Potentiation of Cisplatin-Induced Lipid Peroxidation in Kidney Cortical Slices by Glutathione Depletion

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Abstract—Effects of cis-diamminedichloroplatinum II (cisplatin), an antitumor agent with a dose-limiting effect of nephrotoxicity, on lipid peroxides and glutathione (GSH) were examined in rat kidney cortical slices treated with or without diethylmaleate (DEM), a GSH depletor, in vitro. DEM (3 mM) decreased the GSH level to about 16% of the control with a concomitant increase in lipid peroxides after 90 min of incubation. The same effects were obtained with 1 mM cisplatin 90 min later. Cisplatin (1 mM) with DEM (2 mM) stimulated both the decrease in GSH and the increase in lipid peroxides 90 min after incubation. However, cisplatin with DEM markedly stimulated lipid peroxidation with a small effect on the GSH decrease by cisplatin alone 30 min after incubation, while each drug by itself did not affect lipid peroxidation. The antioxidants N,N'-diphenyl-p-phenylenediamine (DPPD), promethazine, and ascorbic acid abolished cisplatin-induced lipid peroxidation in the presence of DEM. DPPD had no effect on the depletion of GSH caused by cisplatin and DEM. Ascorbic acid and promethazine caused only a slight return towards the control level. The results suggested that cisplatin-induced lipid peroxidation is due to another mechanism in addition to the GSH depletion caused by the antitumor drug.

cis-Diamminedichloroplatinum II (cisplatin) is an effective cancer chemotherapeutic agent used mostly for the treatment of testicular, bladder, and ovarian tumors (1). The free use of cisplatin is prevented by its dose-limiting nephrotoxicity (2–5). The renal damage largely takes the morphological form of the destruction of the pars recta of the proximal tubules (6–8). The exact mechanism of this nephrotoxicity is not yet known.

Previous studies have found that superoxide dismutase and antioxidants ameliorate the nephrotoxicity of cisplatin (9–11). Lipid peroxidation (as assessed by the concentration of malondialdehyde, MAD) are increased in renal tissue by cisplatin, and the enhanced formation of lipid peroxides is prevented by antioxidant (12, 13). These results suggested that free radicals or lipid peroxidation generated by cisplatin are responsible for the nephrotoxicity.

Reduced glutathione (GSH), a tripeptide that contains thiol, is present in millimolar concentrations in mammalian cells, and it is generally known as a potent factor in the control of the lipid peroxidation (14–16), which is thought to have a major role in the promotion of cell damage (17–19). In the liver, lipid peroxidation is enhanced when the tissue GSH level is lowered both in vivo and in vitro (20–22). Depletion of GSH potentiates the hepato- and nephrotoxicity caused by certain drugs (23–28); these drugs per se can decrease the GSH level in target organs. Therefore, one possible mechanism for cisplatin nephrotoxicity is lipid peroxidation in renal tissue depleted of GSH, if the antitumor drug markedly depletes the GSH level in such cells.

The present experiments were done to study the effect of cisplatin on the GSH level using renal cortical slices in vitro, which is a...
useful technique for studying the direct effects of a drug on a tissue. We also investigated how lipid peroxidation caused by cisplatin in renal tissue was changed by diethylmaleate (DEM), a GSH depletor, which conjugates with GSH and decreases the intracellular GSH concentration (29).

**Materials and Methods**

**Preparation of renal slices:** Male Sprague-Dawley rats weighing 180–280 g were used. The animals were housed in a room with controlled temperature (24±1 °C), humidity (55±10%), and light (12-hr dark, 12-hr light cycle) and given feed and water ad libitum. Kidney cortical slices were prepared from rats with the use of a razor blade as already described (30). Unless otherwise stated, the slices were placed in a chilled saline medium adjusted with NaOH to pH 7.4. The medium contained the following: 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’-2-ethanesulfonic acid (Hepes).

**Measurement of lipid peroxidation and GSH in the slices:** The slices were added to 10 ml of the above medium and incubated at 37°C under a 100% oxygen atmosphere. After incubation, the slices were removed, weighed and homogenized in Tris-HCl buffer (pH 7.4) for the measurement of lipid peroxides or in 100 mM phosphate buffer (pH 7.4) for the measurement of GSH. Lipid peroxidation in the homogenate was monitored by measurement of the production of MDA with the thiobarbituric acid assay described by Buege and Aust (31). The protein in the phosphate-buffered homogenates was precipitated by the addition of an equal volume of 4% sulfosalicylic acid and centrifugation. GSH as a nonprotein sulfhydryl was assayed in an appropriately-sized portion of supernatant by the method of Ellman (32).

**Chemicals and statistics:** Cisplatin was purchased from the Sigma Chemical Co. (St. Louis, MO). DEM was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and dissolved in dimethyl sulfoxide (DMSO), which was added to the medium at the final concentration of 2%. This concentration of DMSO had no effect on the factors we measured. All other chemicals were of reagent grade. Values are expressed as means±S.E. Statistical analysis was done by Student’s t-test.

**Results**

In the first experiments, different concentrations of DEM, a GSH depletor, were plotted against the corresponding GSH and

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![Graph](image-url)

Fig. 1. Effect of the diethylmaleate (DEM) concentration on malondialdehyde (A) and GSH (B) levels in rat kidney cortical slices. Slices were incubated at 37°C for 90 min in medium containing the concentration of DEM shown in the figure. Values represent means±S.E. of four experiments. *P<0.002 and **P<0.001, compared to the control value without DEM.
MDA levels after 90-min incubation to evaluate the relationship between lipid peroxidation and GSH levels in rat kidney cortical slices (Fig. 1) DEM decreased GSH in a dose-dependant way. When the GSH level in the slices reached 0.36±0.02 μmoles/g tissue with 3 mM DEM, lipid peroxidation increased. So, the threshold value of GSH for the onset of lipid peroxidation was 16% of the control value before incubation.

Because DEM used at concentrations up to 2 mM led to no increase in lipid peroxides but caused a decrease in GSH concentrations, the next experiments were designed to study the effects of cisplatin on lipid peroxidation in GSH-depleted slices in the presence of 2 mM DEM. By 90 min, cisplatin-induced lipid peroxidation was enhanced by 2 mM DEM, and the depletion of GSH by cisplatin and by DEM was further enhanced by the combination of the two (Fig. 2). By 30 min, incubation of the slices with this combination of cisplatin and DEM resulted in lipid peroxidation, although each drug by itself did not. The GSH levels in the slices were 0.62±0.06 and 0.10±0.03 μmoles/g tissue at 30 and 90 min after incubation, respectively, when both drugs were present.

The antioxidants N,N'-diphenyl-p-phenylene diamine (DPPD) (33, 34), promethazine...
zine (35) and ascorbic acid (36) almost completely abolished lipid peroxidation generated by the coexistence of cisplatin and DEM, although each drug alone slightly reduced the level of lipid peroxides in the control slices. DPPD had no effect on the depletion of GSH caused by cisplatin and DEM, and ascorbic acid or promethazine only brought the level slightly toward the control level (Fig. 3).

Discussion

GSH is involved in protecting cells from cytotoxic damage by many compounds (37). Nephrotoxicity caused by a wide variety of drugs may be due to at least in part to depletion of intracellular GSH. However, several authors have shown that the administration of cisplatin to rats resulted in an increase, not a decrease, in the renal GSH level (38-40) or had no effect on the renal GSH level (41). Here, the presence of cisplatin in the incubation medium decreased the GSH level in the slices to below control values. So, these results suggest that a direct effect of cisplatin on the kidney results in the depression of the GSH level.

DEM, a GSH depletor, markedly decreased GSH in the slices dose-dependently and resulted in lipid peroxidation when GSH in the slices was depleted by 16% or more. Treatment of the slices with cisplatin decreased the GSH level by 16% at 90 min after incubation and concomitantly enhanced lipid peroxidation to almost the same extent as did the GSH depletor. These results suggest that when GSH stores in renal cortical cells are critically depleted by cisplatin, the insufficient GSH cannot protect the cells from peroxidative damage. By 90 min, cisplatin-induced lipid peroxidation was enhanced by the presence of 2 mM DEM, which alone had no effect on lipid peroxidation; concomitantly, GSH in the slices decreased more when both cisplatin and DEM were present than with each compound alone. In this case, the more striking GSH depletion per se may account for the enhanced lipid peroxidation. By 30 min, cisplatin or DEM alone had no effect on lipid peroxidation; however, cisplatin with DEM markedly stimulated lipid peroxidation when the GSH level in the slices decreased to 25% of the control value (time zero). Treatment of the slices with 2 mM DEM alone led to a GSH level of 0.58 μmol/g tissue 90 min after incubation. 25% of the control; such a DEM-induced depletion of GSH in the slices did not lead to lipid peroxidation. These findings indicate that cisplatin induces lipid peroxidation by another mechanism in addition to the cisplatin-induced depletion of GSH itself. The inhibition of cisplatin-induced lipid peroxidation with DEM by antioxidants is further evidence that cisplatin causes renal cells to generate free radicals that react with membrane lipids to induce lipid peroxidation and cellular damage.

Because GSH is a co-factor for glutathione peroxidase, which reduces peroxides as part of a defense mechanism against oxidative damage (42), increases in the enzyme activity would be related to the level of GSH reduced by cisplatin or a decrease in its activity would lead to generation of lipid peroxidation. However, no effect on the enzyme activity was seen at the therapeutic dose and concentration used in this study of cisplatin (40), indicating that no relationship can be found between the GSH depletion induced by cisplatin and GSH peroxidase activity or between cisplatin-induced lipid peroxidation and the enzyme activity. Other GSH-dependent enzymes, glutathione S-transferases, are unaffected by the addition of cisplatin to the medium (39), indicating that there is no relationship between the GSH depletion induced by cisplatin and the enzyme activities. γ-Glutamylcysteine synthetase and γ-glutamyl transpeptidase activities are inhibited by cisplatin (41). Further study is needed to elucidate the relationship between these enzyme activities and GSH status after cisplatin treatment.

Cisplatin reacts with sulfhydryl groups (43), suggesting the depressed GSH levels are caused directly by the antitumor drug. However, Levi et al. (38) found that it does not interact directly with compounds that contain thiol in vitro. Our results do not show whether cisplatin is bound to GSH or not. Wendel et al. (24) reported that GSH participates in the inhibition of lipid peroxidation, since the process is accompanied by a loss of hepatic GSH. One suggestion is that
GSH, an endogenous antioxidant, protects the renal cortex from cell injury by detoxifying peroxides caused by cisplatin, resulting in a decreased GSH level in the slices. In this case, in addition to a decline in the total GSH, reduced GSH would be consumed to shift oxidized GSH, which was not assayed in this study. However, our results indicate that the fall in GSH caused by cisplatin may not be due to this, because of the very slight effect of antioxidants on cisplatin-induced decreases in GSH level in spite of the abolishment of cisplatin-induced lipid peroxidation by the antioxidants.

In summary, cisplatin-induced decreases in GSH levels per se may lead to lipid peroxidation in renal cortical slices in vitro. In addition to this lack of GSH as one defense factor against peroxides, cisplatin may produce free radicals that react with membrane lipids, causing the generation of lipid peroxides; such production of free radicals by cisplatin is probably promoted in the condition of the decrease in GSH levels to some extent.

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