J. Pharm. Pharmacol. 1998, 50: 687–691
Received October 10, 1997
Accepted February 4, 1998

Protective Effects of Selenomethionine against Cisplatin-induced Renal Toxicity in Mice and Rats

MAHADEV RAO AND M. N. A. RAO*

Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Manipal 576 119, India

Abstract

The effect of selenomethionine on the toxicity of cisplatin has been studied in mice and rats.

When selenomethionine (0·5–4 mg kg^{-1}) was administered intraperitoneally to mice 1 h before intraperitoneal cisplatin (6 mg kg^{-1}), the toxicity of cisplatin, as measured by loss of body weight and blood urea nitrogen and serum creatinine levels, was reduced significantly. The protection was dose-dependent but less when given orally. Similar results were obtained with rats. Deterioration of renal function was characterized by reduced creatinine clearance, and increased excretion of urinary protein was significantly reversed. Partial but significant protection was also observed against capsulation-induced reduction of white blood-cell count. Protective properties were further demonstrated by increased survival of mice pretreated with selenomethionine compared with the lethality observed for animals given cisplatin only.

These results suggested that selenomethionine protects against cisplatin-induced renal and other toxicity. The study has many clinical implications in cancer chemotherapy and needs further investigation.

Although cisplatin is a potent anticancer agent against solid tumours of the testes, ovaries, breasts, lungs, bladder, etc. (Rozeneweig et al 1977), its clinical use is limited by its renal toxicity (Madias & Harringon 1978; Goldstein & Mayor 1983). Although the mechanism of cisplatin renal toxicity is not clear, it has been suggested that oxygen free-radicals play an important role (McGinness et al 1978; Bull et al 1988; Baldew et al 1989; Inselmann et al 1995). Cisplatin is known to cause increased lipid peroxidation in renal cortical slices (Nakano & Gemba 1989; Zhong et al 1990; Zhang & Lindup 1993; Inselmann et al 1995). It has been reported that the administration of free-radical scavengers and antioxidants such as superoxide dismutase (McGinness et al 1978), sodium selenite (Baldew et al 1989), or hydroxyethylrutoside (Bull et al 1988) afford partial protection of the kidney against cisplatin toxicity.

Selenium is an essential trace element known to play an important role in chemoprevention of cancer (Hocman 1988). Lower plasma selenium levels are reported to be correlated with higher incidence of cancer (Willet et al 1983). Selenium (as sodium selenite) is also reported to reduce the lethality and nephrotoxicity of cisplatin without reducing its antitumour activity (Berry et al 1983; Baldew et al 1989). Selenomethionine, a component of many selenoproteins found in mammalian tissues (Zwelling & Kohn 1982), is more active than sodium selenite in attenuating the oxidation and nitration reactions of peroxynitrite (Briviba et al 1996).

In this study we have investigated the effect of selenomethionine on cisplatin-induced renal toxicity in mice and rats. We have also studied the effect of selenomethionine on the lethality of cisplatin in mice and the cisplatin-induced reduction of the white blood-cell count in rats. The study shows that selenomethionine partially but significantly protects both mice and rats against cisplatin toxicity.

Materials and Methods

Animals

The study was performed on male Wistar rats, 8–10 weeks, 200–240 g, and Swiss mice of both sexes, 8–9 weeks, 20–25 g. They were maintained on a standard diet (Lipton's India, Calcutta) and water was freely available.

* Present address: Divis Laboratories Ltd, 7-1-77/E/1/303, D. K. Road, Ameerpet, Hyderabad 500 016, India.

Correspondence: Department of Pharmaceutical Chemistry, M. Rao, College of Pharmaceutical Sciences, Manipal 576 119, India.

Drugs
Cisplatin was obtained from Sigma (St Louis, MO). L-(+)-Selenomethionine was a gift from Sami Chemicals and Extracts (Bangalore, India).

Renal toxicity in mice
Mice were randomly divided into groups of ten animals. Graded doses of selenomethionine was given orally or intraperitoneally 1 h before intraperitoneal administration of cisplatin. On day 5 blood was collected from the retrorbital venous plexus for measurement of levels of blood urea nitrogen (by the diacetylmonoxime method) and serum creatinine (by the alkaline picrate method) by use of commercial kits (Ranbaxy Diagnostics, New Delhi, India). The body weight of the animals was recorded daily.

Renal toxicity and functional study in rats
Four groups of rats (n = 8) were used to study the effect of selenomethionine on cisplatin-induced renal toxicity and changes in renal function. Group 1 animals received cisplatin (3 mg kg^{-1}) intraperitoneally. Groups 2 and 3 received intraperitoneal selenomethionine (1 and 4 mg kg^{-1}, respectively) 1 h before intraperitoneal cisplatin (3 mg kg^{-1}). Group 4 acted as vehicle (distilled water) control. The body weight was recorded daily and on day 5 blood was collected from the retrorbital venous plexus for measurement of blood urea nitrogen, serum creatinine, and white blood-cell count. Blood urea nitrogen and serum creatinine were measured by use of commercial kits. The white blood-cell count was performed with a haemocytometer. Urine was collected on day 5 for 6 h (initiated at 0800 h), by use of metabolic cages, and analysed for creatinine and protein (Godkar 1994). Creatinine clearance was calculated by use of the formula: Creatinine clearance = urinary creatinine × urinary volume h^{-1}/serum creatinine. The body weight of the animals was recorded daily.

Lethality to mice
Mice of both sexes (n = 20) were treated intraperitoneally with selenomethionine, 1 and 4 mg kg^{-1}, 1 h before intraperitoneal administration of cisplatin, 6, 8 and 10 mg kg^{-1}. Lethality was measured on day 10.

Statistical analysis
Values are expressed as means ± s.e.m. Results were compared by analysis of variance followed by Student's Newman–Keuls test. Statistical significance was set at $P < 0.05$.

Results

Protective effect in mice
Table 1 lists the effects of oral administration of selenomethionine on cisplatin-induced elevation of blood urea nitrogen and serum creatinine and on the reduction of body weight in mice. The control animals gained 3·0 g body weight on average (n = 10) during the five days. The body weight of animals which received intraperitoneal cisplatin (6 mg kg^{-1}) decreased by 4·0 g. However, when selenomethionine, 1 or 4 mg kg^{-1}, was given 1 h before cisplatin, the reduction in the body weight was 2·3 and 1·3 g, respectively. Levels of blood urea nitrogen and serum creatinine increased significantly in cisplatin-treated animals (91·6 and 1·69 mg dL^{-1} compared with 16·9 and 0·56 mg dL^{-1} for control animals). The extent of the elevation was reduced significantly when selenomethionine was given orally 1 h before cisplatin.

Much higher protection was observed when selenomethionine was given intraperitoneally instead of orally (Table 2). Selenomethionine at 1, 2 and 4 mg kg^{-1} reversed the loss of body weight observed for cisplatin-treated animals. Similar results were obtained for blood urea nitrogen and serum creatinine. When selenomethionine, 4 mg kg^{-1}, was given 1 h before cisplatin the level of blood urea nitrogen increased to 43·2 ± 5·0 mg dL^{-1} compared with 91·6 ± 4·0 mg dL^{-1}

Table 1. Protective effect of oral selenomethionine against cisplatin-induced elevation of blood urea nitrogen and of serum creatinine, and against body weight reduction in mice.

Treatment	Change in body weight (g)	Blood urea nitrogen (mg dL^{-1})	Serum creatinine (mg dL^{-1})
Control	3·0 ± 0·7	16·9 ± 0·9	0·56 ± 0·03
Cisplatin	− 4·0 ± 0·9*	91·6 ± 4·0*	1·69 ± 0·08*
Cisplatin + selenomethionine			
1·0 mg kg^{-1}	− 2·3 ± 1·1*	68·5 ± 4·0*,**	1·28 ± 0·13*,**
4·0 mg kg^{-1}	− 1·3 ± 1·1*	71·3 ± 6·0*,**	1·17 ± 0·12*,**

Selenomethionine was given orally 1 h before intraperitoneal cisplatin (6·0 mg kg^{-1}) and measurements were taken on day 5. Values are expressed as mean ± s.e.m., n = 10. * $P < 0.05$ significantly different from result for control. ** $P < 0.05$ significantly different from result for animals treated with cisplatin alone.

Table 2. Protective effect of intraperitoneal selenomethionine against cisplatin-induced elevation of blood urea nitrogen and of serum creatinine, and against body weight reduction, in mice.

Treatment	Change in body weight (g)	Blood urea nitrogen ($mg\,dL^{-1}$)	Serum creatinine ($mg\,dL^{-1}$)
Control	3·0 ± 0·7	16·9 ± 0·9	0·56 ± 0·03
Cisplatin	− 4·0 ± 0·9*	91·6 ± 4·0*	1·69 ± 0·08*
Cisplatin + selenomethionine			
$0·5\,mg\,kg^{-1}$	− 3·0 ± 1·2*	76·9 ± 6·3*	1·37 ± 0·13*
$1·0\,mg\,kg^{-1}$	− 0·5 ± 1·5*	52·0 ± 5·4*,**	0·90 ± 0·09*,**
$2·0\,mg\,kg^{-1}$	0·0 ± 0·9*,**	46·9 ± 5·0*,**	0·80 ± 0·09*,**
$4·0\,mg\,kg^{-1}$	0·3 ± 1·0*,**	43·2 ± 5·0*,**	0·80 ± 0·08*,**

Selenomethionine was given intraperitoneally 1 h before intraperitoneal cisplatin ($6·0\,mg\,kg^{-1}$), and measurements were taken on day 5. Values are expressed as mean ± s.e.m., n = 10. * $P < 0·05$ significantly different from result for control. ** $P < 0·05$ significantly different from result for animals treated with cisplatin alone.

for animals given cisplatin alone (control = 16·9 ± 0·9 $mg\,dL^{-1}$). This amounted to approximately 65% protection. Serum creatinine levels increased from 0·56 $mg\,dL^{-1}$ in the control to 1·69 $mg\,dL^{-1}$ on treatment with cisplatin. When selenomethionine, 4 $mg\,kg^{-1}$, was given, serum creatinine increased to 0·8 $mg\,dL^{-1}$ only; this amounted to approximately 78% protection. Protection was observed for selenomethionine doses of 1 and 2 $mg\,kg^{-1}$ and a further increase in dose (2 and 4 $mg\,kg^{-1}$) did not result in a significant increase in protection. Similarly, multiple doses of selenomethionine (1 $mg\,kg^{-1}$ for 3 or 5 days) did not lead to improved protection compared with a single dose (data not shown).

Protective effects in rats

The study was extended to rats (Table 3). Selenomethionine resulted in significant protection when given intraperitoneally 1 h before cisplatin. The body weight of cisplatin-treated animals decreased by 12·5 g whereas that of control animals increased by 12·5 g during the same period. When selenomethionine was given intraperitoneally 1 h before cisplatin there was no decrease in body weight. Animals treated with selenomethionine (1 or 4 $mg\,kg^{-1}$) gained 1·7 g weight on average.

Blood urea nitrogen levels increased to 53·3 $mg\,dL^{-1}$ when the animals were treated with cisplatin (control = 14·6 $mg\,dL^{-1}$). When selenomethionine 4 $mg\,kg^{-1}$ was given, the increase was only to 30·6 $mg\,dL^{-1}$. In cisplatin-treated animals serum creatinine increased to 1·45 $mg\,dL^{-1}$ compared with 0·61 $mg\,dL^{-1}$ in control animals. Pretreatment with selenomethionine, 4 $mg\,kg^{-1}$, significantly arrested this elevation (0·79 $mg\,dL^{-1}$).

Table 4 lists the effects of selenomethionine on cisplatin-induced deterioration of renal function. Although urinary volume remained unchanged, cisplatin-treated animals excreted a large amount of protein (17·8 $mg\,day^{-1}$ compared with 8·1 $mg\,day^{-1}$ for controls). Pretreatment with selenomethionine (1 or 4 $mg\,kg^{-1}$) reduced protein excretion to almost normal (8·6 and 9·0 $mg\,day^{-1}$, respectively). The effect on creatinine clearance was similar. Renal failure was characterized by the reduction in creatinine clearance in cisplatin-treated animals (8 $mL\,h^{-1}/100\,g$ body weight) compared with the control (15·7 $mL\,h^{-1}/100\,g$). Pretreatment with selenomethionine (1 or 4 $mg\,kg^{-1}$) resulted in creatinine clearance similar to that for control animals (14·7 $mL\,h^{-1}/100\,g$).

Effect on white blood-cell count

Intraperitoneal treatment with cisplatin, 3 $mg\,kg^{-1}$, resulted in a reduction in the white blood-cell count in rats on day 5 (Table 4). From $7·0 \times 10^3$ in control animals, it decreased to $3·2 \times 10^3$. Pretreatment with intraperitoneal selenomethionine 1 h before administration of cisplatin resulted in partial but significant protection.

Effect on lethality

Table 5 lists the effects of selenomethionine on the lethality of cisplatin to mice. Cisplatin (6 $mg\,kg^{-1}$, i.p.) resulted in the death of 12 out of 20 animals (40% survival). Intraperitoneal pretreatment with selenomethionine, 1 or 4 $mg\,kg^{-1}$, 1 h before administration of cisplatin resulted in increased survival (55 and 65%, respectively). A similar increase in survival was observed for animals treated with larger doses of cisplatin.

Discussion

Although cisplatin is an important anti-neoplastic agent useful in treating many types of solid tumour, its use is limited by its nephrotoxicity. Although the mechanism of the nephrotoxicity is not clear, oxygen free-radicals have been implicated and many compounds with antioxidant properties are reported to protect experimental animals against cisplatin toxicity. This study shows that seleno-

Table 3. Protective effect of selenomethionine against cisplatin-induced elevation of blood urea nitrogen and of serum creatinine, and against body weight reduction, in rats.

Treatment	Change in body weight (g)	Blood urea nitrogen ($mg\,dL^{-1}$)	Serum creatinine ($mg\,dL^{-1}$)
Control	12·5 ± 2·1	14·6 ± 0·9	0·61 ± 0·05
Cisplatin	− 12·5 ± 2·1*	53·3 ± 4·0*	1·45 ± 0·15*
Cisplatin + selenomethionine			
$1·0\,mg\,kg^{-1}$	1·7 ± 4·4*	33·2 ± 3·1*,**	0·83 ± 0·06**
$4·0\,mg\,kg^{-1}$	1·7 ± 4·9*	30·6 ± 3·4*,**	0·79 ± 0·08**

Selenomethionine was given intraperitoneally 1 h before intraperitoneal cisplatin ($3·0\,mg\,kg^{-1}$) and measurements were taken on day 5. Values are expressed as mean ± s.e.m., n = 8. * $P < 0·05$ significantly different from result for control. ** $P < 0·05$ significantly different from result for animals treated with cisplatin alone.

Table 4. Effect of selenomethionine on cisplatin-induced changes in renal function and on white blood cell count in rats.

Treatment	Urinary volume ($mL\,h^{-1}/100\,g$)	Creatinine clearance ($mL\,h^{-1}/100\,g$)	Urinary protein ($mg\,day^{-1}$)	White blood cell count ($\times 10^3$)
Control	1·0 ± 0·1	15·7 ± 1·5	8·1 ± 1·5	7·0 ± 0·6
Cisplatin	1·1 ± 0·1	8·0 ± 0·7*	17·8 ± 2·3*	3·2 ± 0·2*
Cisplatin + selenomethionine				
$1·0\,mg\,kg^{-1}$	1·2 ± 0·1	14·8 ± 1·8**	8·6 ± 1·5†	5·2 ± 0·4*,**
$4·0\,mg\,kg^{-1}$	1·3 ± 0·1	14·7 ± 1·4**	9·0 ± 0·9†	4·8 ± 0·5*,**

Selenomethionine was given intraperitoneally 1 h before intraperitoneal cisplatin ($3·0\,mg\,kg^{-1}$) and blood was collected on day 5 for measurement of white blood cell count. Urinary volume, urinary protein and creatinine clearance were measured on day 5. * $P < 0·05$ significantly different from result for control. ** $P < 0·05$ significantly different from result for animals treated with cisplatin alone.

methionine, which is also an antioxidant, significantly protects mice and rats against cisplatin-induced renal toxicity. Pretreatment of animals 1 h before administration of cisplatin resulted in less renal toxicity, as characterized by blood urea nitrogen and serum creatinine. The extent of protection was more when the selenomethionine was given intraperitoneally rather than orally. Multiple doses on different days did not improve the protection compared with that resulting from a single dose. Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in the renal tubules (Howe-Grant & Lippard 1980; Heidmann et al 1985). Because this renal damage occurs in the first hour after administration (Har et al 1972; Elferink et al 1986), it is important that the protective agent is present at a sufficient concentration in renal tissue before the damage occurs. This might explain why selenomethionine must be given in advance of cisplatin if it is to be effective and might also explain why improved protection did not result when selenomethionine was given in multiple doses after administration of cisplatin. Selenomethionine also protected against the cisplatin-induced reduction in white blood-cell count in rats and lethality in mice.

Table 5. Effect of selenomethionine on the lethality of cisplatin to mice.

Cisplatin* ($mg\,kg^{-1}$)	Selenomethionine ($mg\,kg^{-1}$)	Survival (%)
6	0	40
6	1	55
6	4	65
8	0	15
8	1	40
8	4	40
10	0	0
10	1	20
10	4	20

Selenomethionine was given intraperitoneally 1 h before intraperitoneal cisplatin (n = 20) and lethality was measured after 10 days.

Selenium is known to protect glutathione by forming the selenodiglutathione complex (Milner & Hsu 1981). Selenomethionine might be acting in a similar manner because sulphydryl groups are implicated in cisplatin nephrotoxicity (Howe-Grant & Lippard 1980; Heidmann et al 1985). It is interesting to note that when selenium is given intraperitoneally 1 h before intraperitoneal cisplatin it did not reduce the anti-tumour activity in BALB/C mice inoculated with MPC11 plasmacytoma or Prima breast tumour cells (Badmaev et al 1996). Selenium has anti-neoplastic activity against several tumours (Greeder & Milner 1980; Milner

1985) and reduces the renal toxicity of cisplatin in cancer patients (Hu et al 1997). Because selenomethionine is both a source of selenium and protects against cisplatin-induced renal and other toxicity, this study has many clinical implications in cancer chemotherapy and warrants further investigation.

Acknowledgements

This work was supported by a grant from Department of Atomic Energy, Government of India. We thank Sami Chemicals and Extracts, Bangalore for the selenomethionine sample and other technical help.

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