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Glycine reduces early renal parenchymal uptake of cisplatin

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Glycine reduces early renal parenchymal uptake of cisplatin. We evaluated the effect of glycine infusions on the early renal uptake of cisplatin, measured one hour after cisplatin was injected, as well as five days following cisplatin administration. Glycine (1.25 mmol per 100 g body wt) markedly attenuated the early uptake of platinum by the kidney, an effect not observed with control infusions of saline or of L-alanine. The kidney content of platinum at five days, on the other hand, was similar in glycine-treated animals and saline controls. Early inhibition of renal uptake of platinum may be responsible for glycine's protective action in experimental cisplatin nephrotoxicity.

Reports of the cytoprotective effect of glycine, alanine, and structurally related compounds from hypoxic and toxic insults *in vitro* [1–7] have recently been extended to cisplatin nephrotoxicity *in vivo* [8]. Glycine infusion (75 mg/kg/hr) markedly attenuated the proximal (S3) tubular injury and deterioration of renal function seen five days after the administration of cisplatin (3.5 or 5 mg/kg). Similar protection was not afforded by infusions of L-alanine [8]. This finding is of clinical interest, since nephrotoxicity remains the major adverse effect of cisplatin chemotherapy, in spite of various protective measures [9].

Although glycine-related cytopreservation has been studied extensively [10–14], its mechanism still remains unclear. Physiological concentrations of glycine (0.25 to 2 mM) appear to blunt hypoxic and toxic tubular damage *in vitro* [1–8, 11, 13–15], but cytoprotection *in vivo* has been more difficult to demonstrate. Much higher plasma levels induced at the time of the injection of cisplatin (3.5 to 8 mM) were required to prevent cisplatin nephrotoxicity in intact rats [8]. Moreover, this finding was anomalous, since comparable glycine infusions failed to modify renal injury produced *in vivo* by ischemia-reflow or radiocontrast injection [16]. The cytoprotection observed with glycine *in vitro*, in isolated cells or perfused organs, might reflect replenishment of cellular stores of glycine that are normally present during life but are depleted in the course of organ perfusion with artificial media or the preparation of isolated tubules and cells [15]. We wondered if glycine infusions in intact rats might modify the early uptake of cisplatin by the kidney, thereby blunting its toxic effect. Though glycine did not affect early urinary and plasma contents of platinum or platinum levels in kidneys measured five days after injection [8], we now report that the infusion of glycine markedly attenuates early accumulation of platinum in the kidney, suggesting a mechanism for glycine's protective effect.

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Methods

In vivo *studies*

General. Male Sprague-Dawley rats (210 to 330 g) were used for all experiments. They were given regular chow and tap water *ad libitum*. Under Inactin anesthesia (100 mg/kg) the right jugular vein was cannulated with two polyethylene tubes (PE-50, Clay-Adams, Parsippany, New Jersey, USA) for the infusion of cisplatin and amino acids. The bladder was cannulated by a polyethylene tube for urine collection as previously described [17], and the rats were kept in heated chambers (37°C) throughout the experiment. At the end of the infusion period the rats were sacrificed and plasma, red blood cells and tissue samples from kidney, liver and gut were obtained for the determination of platinum content. In another set of experiments rats were anesthetized with phenobarbital (65 mg/kg) and their bladders were not cannulated. At the end of the infusion period, the venous cannulae were removed, the rats were allowed to recover and were sacrificed five days later for the determination of organ platinum contents.

Cisplatin injection. Five mg/kg cisplatin were injected over 60 seconds through a jugular cannula. A preparation used in clinical practice was utilized (Platinol, Bristol-Myers Co., Evansville, Indiana, USA), dissolved in distilled water, each milliliter containing 1.67 mg cisplatin, 16.7 mg of mannitol and 15 mg of sodium chloride.

Amino acid infusion. Glycine was infused at a rate of 75 mg (1 mmol) per 100 g body wt, in 1.06 ml saline per hour. L-alanine was given in an equimolar dose (89 mg/100 g body wt/1.56 ml saline/hr). The lower solubility of alanine necessitated the difference in solute volume. Control rats were given 1.06 ml saline/hr.

The infusion of glycine, alanine, or saline was initiated through the second cannula 15 minutes before the cisplatin injection and was carried out for 75 minutes.

Cisplatin measurement

Heparinized blood samples were centrifuged to obtain plasma and erythrocytes. The entire right kidney and samples of tissue obtained from liver and gut were weighed. All specimens were

Table 1. Platinum content of organs, red blood cells, plasma and urine

Experimental group	N		Plasma	RBC	Kidney	Liver	Gut	Urine
			μg/ml	*μg/gww*				*μg/hr*
Controls	9	60 min	1.03 ± 0.06	1.7 ± 0.2	9.2 ± 0.5	3.2 ± 0.2	1.1 ± 0.0	327 ± 25
	4	5 days	0.12 ± 0.01	1.6 ± 0.1	6.0 ± 0.2[b]	1.7 ± 0.1	0.5 ± 0.1	
Glycine	9	60 min	0.98 ± 0.10	1.5 ± 0.2	5.6 ± 0.2[c]	3.1 ± 0.2	1.2 ± 0.1	391 ± 45
	4	5 days	0.09 ± 0.01[a]	1.4 ± 0.2	5.4 ± 0.1[a]	1.6 ± 0.1	0.5 ± 0.0	
Alanine	4	60 min	1.5 ± 0.29 ($N = 5$)		11.2 ± 2.2			348 ± 41

[a] $P < 0.05$ vs. control, 5 days (Student's *t*-test)
[b] $P < 0.002$ vs. 60 min (Student's *t*-test); $N = 8$ for kidney platinum content in rats given glycine
[c] $P < 0.05$ vs. others at 60 min (ANOVA)

then immediately frozen (−20°C) and analyzed later in duplicate for platinum levels with a Perkin Elmer model 1100 atomic absorption spectrometer, as described elsewhere [18].

Statistics

Values are presented as the means ± SE. Non-paired Student's *t*-test and one-way analysis of variance with the Newman Keuls test were applied for the comparisons of platinum content. Statistical significance was set at $P < 0.05$.

Results

Table 1 summarizes the results of platinum contents in tissues, blood components and urine in the various experimental groups. Sixty minutes after the injection of cisplatin, the platinum contents of plasma, red cells, liver and gut were comparable in rats treated with glycine and with saline. By contrast, kidney platinum content was markedly lower in rats given glycine than in saline-infused controls (5.6 ± 0.2 vs. 9.2 ± 0.5 μg/g wet weight; $P < 0.01$, ANOVA), or in rats infused with alanine (11.2 ± 2.2 μg/g wet weight). Urinary excretion of cisplatin was not significantly different among the three groups. In Figure 1, urinary excretion of platinum during the first hour is plotted against the kidney content of platinum at the end of that time. At comparable rates of platinum excretion, the kidneys of glycine-treated rats uniformly contained less platinum than the kidneys of control rats given saline.

Five days later, plasma platinum levels were slightly lower in the glycine group, as compared to controls (0.09 ± 0.2 vs. 0.12 ± 0.01 μg/ml, $P = 0.04$, Students' *t*-test), in concert with our previous report, probably because renal excretory function is better preserved in glycine-treated animals, permitting more platinum to be excreted [8]. Platinum contents of liver, gut, and red cells were unaffected by glycine. Kidney platinum was slightly lower in the glycine group after five days, as compared to controls (5.4 ± 0.1 vs. 6.0 ± 0.2 μg/gww, $P = 0.03$), but whereas it fell by some 35% from the first to the fifth day in controls ($P < 0.002$), it remained unchanged in rats treated with glycine.

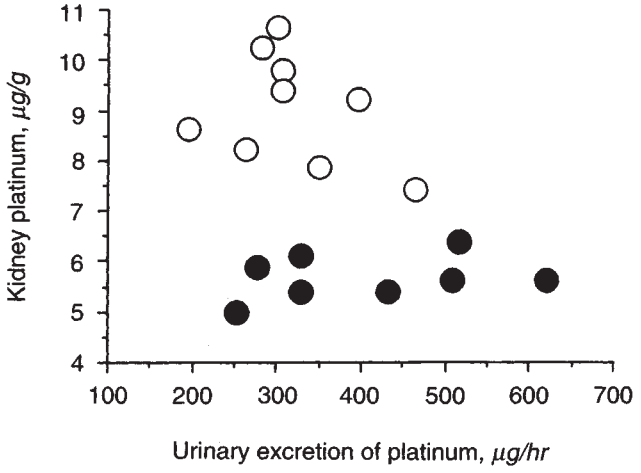

Fig. 1. *Urinary excretion of platinum is plotted against kidney content of platinum at the end of one hour following cisplatin infusion in rats treated with glycine (●) or saline (○).* At comparable rates of platinum excretion, the kidneys of glycine-treated rats accumulated less platinum than those of saline-treated rats.

Discussion

High plasma and parenchymal levels of glycine are required for renal salvage at the time of injection of cisplatin, since delayed glycine infusion is not protective [8]. As toxic intracellular platinum species are probably formed early after injection and quickly converted to inactive forms [19], early, rather than late tissue platinum content may better predict cellular damage. In this perspective, the low kidney platinum content observed in glycine-treated rats shortly after injection of cisplatin may explain the protective effect of glycine. It is notable that although the early (1 hr) kidney content of platinum was almost halved by glycine, plasma levels of platinum and urinary excretion of platinum were not significantly different from those of control rats given saline. In contrast, L-alanine did not reduce the early platinum content of the kidney, and was not protective [8]. The effect of glycine on the platinum content of the kidney was specific in that levels of platinum in other organs (liver and gut) were unaffected by glycine. After five days, the platinum content of the kidney in glycine-treated rats was only marginally lower than in control animals given saline, presumably reflecting the delayed accumulation of non-toxic platinum species as glycine levels in the plasma fell to their physiological range. *In vitro* studies of cytoprotection by glycine have consistently shown that the amino acid must be present at the time that cells would otherwise undergo lethal membrane damage. Since cell necrosis is not seen for 24 to 48 hours after cisplatin administration, it is likely that if glycine is provided only at the time cisplatin is initially administered, its cytoprotective effect is achieved by acting on an earlier event such as the uptake or activation of cisplatin, as suggested by these experiments.

Experimental cisplatin nephrotoxicity can be blunted by prehydration, saline diuresis, or treatment with furosemide, mannitol and other diuretics which, however, do not lower the plasma level or renal content of platinum [19]. The present experiments suggest that inhibition of the renal uptake of platinum might also be useful in preventing renal injury from cisplatin. Cisplatinum is actively transported into cells lining the straight portion of the proximal tubule (S3), leading to accumulation that is most marked in the region of the corticomedullary junction. The selective early uptake of cisplatin by these cells presumably accounts for the sharply specific localization of early cisplatin toxicity to S3 segments in the outer medulla and medullary rays [19]. The mechanism of uptake across the basolateral border probably involves the organic cation transporter responsible for secretion of an array of positively-charged substituted nitrogenous bases. Accumulation of cisplatin by kidney slices is inhibited by drugs that specifically block this transport pathway, like tolazoline and mepiperphenidol [20]. The inhibitors of organic cation transport, quinine and cyanine, protect against cisplatin nephrotoxicity when infused into the renal portal circulation of chickens [21]. While glycine is also actively transported by renal cells [22–24], it is not known whether it utilizes the organic cation transporter. The present findings suggest the possibility that glycine might interfere with cisplatin uptake by renal tubular cells in the S3 segment, perhaps by affecting a cation transporter.

In summary, glycine infusions markedly reduce early renal accumulation of cisplatin. This may explain their amelioration of cisplatin nephrotoxicity.

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