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L-Arginine Ameliorates Kidney Function and Urinary Bladder Sensitivity in Experimentally-induced Renal Dysfunction in Rats

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Effects of L-arginine and NG-nitro-L-arginine methyl ester (L-NAME) on the renal dysfunction that is induced by cisplatin (CDDP) were investigated. A single dose of CDDP (7.5 mg/kg i.p.) induced renotoxicity, which was manifested by increasing the sensitivity of isolated urinary bladder rings to acetylcholine (ACh), together with a significant elevation of serum urea and creatinine, and a severe decrease in serum albumin. Moreover, renal dysfunction was further confirmed by a significant decrease of enzyme activities, such as glutathione peroxidase, GSH-Px (E.C 1.11.1.9), catalase (E.C 1.11.1.6), as well as a significant increase in lipid peroxides that were measured as malondialdhyde (MDA) in kidney tissue homogenates. The administration of L-arginine (70 mg/kg/d p.o in drinking water 5 d before and 5 d after the CDDP injection) significantly ameliorated the renotoxic effects of CDDP, as judged by restoring the normal responses of isolated bladder rings to Ach, and also by an improvement in a range of renal function indices, which included serum urea and creatinine concentrations and kidney weight. In addition, L-arginine prevents the rise of MDA, as well as a reduction of GSH-Px and catalase activities in kidney tissues homogenates. On the other hand, the administration of L-NAME (4 mg/kg/d p.o) resulted in no protection against renal dysfunction that was induced by CDDP treatment. The findings of this study suggest that L-arginine can attenuate kidney injury that is produced by CDDP treatment. In addition, L-arginine may be a beneficial remedy for CDDP-induced renal toxicity, and could be used to improve the therapeutic index of CDDP.

Keywords: Cisplatin, Rat, Creatinine, Kidney, L-Arginine, Oxidative stress, Urea

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Introduction

Cisplatin (Cis-dichlorodiammineplatinum [II]; CDDP) is a prominent member of the effective broad spectrum antitumor drugs. It is effective against several human tumors, e.g. the testis, ovary, head, neck, and lung (Prestayko *et al.*, 1980; Loehrer and Einhorn, 1984), as well as in animal tumor models (Rosenberg, 1977). However, the clinical usage of CDDP is restricted, due to its adverse side effects, including renotoxicity and myelo-suppression (Von-Hoff *et al*., 1979; Goldstein and Mayor, 1983). Consequently, there is great interest in developing new methods to abrogate renal damage (Walker and Gale, 1981). In animals, the administration of the calcium antagonist, nifedipine (Deray *et al.*, 1988), treatment with adenosine antagonists (Knight *et al*., 1991), and L-histidinol (Badaray *et al*., 1997) have all been shown to reduce CDDP-induced nephrotoxicity.

The pathogenesis of CDDP-induced nephrotoxicity is uncertain. However, Offerman *et al.* (1984) recorded a 16% fall in effective renal plasma within 3 h of treatment with CDDP, and proposed that changes in haemodynamics play a role in nephrotoxicity that is induced by CDDP. This has been confirmed by Li *et al.* (1994a) who showed a 50% reduction in the renal blood flow and glomerular filtration rate in rats over a 2-h period following an injection of CDDP. However, lipid peroxidation and free radical generation in the tubular cells were suggested as being responsible for the nephrotoxicity (Hannemann and Baumann, 1988; Ishkawa *et al*., 1990; Vermeulen and Baldew, 1992). In addition, protein synthesis inhibition in the tubular cells by CDDP cannot be excluded (Tay *et al*., 1988).

Nitric oxide (NO) is one of the smallest biologically active molecules that are produced from L-arginine by the nitric oxide synthase enzyme (NOS) (de-Belder and Radomski, 1994; Choi *et al*., 2002). There are three isoforms of NOS: the endothelial type (eNOS), the neuronal type (nNOS), and the isoform that is expressed de novo by exposure to pro-inflammatory cytokins, the inducible type (iNOS). Under pathological conditions, iNOS catalyzes an inadequate

quantity of inducible NO (iNO). The overproduction of iNO has been implicated in the pathogenesis of a variety of inflammatory and immunologically-mediated diseases (Misko *et al*., 1993).

A previous report showed that L-arginine, the substrate for NOS, increases renal blood flow and the glomerular filtration rate in normal rats through a NO-mediated mechanism (Cernadas *et al*., 1992). In addition, the study of the perfused kidney of the rat showed that a L-arginine injection produced vasodilatation (Bhardwaj and Moore, 1989). It was demonstrated that the administration of L-arginine to rats greatly ameliorated the nephrotoxicity that was induced by the CDDP treatment (Li *et al.*, 1994b). On the other hand, the administration of L-NAME, the NOS inhibitor, did not attenuate the nephrotoxicity that was induced by the CDDP treatment (Li *et al.*, 1994a). However, this was disputed by Srivastava, *et al.* (1996) who demonstrated that the pretreatment with L-NAME markedly reduced the renal and gastero-intestinal toxicities that are induced by CDDP. This uncertain effect of L-NAME was the main drive of this study, which re-evaluated the effect of L-NAME in CDDP-induced renal toxicity. In addition, L-NAME was also used to confirm the results of L-arginine. Therefore, the present work was conducted to evaluate the effects of L-arginine and L-NAME on renal toxicity that is induced by CDDP. This goal will be achieved by studying the effect of CDDP on the responses of isolated rat urinary bladder rings to ACh, and on some biochemical parameters that are related to renal functions. Furthermore, the possible protective effects of L-arginine and L-NAME on the CDDP-induced renal dysfunction will also be investigated.

Materials and Methods

Materials L-arginine and L-NAME were purchased from Sigma (St. Louis, USA), while cisplatin (CDDP) was obtained from F. H. Faulding & Co. Limited (Lexia Place Mulgrave-Victoria 3170-Australia). Thiobarbituric acid (TBA) was a product of Fluka (Buchs, Switzerland). All of the remaining chemicals were of the highest commercially available grade.

Animals Male Swiss albino rats weighing 200-250 g were used in all of the experiments. They were obtained from the Experimental Animal Care Center of King Saud University, Riyadh, KSA. The animals were maintained under standard conditions of temperature 24 ± 1°C and 55 ± 5% relative humidity with regular 12 h light/12 h dark cycles. They were allowed free access to standard laboratory food (Purina Chow) and water.

Experimental protocol The animals were divided at random into six groups of 10 rats each. The first group (control) received vehicles used for CDDP (physiological saline solution, i.p). The second group, received L-arginine in drinking water (70 mg/kg/d p.o). The third group received L-NAME in drinking water (4 mg/kg/d p.o). The calculated doses of L-arginine and L-NAME were based on the average daily intake of water. The fourth group was injected with CDDP (7.5 mg/kg i.p). The fifth group received L-arginine in drinking water for 5 consecutive days, before and after the CDDP injection (7.5 mg/kg i.p.). The last group received L-NAME in drinking water for 5 consecutive days, before and after the CDDP injection. Based on preliminary data from our laboratory, the selected concentrations of L-arginine and L-NAME, and the schedule of doses, were chosen.

On the fourth day after the beginning of the CDDP injection, all of the animals were housed separately in metabolic cages to collect 24-h urine. One day later, the blood samples were taken by cardiac puncture, under light ether anesthesia, into non-heparinized tubes. Serum was separated by centrifugation for 5 min at 4,000 rpm and stored at −20°C until analysis.

Isolation and preparation of kidney homogenates and urinary bladder rings Following the collection of blood samples, the rats were sacrificed by cervical dislocation; the lower abdomen was opened and the kidneys were quickly isolated, washed with saline, blotted dry on filter paper, and weighed. Thereafter, 10% (w/v) homogenate of the kidney was made in an ice cold saline using a Branson sonifier (250, VWR Scientific, Danbury, USA). At the same time, the urinary bladder was exposed, the connective tissue and accompanying blood vessels were cut away, and the bladder was cut into rings and placed in a warm physiological salt solution (PSS). The composition of the PSS (Mostafa *et al.*, 2000) in g/l was as follows: NaCl, 6.9; $NaHCO_3$, 2.1; KCl, 0.35; $MgSO_4$, 0.15; KH_2PO_4, 0.16; $CaCl_2$, 0.28, and glucose, 2.0. The rings were mounted horizontally between a clamp and a force transducer for measurement of the isotonic tension using a Statham transducer in an organ bath that was filled with 10 ml of the PSS at a temperature of 37°C, and gassed with 95% O_2-5% CO_2.

The rings were allowed to equilibrate for 30 min prior to the experiment under a resting load of 1g (Nakamura *et al.*, 1992). During this time, the bath solution was replaced every 5 min. The isometric tension was recorded by means of a Statham transducer that was connected to a physiograph (NARCO Bio-system).

Concentration-response curves to ACh were constructed. The rings were exposed to different concentrations of ACh (10^{-7}-10^{-5} M) in a non-cumulative manner. Exposure to each concentration of ACh was maintained until the maximal response to that concentration was reached. The rings were then repeatedly washed with several changes of PSS. The rings were allowed to return to baseline before the next ACh concentration was added. The responses of the bladder rings were calculated as g tension/g tissue.

Measurement of serum biochemical parameters Serum creatinine and urea concentrations were determined colorimetrically as described by Bartles *et al.* (1972) and Patton & Crouch (1977), respectively, using commercially available diagnostic kits (bioMçrieux-RCS, Lyon, France).

Determination of glutathione content, lipid peroxides, and enzyme activities in kidney homogenates Glutathione contents and lipid peroxides (Malondialdhyde [MDA] production) in the kidney tissue homogenates were determined according to the methods of Ellman (1959) and Ohkawa *et al.* (1979), respectively. Catalase and glutathione peroxidase (GSH-Px) activities were

measured in the kidney homogenates according to Higgins *et al.* (1978) and Kraus & Ganther (1980), respectively.

Statistical analysis Data are expressed as (means ± SEM). A statistical comparison between the different groups was performed using a one-way analysis of the variance (ANOVA) that was followed by a Tukey-Kramer multiple comparison test to judge the difference between the various groups.

Results

Effects of L-arginine and L-NAME on CDDP-induced changes in the sensitivity of isolated urinary bladder rings to ACh The effects of CDDP, L-arginine, and L-NAME on the responses of bladder rings to ACh are shown in Figs. 1 and 2. The average increments in the control urinary bladder rings tension after ACh of 10^{-7}, 10^{-6}, and 10^{-5} M were 23.9 ± 2.1, 37.8 ± 3.1, and 80.9 ± 6.4 g tension/g tissue, respectively. Treatment with CDDP significantly enhanced the responsiveness of the rings towards ACh, as compared with the control group. Thus, the average increments in tension in response to ACh of 10^{-7}, 10^{-6}, and 10^{-5} M were 47.8 ± 6.8, 110.6 ± 15.4, and 170.6 ± 22.3 g tension/g tissue, respectively. Treatment of rats with L-arginine did not significantly alter the responses of the isolated urinary bladder rings to ACh when compared with the control, as shown in Fig. 1. Urinary bladder rings, isolated from rats concurrently treated with L-arginine and CDDP, showed a significant reduction in their responsiveness to ACh when compared to the CDDP group. The average increments in their tensions in response to ACh of 10^{-7}, 10^{-6}, and 10^{-5} M were 28 ± 2.6, 46.1 ± 4.2, and 118.6 ± 8.3 g tension/g tissue, respectively. Meanwhile, the responses of the rings that were isolated from the rats that were treated with both L-arginine and CDDP to ACh were not significantly different from the responses of the control group, except for the higher ACh concentration (Fig. 1).

On the other hand, treatment of the rats with L-NAME alone significantly enhanced the responsiveness of their isolated urinary bladder rings to ACh when compared to the control group. The enhancement was quite comparable to the increases that were induced by CDDP (Fig. 2). Treatment of the rats with both L-NAME and CDDP simultaneously failed to reduce the elevated responses of their isolated bladder rings to ACh that was induced by CDDP. The responses of the rings were not significantly different from that of the CDDP group, and significantly higher than the responses of the control group to ACh (Fig. 2). Therefore, the average increments in their tensions in response to ACh of 10^{-7}, 10^{-6}, and 10^{-5} M were 35 ± 6.8, 111.8 ± 21.9, and 164 ± 33.7 g tension/g tissue, respectively.

Effects of L-arginine and L-NAME on CDDP-induced changes in serum biochemical parameters Table 1 demonstrates the effect of L-arginine (70 mg/kg/d p.o) and L-NAME (4-mg/kg p.o) on the kidney dysfunction that was induced by a single injection of CDDP (7.5 mg/kg i.p). Intraperitoneal administration of CDDP caused abnormal renal function in all of the rats. Serum urea and creatinine were significantly increased, 2.8- and 2.9-fold of the control values, respectively. Also, there was a 31% decrease in serum albumin.

Pretreatment of the animals with L-arginine (70 mg/kg/d p.o), 5 days before and after a single injection of CDDP,

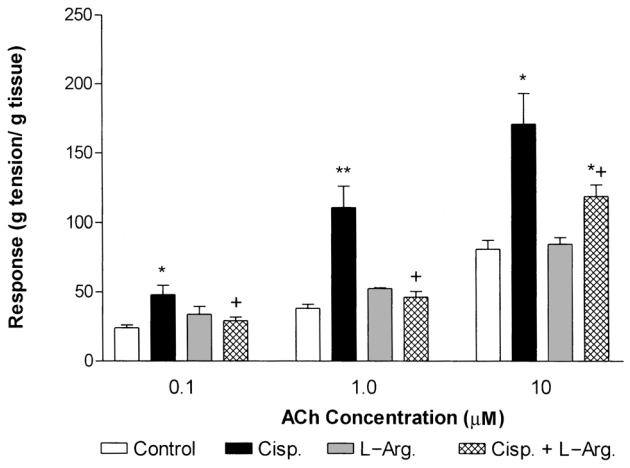

Fig. 1. Effects of CDDP (Cisp), L-arginine (L-Arg.), and their combinations on the responsiveness of isolated urinary bladder rings to ACh. L-arginine (70 mg/kg/d p.o) was given in drinking water for 5 consecutive days before and after CDDP (7.5 mg/kg i.p) administration. Data are expressed as mean values ± SEM. n=10. *Significant difference from control group. P<0.05. **Significant difference from control group. P<0.01. +Significant difference from CDDP group. P<0.05

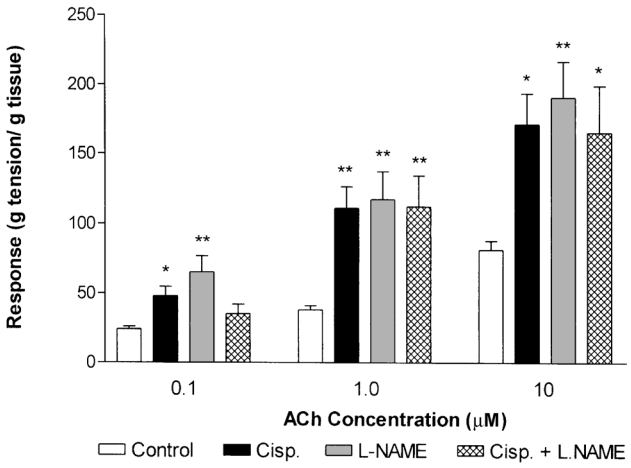

Fig. 2. Effects of CDDP (Cisp), L-NAME, and their combinations on the responsiveness of isolated urinary bladder rings to ACh. L-NAME (4 mg/kg/d p.o) was given in drinking water for 5 consecutive days before and after CDDP (7.5 mg/kg i.p) administration. Data are expressed as mean values ± SEM. n=10. *Significant difference from control group. P<0.05 **Significant difference from control group. P<0.01

Table 1. Effect of L-NAME and L-arginine on CDDP-induced changes in rat serum biochemical parameters urea, creatinine and albumin concentrations

Parameters	Control	L-NAME	L-arginine	CDDP	CDDP+ L-NAME	CDDP+ L-arginine
Urea (mmol/l)	6.5 ± 0.12	7.2 ± 0.32	6.6 ± 0.15	18.6 ± 2.7**	17.2 ± 2.7**	8.7 ± 1.03##
Creatinine (μmol/l)	90 ± 6	96 ± 12	85 ± 6	262 ± 16***	272 ± 13***	160 ± 27*###
Albumin (g/l)	45 ± 3.5	38 ± 1.4	41 ± 2.2	31.1 ± 3**	29 ± 3.7*	39 ± 2.6

All data represent mean values ± SEM.
L-arginine (70 mg/kg/d p.o) and L-NAME (4 mg/kg/d p.o) were given in drinking water for 5 consecutive days before and after CDDP administration. Blood samples were obtained 5 days after CDDP (7.5 mg/kg i.p).
Significant difference from control group. *P<0.05, **P<0.01, ***P<0.001
Significant difference from CDDP group. #P<0.05, ##P<0.01, ###P<0.001

Table 2. Effect of L-NAME and L-arginine pretreatment on CDDP induced changes on relative kidney weight, lipid peroxides (MDA), glutathione content, catalase and GSH-Px

Parameters	Control	L-NAME	L-arginine	CDDP	CDDP+ L-NAME	CDDP+ L-arginine
Kidney weight % of body wt.	0.8 ± 0.03	0.8 ± 0.02	0.78 ± 0.02	1.1 ± 0.08**	1.1 ± 0.08*	0.8 ± 0.06#
MDA (nmol/g tissue)	103 ± 5.8	105 ± 3.6	90 ± 5.6	131 ± 6.7*	117.6 ± 3	75 ± 7.3*##
GSH (μmol/g tissue)	4.0 ± 0.38	3.4 ± 0.38	4.06 ± 0.4	2.27 ± 0.27*	2.06 ± 0.5*	3.7 ± 0.36
Catalase (mmol/min/g)	59 ± 4.7	45.6 ± 6.2	68 ± 8.7	30 ± 4.8*	34.6 ± 1.2	43.7 ± 3.6
GSH-Px (μmol/min/g)	131 ± 8	80.4 ± 6.5*	117 ± 5.5	79.3 ± 10.5*	77 ± 10*	94 ± 1.5*

All data represent mean values ± SEM.
L-arginine (70 mg/kg/d p.o) and L-NAME (4 mg/kg/d p.o) were given in drinking water for 5 consecutive days before and after CDDP administration. Blood samples were obtained 5 days after CDDP (7.5 mg/kg i.p).
Significant difference from control group. *P<0.05, **P<0.01, ***P<0.001
Significant difference from CDDP group. #P<0.05, ##P<0.01, ###P<0.001

significantly reduced the elevated level of serum urea and creatinine. In addition, L-arginine tended to normalize the decreased level of albumin. However, an oral supplementation of L-NAME (4 mg/kg/d p.o) did not attenuate the kidney injury that was induced by CDDP treatment.

Effects of L-arginine and L-NAME on CDDP-induced changes in glutathione content, lipid peroxides and enzyme activities in kidney homogenates Table 2 shows the effect of an oral supplementation of L-arginine and L-NAME on CDDP-induced changes in lipid peroxides that was measured as MDA, antioxidant enzymes, and glutathione content in kidney homogenates.

A single injection of CDDP induced acute renal damage. This was manifested by a significant increase in kidney weight as a percent of body weight and lipid peroxides. In addition, there was a significant decrease in glutathione content by 48%. Moreover, 49% and 42% reductions in catalase and GSH-Px activities, respectively, were also observed. Pretreatment with L-arginine (70 mg/kg/d p.o) caused significant decreases in kidney weight, and prevented a significant increase in lipid peroxides. In addition, glutathione content was greatly improved. Moreover, L-arginine prevented a significant decrease in antioxidant enzyme activities; catalase and glutathione peroxidas. The administration of L-NAME (4 mg/kg/d p.o) did not ameliorate the nephrotoxicity that was induced by the CDDP administration.

Discussion

In the present study, the renal dysfunction that was induced by CDDP (7.5 mg/kg i.p) was confirmed by a significant change in the sensitivity of isolated urinary bladder to increasing concentrations of ACh, and by the marked elevations of serum urea and creatinine concentration as well as a significant decrease in albumin. To our knowledge, this is the first study to show that CDDP significantly increases the responsiveness of isolated urinary bladder to ACh. Renal dysfunction was also reflected in the kidney as a depletion of glutathione content, which was mainly accompanied by a marked reduction in enzyme activities, GSH-Px and catalase, in addition to a significant elevation of lipid peroxides.

In the present study, the treatment of rats with L-arginine alone (70 mg/kg/d p.o) for 10 days induced no changes in the sensitivity of the isolated urinary bladder rings to ACh or in the measured biochemical parameters. However, providing L-arginine to the CDDP-treated animals with drinking water

(70 mg/kg/d p.o) for 5 consecutive days before the treatment, then continuing for another 5 consecutive days resulted in a significant reduction in the CDDP-induced elevated urinary bladder responses to ACh. In fact, the responses of the rings that were isolated from the rats that were treated with both L-arginine and CDDP to ACh were normalized and returned back to normal value, except with the higher ACh concentration. It seems that the dose of L-arginine that was supplied in the drinking water was not sufficient to normalize the bladder response to high concentrations of ACh (10 μM). Also, by increasing the dose of L-arginine, a complete normalization may be achieved. Therefore, by producing more NO in the renal system, L-arginine probably antagonizes the constrictor effect of ACh on the urinary bladder and protects the urinary bladder from hypersensitivity to Ach that is induced by the CDDP treatment. Similarly, concurrent treatment of rats with L-arginine and CDDP produced an improvement in the studied indices of renal function when compared to the CDDP-treated animals. This protection was evidenced in the serum since the elevated level in both urea and creatinine concentrations were markedly lower than those that were elicited by the nephrotoxicant, and the severe decrease in albumin tended to be normalized. In addition, the attenuation of the renotoxicity was also observed in the kidney. L-arginine prevents a rise in lipid peroxides and a significant decrease in catalase and GSH-Px activities in the kidney tissues. This finding agrees with an earlier work (Li *et al.*, 1994b) that showed that i.v. administration of L-arginine in a CDDP injection produced a significant protection of renal function. In addition, it was demonstrated that L-arginine ameliorates the renal injury that is induced by lead acetate. Dehpour *et al.*, 1999, revealed that the concurrent perfusion of L-arginine with lead acetate in the perfusion fluid to the rat kidney significantly decreased the nephrotoxicity that was induced by lead acetate.

Reportedly, L-arginine increases the renal blood flow and glomerular filtration rate in normal rats through an NO-mediated mechanism (Cernadas *et al.*, 1992). Therefore, the possible protective effect that is afforded by L-arginine is due to antagonism of CDDP's effect on renal haemodynamics. However, D-arginine, which is not a substrate for NO and has no vasodilating effect, is also reported to significantly ameliorate the renal dysfunction that is induced by CDDP; however, this protection was not as great as that produced by the same dose of L-arginine (Li *et al.*, 1994b). Moreover, the results of the present study demonstrate that L-argnine blunts the rise of lipid peroxide, besides a significant decrease in catalase and GSH-Px activities. In addition, Sofirstein *et al.*, 1987, showed that the necrosis of the proximal tubule that is induced by the CDDP treatment was lessened by L-arginine. It could be concluded that L-arginine has an additional effect, a cyto-protective effect. However, it is currently difficult to assess which of these properties of L-arginine is responsible for the protecting effect against renal dysfunction that is induced by CDDP.

The rationale for the L-arginine dose schedule in this study was to maintain a steady sufficient plasma concentration of L-arginine before, during, and after the critical period of CDDP-induced toxicity. The biochemical changes that occur in the kidney within a few hours of CDDP administration are indeed of crucial importance in determining the extent of a nephrotoxic lesion (Borch and Pleasants, 1979).

In the present study, the treatment of rats with L-NAME alone (4 mg/kg/d p.o.) for 10 days induced a significant increase in the responsiveness of their urinary bladder rings to ACh in a way that is quite comparable to the increases in the responses of the rings that are isolated from the rats that were treated with CDDP alone to same concentrations of ACh. This finding may indicate that both CDDP and L-NAME act through the same mechanism to induce an increase in the response of the bladder rings to ACh. L-NAME, a NOS inhibitor, reduces the amount of NO in the renal system that may act normally to suppress the contractions of the urinary bladder that is induced by ACh. Similarly, CDDP may also increase the responses of the urinary bladder rings to ACh by inhibiting a normal release of NO as a result of damaging the epithelial cell layers that line the renal system (Sofirstein *et al.*, 1987). Furthermore, the treatment of rats with L-NAME alone (4 mg/kg/d p.o.) for 10 days induced a significant decrease in GSH-Px activity in kidney homogenates. In addition, there was an insignificant decrease in catalase activity and reduced glutathione. However, L-NAME induced no changes in the serum biochemical parameters. Administrating L-NAME in drinking water (4 mg/kg/d p.o) for 5 consecutive days before and then continuing for another 5 consecutive days after a single dose of CDDP (7.5 mg/kg i.p) resulted in no protection against kidney damage that is induced by treatment with CDDP. This finding supports the previous study that demonstrated that L-NAME administration did not protect the kidney function against nephrotoxicity that is induced by CDDP (Li *et al.*, 1994a). In addition, the administration of L-NAME did not ameliorate the renal dysfunction in another model of kidney damage. Dehpour *et al.* (1999) studied the effect of the concurrent perfusion of L-NAME with lead acetate and concluded that L-NAME increased renal injury that is induced by lead. Moreover, Wang *et al.* (2001) reported the co-administration of L-NAME with FK-506, and concluded that the renal function was significantly worsened when compared to FK-506 without the L-NAME group. However, Srivastava *et al.* (1996) showed that pretreatment with L-NAME markedly reduced the renal and gastro-intestinal toxicities that are induced by CDDP. This discrepancy could be due to the difference in the dose of L-NAME that was used or the experimental design.

In conclusion, the results of the present study may indicate that L-arginine is beneficial as a protective agent against kidney damage that is induced by CDDP in rats. In contrast, L-NAME did not ameliorate the kidney dysfunction that was induced by CDDP administration. Further studies are needed

to elucidate the mechanism(s) of protection and the effect of L-arginine on the anti-tumor activity of CDDP.

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