Prevention of Renal Failure in Rats Receiving \textit{cis}-Diamminedichloroplatinum(II) by Administration of Furosemide

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SUMMARY

The clinicopathological signs of renal failure induced in rats by weekly i.v. administration of \textit{cis}-diamminedichloroplatinum(II) were prevented by pretreatment with furosemide. Weight loss, anemia, and generalized toxic effects of the drug were not effected by furosemide.

INTRODUCTION

\textit{DDP} is a cancer chemotherapeutic agent with toxic effects in the hematopoietic, auditory, intestinal, and renal tissues of man and laboratory animals (2-5, 7-10, 13-16). After an acutely toxic single dose, rats developed diarrhea, leukopenia, and azotemia and eventually died (4, 13) as a result of the combination of some of these toxic effects. Rats receiving weekly doses of the drug developed uremia and renal failure (13). From the results of phase I and phase II human studies and experimental studies in rats, the dose-limiting toxicity of chronic administration of DDP is nephrotoxicity (7, 13, 16). As a result, many antitumor analogs of DDP are being developed in an effort to identify a less nephrotoxic platinum drug with acceptable antitumor activity.

These studies were designed to characterize a method for modification or prevention of nephrotoxicity of DDP in rats. Previous studies from our laboratory have revealed that furosemide modifies the acute nephrotoxicity and platinum distribution after a single administration of DDP (14). This paper describes the effects of furosemide administration on the renal failure induced by DDP.

MATERIALS AND METHODS

Seventy-five male F344 rats, 4 weeks old, were obtained from a commercial supplier (Charles River Breeding Laboratories, Wilmington, Mass.), placed in polycarbonate cages with filter tops and an automatic watering system, and fed NIH mouse and rat ration \textit{ad libitum}. Starting at 6

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart1.png}
\caption{Furosemide protection against renal failure induced by DDP. F344 rats were given DDP, 3 mg/kg i.v. weekly. Furosemide was given s.c. at a dose level of 12.5 mg/kg 30 min before DDP. Groups of 5 rats were sacrificed from 0 to 6 weeks after initial drug administrations. All values are mean ± S.E. The elevations of the renal weight and blood urea nitrogen values of rats given DDP are significantly different (p < 0.05) from those of rats in the 2 other treatment groups.}
\end{figure}
weeks of age, 25 rats received i.v. injections of DDP at a dose level of 3.0 mg/kg once weekly for 6 weeks. An additional 25 rats received furosemide once weekly s.c. for 6 weeks at a dose level of 12.5 mg/kg 30 min before i.v. administration of the platinum drug. Twenty-five control rats received i.v. injections of 0.9% NaCl solution 30 min after furosemide administration. Rats were weighed weekly and sacrificed in groups of 5 at 0, 2, 4, and 6 weeks after the initiation of drug treatment. Heparinized blood obtained from the abdominal aorta was used for determination of the packed cell volume, and additional blood was allowed to clot for serum collection. Sera were analyzed for blood urea nitrogen and serum creatinine concentrations (6, 11). Both kidneys of all sacrificed rats were weighed, and renal weight was recorded as percentage of body weight. Tissues were fixed in Telyesnichtky’s acetic acid-alcohol-formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

For each sacrificed group of 5 rats, the mean ± S.E. was determined for hematocrit, renal weight, blood urea nitrogen, and creatinine. The significance of differences was calculated using Student’s t test.

RESULTS

Rats given DDP alone gained weight at a rate similar to that of controls until the 3rd week when they started to lose weight (Chart 1). The 1st rat in this group died 5 weeks after the initial dose, while several others subsequently died during the next week. Signs of illness were weight loss and roughening of the hair coat. Rats receiving DDP and furosemide gained weight at a rate slightly lower than that of the controls until 5 weeks, when they also began losing weight. A few rats died during the 6th week. Control rats gained weight at a normal rate and none died.

The mean packed cell volume of rats treated with DDP or DDP and furosemide was reduced progressively and was statistically different (p < 0.05) from that of the controls from the 2nd to 4th week until it reached a mean of 22 to 25% by Week 6 (Chart 1). Control rats had mean values of 40 to 50% throughout the experiment.

Blood urea nitrogen and serum creatinine levels were elevated progressively and significantly (p < 0.05) in rats receiving DDP (Chart 1) but much less so in rats given DDP and furosemide. Several rats receiving DDP only became comatose during Week 5 or 6 and had blood urea nitrogen values of 200 to 400 mg/100 ml. Creatinine values paralleled the rise in blood urea nitrogen and reached 3 to 8 mg/100 ml by 6 weeks in most rats receiving DDP.

At necropsy, rats given DDP had progressively enlarged kidneys (Chart 1), whereas kidneys of those receiving DDP and furosemide were not significantly increased in size. Several comatose rats that developed high blood urea nitrogen levels after administration of DDP alone had gastric hemorrhages at necropsy. No other gross lesions were seen.

Renal lesions were progressive and severe in rats receiving DDP but were only mild in rats given DDP and furosemide. Markedly cystic, renal tubular lesions, most prominent at the outer stripe of the medulla (Fig. 1), were observed in rats with high blood urea nitrogen levels. Atrophy of tubules in the cortex also was noted. Furosemide protected against these cystic renal lesions, although mild regenerative tubular lesions were noted in the outer stripe of the medulla in rats receiving this drug and DDP (Fig. 2). The bone marrow of rats of both treatment groups was depleted to various degrees of all types of hematopoietic cells by 6 weeks. Intestinal lesions were mild in rats of both groups. Rats receiving furosemide and 0.9% NaCl solution did not have any histological renal lesions or lesions in other tissues.

DISCUSSION

The weekly administration of DDP to F344 rats induced progressive renal lesions, uremia, and death from renal failure 6 weeks after initial drug administration. Rats receiving furosemide 30 min before DDP administration had significantly less renal damage by 6 weeks and did not die from
renal failure. The prior administration of furosemide modified the acute nephrotoxic effects of DDP, whereas simultaneous administration of DDP and furosemide did not (14). Although rats of both treatment groups developed anemia, weight loss was more marked in those given DDP only. It was previously demonstrated that furosemide modified the acute nephrotoxicity of DDP after a single injection (14). From the results of these and other studies (14), it was noted that furosemide modified the plasma clearance of platinum and the excretion of platinum in the urine. Also, furosemide may effect the target tubule cells of DDP toxicity (1, 2). The experimental method for modifying acute nephrotoxicity of DDP has been applied to rats in this chronic study. The results of this study demonstrate that furosemide pretreatment protects significantly against the development of DDP-induced nephrotoxicity in rats as measured by serum blood urea nitrogen and creatinine and histopathological lesions in the kidney. The practical application of this technique to human patients remains to be tested thoroughly (7). Previous reports have indicated that mannitol diuresis and furosemide administration may affect nephrotoxicity of DDP in humans (3, 7, 8). Since it has been postulated that furosemide administration in rats may not protect against DDP nephrotoxicity through its diuretic effect (14), the simultaneous administration of a diuretic drug (mannitol) and one that may act in a completely different protective way may be undesirable. Further studies are required to elucidate the mechanism of the protective action of mannitol alone and furosemide alone against the nephrotoxicity of DDP.

REFERENCES

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