Pergamon

Biochemical Pharmacology, Vol. 47, No. 12, pp. 2298–2301, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)E0109-X

Effect of arginine on cisplatin-induced acute renal failure in the rat

(*Received* 29 *November* 1993; *accepted* 24 *February* 1994)

Abstract—The effect of arginine on the nephrotoxicity produced by cisplatin (6.0 mg/kg i.v.) was investigated in the rat. Intravenous administration of L-arginine at doses of 0.26–2.63 g/kg at the time of cisplatin injection produced significant protection of renal function as evidenced by reductions in plasma urea and creatinine concentrations, decreased polyuria and increases in the plasma clearance of [^{3}H]inulin and [^{14}C]-*p*-aminohippurate. Administration of D-arginine (2.63 g/kg i.v.) also significantly ameliorated the renal dysfunction induced by cisplatin although this protective effect was not as great as produced by the same dose of L-arginine. D-arginine, by contrast to its L-isomer, is reported to have little or no effect on renal haemodynamics. Consequently, the results of this study indicate that the protective effect of L-arginine in cisplatin nephrotoxicity involves both haemodynamic and non-haemodynamic components.

Key words: nephrotoxicity; polyuria; amino acid; inulin; *p*-aminohippuric acid; urea

Cisplatin is an important antineoplastic drug, being particularly effective for treatment of testicular and ovarian cancers. Its use, however, is circumscribed in some patients by its nephrotoxic effects which are evident in about 20% of patients despite the use of saline hydration and diuretics [1]. Consequently, there is much interest in developing new methods to abrogate renal damage. In animals, administration of diethyldithiocarbamate [2], treatment with adenosine antagonists [3], and the calcium antagonist nifedipine [4] have all been shown to reduce cisplatin-induced nephrotoxicity. Recently, Heyman *et al.* [5] demonstrated the amino acid glycine could ameliorate cisplatin-induced ARF* in the rat. NO may play an important role in this protective effect since the beneficial actions of glycine can be partially blocked by co-administration of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester [6]. As the guanidine moiety of L-arginine is the source of NO [7], we have investigated the ability of this amino acid to prevent cisplatin-induced ARF in the rat.

Materials and Methods

Materials. L- and D-arginine hydrochloride, inulin, *p*-aminohippuric acid and cisplatin were purchased from the Sigma Chemical Co. (Poole, U.K.). [^{3}H(*G*)]Inulin (201 mCi/g) and *p*[glycyl-l-^{14}C]aminohippuric acid (43 mCi/mmol) were obtained from DuPont NEN Research Products (Stevenage, U.K.). The stated radiochemical purity of each isotope was greater than 98%. Reagent kits for assay of creatinine and urea were bought from Pierce & Warriner (U.K.) and BDH Ltd (Poole, U.K.), respectively.

Induction of ARF. Male Wistar albino rats (200–250 g) were injected via the tail vein with one of the following: (1) saline (0.9% w/v NaCl, 3 mL/kg); (2) cisplatin (6 mg/kg, 2 mg/mL in saline); (3) cisplatin (6 mg/kg) plus L-arginine (either 0.26, 0.66, 1.32 or 2.63 g/kg in distilled water); (4) cisplatin (6 mg/kg) followed by D-arginine (2.63 g/kg) and (5) cisplatin (6 mg/kg) plus vehicle for arginine (distilled water, 5 mL/kg). Arginine solutions were adjusted to pH 7.0 with 1 M NaOH. The 2.63 g/kg dose of arginine is equimolar with 1 g/kg of glycine which we have previously shown to protect against cisplatin-induced ARF [6]. Vehicle and arginine solutions were administered over a total of 3 min, with the dose of cisplatin given at the mid-point.

A blood sample (0.75 mL) was taken from the tail vein 3 days following the various treatments (day 4) after which rats were placed in metabolic cages for a 24 hr urine collection. Animals were also placed in metabolic cages for urine collection over 24 hr on day 7. On day 8, rats were anaesthetised and the plasma clearances of [^{3}H]inulin, C_{IN} (100 mg/kg, 20 μCi/kg, i.v.) and [^{14}C]-*p*-aminohippuric acid, C_{PAH} (40 mg/kg, 4 μCi/kg, i.v.) were then determined by the single injection method of Hall *et al.* [8]. At the end of the experiment, a final blood sample (1 mL) was taken from the carotid artery and rats were killed with an overdose of anaesthetic.

Analysis of plasma and urine samples. Plasma concentrations of creatinine and urea were measured by reaction with alkaline picrate and diacetyl monoxine, respectively. Urine sodium was assayed with a Corning 480 flame photometer.

Statistical methods. Values are expressed as means ±SEM. Comparison of means between groups was initially made by ANOVA, with means compared by Scheffe's test.

Results and Discussion

Effect of cisplatin. Table 1 shows rats given a single bolus dose of cisplatin (6 mg/kg) exhibited marked increases in plasma creatinine and urea concentrations, and a polyuria accompanied by reduced sodium excretion. Moreover, both C_{IN} and C_{PAH} were significantly ($P < 0.001$) diminished when compared to saline-injected rats (Fig. 1). These results are similar to those obtained previously [3] and they suggest cisplatin at a dose of 6 mg/kg severely impairs renal function.

Effect of vehicle treatment on cisplatin-injected rats. Treatment with the vehicle for arginine (distilled water 5 mL/kg) had no significant ($P > 0.05$) effects on plasma urea concentrations on either days 4 or 8 or creatinine levels on day 4, although creatinine concentrations on day 8 were significantly ($P < 0.05$) higher than in the group given cisplatin alone (Table 1). Furthermore, there were no statistically significant differences between rats injected with cisplatin and the cisplatin-vehicle treated group with respect to either 24 hr urine volume or sodium excretion. Although vehicle treatment had no significant effect on C_{PAH}, C_{IN} was significantly ($P < 0.05$) lower compared to

* Abbreviations: ARF, acute renal failure; NO, nitric oxide; C_{IN}, plasma clearance of [^{3}H]inulin; C_{PAH}, plasma clearance of [^{14}C]-*p*-aminohippurate; ANOVA, one way analysis of variance.

Table 1. Effect of treatment with L-arginine and D-arginine on plasma urea and creatinine concentrations, urine output and sodium excretion in cisplatin-injected rats

	Day(s)	Saline	CP	CP + V	CP + $A_{0.26}$	CP + $A_{0.66}$	CP + $A_{1.32}$	CP + $A_{2.63}$	CP + $DA_{2.63}$
Plasma urea	4	36 ± 1	133 ± 4†	138 ± 6	94 ± 4‖	90 ± 8‖	49 ± 7¶	68 ± 6¶**	83 ± 8¶
(mg 100 mL^{-1})	8	30 ± 2	128 ± 7†	141 ± 13	82 ± 2‖	74 ± 3‖	37 ± 3¶	69 ± 10¶**	99 ± 3‖
Plasma creatinine	4	0.56 ± 0.02	1.91 ± 0.11†	1.89 ± 0.09	1.20 ± 0.07§	1.12 ± 0.11‖	1.02 ± 0.06‖	1.05 ± 0.08‖	1.24 ± 0.03§
(mg 100 mL^{-1})	8	0.63 ± 0.08	1.94 ± 0.26†	2.52 ± 0.11‡	1.25 ± 0.05‖	1.58 ± 0.20‖	0.88 ± 0.06¶	0.92 ± 0.08¶**	1.39 ± 0.13
Urine output	4–5	3 ± 1	17 ± 1†	16 ± 1	15 ± 2	11 ± 0.4§	9 ± 1§	7 ± 1‖**	10 ± 1§
(mL 100 g^{-1} 24 hr^{-1})	7–8	3 ± 1	15 ± 1†	12 ± 1	9 ± 1§	9 ± 1§	5 ± 0.3¶	6 ± 1¶**	10 ± 1
Sodium excretion	4–5	0.65 ± 0.04	0.43 ± 0.05*	0.36 ± 0.04	0.41 ± 0.07	0.50 ± 0.05	0.69 ± 0.04§	0.50 ± 0.08**	0.86 ± 0.04§
(mmol 100 g^{-1} 24 hr^{-1})	7–8	0.86 ± 0.05	0.40 ± 0.05*	0.53 ± 0.08	0.35 ± 0.04	0.58 ± 0.04	0.55 ± 0.07	0.37 ± 0.03††	0.76 ± 0.09§

Results shown are means ±SEM (N = 8). Key: saline (0.9% NaCl, 3 mL/kg); CP (cisplatin 6.0 mg/kg); CP + V (cisplatin 6.0 mg/kg + distilled water 5 mL/kg); CP + A (cisplatin 6.0 mg/kg + L-arginine at doses of 0.26, 0.66, 1.32 and 2.63 g/kg, subscript indicates dose); CP + $DA_{2.63}$ (cisplatin 6.0 mg/kg + D-arginine 2.63 g/kg).

* $P < 0.01$, † $P < 0.001$ relative to saline group (*t*-test); ‡ $P < 0.05$ relative to CP group; § $P < 0.05$, ‖ $P < 0.01$, ¶ $P < 0.001$ relative to CP + V; ** $P < 0.05$, †† $P < 0.01$ relative to CP + $DA_{2.63}$ (ANOVA).

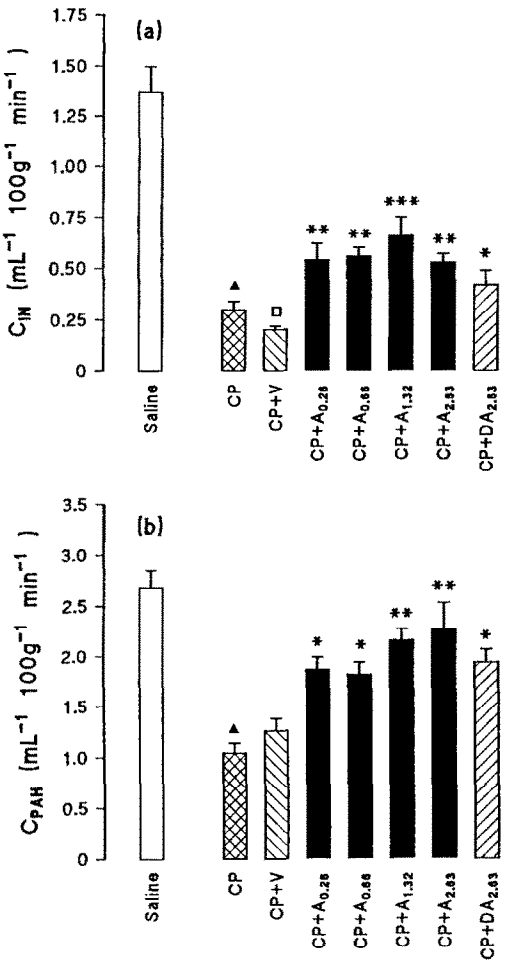

Fig. 1. Clearance of (a) [^{3}H]inulin (C_{IN}) and (b) [^{14}C]-*p*-aminohippuric acid (C_{PAH}) in anaesthetised rats 7 days following treatment with cisplatin (6.0 mg/kg) and arginine. Columns represent means with vertical bars SEM. Key: saline (0.9% NaCl, 3 mL/kg); CP (cisplatin); CP + V (cisplatin + distilled water 5 mL/kg); CP + A (cisplatin + L-arginine at doses of 0.26, 0.66, 1.32 and 2.63 g/kg, subscript indicates dose); CP + $DA_{2.63}$ (cisplatin + D-arginine 2.63 g/kg). (▲) $P < 0.001$ relative to saline-treated animals (*t*-test); (□) $P < 0.05$ relative to CP group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ relative to CP + V group (ANOVA).

cisplatin-injected animals (Fig. 1). Thus, vehicle treatment had no detectable beneficial effects on cisplatin-induced nephrotoxicity.

Effect of arginine treatment on cisplatin-injected rats. All four doses of L-arginine, ranging from 0.26 to 2.6 g/kg, significantly ($P < 0.05$) reduced plasma urea and creatinine levels in cisplatin-injected rats (Table 1). In addition, urine output on days 7–8 was decreased ($P < 0.05$) in L-arginine treated rats, when compared to vehicle treated animals. However, though sodium excretion tended to be higher in L-arginine treated rats, these changes, with the exception of rats given 1.32 g/kg (days 4–5) were not statistically different from data for vehicle treated rats. Figure 1 shows treatment with all doses of L-arginine significantly ($P < 0.05$) increased both C_{IN} and C_{PAH}. The greatest protection of renal function was produced by 1.32 g/kg as administration of this dose resulted in: the lowest plasma creatinine and urea levels, maximum reduction in cisplatin-induced polyuria and largest increase in sodium excretion (Table 1) and C_{IN} (Fig. 1).

It is clear that L-arginine, like glycine [5, 6] is able to ameliorate cisplatin-induced ARF in the rat. However, a comparison of the beneficial effects of glycine [6] with L-arginine indicates that glycine has a greater capacity to reduce the severity of cisplatin nephrotoxicity. For example, 1 g/kg of glycine (a dose that produces maximum protection of cisplatin nephrotoxicity, unpublished studies) administered to cisplatin-injected rats produced a reduction in plasma creatinine levels of 70% and an increase of 190% in C_{PAH} in comparison to values noted in rats given cisplatin only [6]. By comparison, treatment with the most effective dose of L-arginine (1.32 g/kg) resulted in a lowering of plasma creatinine levels of about 50% and an elevation of C_{PAH} of 120%.

D-Arginine (2.63 g/kg) also diminished the severity of cisplatin-induced ARF since, in comparison to vehicle-treated rats, there were significant ($P < 0.05$) reductions in urea (days 4 and 8), creatinine (day 4) and urine output (days 4–5), and increases in sodium excretion, C_{IN} and C_{PAH} (Table 1 and Fig. 1). A comparison of the effects of D-arginine with the equivalent dose of L-isomer revealed L-arginine to be significantly ($P < 0.05$) more effective at reducing plasma creatinine and urea levels, and the polyuria whereas D-arginine significantly ($P < 0.05$) increased sodium excretion to a greater extent than the L-amino acid (Table 1). L-Arginine appeared to enhance both C_{IN} and C_{PAH} to a greater extent than the D-isomer, but this difference was not statistically significant ($P > 0.05$) (Fig. 1).

The pathogenesis of cisplatin's nephrotoxic effects is not fully understood. However, Offerman *et al.* [9], in a study of patients with testicular carcinoma, recorded a fall of 16% in effective renal plasma flow within 3 hr of treatment with cisplatin and proposed that changes in renal haemodynamics play a role in cisplatin-induced nephrotoxicity. We have observed reductions of 50% in renal blood flow and glomerular filtration rate in rats over a 2 hr period following injection of 6 mg/kg cisplatin [6] and Winston and Safirstein [10] found a 36% decrease in renal blood flow and a 62% increase in renal vascular resistance 72 hr after administration of cisplatin. L-Arginine increases renal blood flow and glomerular filtration rate in normal rats through an NO-mediated mechanism [11]. Thus, it is possible the protection afforded by L-arginine is due to antagonism of cisplatin's renal haemodynamic effects. However, D-arginine which is not a source for NO [7] also had beneficial effects in cisplatin nephrotoxicity. In the conscious dog, infusion of L-arginine (cumulative dose ~0.4 g/kg) evoked an increase in renal blood flow of 84% whilst the equivalent dose of D-arginine produced an elevation in renal blood flow of 13% [12]. A study of the perfused kidney of the rat showed that bolus injection of L-arginine chloride produced vasodilation (ED_{50} 14 ± 1.2 μmol) although D-arginine chloride at doses as high as 170 μmol did not affect perfusion pressure [13]. Whilst it is difficult to relate the above studies [12, 13] to the present work in which isomers of arginine were given to anaesthetised rats as bolus doses of up to 2.63 g/kg, these investigations suggest that D-arginine, by comparison to its L-isomer, is likely to have minimal effects on renal blood flow in the rat. The beneficial effects of D-arginine in maintaining C_{IN} and C_{PAH} were similar to the equivalent dose of L-arginine although the protective effects of D-arginine were less pronounced with respect to plasma urea and creatinine and the polyuria. One explanation for these findings is that there are two components to the protective effect of L-arginine, a haemodynamic effect and a non-

haemodynamic cytoprotective effect. In addition to any renal haemodynamic changes, cisplatin nephrotoxicity is characterized by necrosis of the S_3 segment of the proximal tubule [14]. Such damage may be lessened by any cytoprotective effect of L-arginine. A non-haemodynamic protective effect of amino acids has been demonstrated previously since glycine and alanine maintain cell viability in suspensions of proximal tubules subjected to hypoxia or anoxia [15]. Recently Garza-Quintero *et al.* [16] proposed that the mechanism which underlies this cytoprotective action of glycine is its ability to maintain the structural stability of proximal tubular cell membranes [16].

In conclusion, L-arginine provides protective effects in cisplatin nephrotoxicity by a mechanism which might involve both haemodynamic and non-haemodynamic components.

Acknowledgements—QL wishes to thank the Henry Lester Trust and the Great Britain China Educational Trust for their financial support.

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REFERENCES

1. Garnick MB, Mayer RJ and Abelson HT, Acute renal failure associated with cancer treatment. In: *Acute Renal Failure* 2nd Edn (Eds. Brenner BM and Lazarus JM), pp. 621–657. Churchill Livingstone, New York, 1988.
2. Gandara DR, Perez EA, Wiebe V and De Gregorio MW, Cisplatin chemoprotection and rescue: pharmacologic modulation of toxicity. *Semin Oncol* **18**: 49–55, 1991.
3. Knight RJ, Collis MG, Yates MS and Bowmer CJ, Amelioration of cisplatin-induced acute renal failure with 8-cyclopentyl-1,3-dipropylxanthine. *Br J Pharmacol* **104**: 1062–1068, 1991.
4. Deray G, Dubois M, Beaufils H, Cacoub P, Anouar M, Jaudon MC, Baumelou A, Jouanneau C and Jacobs C, Effects of nifedipine on cisplatinum-induced nephrotoxicity in rats. *Clin Nephrol* **30**: 146–150, 1988.
5. Heyman SN, Rosen S, Silva P, Spokes K, Egorin MJ and Epstein FH, Protective action of glycine in cisplatin nephrotoxicity. *Kidney Int* **40**: 273–279, 1991.
6. Li Q, Bowmer CJ and Yates MS, The protective effect of glycine in cisplatin nephrotoxicity: inhibition with N^G-nitro-L-arginine methyl ester. *J Pharm Pharmacol*, **46**: 346–351, 1994.
7. Moncada S, Palmer RMJ and Higgs EA, Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* **43**: 109–141, 1991.
8. Hall JE, Guyton AC and Farr BM, A single-injection method for measuring glomerular filtration rate. *Am J Physiol* **232**: F72–F76, 1977.
9. Offerman JJG, Meijer S, Sleijfer DT, Mulder NH, Donker AJM, Koops HS and van der Hem GK, Acute effects of *cis*-diamminedichloroplatinum (CDDP) on renal function. *Cancer Chemother Pharmacol* **12**: 36–38, 1984.
10. Winston JA and Safirstein R, Reduced renal blood flow in early cisplatin-induced acute renal failure in the rat. *Am J Physiol* **249**: F490–F496, 1985.
11. Cernadas MR, López-Farré A, Riesco A, Gallego MJ, Espinosa G, Digiuni E, Hernando L, Casado S and Caramelo C, Renal and systemic effects of amino acids administered separately: comparison between L-arginine and non-nitric oxide donor amino acids. *J Pharm Exp Ther* **263**: 1023–1029, 1992.
12. Murakami M, Suzuki H, Ichihara A, Naitoh M, Nakamoto H and Saruta T, Effects of L-arginine on systemic and renal haemodynamics in conscious dogs. *Clin Sci* **81**: 727–732, 1991.
13. Bhardwaj R and Moore PK, The effect of arginine and nitric oxide on resistance blood vessels of the perfused rat kidney. *Br J Pharmacol* **97**: 739–744, 1989.
14. Safirstein R, Winston J, Moel D, Dikman S and Guttenplan J, Cisplatin nephrotoxicity: insights into mechanism. *Int J Androl* **10**: 325–346, 1987.
15. Weinberg JM, The cell biology of ischemic renal injury. *Kidney Int* **39**: 476–500, 1991.
16. Garza-Quintero R, Weinberg JM, Ortega-Lopez J, Davis JA and Venkatachalam MA, Conservation of structure in ATP-depleted proximal tubules: role of calcium, polyphosphoinositides, and glycine. *Am J Physiol* **265**: F605–F623, 1993.

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