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Seleno L-Methionine Acts on Cyclophosphamide-Induced Kidney Toxicity

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Abstract The anticancer drug cyclophosphamide (CP) has nephrotoxic effects besides its urotoxicity, which both in turn limit its clinical utility. The nephrotoxicity of CP is less common compared to its urotoxicity, and not much importance has been given for the study of mechanism of CP-induced nephrotoxicity so far. Overproduction of reactive oxygen species (ROS) during inflammation is one of the reasons of the kidney injury. Selenoproteins play crucial roles in regulating ROS and redox status in nearly all tissues; therefore, in this study, the nephrotoxicity of CP and the possible protective effects of seleno L-methionine (SLM) on rat kidneys were investigated. Forty-two Sprague–Dawley rats were equally divided into six groups of seven rats each. The control group received saline, and other rats were injected with CP (100 mg/kg), SLM (0.5 or 1 mg/kg), or CP+ SLM intraperitoneally. Malondialdehyde (MDA) and glutathione (GSH) levels in kidney homogenates of rats were measured, and kidney tissues were examined under the microscope. CP-treated rats showed a depletion of renal GSH levels (28% of control), while CP+SLM-injected rats had GSH values close to the control group. MDA levels increased 36% of control following CP administration, which were significantly decreased after SLM treatment. Furthermore, these biochemical results were supported by microscopical observations. In conclusion, the present study not only points to the therapeutic potential of SLM in CP-induced kidney toxicity but also indicates a significant role for ROS and their relation to kidney dysfunction.

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Introduction

Cyclophosphamide (CP) is a drug with a wide spectrum of clinical uses, and it has been proved to be effective in the treatment of cancer (lymphoma, acute and chronic leukemias, and multiple myeloma) and non-malignant disease states such as rheumatoid arthritis [1]. However, this drug may induce acute inflammation in the urinary bladder (cystitis) and causes renal damage [2, 3] and liver damage [4], thereby limiting its therapeutic window. Ifosfamide and CP have to be activated through a metabolic step in vivo in which numerous metabolites are known [5]. Studies have shown that CP can also cause nephrotoxicity besides its urotoxic effects [3, 4]. The nephrotoxicity of CP was generally overlooked because plasma creatinine, an indicator of glomerular function of the kidney, is not altered significantly in patients on CP chemotherapy. However, in a recent study, Sugumar et al. [6] have demonstrated in a rat model that CP induces renal damage microscopically but a reliable biochemical marker of renal dysfunction, the plasma creatinine, remained unaltered. CP can result in glomerular dysfunction and tubular dysfunction [6, 7]. The nephrotoxicity is considered to be dose dependant and includes a variable reduction of glomerular filtration rate along with tubular dysfunction.

Phosphoramide mustard (PAM) and acrolein (ACR) are two active metabolites of cyclophosphamide produced by the liver microsomal enzymes [8]. Cyclophosphamide's antineoplastic effects are associated with PAM, while ACR is linked with its toxic side effects. ACR interferes with the tissue antioxidant defense system [9], produces highly reactive oxygen-free radicals [10], and are mutagenic to mammalian cells [11]. Antioxidant agents like amifostine, a thiol antioxidant, have cytoprotective action against platinum-induced renal toxicity. However, amifostine is not used due to its toxic effects: hypocalcaemia, anxiety, and hypotension [12].

Seleno L-methionine is a nutritionally essential trace element representing the major nutritional source of selenium for higher animals and humans [13]. Se is a constituent of glutathione peroxidase (GSH-Px), an enzyme that uses glutathione (GSH) to remove hydrogen peroxide as well as fatty acid hydroperoxides, thereby preventing hydroxyl radical formation [14]. The level of MDA, a marker of lipid peroxidation, was found to be higher in tumor than in non-tumor tissues [15]. Selenium was found to be highly protective against toxicity induced by a variety of chemotherapeutic agents [16]. The aim of the present study was to investigate the potential protective effects of pharmacological dosages of seleno L-methionine against CP-induced kidney damage in rats.

Materials and Methods

Chemicals and Drugs

CP (Endoxan, Cyclophosphamide Monohydrate, C0768) was purchased from Sigma-Aldrich, Taufkirchen, Germany. Se (Seleno-L-Methionine, S3132) was purchased from Sigma, Germany.

Experimental Protocol

A total of 42 Sprague–Dawley rats of either sex, 200±20 g, were provided by the Experimental Animal Research Facility, Eskisehir Osmangazi University, Eskisehir, Turkey. The rats were housed under conditions of controlled temperature (22°C) and acclimatized to 12:12 h light/dark cycle. Animals were given food and water ad libitium. Animal experiments were conducted according to the guidelines of Institutional Animal Ethics Committee (Date-Protocol no: 16.06.2009-117/2009).

The rats were randomly divided into the following experimental groups, each including seven animals: Group 1 served as the vehicle (normal saline) treated controls. Group 2 animals received CP i.p. dissolved in saline, in a single dose of 100 mg/kg b.w. Groups 3 and 4 were treated with 0.5 or 1 mg/kg seleno L-methionine (SLM) for six consecutive days. The animals of group 5 or 6 received respective SLM for 6 days and then a single dose of CP administered on the fourth day [17]. Animal groups and their treatments are summarized in Table 1.

Sample Collection and Biochemical Assays

All animals were anesthetized with an i.p. injection of 60 mg sodium pentobarbitone per kilogram of b.w. and then killed by cervical dislocation 24 h after the final saline, CP, or SLM injections. The kidneys were removed intact, washed with saline, and blotted dry on a filter paper. The kidneys were then cut into two equal pieces. One half was stored at −20°C to measure kidney MDA and GSH contents; the rest was fixed for 24 h in 10% buffered formaldehyde (Table 2).

Histological Procedures

For light microscopy, tissues were processed routinely for paraffin embedding, and 5-μm serial cross sections were stained and examined for the presence of necrosis, edema,

Table 1 Groups of Rats (n=7) and Their Treatments

Groups	Days						
	1	2	3	4	5	6	7
Control	Saline	Saline	Saline	Saline	Saline	Saline	X
CP	Saline	Saline	Saline	CP	Saline	Saline	X
0.5 SLM	SLM (0.5 mg/kg)	SLM (0.5 mg/kg)	SLM (0.5 mg/kg)	SLM (0.5 mg/kg)	SLM (0.5 mg/kg)	SLM (0.5 mg/kg)	X
1 SLM	SLM (1 mg/kg)	SLM (1 mg/kg)	SLM (1 mg/kg)	SLM (1 mg/kg)	SLM (1 mg/kg)	SLM (1 mg/kg)	X
CP+0.5 SLM	SLM (0.5 mg/kg)	SLM (0.5 mg/kg)	SLM (0.5 mg/kg)	SLM (0.5 mg/kg) +CP	SLM (0.5 mg/kg)	SLM (0.5 mg/kg)	X
CP+1 SLM	SLM (1 mg/kg)	SLM (1 mg/kg)	SLM (1 mg/kg)	SLM (1 mg/kg) +CP	SLM (1 mg/kg)	SLM (1 mg/kg)	X

CP cyclophosphamide (100 mg/kg), *SLM* seleno L-methionine, *X* killed

Table 2 MDA and GSH Levels in Kidney Tissue of Rats Treated with CP, SLM, and SLM Followed by CP

Groups (n=7)	MDA (nmol/g protein)	GSH (μmol/g protein)
Control	14.22±1.36	38.20±1.66
CP	22.17±1.25[a]	27.79±0.97[a]
0.5SLM	14.35±0.93[b,c]	40.81±2.14[a,b,c]
1SLM	14.81±0.79[b,d]	41.14±1.36[a,b,d]
CP+0.5SLM	17.36±1.13[a,b]	36.86±0.88[b]
CP+1SLM	19.37±0.96[a,b,c]	36.87±1.12[b]

Values are shown as mean ± SD

[a] Significantly different from controls (p<0.05)

[b] Significantly different from CP group (p<0.05)

[c] Significantly different from CP+0.5 SLM group (p<0.05)

[d] Significantly different from CP+1 SLM group (p<0.05)

hemorrhage, tubular degeneration, mononuclear/polimorphonuclear cell infiltration, and narrowing of Bowman's capsule space [18].

Malondialdehyde

MDA content was measured as described by Ohkawa et al. in 1979 [19]. Kidney tissues were homogenized in appropriate buffers and used for the following assays. The mixture consisted of 0.8 ml of sample (1 mg), 0.2 ml of 8.1% sodium dodecylsulfate, 1.5 ml of 20% glacial acetic acid adjusted to pH=3.5, and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid. The mixture was diluted to 4 ml with distilled water and heated at 95°C for 60. Distilled water (1 ml) and 5 ml n-butanol and pyridine mixture (15:1) were added and centrifuged at 2,000×g for 10 min. The absorbance of the organic layer was measured at 532 nm. Amount of thiobarbituric reacting substances formed is calculated from standard curve prepared using 1, 1′, 3, 3′ tetramethoxy propane, and the values were expressed as nmol MDA/g tissue.

Glutathione

Tissue levels of acid-soluble thiols, mainly GSH, were determined colourimetrically at 412 nm. Briefly, 0.5 ml of the previously prepared homogenate was added to 0.5 ml of 5% trichloroacetic acid, and after centrifugation at 750×g for 5 min, the supernatant (200 μl) was added to a tube containing 1,750 μl of 0.1 mol/l potassium phosphate buffer, pH=8, and 50 μl 5,5′-dithiobis-(2-nitrobenzoic acid) reagent. Tubes were mixed, and the yellow color that developed was measured against a standard curve of GSH. The protein thiol (protein-SH) content was expressed as μmol/g of tissue.

Statistics

The results were expressed as means ± SD and p<0.05 accepted as statistically significant. Statistical analyses were performed using one-way analysis of variance for MDA and GSH levels. The Tukey's test was then performed to analyze the two groups consecutively.

Results

Biochemical Results

CP significantly increased MDA relative to the control (36%, $p<0.05$), which was significantly restored in the kidneys of the animals pretreated with SLM (0.5 or 1 mg/kg/day) as opposed to those receiving only CP tissue (see Fig. 1). CP also produced a significant reduction in GSH (28%) of the kidney relative to the control ($p<0.05$). Groups receiving SLM (0.5 or 1 mg/kg/day) showed a significant restoration in GSH concentration of the kidney. The group of rats having SLM alone showed no significant alteration in the levels of MDA and GSH (see Fig. 2).

Microscopic Evaluation

Microscopical observations also provided supportive evidence for the biochemical results as seen in Fig. 3. Medullar regions showed normal histology in all sections of the groups. The kidneys of the control and the groups having SLM alone showed normal histology. Kidney tissues of CP-administered rats showed desquamation of tubular epithelial cells with accumulation of eosinophilic granular material within the lumen of the tubules and narrowing of Bowman's capsule spaces. In the CP+SLM groups, SLM decreased the degeneration of the tubules and the kidney corpuscles looked normal.

Discussion

CP has been associated with various kinds of urinary tract toxicities [20, 21], but kidney toxicity has only rarely been reported. In 1961, Philips et al. reported that renal papillary

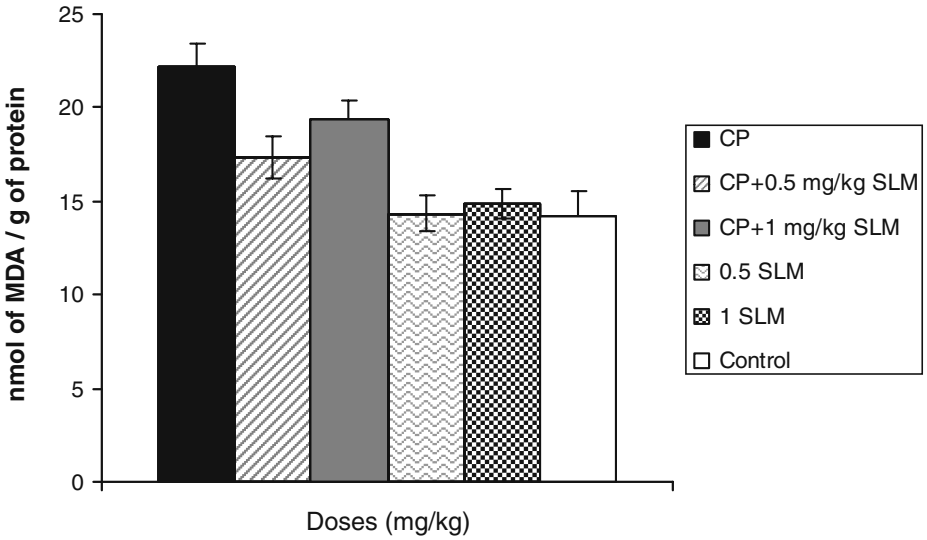

Fig. 1 MDA levels of the kidney. CP administration significantly increased MDA level relative to the control (36%), which was significantly restored kidney in animals pretreated with SLM (0.5 or 1 mg/kg/day) as opposed to those receiving only CP tissue ($p<0.05$)

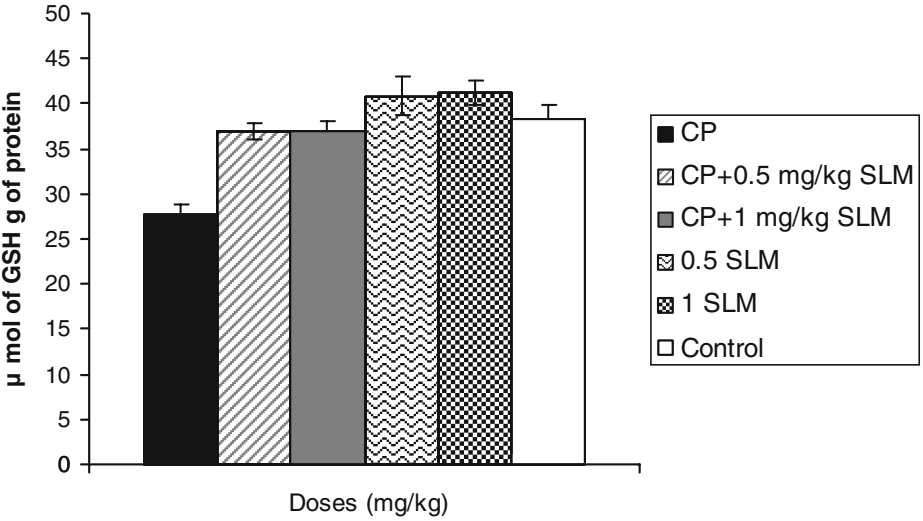

Fig. 2 CP produced a significant reduction in GSH level (28%) in the kidney relative to the control ($p<0.05$). Groups receiving SLM (0.5 or 1 mg/kg/day) showed a significant restoration in GSH concentration in the kidney ($p<0.05$)

necrosis had developed after a single i.p. injection of CP (222 mg/kg) in male rats [22]. Since then, there have been a few publications concerning the nephrotoxicity of CP in animal experiments, but these have only mentioned renal tubular necrosis [23] or glomerular changes [24].

In the present study, it was shown that treatment with only CP caused nephrotoxicity in rats, as evidenced by the high biochemical parameters such as high lipid peroxidation (MDA) and low antioxidant activity (GSH) and by histological changes as well. Many studies have reported structural changes in the kidney [5, 25, 26]. The level of kidney MDA in the CP-treated group was significantly higher compared with their levels in the controls. Increased MDA (36%) levels indicated that lipid peroxidation, mediated by ROS, was an important contributing factor in the development of CP-mediated tissue damage ($p<0.05$). However, pretreatment with SLM significantly prevented CP-induced lipid peroxidation in the kidney tissues, implicating an antioxidant effect from this trace element. This was probably due to less damage having occurred from oxygen-free radicals.

GSH is the most important molecule for maintaining cell integrity and participation in cell metabolism [27]. The significant reduction in GSH levels promoted by CP represents an alteration in the cellular redox state, suggesting that the cells could be more sensitive to ROS. This leads to a reduction in effectiveness of the antioxidant enzyme defense system [28]. In this study, GSH levels in the kidney tissues of rats treated with CP were lower (28%) than the control groups ($p<0.05$). On the other hand, an increase in GSH levels in the kidney tissues indicated that pretreatment with SLM caused the increase as a response to oxidative stress.

Se is recognized to have a capacity for conferring tolerance to the toxic manifestations of various heavy metal exposures, including cadmium, mercury, lead, and arsenic [29, 30] in addition to role as an anticancer agent [31]. Moreover, Se has protective effects against mercury toxicity in rat kidney and Se also used to diminish the toxic effects of the cadmium on the antioxidant enzyme system, which in turn affects the membranes structures such as

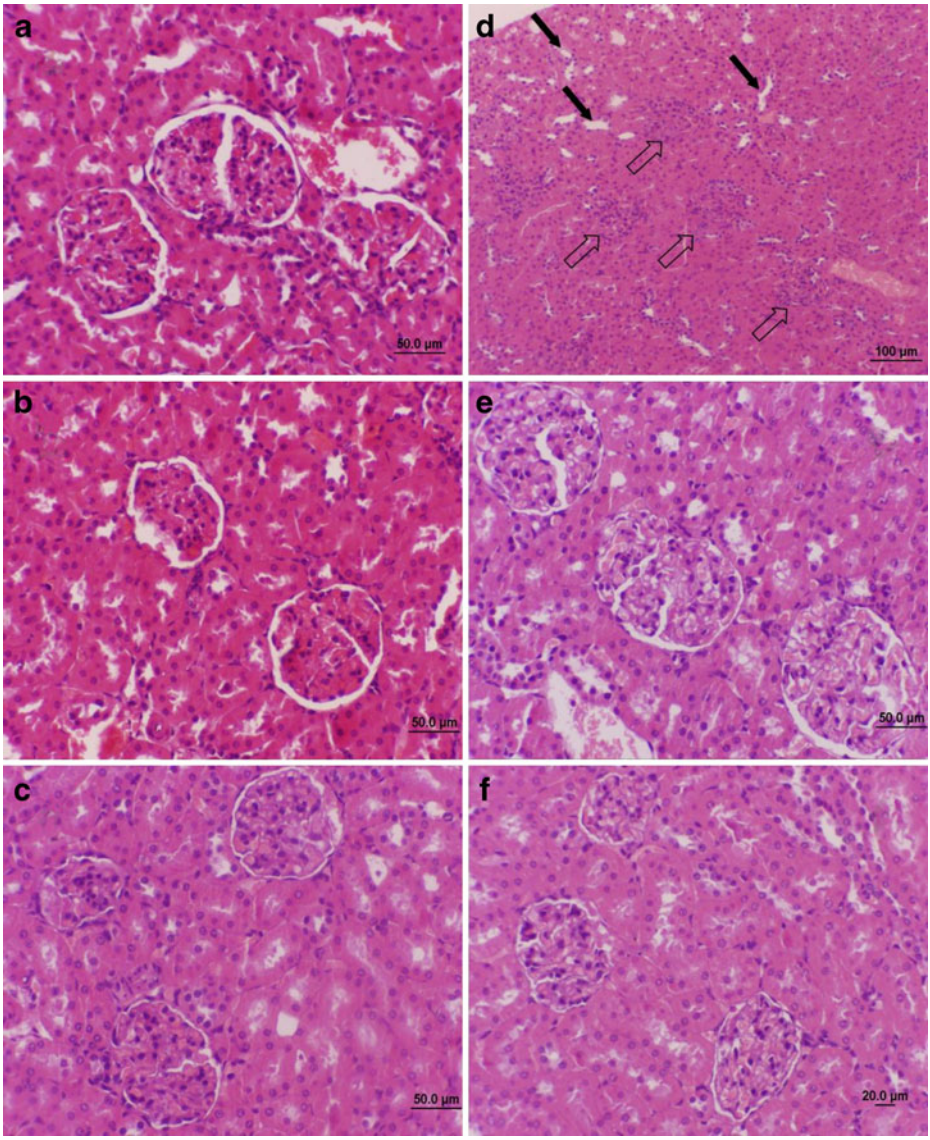

Fig. 3 Micrographs of kidney cortexes of the rats from **a** control, **b** 0.5 SLM, and **c** 1 SLM groups showing normal histology; **d** CP group showing degenerated tubules (*black arrows*) and narrowing of Bowman's capsule spaces (*white arrows*); **e** CP+0.5 SLM and **f** CP+1 SLM showing almost normal architecture. All sections were stained with H&E

mitochondria and endoplasmic reticulum. On the other hand, Se deficiency reducing GSH-Px activity has been reported in the diet of glomerular disease and in the diet of tubular epithelium in normal rats [32]. Concurrent or prior injections of Se were found to protect against many of the acute effects of cadmium, e.g., testicular necrosis, placental hemorrhage, teratogenecity, and damage to the lactating mammary glands [31–34].

These results correlated well with the microscopical results from the kidneys, which revealed tubular necrosis in the renal cortex. The kidneys of the control and SLM groups

showed normal histology. However, rats given CP had a moderately severe glomerular congestion and degeneration and dilatation in Bowman's space and in the collective tubules in the kidney sections. On the other hand, the tubule cells from rats in the CP+SLM groups were nearly normal in histological architecture, except for a minor desquamation of the kidney epithelial cells. Similar findings were also reported by Tripathi and Jena [35], and Babu et al. [25], demonstrating the structural changes in the kidney tissues of CP-treated animals and its healing by different agents.

In this study, we have shown that experimental CP administration caused an increase in lipid peroxidation in rats, which we believe is a result of the oxidative stress caused by CP, and SLM can act as a protective pharmacological agent or decrease the side effects of CP-induced kidney toxicity.

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