**Direct Amifostine Effect on Renal Tubule Cells in Rats**

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**ABSTRACT**

Clinical trials indicate that amifostine offers protection against cisplatin-induced nephrotoxicity. It is unclear whether a direct pharmacological effect on renal tubular cells is involved. We investigated the effect of amifostine pretreatment on the tubular apparatus and evaluated its nephroprotective potential. A total of 32 rats were treated by i.p. administration of 0.9% saline solution (group 1), 5 mg/kg cisplatin (group 2), 25 mg/kg amifostine (group 3), and 25 mg/kg amifostine followed by 5 mg/kg cisplatin (group 4) after 30 min. We recorded elevation of *N*-acetyl-β-D-glucosaminidase (NAG) in 24 h pooled urine as a specific marker for tubular lesions, renal leakage of magnesium as an unspecific nephrotoxicity marker, and survival over a 10-day observation period. A significant ($P < 0.002$) increase in urinary NAG after treatment was documented only in cisplatin-treated group 2 [day 2 (mean ± SE), 9.3 ± 2.1 units/gram creatinine; day 4, 70.6 ± 16 units/gram creatinine; normalization at day 8]. Treatment with amifostine before cisplatin administration resulted in a slight urinary NAG leakage (day 2, 2.8 ± 1.8 units/gram creatinine; day 4, 13.8 ± 13 units/gram creatinine; normalization at day 6). No increase in urinary enzyme levels was seen in the other groups, and there were no significant differences in urinary magnesium between all groups. Four of eight rats in the cisplatin-treated group and one of eight rats in the amifostine plus cisplatin-treated group died.

**INTRODUCTION**

Amifostine (Ethylol; WR-2721) is a prodrug converted by alkaline phosphatase to an active sulfhydryl compound (WR-1065). The substance selectively protects normal cells from antineoplastic drug toxicity by scavenging free radicals, donating hydrogen ions to free radicals, depleting oxygen, and binding to active derivatives of antineoplastic agents (1). Experimental and clinical trials show that amifostine does not alter antitumor activity in chemotherapy (2, 3). There is overwhelming animal and clinical evidence of a clinically highly significant nephroprotective effect against dose-limiting cisplatin toxicity. This evidence includes lower urea increases in mice (4), improved resistance in rats against cisplatin nephrotoxicity (5), tolerance of higher cisplatin doses without renal complications, and tolerance of shorter intervals before the next chemotherapy cycle (6–8). Evaluation from animal and clinical studies has been based on unspecific and fairly insensitive parameters such as serum creatinine and creatinine clearance. Systematic studies of the impact of amifostine on the renal tubular system have not been carried out, and there are no hard data on the quality or potential extent of the effect of the drug on this functionally important renal segment.

Our study was therefore performed to establish whether nephroprotection is based on a direct pharmacological effect of amifostine on renal tubule cells and to investigate the extent of this effect.

**MATERIALS AND METHODS**

**Chemicals.** Platinex (0.5 mg/ml cisplatin in 0.9% saline solution) was obtained from Bristol (Munich, Germany). Ethylol (500 mg of amifostine and 500 mg of mannitol) was reconstituted immediately before injection with 9.5 ml of 0.9% saline solution (0.9% NaCl).

Four groups of eight inbred male Brown-Norway rats (32 animals) received the following i.p. treatment: (a) group I, 0.5 ml of 0.9% NaCl followed by 4.0 ml of 0.9% NaCl 30 min later; (b) group II, 0.5 ml of 0.9% NaCl followed by 4.0 ml of cisplatin (5 mg/kg) 30 min later; (c) group III, 0.5 ml of amifostine (25 mg/kg body weight) followed by 4.0 ml of 0.9% NaCl 30 min later; and (d) group IV, 0.5 ml of amifostine (25 mg/kg body weight) followed by 4.0 ml of cisplatin (5 mg/kg body weight) 30 min later.

The animals were observed for 10 days after treatment. The tubule-specific lysosomal enzyme NAG (2) was assayed before and after treatment in 24 h pooled urine according to the method of Maruhn et al. (9) in relation to urinary creatinine elimination (Jaffe method). The individual NAG (units/gram creatinine) elevation after treatment was recorded.

In addition, renal leakage of magnesium was measured in relation to urinary creatinine excretion (mM/gram creatinine).

Survival was also documented. The animals were fed a standard diet and kept in metabolic cages on a physiological day-night rhythm.

Statistical evaluation was performed using Student’s *t* test.

**RESULTS**

Marked urinary enzyme elevation was seen only after treatment with cisplatin alone (group II). The elevation was significant ($P < 0.002$) from day 2 (mean ± SE, 9.3 ± 2.1 units/gram creatinine), peaking on day 4/5 (day 4, 70.6 ± 16 units/gram creatinine), and returning to normal on day 8.

Amifostine pretreatment followed by cisplatin treatment (group IV) produced a slight, nonsignificant urinary NAG increase (day 2, 2.8 ± 1.8 units/gram creatinine; day 4, 13.8 ± 13 units/gram creatinine), with normalization of NAG values on day 6.

Treatment with amifostine alone (group III) gave rise to a slight, nonsignificant increase in enzyme excretion on days 1 and 2.

The control group treated with 0.9% NaCl (group I) also displayed a slight, nonsignificant rise in urinary NAG on day 1 (see Figs. 1 and 2 for details).

Direct statistical comparison between group II (cisplatin alone) and group IV (amifostine pretreatment plus cisplatin) disclosed a significant difference ($P < 0.02$) in enzyme depletion from day 2 to day 5.

Statistical analysis was not performed after this point because of the reduced number of animals. Amifostine pretreatment reduced NAG leakage (comparison of means) by 70% on day 2, 85% on day 3, 81% on day 4, and 89% on day 5.

Urinary magnesium excretion did not differ significantly between the groups.

A total of 5 of the 32 rats died: 4 of 8 rats from group II (cisplatin alone) died on days 5 and 6 after treatment; and 1 of 8 rats from group IV (amifostine + cisplatin) died on day 6.

**DISCUSSION**

The nephrotoxicity associated with cisplatin is well documented and constitutes a dose-limiting side effect of cisplatin therapy. More...
to that used in human subjects (910 mg/m² body surface area). The cisplatin (4), using a dose of 25 mg/kg that is proportionately similar with no difficulty and are demonstrably absorbed in sufficient quantum in animals.

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Male rats are a particularly suitable animal model for evaluating tubular lesions because their intrarenal enzyme distribution is similar to that in man (11). We chose inbred male Brown-Norway rats of approximately equal body weight for our study to minimize the effect of interindividual differences. External influences on renal function were minimized through standardized animal care, injection of equal quantities of fluid for administration, and twice daily injection in all animals.

Both amifostine and cisplatin can be injected i.p. in animal models with no difficulty and are demonstrably absorbed in sufficient quantities by this route (2). Amifostine was administered 30 min before cisplatin (4), using a dose of 25 mg/kg that is proportionately similar to that used in human subjects (910 mg/m² body surface area). The cisplatin dose of 5 mg/kg was chosen to approximate a human dose from the upper end of the dose scale.

Urinary excretion of the lysosomal enzyme NAG was measured as a specific indicator of tubule cell damage. NAG is renal tubule specific and is a valid indicator of tubular damage. It has been found to correlate with corresponding morphological tubule lesions arising through other mechanisms (12). To prevent falsification due to individual differences in baseline NAG secretion, the individual post-therapeutic NAG increase was determined in the pooled urine of each animal and set in relation to creatinine clearance to avoid dilution artifacts (11). Method errors (13) due to circadian variation of NAG values and short-term effects of animal care or hydration were minimized by determining enzyme activities from the 24 h pooled urine.

The results show that the administered cisplatin dose induces significant tubulotoxicity over a period of 7 days. Amifostine pretreatment with the clinically recommended dose reduces the tubulotoxicity. The toxicity differences between the animal group treated with cisplatin alone and the animal group receiving amifostine pretreatment were both statistically significant and substantial, amounting to 70–89%. The extent of direct tubular protection by amifostine pretreatment concurs with the results of Treskes et al. (4), who were able to increase the cisplatin dose 2.2-fold in mice before signs of nephrotoxicity expressed by an increase in plasma urea became apparent.

Shorter duration of tubulotoxicity is probably another effect of amifostine. Amifostine treatment shortened the period of urinary NAG elevation by 25%. Statistical analysis of this effect was not performed because of the reduced number of animals. The existence of this effect is suggested by the clinical observation that patients given amifostine need less time to recover from nephrotoxicity between chemotherapy cycles (7).

Renal magnesium excretion was recorded as an additional nephrotoxicity marker. The thick ascending limb of Henle’s loop is the major site of magnesium reabsorption. Increased urinary magnesium leakage is therefore an indicator of cisplatin-induced lesion at this site. Renal magnesium leakage (10) has been observed in the clinical situation as a sign of cisplatin nephrotoxicity and has been found to be present in 40–100% of patients, depending on the dosage and on the number of treatments. Because the determination of total daily magnesium excretion may be subject to artifacts due to the unreliability of measurement of urinary output in small animals in metabolic cages, magnesium was determined in relation to urinary creatinine elimination and not in terms of total daily excretion. In contrast to clinical reports, differences in magnesium excretion between the various treatment groups were not seen in our animal model. Magnesium leakage thus proved less sensitive than urinary NAG excretion.

Amifostine pretreatment also produced impressive survival benefits in our animal study. It is not unlikely that the improved survival in the group receiving amifostine followed by cisplatin relates to the nephroprotective effect of amifostine.

In summary, our results suggest that amifostine exerts a protective effect directly at the site of maximum cisplatin toxicity. At least part of the nephroprotection observed in clinical use can be ascribed to this effect. Amifostine does not fully eliminate nephrotoxicity, but it does significantly reduce the severity and, probably, the duration of nephrotoxicity. Our study further shows that urinary NAG excretion is a suitable marker for evaluating cisplatin nephrotoxicity in animal models at a clinically relevant dose range and demonstrates that urinary NAG levels are reduced by amifostine. Urinary magnesium is not sensitive enough for the purposes of our study. The benefits of amifostine pretreatment for reducing chemotherapy-induced mortality are apparent in this animal model.
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