enzyme necessary for inhibiting the reaction by 50%. Auto-oxidation of pyrogallol in Tris-HCL buffer (50 mM, pH 7.5) was measured by increases in absorbance at 420 nm [27,28].

Assay of nitrite production in peritoneal macrophages

Nitric oxide (NO) produced by macrophages quickly reacts with oxygen to produce nitrate (NO_3^-) and nitrite (NO_2^-) ions. Accumulation of nitrite in the medium was measured spectrophotometrically based on Griess reaction [29]. In brief, 50 µl of Griess reagent mixture of 0.1% N-(1-napthyl) ethylenediamine dihydrochloride, 1% sulphanilamide and 2.5% orthophosphoric acid was reacted with 50 µl of sample (cell-free supernatant) at room temperature for 10 min and the NO_2^- concentration was determined by absorbance at 550 nm in comparison with the sodium nitrite ($NaNO_2$) standards. Data were represented as micromol of nitrite produced in cell free supernatant.

Determination of blood urea nitrogen (BUN) and creatinine levels

Blood samples were collected from mice and centrifuged at 3000 rpm for 5 min for serum separation. Then the BUN and creatinine levels were measured spectrophotometrically by a standard enzymatic method using commercial kits [30,31].

Estimation of protein. Total protein content in tissue homogenate during biochemical analysis assay was measured through Lowry method using Folin-Phenol

reagent. The absorbance of the colour was measured against the colourless blank sample at 660 nm using the Shimadzu160A UV Spectrophotometer [32].

Tissue section preparation and histopathological evaluation

Kidneys were collected from all groups of mice and washed in PBS and soaked on blotting paper to remove the blood. Tissues were then fixed in 10% neutral buffered formalin for 24 h. The tissue samples were dehydrated in ascending concentrations of ethanol, cleared in xylene and embedded in paraffin to prepare the block. Kidney tissues were sectioned, mounted on slides and stained. The 5 µm serial sections were used for staining with haematoxylin-eosin (HE). Stained sections were evaluated by observing the arrangement of kidney architecture, with a Leitz Laborlux D light microscope (Leitz Wetzlar, Germany). Photomicrographs were taken with a still camera (Wild Mikrophot MPS 05) (Leitz Wetzlar) attached to the microscope.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis western blotting for COX-2 and iNOS

Kidney tissues were removed and washed in PBS at 4°C. The whole tissues were cut into pieces and homogenized in five volumes of ice-cold homogenizing buffer (0.1 M NaCl, 0.01 M Tris-Cl, 0.001 M EDTA) containing phenylmethylsulphonyl fluoride (1 mM), aprotinin (1 µg/ml) and leupeptin (0.1 mM) at 13 000 g for 1 h at 4°C. The supernatants were estimated for their protein content using bovine serum albumin as a standard. Western blot analysis was carried out as described by Singh et al. [33] and Laemmli [34]. Two separate gels, one 10% (for COX-2) and another 8% (for iNOS), were run simultaneously. The tissue homogenate equivalent to 50 µg protein was mixed with gel loading buffer (100 mM Tris-Cl, 200 mM β-mercaptoethanol, 4% SDS, 0.2% bromophenol blue and 20% glycerol). The samples were then boiled for 5 min and resolved in polyacrylamide gel along with pre-stained molecular weight markers (Peq Gold protein marker II, PeQ Lab, GmbH).

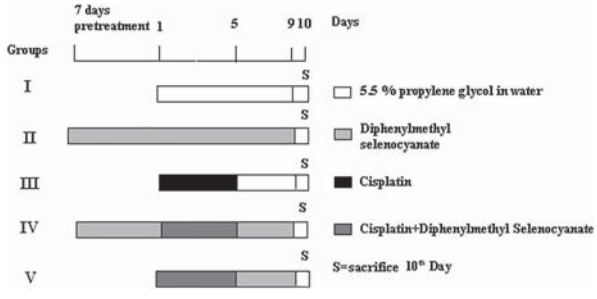

Figure 2. Experimental design to evaluate the effects of diphenylmethyl selenocyanate on the nephrotoxicity of cisplatin.

Electrophoretically resolved proteins were transblotted overnight onto PVDF membrane and subsequently blocked with the blocking buffer (0.1% tween 20 in TBS). The blots were then incubated with the respective primary antibodies, viz., anti-COX-2 and anti-iNOS rabbit polyclonal antibody (Santa Cruz Biotech. Inc.) diluted 1:1000 in blocking buffer. After extensive washing, blots were re-incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (IgG-HRP, Santa Cruz Biotech. Inc.) diluted 1:5000 in blocking buffer. The protein bands were then visualized by using luminol reagent (Santa Cruz Biotech. Inc.) exposed to X-ray film. *β*-actin antibody was (Santa Cruz Biotech. Inc.) used to demonstrate equal loading of sample protein onto the gel.

Statistical analysis

The differences in mean values of different groups were tested and the values are expressed as mean ± SD. The data were analysed using the student's *t*-test and $p < 0.05$ was considered to be significant.

Results

The administration of diphenylmethyl selenocyanate alone did not produce any significant change in the parameters investigated when compared to control. There is no significant change in bodyweights, food or water consumption of these experimental animals post-administration of the diphenylmethyl selenocyanate or pre- or post-administration of the cisplatin vs controls.

Inhibition of microsomal LPO level

The administration of diphenylmethyl selenocyanate alone did not increase lipid peroxidation compared

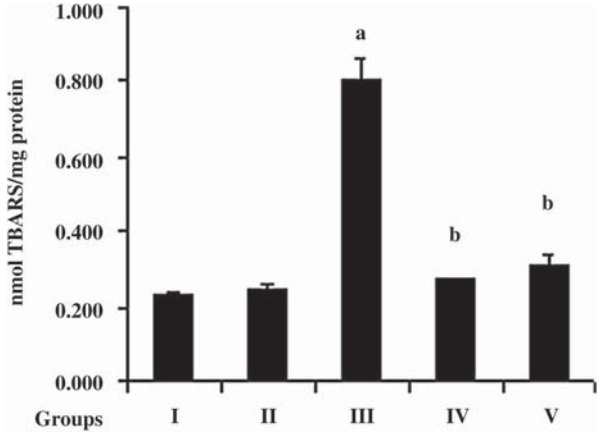

Figure 3. Effect of diphenylmethyl selenocyanate on LPO level in kidney of cisplatin-treated mice. Data represent the mean ± SD. $n = 6$. $^{a}p < 0.05$ with respect to Gr. I (control group), $^{b}p < 0.05$ with respect to Gr. III (cisplatin-treated group).

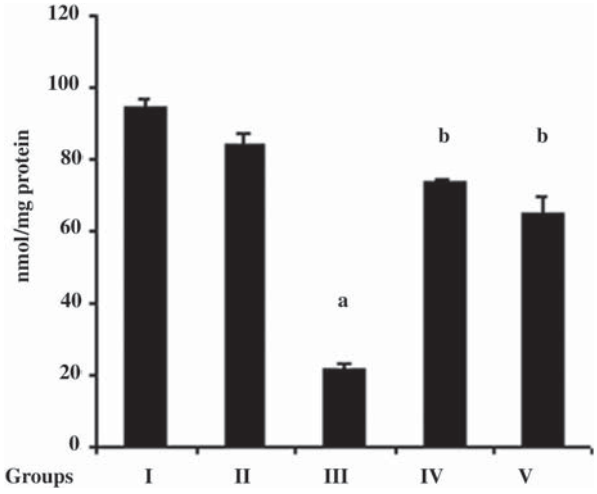

Figure 4. Effect of diphenylmethyl selenocyanate on GSH level in kidney of cisplatin-treated mice. Data represent the mean ± SD. $n = 6$. $^{a}p < 0.05$ with respect to Gr. I (control group), $^{b}p < 0.05$ with respect to Gr. III (cisplatin-treated group).

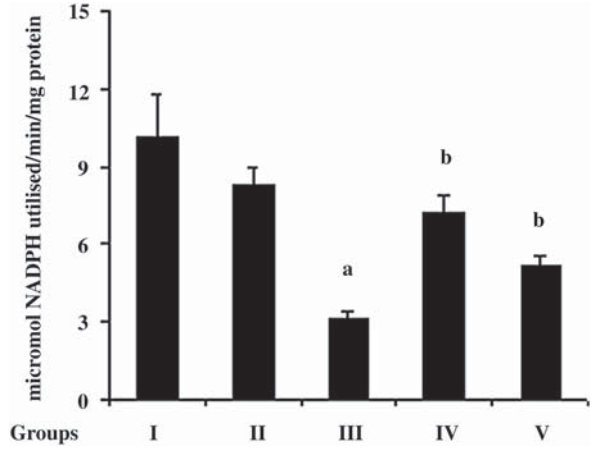

Figure 6. Effect of diphenylmethyl selenocyanate on GPx activity in kidney of cisplatin-treated mice. Data represent the mean ± SD. $n = 6$. $^{a}p < 0.05$ with respect to Gr. I (control group), $^{b}p < 0.05$ with respect to Gr. III (cisplatin-treated group).

to the control group (Gr. I). Injection of cisplatin increased the level of LPO significantly ($p < 0.05$) by 70.5% in Gr. III as compared to Gr. I (Figure 3). Kidney tissues from cisplatin-treated mice showed a statistically significant reduction in the LPO level by 65.7% (Gr. IV) in animals pre-treated with diphenylmethyl selenocyanate and by 61.2% (Gr. V) in the case of concomitant treatment with the selenium compound as compared to the cisplatin-only treated group.

Enhancement of GSH level

The kidney GSH concentration decreased significantly ($p < 0.05$) by 76.3% in cisplatin-treated animals compared to the control (Figure 4). Diphenylmethyl selenocyanate treatment prevented this depletion of GSH by 70.3% (Gr. IV) in the case of pre-treatment and by 66.3% (Gr. V) in the case of concomitant treatment compared to that seen with cisplatin alone (Gr. III).

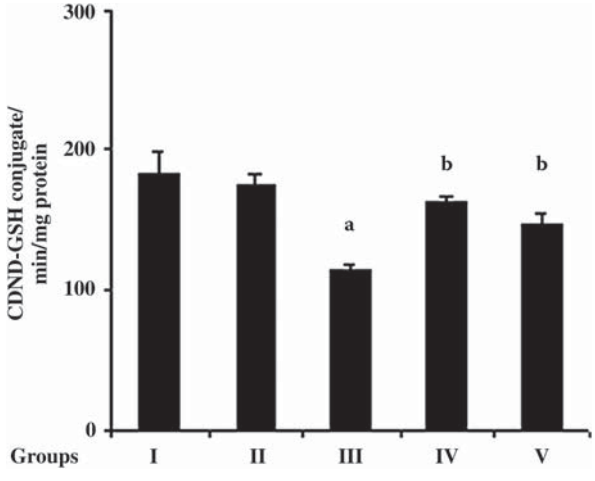

Figure 5. Effect of diphenylmethyl selenocyanate on GST activity in kidney of cisplatin-treated mice. Data represent the mean ± SD. $n = 6$. $^{a}p < 0.05$ with respect to Gr. I (control group), $^{b}p < 0.05$ with respect to Gr. III (cisplatin-treated group).

Modulation of GST activity

The activity of GST in the kidney of animals treated with cisplatin showed a decrease of 37.6% (Gr. III) as compared to the control group (Figure 5). Pre-treatment with diphenylmethyl selenocyanate caused the GST activity to rise sharply by 29.5% (Gr. IV); however concomitant treatment with this compound elevated GST activity by 22.9% (Gr. V) as compared to cisplatin alone (Gr. III).

GPx activity was significantly enhanced

GPx activity in the kidney decreased significantly ($p < 0.05$) by 69.3% (Gr. III) after cisplatin administration (Figure 6). Diphenylmethyl selenocyanate treatment increased the enzyme activity significantly by 56.9% in the case of pre-treatment and by 39.2% in the case of concomitant treatment as compared to the cisplatin-alone treated group.

Increase of CAT activity

CAT activity in the kidney decreased significantly ($p < 0.05$) by 53.9% (Gr. III) in the cisplatin-treated group compared to the control (Figure 7). Administration of diphenylmethyl selenocyanate effectively prevented the decline of CAT activity and restored the level by 43.0% (Gr. IV) in the case of pre-treatment and by 29.3% (Gr. V) in the case of concomitant treatment as compared to cisplatin alone.

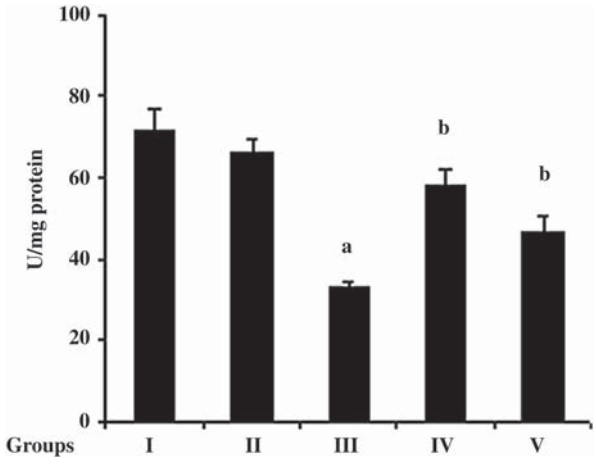

Figure 7. Effect of diphenylmethyl selenocyanate on CAT activity in kidney of cisplatin-treated mice. Data represent the mean ± SD. $n = 6$. $^{a}p < 0.05$ with respect to Gr. I (control group), $^{b}p < 0.05$ with respect to Gr. III (cisplatin-treated group).

Amelioration of SOD activity

Kidney SOD activity decreased significantly ($p < 0.05$) by 52.0% (Gr. III) in cisplatin-treated animals compared to the control (Gr. I) (Figure 8). Administration of diphenylmethyl selenocyanate, 7 days prior to cisplatin treatment, increased the activity of SOD by 45.5% (Gr. IV) and concomitant administration of the Se-compound increased the enzyme activity by 31.7% (Gr. V) as in comparison to the cisplatin-alone treated group.

Modulation of BUN and creatinine levels

Cisplatin caused a marked reduction in renal functions, as characterized by significant increased in BUN and creatinine levels (Table I). BUN and creatinine

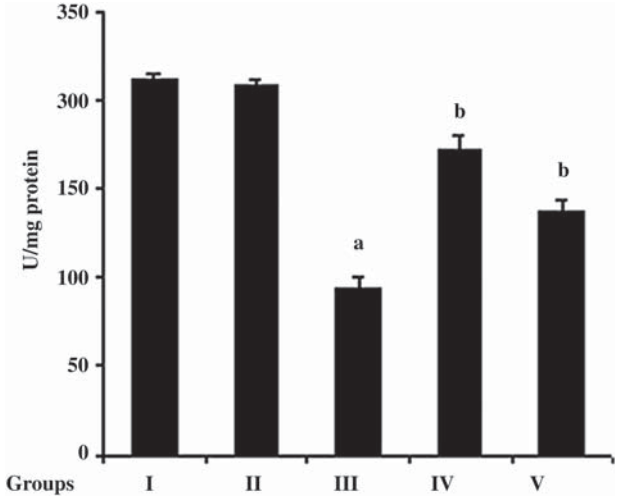

Figure 8. Effect of diphenylmethyl selenocyanate on SOD activity in kidney of cisplatin-treated mice. Data represent the mean ± SD. $n = 6$. $^{a}p < 0.05$ with respect to Gr. I (control group), $^{b}p < 0.05$ with respect to Gr. III (cisplatin-treated group).

Table I. Effect of diphenylmethyl selenocyanate on BUN and creatinine levels in mice treated with cisplatin.

Groups	BUN (mg/dl)	Creatinine (mg/dl)
I	16.9 ± 1.3	0.66 ± 0.05
II	20.3 ± 1.4	0.58 ± 0.04
III	56.8 ± 1.8[a]	3.1 ± 0.31[a]
IV	23.5 ± 0.69[b]	0.75 ± .04[b]
V	27.9 ± 0.78[b]	1.2 ± 0.13[b]

Data represent the mean ± SD. $n = 6$. $^{a}p < 0.05$ with respect to Gr. I (control group), $^{b}p < 0.05$ with respect to Gr. III (cisplatin-treated group).

levels were significantly ($p < 0.05$) elevated by 70.2% and 78.7% (Gr. III) in the cisplatin-treated group as compared to the Control (Gr. I). Pre-treatment with diphenylmethyl selenocyanate reduced the elevated levels of BUN and creatinine by 58.6% and 75.8% (Gr. IV) and concomitant administration of the selenium compound also reduced the levels by 50.8% and 61.2% (Gr. V), respectively, as compared to the cisplatin-alone treated group (Gr. III).

Inhibition of nitrite production in peritoneal macrophages

The nitrite level of the animals treated with cisplatin increased significantly ($p < 0.05$) by 47.6% (Gr. III) as compared to vehicle-treated (Figure 9). The level of nitrite production was decreased significantly ($p < 0.05$) by 35.7% (Gr. IV) in the case of pre-treatment and by 27.6 % (Gr. V) in the case of concomitant treatment as compared to the level of cisplatin alone.

COX-2 and iNOS expression

Expression of COX-2 protein in different experimental groups has been represented in Figure 10A COX-2

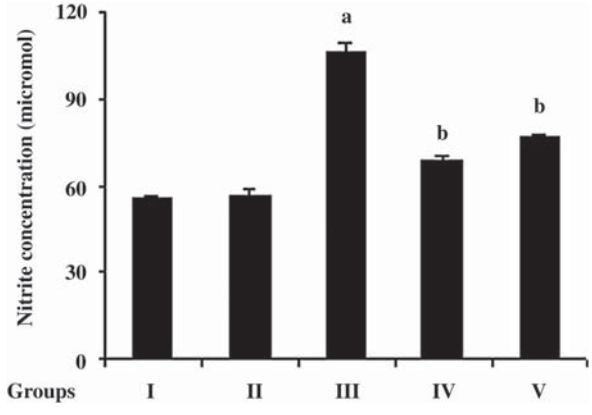

Figure 9. Effect of diphenylmethyl selenocyanate on Nitrite release from peritoneal macrophages of cisplatin treated mice. Data represent the mean ± SD. $n = 6$. $^{a}p < 0.05$ with respect to Gr. I (control group), $^{b}p < 0.05$ with respect to Gr. III (cisplatin-treated group).

immunoreactivity with polyclonal COX-2 antibody was less detectable in the kidney fraction of control mice. In contrast, dark immunoreactive band of COX-2 at a position of 72 kDa (molecular weight of COX-2) was observed in animals treated with cisplatin only (Gr. III). In pre-treatment and concomitant treatment groups, two lanes showed low expression of COX-2.

Expression of iNOS protein in different experimental groups has been represented in Figure 10A. Expression of iNOS was less detectable in the kidney fraction of control mice (Gr. I), whereas a dark expression of the protein was observed in the cisplatin-only treated mice (Gr. III). iNOS expression was less visualized in the case of the animals pre-treated (Gr. IV) and concomitantly (Gr. V) treated with diphenylmethyl selenocyanate.

The relative intensities of bands obtained from western blotting were calculated for COX-2 (Figure 10B) and iNOS (Figure 10C) with the use of the 1D SDS analysis software (Version 4.6.1, Quantityone, Biorad, USA). The relative intensity of iNOS and COX-2 band was increased significantly ($p < 0.05$) in the cisplatin-treated group compared to the control group. However, pre-treatment and concomitant treatment with diphenylmethyl selenocyanate decreased the expression of iNOS and COX-2 significantly ($p < 0.05$) in comparison to the cisplatin-only treated group.

Histopathological examination

The toxic effect of cisplatin was confirmed by the detection of morphological alterations in kidney slices (Figures 11A–E). Diphenylmethyl selenocyanate alone did not affect the kidney tissues. Cisplatin treatment resulted in severe tubular injury reflected by the necrosis of tubular epithelial cells. These alterations were attenuated by treatment with the selenium compound. Pre-treatment with the compound resulted in remarkable improvement in the tubular cell injury as compared to the cisplatin-only treated group.

Discussion

Cisplatin, one of the most active cytotoxic druga used in the treatment of cancer, induces mitochondrial

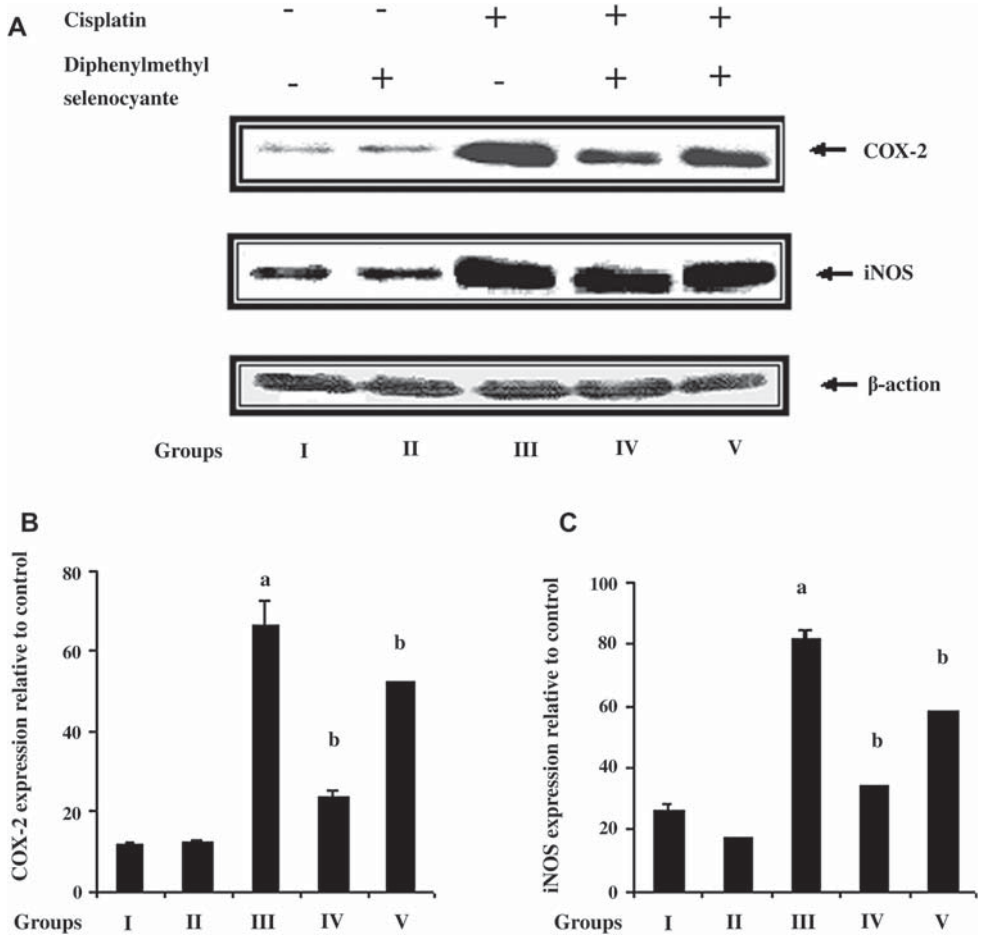

Figure 10. Expression of COX-2 and iNOS protein after immunoblotting in different groups (A). The relative intensities were evaluated for COX-2 (B) and iNOS (C). β-actin was used as internal control for equal loading of proteins. Data represent the mean ± SD. n = 6. [a]$p < 0.05$ with respect to Gr. I (control group), [b]$p < 0.05$ with respect to Gr. III (cisplatin-treated group).

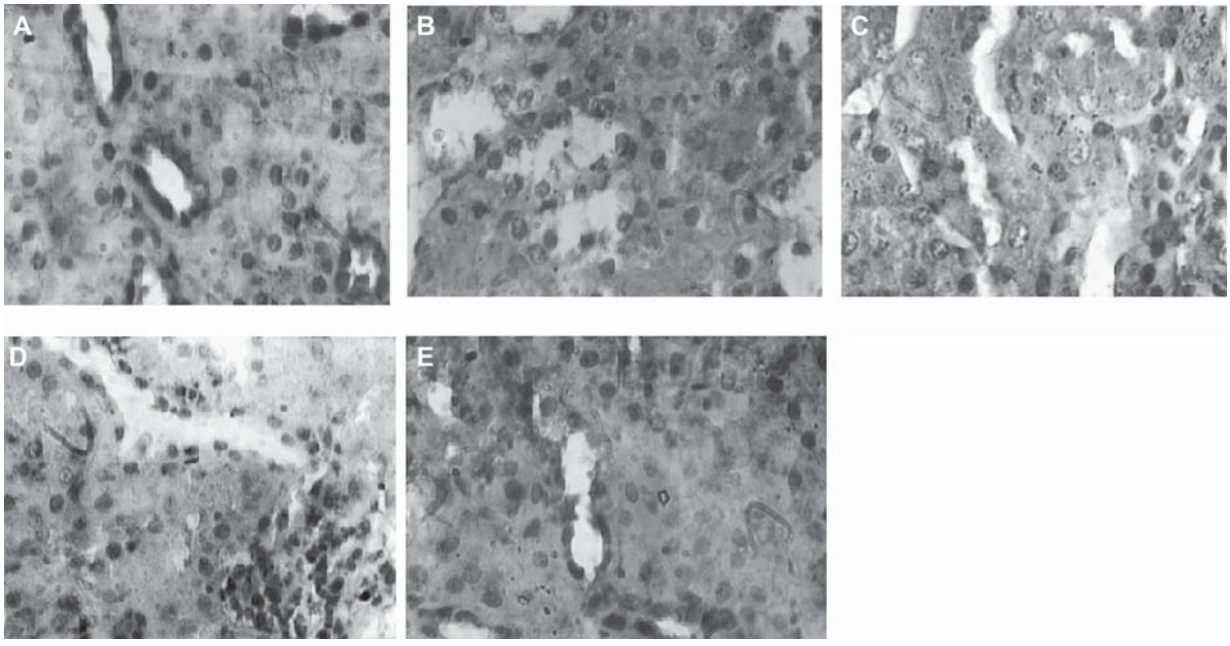

Figure 11. Showing photomicrograph of kidney section of mice stained with haematoxylin and eosin, (A) normal histological appearance of kidney (control); (B) normal tubules of kidney (diphenylmethyl selenocyanate treated); (C) acute tubular epithelial cell necrosis (cisplatin-treated); (D) significant improvement from tubular cell necrosis (pre-treatment); (E) mild tubular epithelial cell necrosis (concomitant treatment).

dysfunctions, particularly the inhibition of the electron transfer system, thereby resulting in enhanced ROS production [35,36] and subsequent tissue damage. The administration of antioxidants prior to cisplatin treatment has been used to protect against nephrotoxicity [37]. In the present study LPO level increased significantly and renal antioxidant status, such as GST, GPx, CAT, SOD activities and GSH levels were decreased significantly in the cisplatin-treated group compared to the vehicle-treated. These observations support the hypothesis that the mechanism of nephrotoxicity in the cisplatin-treated group is related to depletion of the renal antioxidant defense system. LPO constitutes complex chain reaction of free radicals, which leads to the degradation of polyunsaturated fatty acid in cell membranes. Cisplatin generates reactive oxygen species such as superoxide anion and hydroxyl radicals and stimulates renal LPO determined by the increased level of thioberbituric acid reactive substances (TBARS). The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions. The increase in renal TBARS content after cisplatin injection was significantly attenuated by treatment with diphenylmethyl selenocyanate. One of the most important intracellular antioxidant systems is the glutathione redox cycle. GSH form conjugates with electrophilic drug metabolites most often produced by cytochrome P-450 linked monooxygenase. Cisplatin-induced glutathione depletion is a determinant step in oxidative stress in kidney tissue that leads to nephrotoxicity [38]. In the present study cisplatin treatment was able to induce renal glutathione depletion. Our results agree with the other reports pertaining to cisplatin-induced renal GSH depletion [39]. Treatment with the selenium compound prevented the depletion of renal glutathione caused by cisplatin, resulting in values close to those observed in the vehicle-treated group. GST plays an important role in detoxifying/transport of many DNA alkylating agents, carcinogens and environmentally hazardous chemicals by catalysing the conjugation of GSH with these chemicals or their active metabolites [40]. The depleted GST activity was increased by diphenylmethyl selenocyanate treatment, making the cells more effective with respect to detoxification of toxic metabolites. We have also observed depletion in SOD activity after cisplatin administration and this decreased SOD activity could not completely scavenge superoxide anion resulting enhanced lipid peroxidation. CAT and GPx activities were also decreased after cisplatin administration, which in turn decreased the ability of the kidney to scavenge toxic H_2O_2 and lipid peroxides. The inhibition of antioxidant enzyme activity may be because of direct binding of cisplatin to essential sulphydryl groups at the active sites of these enzymes and depletion of copper and selenium which are essential for SOD and GPx activities. The formation of reactive oxygen species and organic peroxide by cisplatin also inactivated the antioxidant enzymes. The administration of diphenylmethyl selenocyanate in cisplatin-intoxicated mice restored SOD, CAT and GPx activity, indicating that this compound can restore these enzyme activities and pre-treating the

animals with the selenium compound restores the antioxidant enzymes activities much more pronouncedly than concomitant treatment.

Blood urea nitrogen (BUN) measures the amount of urea nitrogen, a waste product of protein metabolism, in the blood. Diseased or damaged kidneys cause an elevated BUN because the kidneys are less able to clear urea from the bloodstream. Creatinine is chiefly filtered out of the blood by the kidneys. If the filtering of the kidney is deficient, blood levels rise. Therefore, creatinine levels in blood may be used to calculate the creatinine clearance, which reflects the glomerular filtration rate (GFR). The GFR is clinically important because it is a measurement of renal function. A decreased glomerular filtration rate, as evidenced by increased plasma creatinine levels, has been observed following cisplatin administration [41]. In the present study functional nephrotoxicity indices such as BUN and serum creatinine levels were elevated after administration of cisplatin, which indicates intrinsic acute renal failure. Treatment with diphenylmethyl selenocyanate provided significant protection against cisplatin-induced nephrotoxicity, which was evident from the lowered BUN and creatinine levels.

Nitric oxide (NO) is a gas generated from L-arginine by nitric oxide synthase (NOS) that functions as an intercellular messenger in renal injury and inflammation [42]. Cisplatin treatment induces a significant increase in the activity of calcium-independent NOS in rat kidney and liver tissues, resulting in an increase in serum NO levels as well as in tissue NO formation [43,44]. Furthermore, NO reacts spontaneously with the available superoxide radical to form the more potent and versatile oxidant peroxynitrite. This highly toxic species reacts with GSH, lipids, proteins and DNA. In the present study enhanced production of nitric oxide by peritoneal macrophages was observed after cisplatin treatment compared to vehicle treatment. The data in the present work is also consistent with the other observations implying nitrosative stress as an important mediator in the cisplatin-induced renal injury [45]. Nitric oxide level decreased significantly in the selenium compound treatment groups. The mechanism by which diphenylmethyl selenocyanate ameliorates cisplatin-induced over-expression of iNOS could be strongly related to the inhibition of NO synthesis by iNOS and its conversion to strong electrophillic intermediates $ONOO^-$ and N_2O_3. Cyclooxygenase (COX) is a rate-limiting enzyme in the cellular production of prostaglandins from arachidonic acid [46,47]. COX-2 is an inducible isozyme of COX expressed in response to inflammatory stimuli and a regulator of cell growth [48,49]. In a number of models of chronic renal disease, renal injury increases the expression of COX protein and mRNA [50,51]. In the present study, over-expression of COX-2 in the kidney of the cisplatin-treated group characterized renal interstitial inflammation. Reduced expression of COX-2 in the selenium compound treated groups showed the ability of diphenylmethyl selenocyanate to modulate COX-2 expression and prevent renal cell damage. Cisplatin also provokes loss of tubular epithelial cells by necrosis and apoptosis, followed by inflammatory cell infiltration and fibroproliferative changes [52,53]. Cisplatin toxicity in the proximal tubular cell is morphologically characterized in this study by acute tubular cell necrosis, treatment with diphenylmethyl selenocyanate minimizing tubular injury and, thus, modifying the alterations in renal function and structure the effect was more prominent in the pre-treatment group than the concomitant treatment group. A possible mechanism by which the compound may exert a protective effect in this study is by acting as an antioxidant by quenching free radicals, as cisplatin is able to generate ROS, which inhibits the activities of antioxidant enzymes in renal tissues [54].

Our results suggest that diphenylmethyl selenocyanates have the potential to inhibit the cisplatin-induced nephrotoxicity, preventing cisplatin-induced oxidative stress and acute renal damage in mice through its action by enhancing the GST, GPx, CAT, SOD activities and GSH level and also by controlling iNOS and COX-2 expressions. This study points to a promising use of this organoselenium compound and necessitates further experimental and clinical studies.

Acknowledgements

The authors wish to thank the Director, Dr Jaydip Biswas, for support in this study. We also acknowledge the members of the Department of Cancer Chemoprevention for their help during the experiment.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on Early Online on 4 Oct 2010.