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), toleration of higher cisplatin doses without renal complications, and toleration 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 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).

Results 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.

Approval for the animal experiment series was granted on August 21, 1997 by the Schleswig-Holstein Ministry for Environment, Wildlife and Forestry.

DISCUSSION
The nephrotoxicity associated with cisplatin is well documented and constitutes a dose-limiting side effect of cisplatin therapy. Mor-
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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.

Fig. 1. Day 3 after treatment. Descriptive statistic of the urinary NAG increase (units/gram creatinine). Boxplot, median, interquartile range Q3 to Q1, minimum, and maximum. NaCl, control (0.9% NaCl; group I); CP, cisplatin alone (group II); Ami, amifostine alone (group III); AmiCP, amifostine + cisplatin (group IV).

Fig. 2. Development of the mean urinary NAG leakage from day 1 to day 7 after treatment. NaCl, control (0.9% NaCl; group I); CP, cisplatin alone (group II); Ami, amifostine alone (group III); AmiCP, amifostine + cisplatin (group IV).
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