Use of Cultured Renal Epithelial Cells for the Study of Cisplatin Toxicity

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Abstract—The nephrotoxicity of cisplatin was evaluated in an in vitro system with an established cell line of normal rat kidney, NRK-52E. Leakage of enzymes from the cells into the culture medium increased when they were exposed to 1 μM of cisplatin for 72 hr. The level of lipid peroxides increased in the cells after 48 hr of exposure to cisplatin; the increase was more rapid than the enzyme leakage. This culture system can be used to evaluate drug-induced nephrotoxicity and its mechanism.

The nephrotoxicity of cisplatin (cis-diaminedichloro-platinum II) has been assessed mainly by the measurement of blood urea nitrogen and excretion of several enzymes into the urine of laboratory animals (1-4). Because this process takes a long time, a fast and efficient alternative system for the evaluation of the nephrotoxicity of cisplatin and its analogues is needed. Cisplatin toxicity might be evaluated in an in vitro system with a renal epithelial cell line. Cisplatin causes lipid peroxidation in rat renal slices incubated in vitro (5, 6). The purpose of this study was to assay the activity of enzymes released from cultured renal epithelial cells and to measure the lipid peroxidation in the cells; both values might be indicators of cell injury by exposure to cisplatin in the in vitro system.

NRK-52E (7), a commercially available line of normal rat kidney epithelial cells, was maintained at 37°C in a humidified atmosphere of 5% CO₂ in air in 25-cm² polystyrene tissue-culture flasks (Corning Glass Works, Corning, NY) with Dulbecco's modification of Eagle's medium (DMEM) supplemented with 5% calf serum. The cells were seeded at 2×10⁵/ml, and cisplatin was added 2 hr later to the final concentration of 1 μM. Cell cultures were exposed to cisplatin, and the activities of the cytosolic enzyme, lactate dehydrogenase (LDH), the lysosomal enzyme, N-acetyl-β-glucosaminidase (NAG), and the brush-border enzyme, γ-glutamyl transpeptidase (γ-GTP), were assayed. Cultures of NRK-52E cells, one to four days old, were harvested by scraping them from the flask. The cells were suspended in medium containing 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, and 1.5 mM KH₂PO₄; counted; and then lysed by sonication in the presence of 0.74% sodium dodecyl sulfate. The activities of LDH and γ-GTP in the culture medium were assayed with commercial kits from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Leakage of NAG into the medium was monitored by the method of Maruhn (8) as modified by Nakamura et al. (9). The extent of lipid peroxidation in the cell lysates was estimated by measuring the concentration of malondialdehyde as described by Ohkawa et al. (10). Levels of statistical significance were evaluated with Student's t-test.

Figure 1 shows the leakage of the three enzymes into the culture medium with the exposure of NRK-52E cells to cisplatin. Cisplatin did not have any effect on enzyme leakage up to 48 hr of exposure. With 72 hr of exposure, the activities of all three enzymes in the medium had increased. LDH increased four-fold when cisplatin treatment was for 72 hr. NAG increased less and γ-GTP
Fig. 1. Effects of exposure of cultured renal epithelial cells to cisplatin on the leakage of lactate dehydrogenase (LDH), N-acetyl-β-glucosaminidase (NAG), and γ-glutamyltranspeptidase (γ-GTP) into the culture medium. Each point represents the mean±S.E. of seven to nine experiments. *P<0.001 and **P<0.05, compared to the control at the same time.

Increased the least by 72 hr of exposure. The exposure of NRK-52E cells to various concentrations of cisplatin for 72 hr increased the enzyme leakage in a concentration-dependent manner (data not shown). Malondialdehyde in the cultured cells increased significantly with 48 hr of exposure to cisplatin and remained high to the end of the experiment, at 96 hr (Fig. 2).

Leakage of enzymes into the medium has been used to assess renal injury by drugs in tissue slices (11), primary cultures of cells (12), and isolated tubules (2). The results of this study suggest that measurement of LDH in the culture medium of the rat kidney cell line NRK-52E was a more sensitive indicator of cisplatin toxicity than measurements of the two other enzymes examined, NAG and γ-GTP. With this culture system, it was possible to detect the toxicity of cisplatin at a low concentration (1 μM). This concentration that produced cell injury in vitro was comparable in almost the same order of magnitude to the concentration found in the plasma of rats treated with nephrotoxic doses of cisplatin in vivo (13, 14).

The effect of cisplatin on lipid peroxidation was an early event that occurred before the increase in the activities of the three enzymes in the culture medium. In rat renal slices treated with cisplatin at high enough concentrations (millimolar), lipid peroxidation increased significantly (5, 6). Lipid peroxides in cultured cells were increased by a low concentration of cisplatin (1 μM). These results suggest that lipid peroxidation in NRK-52E cells may contribute to the cell injury caused by cisplatin; however, further studies will be required in order to establish an exact cause-and-effect relationship.

This culture system could be used to screen cisplatin analogues for nephrotoxicity and for studies of the mechanism of this effect.

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