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PROTECTION AGAINST CISPLATIN-INDUCED NEPHROTOXICITY IN THE RAT BY INDUCERS AND AN INHIBITOR OF GLUTATHIONE *S*-TRANSFERASE

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Abstract—In an attempt to decrease cisplatin-induced nephrotoxicity, glutathione *S*-transferase (GST) inducers and a GST inhibitor were combined with cisplatin and administered to rats. t-Stilbene oxide (t-SO) and propylthiouracil (PTU) were the GST inducers, and ketoprofen was the GST inhibitor. Combinations of these GST inducers and the inhibitor with cisplatin decreased cisplatin-induced nephrotoxicity. The drug combinations with cisplatin inhibited the cisplatin-induced increase in urinary total GST activity. The combination of t-SO with cisplatin increased total GST activity in the kidney, compared to levels in the cisplatin only group. The t-SO combination recovered the cisplatin alone-induced decrease in GST-α activity to control levels. However, glutathione peroxidase (GSHpx) activity after the t-SO combination was ever further reduced compared to the cisplatin alone-induced decrease. The combination of PTU, with cisplatin increased total GST, GST-α and GSHpx activity, compared to the cisplatin alone group. However, PTU severely decreased the glutathione (GSH) level. The combination of ketoprofen with cisplatin normalized GST-μ and -α activity, and elevated the cisplatin-induced decrease of GSHpx activity and GSH. These findings suggest that ketoprofen decreases cisplatin-induced nephrotoxicity.

Key words: ketoprofen; cisplatin; nephrotoxicity; BUN; glutathione *S*-transferase-α; glutathione peroxidase

Cisplatin is an important agent in cancer chemotherapy; however, as its nephrotoxicity parallels its antitumor activity, various attempts have been made to increase its clinical usefulness by reducing its toxicity. Combinations of various drugs with cisplatin have been used to this end [1–6]. However, the reduction of cisplatin nephrotoxicity by drugs which act on the enzymes that are involved in this nephrotoxicity has not been reported. We have previously shown that cisplatin-induced nephrotoxicity in rats was due to a decrease of GST†-α activity in the kidney after cisplatin administration [7, 8]. Thus, we speculated that is possible to reduce the nephrotoxicity of cisplatin by administering GST inducers or inhibitors.

In this study, we used t-SO [9] and PTU [10] as GST inducers. We compared the effect of CPH [11], which produces renal damage and then induces GST activity, with the effect of cisplatin.

We used ketoprofen as a GST inhibitor. This agent is reported to inhibit GST-π activity [12]; it is a tumor marker [13] *in vitro*, and is used as an anti-inflammatory drug clinically. The inhibitory effect of ketoprofen on GST-π activity has been confirmed *in vitro* only.

In this study, we determined nephrotoxic markers in rat serum and urine after the administration of combinations of cisplatin with GST inducers or the GST inhibitor, ketoprofen. By examining the effect of these drugs on cisplatin-induced nephrotoxicity, we attempted to determine whether there was a relationship between GST isoenzyme and the decrease that these agents produced in cisplatin nephrotoxicity. We isolated GST isoenzymes by *S*-hexylglutathione affinity chromatography, and we determined GST isoenzyme activity and the level of Pt binding in GST isoenzyme fractions.

MATERIALS AND METHODS

Chemicals. Cisplatin and ketoprofen for injection were purchased from Nippon Kayaku, Ltd (Tokyo, Japan) and Chugai Pharmaceutical Co., Ltd (Tokyo, Japan), respectively. GSH, S-hexylGSH, PTU, CPH and GST were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). t-SO was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, U.S.A.). CDNB was obtained from Wako Pure Chemical Industries, Ltd (Tokyo, Japan), and cumene hydroperoxide was obtained from Nakarai Tesque, Inc. (Kyoto, Japan). BUN level and GOT and GPT activity determination kits were purchased from Wako Pure Chemical Industries.

Animal experiments. Male Wistar rats, 6 weeks old and weighing 120–140 g, were obtained from Japan SLC Co. Ltd (Hamamatsu). They were housed in a room maintained at a temperature of 25 ± 1° and 55 ± 5% relative humidity and were allowed standard laboratory feed and water *ad lib*. The animals were divided into nine groups: control, t-

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† Abbreviations: GST, glutathione *S*-transferase; PTU, propylthiouracil; t-SO, t-stilbene oxide; CPH, cephaloridine; CDNB, 1-chloro-2,4-dinitrobenzene; BUN, blood urea nitrogen; GSHpx, glutathione peroxidase; GSH, glutathione; LPO, lipid peroxide.

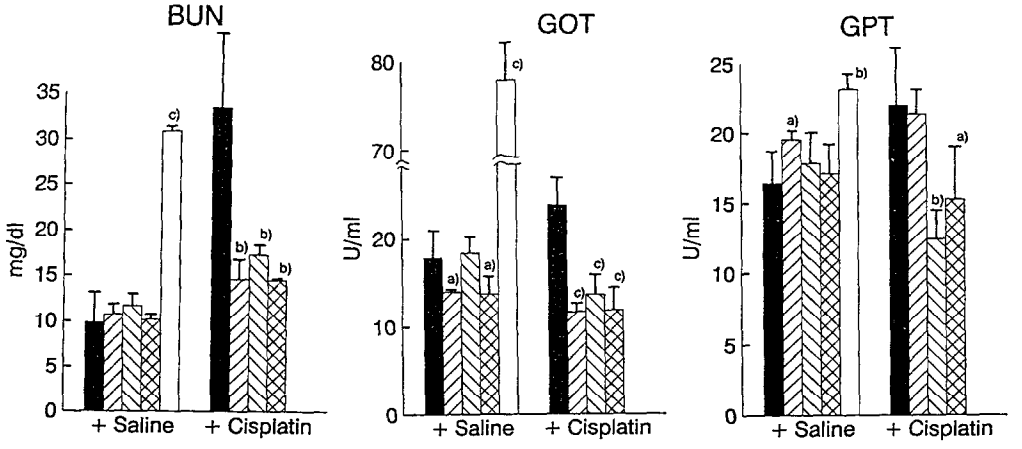

Fig. 1. Effects of GST inducers and inhibitor on cisplatin-induced changes in BUN level and GOT and GPT activity. Wistar rats received an intraperitoneal injection of each drug. Each column represents the mean ± SD of six rats. Significant differences from saline values in each group are indicated by (a) $P < 0.05$, (b) $P < 0.01$, and (c) $P < 0.001$.
■ Saline, ⁒ t-SO, ⁖ PTU, ⊠ KP, □ CPH.

SO, PTU, ketoprofen, CPH, cisplatin, cisplatin + t-SO, cisplatin + PTU and cisplatin + ketoprofen. t-SO (400 mg/kg/day), PTU (50 mg/kg/day), ketoprofen (10 mg/kg/day) and CPH (750 mg/kg/day) were injected intraperitoneally for 8 days from the day before single administration of cisplatin (5.0 mg/kg, i.p.). Urine was collected for 24 hr before killing. The animals were killed by cervical dislocation on the day after the last injection of the drug. Blood samples were taken from the heart, and the liver and kidney were quickly removed.

Enzyme assay. GST and glutathione peroxidase (GSHpx) activity were determined according to the method of Habig *et al.* [14] and Hafeman *et al.* [15], respectively. The GST activity, determined using CDNB and cumene hydroperoxide as substrates, was defined as the total activity and isoenzymes activity, respectively.

LPO, GSH and Pt levels. Determination of LPO, GSH and Pt levels in the tissue samples was carried out as described in our previous studies [8, 16].

Isolation of GST isoenzymes by S-hexylGSH affinity chromatography. Isolation of GST isoenzymes was carried out according to the method of Hayes [17]. The first, second and third peaks represented the α, μ and π fractions, respectively.

Statistical analysis. The statistical significance of differences was evaluated with ANOVA and Student's *t*-test.

RESULTS

Effects of GST inducers and inhibitor on changes in BUN levels and GOT and GPT activity in rat serum after cisplatin administration

As shown in Fig. 1, BUN levels were not significantly changed in the t-SO, PTU and ketoprofen only groups, compared to levels in the control group. t-SO, PTU and ketoprofen significantly decreased, by about 50% ($P < 0.01$), the cisplatin-induced increase (3.4-fold of control, $P < 0.001$) in BUN level. GOT activity in the t-SO, PTU and ketoprofen combination groups was about half that in the cisplatin alone group ($P < 0.001$). PTU and ketoprofen reduced the increased GPT activity in the serum of rats treated with cisplatin, but t-SO had no effect. In the CPH alone group, BUN level and GOT and GPT activity increased.

Effects of GST inducers and inhibitor on changes in GST activity in rat urine after cisplatin treatment

Total GST activity was not detected in the control, t-SO, PTU and ketoprofen alone groups. However, there was a significant increase, to 0.80 ± 0.18 U/day ($P < 0.001$), after cisplatin administration. After the administration of t-SO, PTU and ketoprofen, this activity was 80.5%, 92.6% and 75.5% ($P < 0.05$), respectively, of the cisplatin-treated levels. In contrast, total GST activity in the CPH alone group increased significantly, to 0.58 ± 0.06 U/day ($P < 0.001$).

Effects of GST inducers and inhibitor on changes in GST activity in rat tissues after cisplatin treatment

(1) *Kidney (Fig. 2).* (a) Total GST activity: In t-SO, PTU and ketoprofen alone groups, the activity was significantly elevated, to 2.1 times ($P < 0.001$), 1.4 times ($P < 0.01$) and 1.3 times ($P < 0.05$) the normal level, respectively. There was no effect on total GST activity after cisplatin administration. However, t-SO, PTU or ketoprofen combined with cisplatin significantly increased this activity, to 2.4 times ($P < 0.001$), 2.0 times ($P < 0.001$) and 1.5 times ($P < 0.01$) that in the cisplatin only group.

(b) GST-α activity: GST-α activity in the t-SO alone group increased to 1.6 times ($P < 0.01$) the control level. This activity in the cisplatin alone group was 66.6% ($P < 0.01$) of the control level. In contrast, in the t-SO, PTU and ketoprofen combinations with cisplatin, this activity was

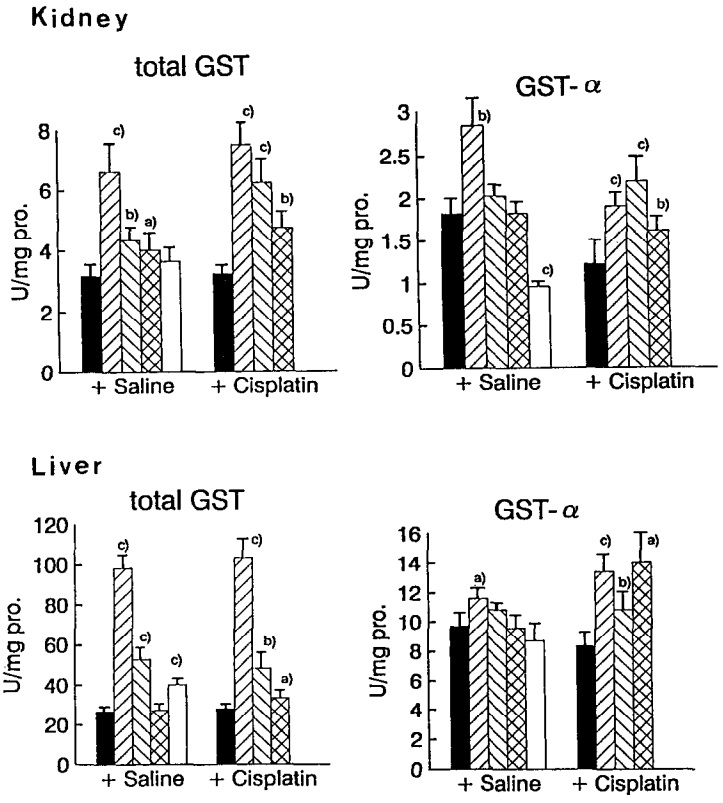

Fig. 2. Effects of GST inducers and inhibitor on cisplatin-induced changes in renal and hepatic GST activity.

increased to 1.6 times ($P < 0.001$), 1.8 times ($P < 0.001$) and 1.3 times ($P < 0.01$), respectively, that in the cisplatin alone group. CPH caused 52.5% inhibition.

(2) *Liver (Fig. 2).* (a) Total GST activity: In the t-SO and PTU alone groups, total GST activity was increased ($P < 0.001$), compared to the control level. Cisplatin had no effect on this activity, whereas in the t-SO, PTU and ketoprofen combination groups, the activity was increased to 3.7 times ($P < 0.001$), 1.7 times ($P < 0.01$) and 1.2 times ($P < 0.05$) respectively, that in the cisplatin only group.

(b) GST-α activity: In the t-SO only group, GST-α activity was elevated ($P < 0.05$). In the t-SO, PTU and ketoprofen combination with cisplatin group, this activity was increased to 1.6 times ($P < 0.001$), 1.3 times ($P < 0.01$) and 1.7 times ($P < 0.05$), respectively, the level of the cisplatin alone group, whose level was 86.0% ($P < 0.05$) of control activity.

Effects of GST inducers and inhibitor on changes in GSHpx activity and GSH and LPO levels in rat tissues after cisplatin administration

The effects of these drugs on cisplatin-induced changes in GSHpx activity and GSH and LPO levels are shown in Figs 3 and 4. In the kidney (Fig. 3), GSHpx activity was significantly decreased after cisplatin alone administration ($P < 0.001$), whereas in the PTU and the ketoprofen combination groups, the activity was higher than that in the cisplatin alone group. In the t-SO, PTU and ketoprofen groups, GSH levels were significantly decreased compared to the control level. In the cisplatin alone group, the level was 69.0% ($P < 0.01$) of the control level. However, in the t-SO and ketoprofen combination groups, the level was higher than that in the cisplatin only group. LPO levels in the PTU alone group were decreased (71.6% of control level, $P < 0.05$). In the cisplatin group, the level was increased. In contrast, after t-SO, PTU and ketoprofen administration, this level decreased by 33.3% ($P < 0.05$), 49.7% ($P < 0.05$) and 37.5% ($P < 0.05$), respectively.

In the liver (Fig. 4), GSHpx activity was significantly inhibited by cisplatin. This activity in the t-SO combination group was decreased more than that in the cisplatin only group. On the other hand, the activity in the ketoprofen combination group was elevated, to 1.2 times ($P < 0.01$) that in the cisplatin only group. Cisplatin alone had no effect on GSH levels. The combinations of both PTU and ketoprofen with cisplatin increased this level. Cisplatin induced an increase in the LPO level, and the LPO level in the t-SO combination group was also increased. However, the level in the PTU combination group was decreased by 27.7% ($P < 0.001$). Neither ketoprofen alone nor the ketoprofen combination had any effect on LPO level.

GST isoenzyme fraction and Pt binding level

Figure 5 shows the elution curves of renal GST

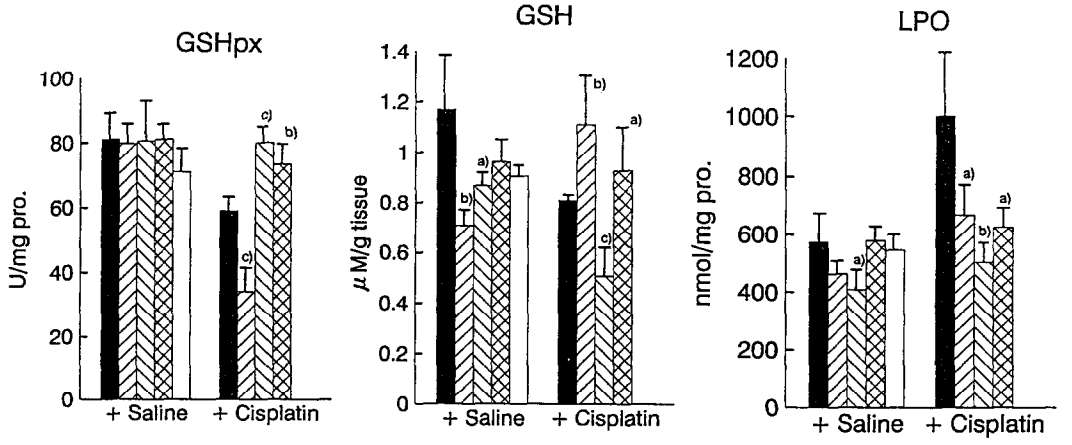

Fig. 3. Effects of GST inducers and inhibitor on cisplatin-induced changes in renal GSHpx activity and GSH and LPO levels.

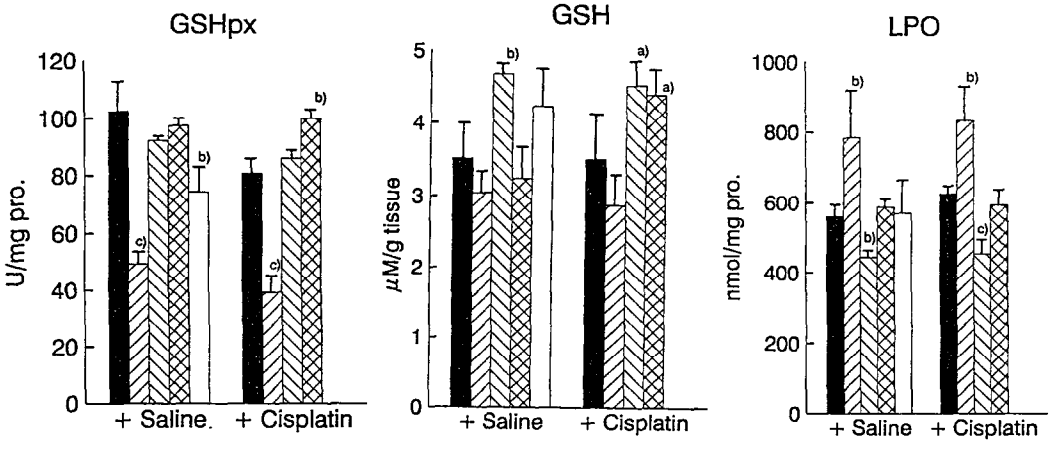

Fig. 4. Effects of GST inducers and inhibitor on cisplatin-induced changes in hepatic GSHpx activity and GSH and LPO levels.

isoenzymes in control rats, in those treated with cisplatin alone, and in those treated with the cisplatin and ketoprofen combination. Isolation was done by *S*-hexylGSH affinity chromatography, and enzyme activity was determined with CDNB as the substrate.

Compared to control levels, GST-α activity in the cisplatin alone group decreased by 20.0%, GST-μ activity increased to 2.2 times and GST-π activity did not change in the ketoprofen combination group. On the other hand, GST-α activity increased to 1.4 times the level in the cisplatin alone group, GST-μ activity decreased by 25.8% and GST-π activity did not change. In the cisplatin alone group, the Pt level in the GST-α, -μ and -π fractions was 0.154, 1.014 and 0.371 ppm, respectively. In the ketoprofen combination group, the Pt level in the GST-α and -μ fractions increased to 4.0 times and decreased to 1/4, respectively, that in the cisplatin alone group.

DISCUSSION

The cisplatin induced increase in BUN reflected the severe nephrotoxicity induced by cisplatin. The t-SO, the PTU and the ketoprofen combinations inhibited this cisplatin-induced increase in BUN levels. The effects of these drugs on GOT and GPT activity were similar to their effects on BUN levels. These findings suggest that these GST inducer and inhibitor combinations decreased cisplatin-induced nephrotoxicity. In the CPH group, these parameters were elevated and nephrotoxicity, similar to that of cisplatin, was exhibited.

We examined the effects of the GST inducer and inhibitor combinations, which decreased cisplatin-induced nephrotoxicity, as shown above, on urinary total GST activity. It has been reported that urinary GST activity is not detected in control animals but elevated in animals with cisplatin-induced nephrotoxicity [8, 18]. We found that the t-SO, PTU and ketoprofen combinations inhibited the cisplatin-induced increase in urinary total GST activity, ketoprofen was the most effective of these drugs. On the other hand, urinary total GST activity

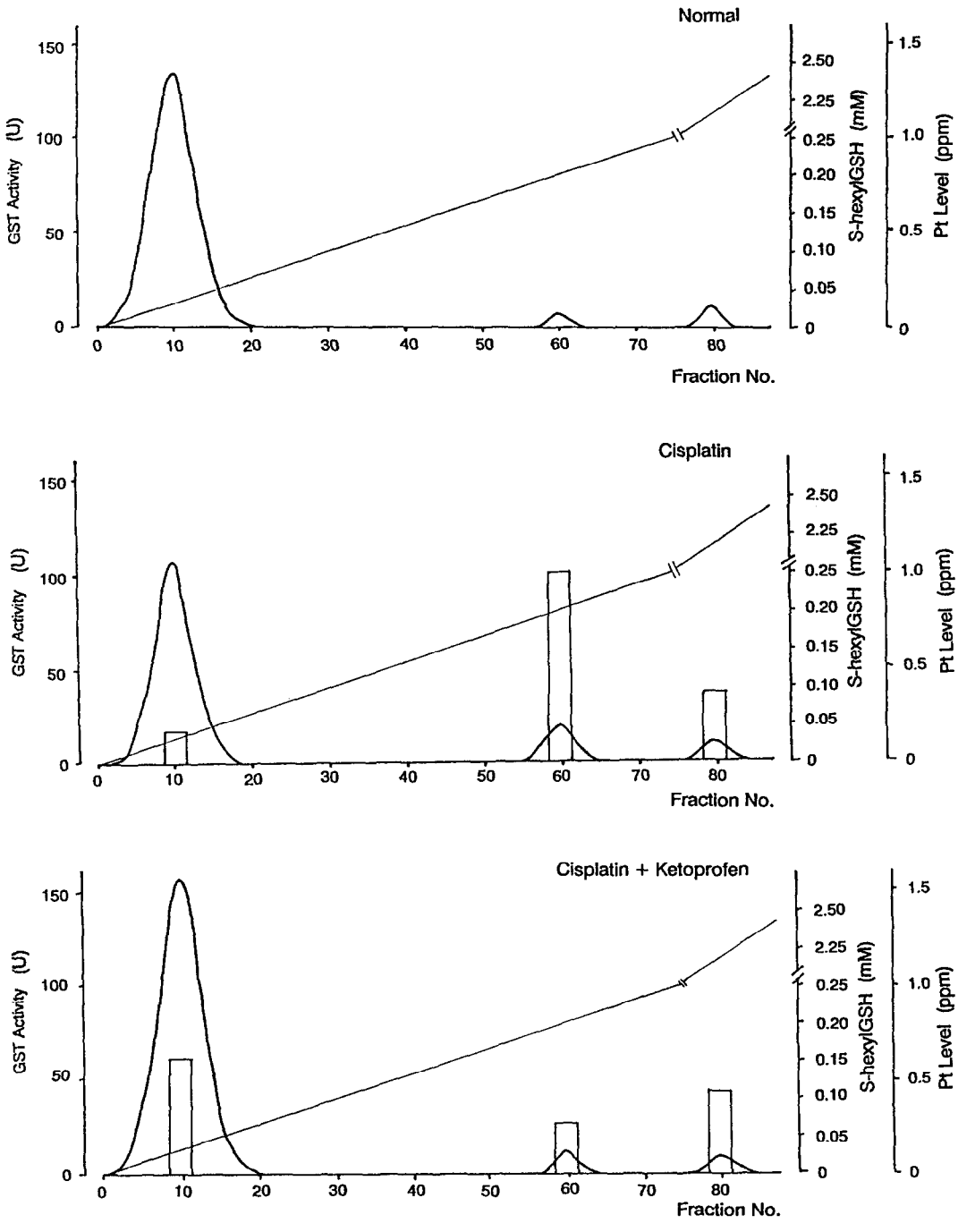

Fig. 5. Isolation of renal GST isoenzymes and cisplatin concentration by *S*-hexylGSH affinity chromatography. Chromatography of renal GST isoenzymes was performed at 3° on a 1.0 × 10.0 cm column. The column was eluted at 45.0 mL/hr and 3.0 mL fractions were collected.
——: GST activity 凵: Pt level: ——: *S*-hexylGSH.

increased after CPH administration. Therefore, it appears that not all GST inducers are effective in reducing cisplatin-induced nephrotoxicity.

The changes in tissue GST activity induced by these drug combinations indicated that this activity was related to the mechanism of cisplatin-induced nephrotoxicity. The determination of GSHpx activity and GSH and LPO levels showed whether these drugs were effective inhibitors of cisplatin-induced nephrotoxicity.

t-SO administration, induced total GST and GST-α, activity in the kidney

These results indicated that t-SO, as a GST inducer in the kidney, is the most effective of these drugs. The combination of t-SO with cisplatin increased total GST activity, compared with the cisplatin only group. The t-SO combination recovered the cisplatin alone-induced decrease in GST-α activity to normal levels. However, GSHpx activity after t-SO combined administration was further reduced below the level of the cisplatin alone-induced decrease.

It appeared that PTU alone increased total GST activity but had no effect on GST-α and GSHpx activity. The combination of PTU with cisplatin increased total GST activity, compared with that in the cisplatin alone group, and recovered the cisplatin-induced decrease in GST-α and GSHpx activity to normal levels. It would be expected that LPO levels would decrease if activity of lipid peroxidation-protecting enzymes were elevated. However, PTU alone and the PTU combination severely decreased the GSH level. These findings indicate that PTU is not a suitable agent to reduce cisplatin-induced nephrotoxicity.

Niitsu *et al.* [12] have reported that a ketoprofen combination enhanced the activity of an antitumor agent by specific inhibition of GST-π activity *in vitro*. We found that ketoprofen alone did not induce total GST and GST-α activity. The combination of ketoprofen with cisplatin normalized GST-μ and -α activity. Furthermore, the ketoprofen combination elevated the cisplatin-induced decrease of GSHpx activity. The changes in GST-α activity were similar to those in BUN levels, i.e. there were increases with cisplatin-induced nephrotoxicity and decreases with the ketoprofen combination. To date, the antioxidants used to reduce cisplatin-induced nephrotoxicity have not been shown to recover the changes in these enzymes induced by cisplatin [6]. We found here, however, that the ketoprofen combination recovered the cisplatin-induced changes in the activity of these enzymes and in LPO levels, as well as elevating the cisplatin-induced decrease in GSH levels. In light of these findings, it appears that ketoprofen is the most effective of these drugs in reducing cisplatin-induced nephrotoxicity. Furthermore, the Pt binding of the GST isoenzyme fraction indicated that the ketoprofen combination increased the Pt binding of GST-α and decreased the Pt binding of GST-μ, which is the main isoenzyme in metabolism of cisplatin after cisplatin alone administration. These findings indicate that the decrease of GST-α activity and the increase of GST-μ activity induced by cisplatin affect cisplatin metabolism, and the recovery of activity of both GST isoenzymes induced by the ketoprofen combination was by the same mechanism of decreases cisplatin-induced nephrotoxicity, in terms of cisplatin metabolism. As ketoprofen had no effect on cisplatin metabolism in terms of change in GST-π, a tumor marker, it is possible that ketoprofen combination prevents the antitumor activity of cisplatin.

Our proposed mechanism for cisplatin-induced nephrotoxicity follows. The combination of cisplatin with ketoprofen recovered the decrease of GST-α activity and GST-α is mainly involved in cisplatin metabolism. Furthermore, the ketoprofen combination normalized the cisplatin-induced changes in GSHpx activity and LPO levels,and decreased the cisplatin-induced nephrotoxicity.

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