The Iron Chelator Deferoxamine Prevents Cisplatin-Induced Lipid Peroxidation in Rat Kidney Cortical Slices

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ABSTRACT—We evaluated the effect of the iron chelator deferoxamine on lipid peroxidation produced by the nephrotoxic antineoplastic drug cisplatin in rat kidney cortical slices. The addition of deferoxamine to the incubation medium prevented such lipid peroxidation in the incubated slices. Treatment of rats with deferoxamine inhibited the increase in lipid peroxidation caused by cisplatin in the medium. These results suggest that iron may be a causal agent of cisplatin-induced lipid peroxidation.

cis-Diamminedichloroplatinum II (cisplatin), a nephrotoxic antineoplastic drug, stimulates lipid peroxidation in renal cortical slices (1, 2). Lipid peroxidation occurs when reactive oxygen species react with polyunsaturated fatty acids in biological membranes (3). The Haber-Weiss reaction, in which superoxide radicals and hydrogen peroxide yield hydroxyl radicals, is accelerated by transition metals, particularly iron (4). The highly reactive hydroxyl radicals eventually initiate lipid peroxidation. Deferoxamine, a potent iron chelator, inhibits the Haber-Weiss reaction (5) and thus can be used to study a process that seems to involve reactive oxygen species, especially hydroxyl radicals.

The purpose of this study was to examine the effect of deferoxamine on lipid peroxidation in renal cortical slices incubated with cisplatin. We wanted to find whether iron chelation by deferoxamine would inhibit the lipid peroxidation caused by cisplatin.

Male Sprague-Dawley rats weighing 180–250 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Slices were prepared from kidney cortices of the rats with the use of a razor blade. In the first experiment, we incubated kidney cortical slices prepared from untreated rats at 37°C in medium with cisplatin, deferoxamine, or both. In the next experiments, slices prepared from rats 30 min after a single injection of deferoxamine (300 mg/kg, i.v.) were incubated at 37°C in medium with cisplatin or ethylenediaminetetraacetate (EDTA)-iron complex to evaluate the in vivo effects of the iron chelator. Ferric-EDTA or ferrous-EDTA was prepared by mixing equimolar amounts of Na2H2EDTA and FeCl3 or FeCl2 (6).

Slices were incubated in a medium containing 137 mM NaCl, 5.9 mM KCl, 1.5 mM CaCl2, 1.2 mM MgCl2, 11.5 mM glucose, and 5.8 mM N-2-hydroxyethyl-piperazine-N'-ethanesulfonic acid titrated with NaOH to pH 7.4, as described previously (7). When the incubation was over, the slices were removed from the medium, weighed, and homogenized in 20 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl and 3 mM EDTA. Lipid peroxidation in homogenates of the incubated slices was monitored by measuring the malondialdehyde production by the thiobarbituric acid assay de-
scribed by Buege and Aust (8). Glutathione (GSH) as a nonprotein sulfhydryl in the homogenates was assayed by the method of Ellman (9).

Cisplatin and deferoxamine mesylate were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were of the highest purity available from commercial sources. Data are expressed as means ± S.E. Statistical analyses were done by Student's *t*-test.

The effects of cisplatin and deferoxamine on the malondialdehyde (lipid peroxide) and GSH levels in rat kidney cortical slices are shown in Fig. 1. Incubation of the slices with 2 mM cisplatin for 90 min significantly increased the malondialdehyde level in the slices from control values of 31.5 ± 1.2 nmol/g wet wt. to 42.4 ± 1.1 nmol/g wet wt. Deferoxamine (0.1 mM) completely prevented the lipid peroxidation caused by cisplatin. Deferoxamine alone had no effect on lipid peroxidation in the slices. Cisplatin markedly decreased the GSH level in the slices during the incubation. Deferoxamine had no effect on the decrease in GSH level caused by cisplatin. Cellular GSH is well-known to be important in the protection of cells from free radicals and peroxides. However, the prevention by deferoxamine of cisplatin-induced lipid peroxidation was not accompanied by a recovery of the decrease in the GSH level in this study. The next experiments were done to study the effect of treating rats with deferoxamine on lipid peroxidation in kidney cortical slices incubated in the presence of cisplatin or iron in the medium (Fig. 2). Lipid peroxidation was increased in the slices from the control-group that were incubated not only in the presence of cisplatin in the medium as mentioned above but also in the presence of ferrous ion chelated with EDTA, although Fe^{3+}-EDTA did not affect lipid peroxidation in the slices. Deferoxamine treatment completely prevented this increase in lipid peroxidation in the slices incubated with cisplatin or Fe^{2+}-EDTA. The deferoxamine treatment itself had no effect on the level of lipid peroxidation in the incubated slices.

The results showed that the addition of deferoxamine to the medium completely prevented the increase in lipid peroxidation produced by cisplatin in rat kidney cortical slices without any effect on the level of the cellular antioxidant GSH. This was also shown by the improved effect obtained in the slices incu-
bated in the presence of not only cisplatin but also Fe$^{2+}$-EDTA when deferoxamine was injected into the rats. So, we excluded a direct effect of deferoxamine on the assay of malondialdehyde levels and demonstrated that deferoxamine, which was previously administered to rats, inactivated ferrous ion by chelating it. Deferoxamine, which has a high affinity for iron (10), inactivates this important catalyst, which is responsible for the generation of hydroxyl radicals in the Haber-Weiss and Fenton reactions (5). These results can be explained by the hypothesis that iron plays a significant role in cisplatin-induced lipid peroxidation in renal cells. Most of the iron in tissues is stored safely in cells as ferric hydroxide micelles within ferritin molecules (11). Reduction of the iron stored as ferritin in the form of ferric iron to the ferrous form results in its mobilization (4). Release of iron from ferritin can be mediated by xanthine oxidase, primarily via superoxide radicals (12, 13). Iron is released from its storage pool and redistributed to reactive pool for catalysis of oxygen-derived free radical-mediated lipid peroxidation within renal tissues during ischemia and subsequent reperfusion of the kidney (14, 15), whereby oxygen free radicals probably contribute to kidney damage. Perhaps iron can be released from ferritin in renal cells by cisplatin. Our results suggest that the beneficial effect of deferoxamine on the cisplatin-induced lipid peroxidation may be due to its chelation of iron in renal cells, thereby inhibiting hydroxyl radical formation in the cells.

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