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THE EFFECT OF REBAMIPIDE ON CISPLATIN-INDUCED NEPHROTOXICITY IN RATS

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This study aimed to evaluate the protective effect of rebamipide (free radical scavenger) against the nephrotoxic effect induced by cisplatin in normal rats. Twenty-four male Wister albino rats were divided equally into four groups: control, rebamipide, cisplatin and cisplatin plus rebamipide-treated groups. Nephrotoxicity was induced with single intravenous (i.v.) cisplatin dose of 6 mg kg^{-1} and measured through the estimation of kidney weight, serum albumin (Alb), serum creatinine (Cr), blood urea nitrogen (BUN), kidney glutathione (GSH) and malondialdehyde (MDA) production. In the cisplatin-treated group the kidney weight as a percent of the total body weight, serum Alb, serum Cr, BUN, GSH content and MDA amount were: $0.61 + 0.054\%$, $2.84 - 0.24$ g dl^{-1}, 2.99 ± 0.10 mg dl^{-1}, 147.08 ± 7.46 mg dl^{-1}, 3.11 ± 0.238 μmol g^{-1} and 1449.09 ± 127.36 nmol g^{-1}, respectively. All the previous changes were significantly ($P < 0.01$) different from the corresponding values in the control group. In addition, histopathological examination of the kidney tissue revealed degenerative cellular material and apoptotic tubular cells were seen in the renal tubules. Rebamipide treatment (140 mg kg^{-1}, i.p.) for 1 week ameliorated all the previous changes and the results recorded for the cisplatin plus rebamipide-treated group were: $0.45 \pm 0.035\%$, 4.17 ± 0.091 g dl^{-1}, 1.37 ± 0.209 mg dl^{-1}, 72.25 ± 5.14 mg dl^{-1}, 5.063 ± 0.269 μmol g^{-1} and 560.23 ± 21.98 nmol g^{-1} for the previous tests, respectively. Furthermore, significant improvement in the kidney histopathology was observed. The results of this study clearly revealed that rebamipide protected the kidney against the nephrotoxic effect of cisplatin. These results suggest that lipid peroxidation is not the only mechanism by which cisplatin induced nephrotoxicity. More investigations are needed to confirm the effect of rebamipide and at the same time to elucidate the exact mechanism by which cisplatin induces nephrotoxicity.

KEY WORDS: cisplatin nephrotoxicity, rebamipide, free radical scavengers, lipid peroxidation.

INTRODUCTION

Cisplatin is a divalent platinum compound with a potent cell-cycle non-specific killing activity [1]. The drug is effective in the treatment of a wide variety of neoplastic diseases including squamous cell cancer of the head and neck, non-small cell lung cancer as well as breast and ovarian cancers [2]. The dose limiting toxicity for cisplatin is its nephrotoxic effect [3, 4]. Administration of cisplatin is frequently associated with renal insufficiency and tubular dysfunction as a major nephrotoxic side-effect [5, 6]. Cisplatin causes differential toxic effects on renal antioxidants and lipid peroxidation by increasing the hydroxyl radicals formation resulting in glutathione (GSH) depletion and impairing the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Also, it causes an increase of lipid peroxidation and plasma creatinine levels [7, 8]. Several authors elucidate the role of antioxidant system in cisplatin-induced nephrotoxicity and the nephroprotection effects of certain agents such as diethyldithiocarbamate (DDTC), sodium thiosulphate and glutathione esters were studied [9–11]. Rebamipide (2-(4-chlorobenzoylamino)-3-[2(1*H*)-quinolinone-4-yl]-propionic acid), is a newly approved novel antipeptic ulcer drug that has a

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potential antioxidant activity [12–14]. The drug attenuates high glucose-induced nephrotoxicity which is attributable, in part, to its antioxidative property and, in part, to its effect on reversing hyperglycaemia [15]. This study was designed to study the possible effect of rebamipide on the cisplatin-induced nephrotoxicity.

MATERIALS AND METHODS

Twenty-four male Wister albino rats (120 g body wt) were obtained from the animal house at the College of Pharmacy, King Saud University (KSU). Four experimental groups were formed: control, rebamipide-treated control, cisplatin-treated and rebamipide plus cisplatin-treated rats. All rats were maintained on standard rat food and tap water. Cisplatin (Rhone-poulenc Rorer) was obtained from the inpatient pharmacy of King Khalid University Hospital. Rebamipide was supplied as a gift from the Microbiological Research Institute (OTSUKA Pharmaceutical Co. Ltd, Tokushima, Japan). Cisplatin was administered as a single intravenous (i.v.) dose 6 mg kg^{-1} body weight via the tail vein. Rebamipide was administered as a single i.p. daily dose 140 mg kg^{-1} for 1 week. Treatment with rebamipide was started 24 h prior to cisplatin. After that blood samples were collected by heart puncture and the kidneys were removed and homogenized (Biohomogenizer) in normal saline and stored at −20°C until analysed for biochemical parameters (Fig. 1). Another specimen was fixed in 10% neutral formalin and processed for histopathlogical examination.

Lipid peroxidation in the kidney homogenate (malondialdehyde 'MDA' content) was determined spectrophotometrically by the method of Ohkawa *et al.* [16]. Kidney reduced glutathione content (GSH) was measured rapidly after homogenization according to the method of Ellman [17]. Serum creatinine (Cr) level was measured according to the method of Bonses and Taussky [18]. Serum albumin (Alb) and urea nitrogen (BUN) contents were measured according to the methods of Wrenn and Feichtmier [19] and Hallet and Cook [20], respectively. Histopathological studies of the kidney tissue at 7 days after treatment were performed in the pathology laboratory, college of medicine at King Saud University (KSU).

The results were expressed as the means ± standard deviation (SD). Analysis of variance (ANOVA) was used to test the differences between the multiple groups and the P value of 0.05 or less was taken as the criterion for a statistically significant difference.

RESULTS

The effects of rebamipide (140 mg kg^{-1}) given i.p. for 1 week on cisplatin-induced renal dysfunction are shown in Table I. The kidney weight as a percent of total body weight in the cisplatin-treated group (Table I) was 0.61% ± 0.054 *vs* 0.45 + 0.02% in the control group. The concentrations of serum Alb, serum creatinine and BUN in the cisplatin-treated group (Table I) were: 2.84 ± 0.24 g dl^{-1}, 2.99 ± 0.1 mg dl^{-1} and 147.08 ± 7.46 mg dl^{-1}, respectively, and that were representatives 60%, 327% and 297% of the corresponding values in the control group. The

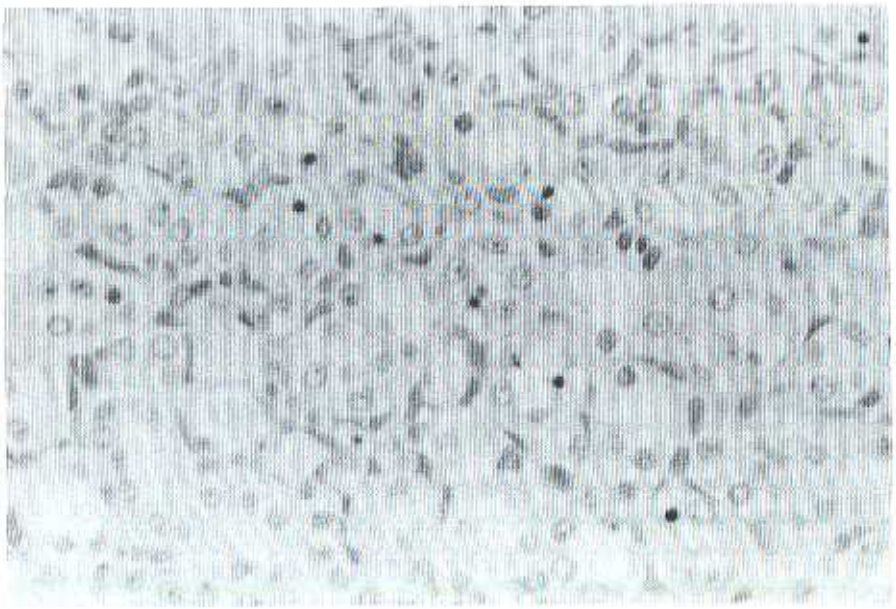

Fig. 1. Section of kidney tissue obtained from a rat treated with saline only. HE ×200.

Table I
The effect of rebamipide on the nephrotoxicity of cisplatin in normal rats (rebamipide, 140 mg kg $^{-1}$, i.p. for 1 week and cisplatin, 6 mg kg $^{-1}$ single i.v. dose)

Parameters	*Control*	*Rebamipide*	*Cisplatin*	*Cisplatin plus rebamipide*
Kidney weight, % of body wt	0.42 ± 0.020 (100%)‡	0.40 ± 0.022 (95%)	0.61 ± 0.054* (145%)	0.45 ± 0.035† (107%)
Serum albumin (g dl^{-1})	4.37 ± 0.143	4.30 ± 0.246	2.84 ± 0.240*	4.17 ± 0.091†
Serum creatinine (mg dl^{-1})	0.673 ± 0.066	0.748 ± 0.060	2.99 ± 0.100*	1.37 ± 0.209*†
Serum urea (mg dl^{-1})	37.17 ± 1.45	41.65 ± 2.27	147.08 ± 7.46*	72.25 ± 5.14*†
Kidney glutathione content (μmol g^{-1})	5.375 ± 0.149	6.070 ± 0.204*	3.110 ± 0.238*	5.063 ± 0.269†
Malondialdehyde (MDA) in (nmol g^{-1})	388.02 ± 32.49	372.19 ± 40.54	1449.09 ± 127.36*	560.23 ± 21.98*†

Note. All data represent mean values ± SD ($n = 6$).
*Different from control at $P < 0.01$.
†Different from cisplatin-treated group at $P < 0.01$.
‡Values in parentheses represent the changes in the kidney wieght taking the control group as 100%.

changes observed as a result of cisplatin treatment were statistically significant ($P < 0.01$) in comparison with the control group. In the presence of rebamipide plus cisplatin-treated group, the average concentrations of serum Alb (4.17 ± 0.091 g dl^{-1}), serum Cr (1.37 ± 0.209 mg dl^{-1}) and BUN (72.25 ± 5.14 mg dl^{-1}) were significantly different from that of the cisplatin group (Table I). At the same time the kidney weight as a percent of the total body weight was 0.45 ± 0.035% which did not differ significantly ($P > 0.05$) from the control group.

The effect of rebamipide treatment on the kidney reduced glutathione (GSH) contents and malondialdehyde (MDA) production as an estimate for lipid peroxidation induced by cisplatin treatment is shown in Table I. Cisplatin treatment resulted in 42% decrease in GSH content (3.11 + 0.238 μmol g^{-1}) and 273% increase in MDA production (1449.09 ± 127.36 nmol g^{-1}) in the rat kidney tissue. The changes observed were statistically significant at a P value of < 0.01 in comparison with the control group. Treatment with rebamipide jeopardized the nephrotoxic effect of cisplatin by reducing MDA production to 560.23 + 21.98 nmol g^{-1} and restoring GSH content to 5.063 + 0.269 μmol g^{-1} which are close to that of the control group. The difference between the amounts of the two products in the cisplatin and rebamipide plus cisplatin group was statistically significant ($P < 0.01$). On the contrary to cisplatin which reduced kidney GSH content, rebamipide treatment

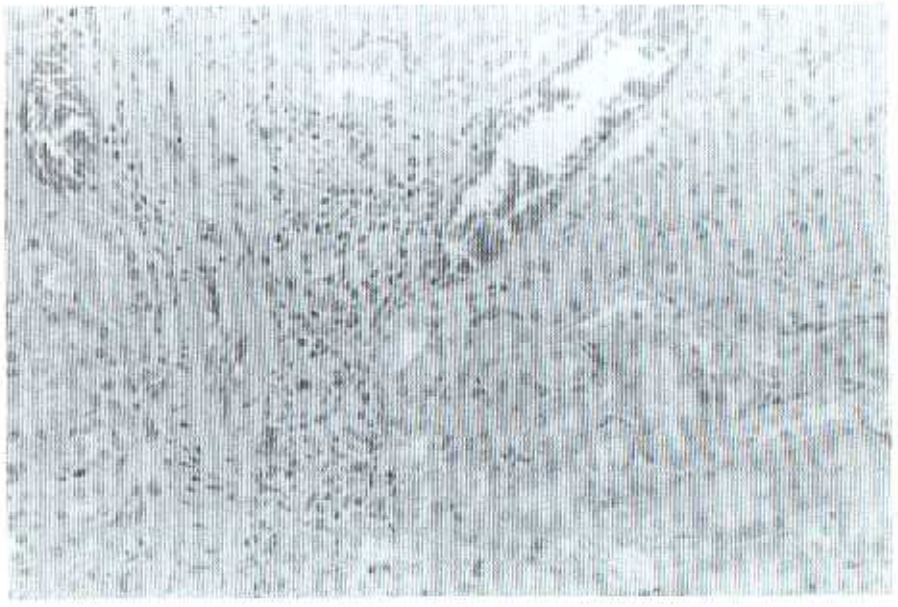

Fig. 2. Section of kidney tissue obtained from a rat 7 days after a single dose of cisplatin (6 mg kg^{-1}, i.v.). Significant large foci of chronic inflammatory cells infiltration in the renal interstitium, degenerative cellular material and apoptotic tubular cells were seen in the renal tubules. HE. ×100.

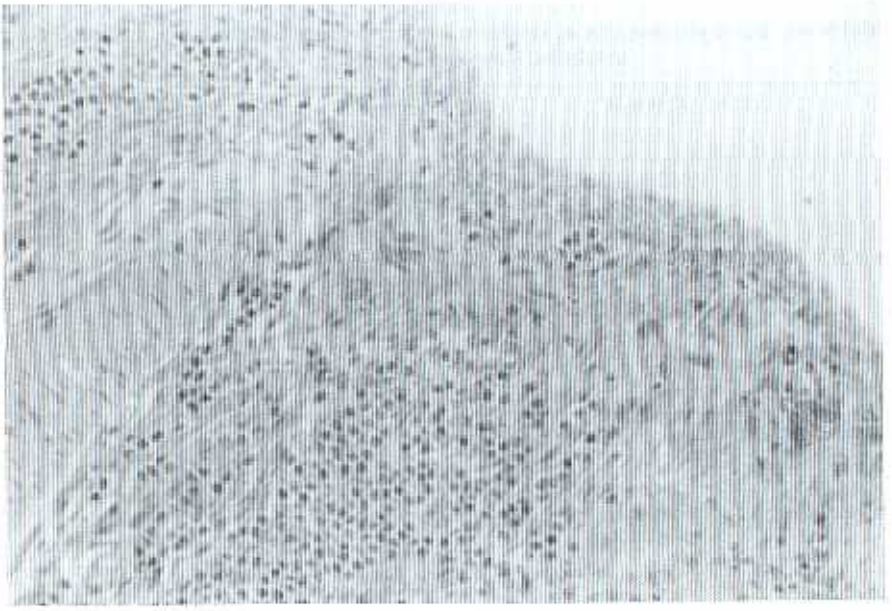

Fig. 3. Section of kidney tissue obtained from a rat 7 days after daily rebamipide treatment (140 mg kg^{-1}, i.p.). No features indicative of tubular damage were noted. HE. ×100.

significantly ($P < 0.05$) increased the GSH content in the kidney of normal rats (6.07 ± 0.204 μmol g^{-1}). Although, rebamipide ameliorated the MDA production by reducing lipid peroxidation and partially restoring the GSH contents in the kidney of rats treated with cisplatin, but still the MDA production was significantly ($P < 0.01$) higher than that in the control group (Table I).

Histopathological study of the kidney tissue at 7 days following cisplatin treatment revealed degenerate cellular material and apoptotic tubular cells were seen in the renal tubules in comparison with the saline treated control (Figs 1 and 2). Concomitant treatment with rebamipide for 1 week ameliorated the nephrotoxic effect of cisplatin and there were no significant pathological changes apart from an occasional hyaline cast in some tubules (Figs 3 and 4).

DISCUSSION

In this study, rebamipide is shown to ameliorate the

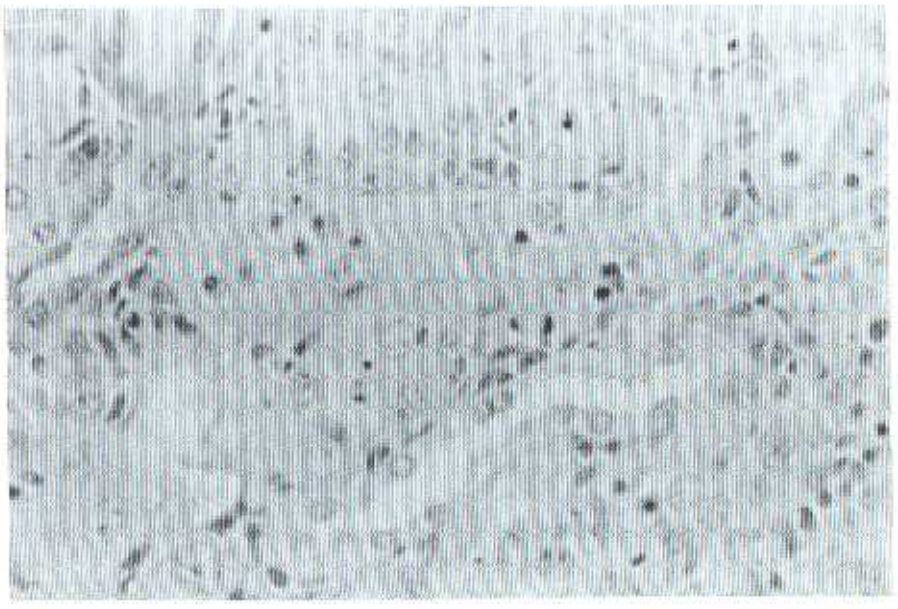

Fig. 4. Section of kidney tissue obtained from a rat 7 days after daily rebamipide treatment (140 mg kg^{-1}, i.p.) carried out 24 h prior to dosing with cisplatin (6 mg kg^{-1}, i.v.). No significant pathology apart from an occasional hyaline cast in some tubules. HE. ×200.

nephrotoxic effect of cisplatin in rats. The changes induced by cisplatin in the following parameters, kidney weight, serum Alb, serum Cr, BUN, kidney GSH contents and MDA production, were significantly ($P < 0.01$) reversed in the presence of rebamipide towards the normal values. The damaging effects of cisplatin on the kidney leading to renal failure have been reported by many investigators [3, 21–24]. The tests utilized in these studies to reflect cisplatin-induced renal dysfunction were the same as in this study and their values were comparable. Several investigators used these tests and the results obtained for serum Alb, serum Cr, BUN and kidney GSH contents and MDA production are in consistent with our findings [4, 22, 23]. Also, cisplatin administration caused a significant increase in the kidney weight as a percent of the total body weight and these results are in accordance with those reported by Hannemann *et al.* [22] and Al-Harbi *et al.* [24]. The increase in the kidney weight could be attributed to the increases in the glomerular volume and cellular degenerative changes including cytoplasmic vaculization of the proximal tubular cells, tubular dilation and pyknotic and hydropic degenerations [25–27]. Literature search did not point to the presence of published studies concerning the protective effect of rebamipide against the nephrotoxic effect of cisplatin or any other cytotoxic drugs. However, several studies involving wide varieties of antioxidants including *N*-acetyl cysteine, glutathione esters, diallyl sulfide and diallyl disulfide, 4-methylthiobenzoic acid, diethyldithiocarbamate and sodium thiosulfate were shown to exert partial protection against the cisplatin-induced nephrotoxicity [5, 7–11].

The cisplatin-induced nephrotoxicity were manifested by the increase in MDA production and the decrease in reduced glutathione contents as well as kidney histopathology (Fig. 2). The changes observed could be due to effects in the renal antioxidant system and lipid peroxidation [8, 10, 21]. In this study, the rats treated only with rebamipide did not differ from the control rats except in the kidney glutathione content which increased significantly. It is possible that in the cisplatin-treated rats, the administration of rebamipide jeopardized the nephrotoxic effect of cisplatin through partial improvement of the renal function tests and amelioration of the kidney-reduced glutathione (GSH) depletion and the increase in malondialdehyde (MDA) production. Zea-Iriate *et al.* [28], stated that rebamipide prevented the impairment of glutathione *S*-transferase and Cu,Zn-superoxide dismutase and restored the glutathione contents. Furthermore, Babu *et al.* [9] reported that cisplatin-induced nephrotoxicity through reduced-glutathione depletion and elevation of lipid peroxidation in the kidney. Histopathologically, administration of cisplatin, 4 or 8 mg kg^{-1} i.p., resulted in proximal straight tubule necrosis observed 3 days after administration [29]. It is possible that in the cisplatin-treated rats, the administration of rebamipide caused the changes in the various laboratory tests via its effect on the reduced glutathione.

Rebamipide ameliorates streptozotocin-induced microalbuminuria and elevated lipid peroxidation in the kidney. Rebamipide attenuates high glucose-induced nephropathy, which is attributed in part, to its antioxidative property and in part to its effect on reversing hyperglycaemia [15]. Considering the known free radical scavenging activity of rebamipide [30], it can be suggested that rebamipide inhibits lipid peroxidation [31].

Nephrology has gained new insights into the role of prostaglandins in kidney function under physiological and pathological conditions. Thromboxane A_2 appears to mediate cisplatin-induced apoptosis of mouse renal cells derived from the terminal proximal tubules by inducing an increase in the level of c-Fos mRNA expression [32].

Thus the protective effect of rebamipide against cisplatin-induced nephrotoxicity may be attributed to the endogenous prostaglandin system involvement through the increase in prostaglandin formation resulting from stimulation of biosynthesis and not inhibition of degradation [33].

In conclusion, rebamipide attenuated the cisplatin-induced nephrotoxicity through its free radical scavenging activity and the study reveals that cisplatin induces its nephrotoxic effect via certain mechanisms beside its free radical generating activity that causes lipid peroxidation. The possibility for the presence of an effect of rebamipide on the disposition of cisplatin can not be eliminated. Further studies concerning the pharmacokinetic and pharmacodynamic interactions with cisplatin as well as the other nephrotoxic cytotoxic drugs are warranted and it needs to extrapolate the results to the human.

REFERENCES

1. Schweitzer VG. Cisplatin-induced ototoxicity: the effect of pigmentation and inhibitory agents. *Laryngoscope* 1993; **103(4 Pt 2)**: 1–52.
2. Gabaizadeh R, Staecker H, Liu W, Kopke R, Malgrange B, Lefevre PP, Van-de-Water TR. Production of both auditory hair cells and auditory neurons from cisplatin induced damage. *Acta-Otolaryngol Stockh* 1997; **117(2)**: 232–38.
3. Goldstein RS, Mayer GH. The nephrotoxicity of cisplatin. *Life Sci* 1983; **32**: 685–90.
4. Ueda H, Sugiyama K, Tashira S, Yokota M. Mechanism of the protective effect of sodium malate on cisplatin-induced toxicity in mice. *Biol Pharm Bull* 1998; **21(2)**: 121–8.
5. Sheikh-Hamad D, Timmins K, Jalali Z. Cisplatin-induced renal toxicity: possible reversal by *N*-acetylcysteine treatment. *J Am Soc Nephrol* 1997; **(10)**: 1640–4.
6. Baliga R, Zhang Z, Baliga M, Ueda N, Shah SV. *In*

vitro and *in vivo* evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. *Kidney Int* 1998; **53(2):** 394–401.
7. Dwivedi C, Abu-Ghazaleh A, Guenther J. Effects of diallyl sulfide and diallyl disulfide on cisplatin-induced changes in glutathione and glutathione-*S*-transferase activity. *Anticancer Drugs* 1996; **7(7):** 792–4.
8. Husain K, Morris C, Whitworth C, Trammel GL, Rybak LP, Somani SM. 4-Methylthiobenzoic acid protection against cisplatin nephrotoxicity:antioxidant system. *Fundam Appl Toxicol* 1996; **32(2):** 278–84.
9. Babu E, Gopalakrishnan VK, Sriganth IN, Gopalakrishnan R, Sakthisekaran D. Cisplatin induced nephrotoxicity and the modulating effect of glutathione ester. *Mol Cell Biochem* 1995; (1): 7–11.
10. Somani SM, Ravi R, Rybak LP. Diethyldithiocarbamate protection against cisplatin nephrotoxicity: antioxidant system. *Drug Chem Toxicol* 1995; **18(2–3):** 151–70.
11. Goel R, Andrews PA, Pfeifle CE, Abramson IS, Kirmani S, Howell SB. Comparison of the pharmacokinetics of ultrafilterable cisplatin species detectable by derivatization with diethyldithiocarbamate or atomic absorption spectroscopy. *Eur J Cancer* 1990; **26(1):** 21–7.
12. Yoshikawa T, Naito Y, Tanigawa T, Kondo M. Free radical scavening activity of the novel antiulcer agent rebamipide studies by electron spin resonance. *Arzneim Forsch* 1993; **43:** 363–6.
13. Kim CD, Hong KW. Preventive effect of rebamipide on gastric lesions induced by ischemic–repertusion in rat. *J Pharmacol Exp Ther* 1995; **275:** 340–4.
14. Lee SM, Kim KH. Rebamipide ameliorates hepatic dysfunction induced by ischemia/reperfusion in rats. *Eur J Pharmacol* 1995; **294:** 41–6.
15. Hunjoo HA, Lee SH, Kim KH. Effects of rebamipide in a model of experimental diabetes and on the synthesis of transforming growth factor-B and fibronectin, and lipid peroxidation induced by high glucose in cultured mesangial cells. *J Pharmacol Exp Ther* 1997; **281(3):** 1457–62.
16. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95:** 351–8.
17. Ellman GL. Tissue sulfahydryl groups. *Arch Biochem Biophys* 1959; **82:** 70–77.
18. Bonsnes RW, Taussky HH. On the colorimetric determination of creatinine by Jaffe reaction. *J Biol Chem* 1945; **158:** 581–91.
19. Wrenn HT, Feichtmeir TV. The quantitation of albumin based on dye linear binding capacity. *Am J Clin Pathol* 1956; **26:** 960–8.
20. Hallet GJ, Cook JG. Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. *Clin Chim Acta* 1971; **35:** 33–7.
21. Matsushima H, Yonemura K, Ohishi K, Hishide A. The role of oxygen free radicals in cisplatin-induced acute renal failure in rats. *J Lab Clin Med* 1998; **131(6):** 518–26.
22. Hannemann J, Duwe J, Baumann K. Iron and ascorbic acid-induced lipid peroxidation in renal microsomes isolated from rats treated with platinum compounds. *Cancer Chemother Pharmacol* 1991; **28(6):** 427–33.
23. Huang Y, Zhou S, Qui L, Wu J, Xu C. Effects of zinc gluconate on the nephrotoxicity and glutathione metabolism disorder induced by cisplatin in mice. *Drug Metabol Drug Interact* 1997; **14(1):** 41–6.
24. Al-Harbi MM, Osman AM, Al-Gharably NM, AL-Bekairi AM, Al-Shabanah OA, Sabah DM, Raza M. Effect of Deferrioxamine on cisplatin-induced nephrotoxicity in normal rats. *Chemotherapy* 1995; **41:** 448–54.
25. Billastre JP, Raguenez-Viotte G. Cisplatin nephrotoxicity. *Toxicol Lett* 1989; **46(1–3):** 163–75.
26. Scott LA, Madan E, Valentovic MA. Attenuation of cisplatin nephrotoxicity by streptozotocin-induced diabetes. *Fundam Appl Toxicol* 1989; **12(3):** 530–9.
27. Sadzuka Y, Shoji T, Takino Y. Change of lipid peroxide levels in rat tissues after cisplatin administration. *Toxicol Lett* 1991; **57(2):** 159–66.
28. Zea-Iriate WL, Makiyama K, Goto S, Murase K, Urata Y, Sekine I, Hara K, Kondo T. Impairment of antioxidants in colonic epithelial cells isolated from trinitrobenzene sulphonic acid-induced colitis rats. Protective effect of rebamipide. *Scand J Gastroenterol* 1996; **31(10):** 985–92.
29. Yamada T. Studies on the mechanisms of renal damages induced by nephrotoxic compounds. *Nippon-Hoigaku-Zasshi* 1995; **49(6):** 447–57.
30. Yoshikawa T, Naito Y, Tanigawa T, Kondo M. Free radical scavenging activity of the novel antiulcer agent rebamipide studied by electron spin resonance. *Arzneim Forsch* 1993; **43:** 363–6.
31. Naito Y, Yoshikawa T, Tanigawa T, Sakurai K, Yamasaki K, Uchida M, Kondo M. Hydroxyl radical scavenging by rebamipide and related compounds: electron paramagnetic resonance study. *Free Radic Biol Med* 1995; **18(1):** 117–25.
32. Jariyawat S, Takeda M, Kobayashi M, Endou H. Thromboxane A_2 mediates cisplatin induced apoptosis of renal tubule cells. *Biochem Mol Biol Int* 1997; **42(1):** 113–21.
33. Kleine A, Kluge S, Peskar BM. Stimulation of prostaglandin biosynthesis mediates gastroprotective effects of rebamipide in rats. *Dig Dis Sci* 1993; **38(8):** 1441–9.