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Amelioration of Cisplatin Nephrotoxicity with Glycine: Dose Dependency in Rats

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Abstract

The effects of glycine (0·1–1·0 g kg^{-1}, i.v.) on the acute changes in renal haemodynamics and nephrotoxicity produced by cisplatin (6·0 mg kg^{-1}, i.v.) were investigated in the rat.

Cisplatin produced decreases of 50% in the clearance of [^{3}H] inulin (C_{IN}) and renal blood flow (RBF), 110 min following its injection. Glycine at a dose of 0·1 g kg^{-1} produced no attenuation of the cisplatin-induced decrease in C_{IN} or RBF. Furthermore, this dose of glycine provided no significant protection of renal function over a 7-day period following cisplatin injection.

By contrast, glycine at a dose of either 0·5 or 1·0 g kg^{-1} markedly attenuated cisplatin-induced falls in C_{IN} and RBF, with the highest dose completely preventing any falls in these indices during the course of the experiment. Treatment with these higher doses of glycine produced prominent protection from the nephrotoxic actions of cisplatin, as evidenced by improvements in a range of indices of renal function which included plasma urea and creatinine concentrations, urine output, sodium excretion, C_{IN} and the clearance of [^{14}C]*p*-aminohippurate.

The results of experiments with an intermediate dose of 0·25 g kg^{-1} glycine revealed some degree of amelioration of acute renal haemodynamic effects of cisplatin, particularly with regard to C_{IN}; whilst in the nephrotoxicity study, 0·25 g kg^{-1} glycine produced a modest but significant reduction in cisplatin-induced acute renal dysfunction.

The results have revealed a clear association between the acute renal haemodynamic effects produced by glycine in cisplatin-injected rats with the longer-term renal protective effects of glycine in cisplatin nephrotoxicity. The findings indicate that glycine's ability to prevent the falls in RBF and glomerular filtration rate produced by cisplatin plays an important role in the protective effect of glycine in cisplatin-induced nephrotoxicity.

Heyman et al (1991) have shown in-vivo that glycine infusion significantly ameliorates the nephrotoxic actions of the anticancer agent cisplatin in the rat. We have confirmed this finding in a recent study in which glycine was administered as a bolus injection and renal function monitored over seven days (Li et al 1994). Furthermore, we noted cisplatin produced a 50% fall in renal blood flow and inulin clearance within 2 h of its injection and that this was prevented by co-administration of glycine (1 g kg^{-1}). Offerman et al (1984), in a study of patients with testicular carcinoma, recorded a fall of 16% in effective renal plasma flow within 3 h of treatment with cisplatin and proposed that changes in renal haemodynamics play a role in cisplatin-induced nephrotoxicity. Similarly, falls in renal blood flow and glomerular filtration rate may contribute to cisplatin nephrotoxicity in the rat, and thus it is possible that glycine's ability to ameliorate cisplatin nephrotoxicity is due to a functional antagonism of the renal vasoconstriction induced by the anticancer agent. However, glycine also has a cytoprotective action as demonstrated by its ability to protect against anoxic injury in-vitro in dispersed rabbit proximal tubules (Weinberg et al 1987). Therefore, glycine's beneficial actions in cisplatin nephrotoxicity may result from both a haemodynamic and a non-haemodynamic cytoprotective action. In the present study, an attempt was made to identify any non-haemodynamic element in glycine's action in cisplatin nephrotoxicity by determining the dose-response relationship for glycine, both with regard to its renal haemodynamic effects in cisplatin-injected animals and its ability to abrogate cisplatin-induced nephrotoxicity. These experiments were conducted to define the threshold doses of glycine which produce, on the one hand, reduction of the acute falls in renal blood flow and glomerular filtration rate induced by cisplatin and, on the other hand, amelioration of cisplatin nephrotoxicity.

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Materials and Methods

Materials

Glycine, inulin, *p*-aminohippuric acid and cisplatin were purchased from Sigma Chemical Co., Poole, UK. [^{3}H(G)]-Inulin (201 mCi g^{-1}) and *p*-[glycyl-1-^{14}C]aminohippuric acid (43 mCi mmol^{-1}) were obtained from DuPont NEN Research Products, Stevenage, UK. The stated radiochemical purity of each isotope was greater than 98%. Reagent kits for assay of creatinine and urea were bought from Pierce & Warriner, Chester, UK, and BDH Ltd, Lutterworth, UK, respectively.

Acute effects of cisplatin on renal function in anaesthetized animals

Male albino Wistar rats, 200–250 g (Leeds University Biomedical Services) were anaesthetized with thiobutabarbitone (180 mg kg^{-1}, i.p.) and cannulae inserted into: the

trachea to facilitate spontaneous ventilation; the left jugular vein for saline infusion and drug administration; and the right carotid artery for blood sampling. The abdomen was opened by a midline incision and a cannula was inserted into the bladder for collection of urine and an ultrasonic perivascular flow probe (model 2SB, Transonic Systems Inc. USA) was placed around the left renal artery. The probe was connected to a small-animal flowmeter (T206 Transonic Systems Inc.) to record mean renal blood flow (RBF). Body temperature was maintained at 37°C using a rectal thermometer and heating lamps. On completion of surgery, 2 mL saline (0.9% w/v NaCl) containing 0·35 μCi mL^{-1} [^{3}H]inulin was administered and this solution was infused for the remainder of the experiment at a rate of 100 μL min^{-1}. A 60-min equilibration period was then allowed for stabilization of urine flow.

Each experiment consisted of a control collection period of 30 min followed by six 20-min clearance periods during which urine was collected into pre-weighed tubes, and a blood sample (0·1 mL) was taken at the midpoint of each urine collection so the renal clearance of [^{3}H]inulin (C_{IN}) could be estimated. Blood samples were centrifuged and the plasma separated for subsequent analysis. The erythrocytes were suspended in an equal volume of isotonic saline and transfused back into the animal. Following the control collection period, rats received intravenously either cisplatin (6 mg kg^{-1}, 2 mg mL^{-1} in saline) or cisplatin (6 mg kg^{-1}) plus glycine (either 0·1, 0·25, 0·50 or 1·0 g kg^{-1} in saline, 5 mL kg^{-1}) in which cisplatin was given at the midpoint of the injection of glycine, which was made over 3 min. Glycine solutions were adjusted to pH 7·0 with 1 M NaOH. The timing of the first test clearance period commenced from the start of the cisplatin or glycine injection.

Chronic effects of glycine on cisplatin-injected rats

Male albino Wistar rats, 200–250 g, were injected via the tail vein with one of the following: saline (3 mL kg^{-1}); cisplatin (6 mg kg^{-1}, 2 mg mL^{-1} in saline); cisplatin (6 mg kg^{-1}) plus vehicle for glycine (saline, 5 mL kg^{-1}) or cisplatin (6 mg kg^{-1}) plus glycine (either 0·1, 0·25, 0·50 or 1 g kg^{-1} in saline, 5 mL kg^{-1}). Vehicle and glycine solutions were administered over a total of 3 min, with the dose of cisplatin given at the mid-point.

Rats were placed in metabolic cages for a 24-h urine collection 2 days following the various treatments (day 3) and then a blood sample (0.75 mL) was taken from the tail vein on day 4. Animals were also placed in metabolic cages for urine collection over 24 h on day 7. On day 8, rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and cannulae were inserted into the trachea, left jugular vein and right carotid artery. Animals were heparinized (200 units kg^{-1}) and the clearances of [^{3}H]inulin (C_{IN}) (100 mg kg^{-1}, 20 μCi kg^{-1}, i.v.) and [^{14}C]*p*-aminohippuric acid (C_{PAH}) (40 mg kg^{-1}, 4 μCi kg^{-1}, i.v.) dissolved in saline, were then determined simultaneously by the single injection method of Hall et al (1977). 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.

Urine and plasma analysis

Urine concentrations of sodium were measured by flame photometry and levels of [^{3}H]inulin and [^{14}C]*p*-aminohippuric acid (8-day nephrotoxicity studies only) were determined by liquid scintillation counting. Plasma levels of creatinine and urea were assayed by standard colorimetric procedures: creatinine by reaction with picrate in alkaline solution and urea by reaction with diacetyl monoxime.

Analysis of results

In acute studies, the response to glycine was calculated as the mean fall in either C_{IN} or RBF at 110 min from control values. This was then expressed as percentage inhibition of the mean fall in C_{IN} or RBF at 110 min noted in rats given cisplatin only.

All other results are expressed as mean ± s.e.m. Comparison of means between groups was made using either a non-paired Student's *t*-test or one-way analysis of variance with means compared by Scheffe's test.

Results

Acute studies

Before cisplatin injection, the values of C_{IN} and RBF were 0·81 ± 0·08 mL min^{-1}/100 g and 5·5 ± 0·4 mL min^{-1} (n = 6), respectively. There were no statistically significant ($P > 0·05$) differences in control values of C_{IN} and RBF between the group of rats which received cisplatin only and the groups of rats which were injected with cisplatin and the various doses of glycine. Following cisplatin injection there was a progressive decline in C_{IN} and RBF such that by 110 min they had decreased by approximately 50% to 0·39 ± 0·08 mL min^{-1}/100 g and 2·8 ± 0·3 mL min^{-1} (n = 6), respectively. Co-administration of glycine produced a dose-related attenuation of the fall in C_{IN} and RBF produced by cisplatin and this is shown in Fig. 1. Glycine at a dose of 0·1 g kg^{-1} produced no inhibition of the cisplatin-induced decline in RBF or C_{IN}, whilst a dose of 1·0 g kg^{-1} completely prevented the decreases in RBF and C_{IN}.

Chronic studies

Cisplatin administration resulted in marked renal dysfunction, as indicated by elevated plasma urea and creatinine concentrations, polyuria, depressed sodium excretion and reduced values for C_{IN} and C_{PAH} in comparison with control rats injected with saline (Tables 1, 2). Treatment with the vehicle for glycine (saline, 5 mL kg^{-1}) produced some beneficial effects on renal function. For example, plasma urea and creatinine on days 4 and 8 were significantly ($P < 0·05$) reduced and urine output on days 4–5 was decreased ($P < 0·05$) in comparison with cisplatin-injected rats (Table 1). In addition, Table 2 shows a significant ($P < 0·05$) increase in C_{PAH} on day 8 after vehicle treatment. It is not surprising that the saline vehicle protects renal function since saline administration has been shown to reduce the incidence of cisplatin nephrotoxicity in man and animals (see Daugaard & Abildgaard 1989).

By comparison with the cisplatin-injected vehicle-treated group, glycine treatment at a dose of 0·1 g kg^{-1} resulted in no significant ($P > 0·05$) improvements in renal function with the exception of a decrease in urine output on days 7–8

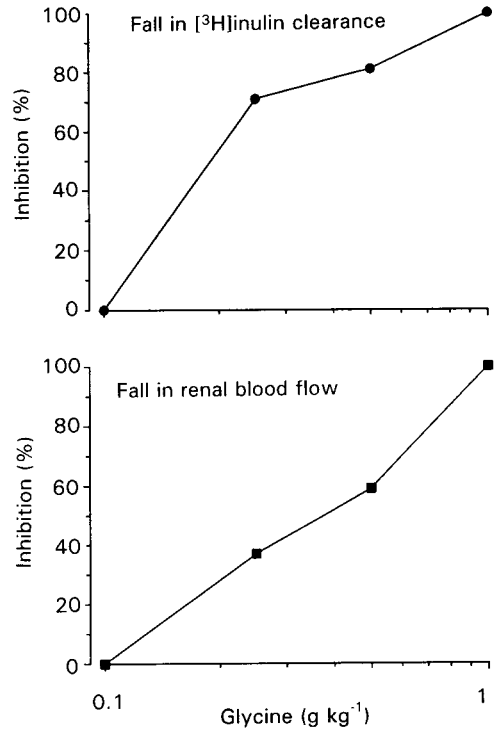

FIG 1. Dose-response relationship of glycine's ability to attenuate the fall in [^{3}H]inulin clearance C_{IN} and renal blood flow (RBF) in rats, 110 min following injection of cisplatin (6 mg kg^{-1}, i.v.). The responses were expressed as percentage inhibition of the mean fall in C_{IN} or RBF noted in the group of rats which received cisplatin only. Each value is derived from experiments in six rats.

(Table 1). An increase in dose of glycine to 0·25 g kg^{-1} resulted in significant protection of renal function with reductions in plasma urea levels of 27% ($P < 0·05$) and 35% ($P < 0·05$) on days 4 and 8, respectively, whilst 24-h urine output was reduced by 50%($P < 0·05$) on days 7–8 compared with cisplatin-injected vehicle-treated rats. Treatment with glycine at a dose of 0·25 g kg^{-1} also produced significant ($P < 0·05$) increases in C_{IN} and C_{PAH} (Table 2). In addition to the beneficial effects noted with 0·25 g kg^{-1}, treatment with 0·5 g kg^{-1} glycine produced a significant

Table 2. [^{3}H] Inulin clearance (C_{IN}) and [^{14}C]*p*-aminohippurate clearance (C_{PAH}) in rats on day 8 following injection of cisplatin (6 mg kg^{-1}, i.v.) or saline (3 mL kg^{-1}).

	C_{IN} (mL min^{-1}/100 g)	C_{PAH} (mL min^{-1}/100 g)
Saline	1·25 ± 0·20	2·9 ± 0·20
Cisplatin 6 mg kg^{-1}	0·30 ± 0·04 ◆	1·0 ± 0·10 ◆
+ saline 5 mL kg^{-1}	0·28 ± 0·06	1·5 ± 0·10†
+ glycine 0·1 g kg^{-1}	0·35 ± 0·04	1·5 ± 0·20
0·25 g kg^{-1}	0·46 ± 0·08*	2·1 ± 0·20*
0·50 g kg^{-1}	0·56 ± 0·16**	2·2 ± 0·30*
1·00 g kg^{-1}	0·77 ± 0·08***	3·0 ± 0·30***

Values are means ± s.e.m. of eight determinations. Groups of cisplatin-injected rats were treated with the vehicle for glycine (saline 5 mL kg^{-1}) or glycine at doses of either 0·1, 0·25, 0·5 or 1·0 g kg^{-1}. ◆ $P < 0·001$ relative to saline (*t*-test); † $P < 0·05$ relative to cisplatin group; *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ relative to cisplatin/vehicle group.

($P < 0·01$) decrease (44%) in plasma creatinine levels on day 4 in comparison with cisplatin-injected rats which received the saline vehicle. Administration of the highest dose of glycine tested (1·0 g kg^{-1}) resulted in marked improvements in the range of indices of renal function measured including Na^+ excretion (Tables 1, 2).

Discussion

The results reveal a clear association between the acute renal haemodynamic effects produced by glycine in cisplatin-injected rats with the longer-term renal protective effects of glycine in cisplatin nephrotoxicity. Glycine at a dose of 0·1 g kg^{-1} produced no attenuation of the cisplatin-induced decline in C_{IN} and RBF, which correlates with a virtual absence of any protection of renal function afforded by 0·1 g kg^{-1} glycine over the 7-day period following cisplatin injection. By contrast, glycine at a dose of either 0·5 or 1·0 g kg^{-1} markedly attenuated cisplatin-induced falls in C_{IN} and RBF, with the highest dose completely preventing any falls in these indices during the course of the experiment. In parallel with these effects, treatment with these higher doses of glycine produced prominent protection from the nephrotoxic actions of cisplatin, with the dose of 1·0 g kg^{-1} showing the greatest beneficial effect. The results of experiments with

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

		Saline	CP	CP + V	CP + $G_{0·1}$	CP + $G_{0·25}$	CP + $G_{0·5}$	CP + $G_{1·0}$
Plasma urea	Day 4	40 ± 2	153 ± 4†††	112 ± 12+	138 ± 14	82 ± 8*	69 ± 14**	45 ± 4***
(mg/100 mL)	Day 8	35 ± 1	128 ± 16†††	96 ± 17+	106 ± 12	62 ± 5*	51 ± 12**	52 ± 7***
Plasma creatinine	Day 4	0·61 ± 0·04	1·91 ± 0·11†††	1·46 ± 0·21+	1·59 ± 0·19	1·24 ± 0·09	0·82 ± 0·11**	0·47 ± 0·04***
(mg/100 mL)	Day 8	0·58 ± 0·06	1·94 ± 0·26†††	1·17 ± 0·19+	1·60 ± 0·19	1·06 ± 0·06	0·91 ± 0·17	0·61 ± 0·07***
Urine output	Days 4–5	3 ± 1	22 ± 1†††	13 ± 2+	12 ± 1	12 ± 1	12 ± 1	4 ± 1***
(mL/100 g/24 h)	Days 7–8	3 ± 1	17 ± 1†††	15 ± 1	11 ± 1*	10 ± 1*	7 ± 1**	5 ± 1**
Na^+ excretion	Days 4–5	0·67 ± 0·04	0·47 ± 0·05†	0·44 ± 0·08	0·35 ± 0·06	0·37 ± 0·05	0·52 ± 0·09	0·62 ± 0·10*
(mmol/100 g/24 h)	Days 7–8	0·70 ± 0·04	0·37 ± 0·05††	0·41 ± 0·09	0·33 ± 0·05	0·42 ± 0·04	0·51 ± 0·09	0·52 ± 0·06*

Results are shown as mean ± s.e.m. (n = 8). Saline (0·9% NaCl, 3 mL kg^{-1}); CP, cisplatin (6·0 mg kg^{-1}, i.v.); CP + V, cisplatin (6.0 mg kg^{-1}, i.v. + NaCl 0·9%, 5 mL kg^{-1}, i.v.); CP + G, cisplatin (6·0 mg kg^{-1}, i.v.) + glycine at doses of either 0·1, 0·25, 0·5 or 1·0 g kg^{-1}, i.v., subscript denotes dose of glycine. †$P < 0·05$, ††$P < 0·01$, †††$P < 0·001$, relative to saline (*t*-test); +$P < 0·05$ relative to CP; *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ relative to CP + V.

an intermediate dose of 0·25 g kg^{-1} revealed some degree of amelioration of acute renal haemodynamic effects of cisplatin, particularly with regard to C_{IN}; whilst in the nephrotoxicity study, 0·25 g kg^{-1} glycine produced a modest but significant reduction in cisplatin-induced acute renal dysfunction. Overall, the findings suggest that the threshold doses of glycine for the attenuation of the acute renal haemodynamic changes produced by cisplatin and limiting its nephrotoxicity are the same, namely 0·25 g kg^{-1}.

The present findings indicate glycine's renal haemodynamic effects, the preservation of RBF and C_{IN} in cisplatin-injected animals, may be the main reason for its beneficial action in cisplatin-induced nephrotoxicity. Thus, although there is in-vitro evidence of glycine's cytoprotective effects in isolated tubules (Weinberg 1991), this aspect of glycine's actions does not appear to play an important role in this form of nephrotoxicity, unless these effects occur in the same dose range as glycine's renal haemodynamic actions. Whilst the protective effects of glycine are considerable, particularly at the highest dose, there is not complete restoration of renal function to levels noted in rats given saline only. For example, at day 8 the fall in C_{IN} is only partially reversed by 1·0 g kg^{-1} glycine. The present findings do not indicate for how long glycine prevents the acute renal haemodynamic effects of cisplatin. Therefore, failure of a bolus dose of glycine to prevent a sustained fall in RBF and glomerular filtration rate produced by cisplatin may explain the incomplete prevention of cisplatin-induced renal dysfunction. Alternatively, the incomplete restoration of renal function may be a reflection of cisplatin's direct nephrotoxic effects (see Safirstein et al 1987) as opposed to its depressant effects on renal haemodynamics.

The initial study conducted by Heyman et al (1991) showed that glycine infusion before cisplatin administration did not affect kidney platinum concentrations measured six days after cisplatin (5 mg kg^{-1}) injection. A later study by Heyman et al (1993) revealed glycine produced a 39% reduction in the renal parenchymal uptake of cisplatin, 1 h following its injection and this effect was selective since platinum concentrations in the liver and gut were unaffected. These findings raise the question of whether the preservation of renal haemodynamics noted in the present study is a direct consequence of glycine's ability to reduce the early renal uptake of cisplatin. Glycine's ability to augment renal blood flow and glomerular filtration rate, however, is not restricted to animals injected with cisplatin since normal animals respond to glycine with increases in renal blood flow and glomerular filtration rate (King et al 1991). Therefore, it might be anticipated that glycine would act as a functional antagonist of cisplatin-induced reductions in renal blood flow and glomerular filtration rate irrespective of any impairment in cisplatin uptake. Nitric oxide (NO) appears to be important in the renal response to certain amino acids including glycine (King et al 1991; Cernadas et al 1992) and we have found in acute experiments that the NO synthase inhibitor N^G-nitro-L-arginine methyl ester, L-NAME (10 μg kg^{-1} min^{-1}), blocks the ability of glycine (1·0 g kg^{-1}) to maintain RBF and C_{IN} in rats injected with cisplatin (Li et al 1994). Furthermore, it was also shown in chronic experiments that a bolus dose of L-NAME (1·0 mg kg^{-1}) reduces the ability of glycine to ameliorate cisplatin nephrotoxicity (Li et al 1994). In these experiments L-NAME, when given alone, did not potentiate cisplatin-induced falls in RBF or C_{IN} or its nephrotoxicity (Li et al 1994). These observations with L-NAME support the proposal that glycine's ability to ameliorate cisplatin nephrotoxicity is primarily a result of a direct renal haemodynamic action rather than inhibition of renal uptake of cisplatin. However, it is possible that both actions contribute to the renal-protective effect of glycine.

In conclusion, the results of this study indicate that glycine's ability to prevent the falls in RBF and glomerular filtration rate produced by cisplatin plays an important role in the protective effect of glycine in cisplatin-induced nephrotoxicity.

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