Effect of N-N'-Diphenyl-p-Phenylenediamine Pretreatment on Urinary Enzyme Excretion in Cisplatin Nephrotoxicity in Rats

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Accepted November 2, 1987

Abstract—Two days after cisplatin was injected into rats, urinary N-acetyl-β-D-glucosaminidase (NAG) and γ-glutamyltranspeptidase (γ-GTP) activities increased. The urinary excretion of NAG continued to rise until 4 days after the injection of cisplatin, the last day examined. However, the increase in urinary γ-GTP excretion which lasted for 2 days returned to its control level 4 days after cisplatin injection. The alkaline phosphatase activity in urine was unaffected by cisplatin injections. The antioxidant N-N'-diphenyl-p-phenylenediamine attenuated these increases in enzyme activities caused by cisplatin. The results of this study suggest that monitoring the change in urinary activities of some enzymes is the method of choice for detecting cisplatin nephrotoxicity and that the increase may involve the generation of free radicals by cisplatin.

Cisplatin (cis-diaminedichloroplatinum II) is an important antineoplastic agent that is toxic to several kinds of healthy tissues, especially that of the kidney. Acute renal failure produced by the administration of this drug has usually been monitored by rises in the concentrations of blood urea nitrogen and creatinine. However, the injection of cisplatin in rats also causes increased production of lipid peroxides (1). Such increases caused by the drug were lessened by treatment of rats with an antioxidant, N-N'-diphenyl-p-phenylenediamine (DPPD), which suggested that there is an interaction of free radicals generated by cisplatin with membrane lipids, causing the production of lipid peroxides (1, 2).

To assess cisplatin nephrotoxicity, different indices can be used, one of which is the measurement of urinary enzymes. Excretion of enzymes in the urine has been monitored to detect the nephrototoxic qualities of some compounds, as reviewed by Plummer and Ngaha (3). Urinary enzymes that have been measured to detect cisplatin nephrotoxicity include N-acetyl-β-D-glucosaminidase (NAG) and alanine aminopeptidase in patients (4) and γ-glutamyltranspeptidase (γ-GTP; 5), alkaline phosphatase, acid phosphatase (6) and glutathione-S-transferase (7) in rats.

Male Sprague-Dawley rats (mean weight, 200 g) received cisplatin intraperitoneally in the dose of 5 mg/kg, and control rats were injected with isotonic sodium chloride. For the experiment with DPPD treatment, the antioxidant (0.5 g/kg) or its vehicle, corn oil, was given intraperitoneally 24 hr before the injection of cisplatin. The animals were kept individually in stainless steel metabolic cages and urine was collected in small test tubes surrounded by ice for 6 hr at 1, 2, 3 or 4 days after the injection of cisplatin. The urine was used for assays of NAG activity by the method of Maruhn (8) as modified by Nakamura et al. (9) and also for assays of γ-GTP and alkaline phosphatase activities with commercial
reagent kits (Wako Pure Chemical Industries, Ltd.). The blood plasma urea nitrogen was assayed by the method of Coulombe and Favreau (10). All results are expressed as means±S.E. Significances of the differences were evaluated by Student's t-test.

We studied the effects of administration of cisplatin on urinary enzyme activities. The results of all experiments are summarized in Fig. 1. These results show that the urinary excretion of NAG became elevated 2 days after injection of cisplatin, and the elevation lasted until 4 days after the injection. The urinary \( \gamma \)-GTP activity was higher than the control activity at 2 and 3 days after cisplatin injection, but was the same as the control at 4 days after the injection. The alkaline phosphatase activities of the treated and control rats were not significantly different throughout all the days examined after the injection.

The results shown in Table 1 indicate that treatment of rats with the antioxidant DPPD before injection of cisplatin significantly attenuated the increases caused by cisplatin not only in the blood plasma urea nitrogen level but also in that of the urinary NAG and \( \gamma \)-GTP activities. DPPD alone had no effect on these levels not only in our preliminary experiments (data not shown) but also in comparison to the control levels in Fig. 1. Because of this, urinary levels of both enzymes in rats treated with DPPD alone were shown as a control in Table 1.

The results of this study suggest that in rats, urinary NAG and \( \gamma \)-GTP measurements are a more sensitive index of cisplatin nephrotoxicity than is measurement of the other urinary enzyme alkaline phosphatase. Feinfeld et al. (7) have shown that there is a correlation between the level of urinary glutathione-S-transferase and serum creatinine. The urinary NAG and \( \gamma \)-GTP activities increased 2 days after the cisplatin injection, which was earlier than the increase in the blood urea nitrogen level 3 days after the injection (2). This suggests that of the enzymes examined, urinary NAG and \( \gamma \)-GTP are the appropriate markers of cisplatin nephrotoxicity.

Free radical scavengers, or antioxidants, partially prevent the increase in blood urea nitrogen caused by cisplatin injection (2, 11, 12). The antioxidant DPPD also partially

Fig. 1. Excretion of enzymes in urine after the intraperitoneal administration of cisplatin. Open columns show the urinary enzyme activities of control rats and shaded columns those of rats given 5 mg/kg cisplatin. Data are the means±S.E. of 5 experiments for the controls and 7 for cisplatin treatment in A and of 10 experiments for the controls and 12 for cisplatin treatment in B and C. Significantly different from the control value on that day, \( *P<0.05, **P<0.01, ***P<0.005 \).
protected against the increases in urinary NAG and γ-GTP activities. Its protective effect is further evidence of free radicals being involved in the pathogenesis of cisplatin nephrotoxicity.

Acknowledgments: We thank Ms. Caroline Latta for help in preparing the manuscript.

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