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Effect of cisplatin on the activities of enzymes which protect against lipid peroxidation

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Abstract—Increases in lipid peroxide in kidneys of rats treated with cisplatin were examined in relation to decreases in the activities of Cu,Zn-superoxide dismutase (Cu,Zn-SOD), Mn-SOD, glutathione peroxidase (GSHpx), glutathione *S*-transferase (GST) and catalase. The activities of catalase, GSHpx and GST in the kidney and the liver were significantly decreased after cisplatin administration. The decrease of GST activity in the kidney was 87.3%, this was the largest decrease among these enzymes in the tissues examined. Cu,Zn-SOD activity significantly decreased only in the kidney. In contrast, the activities of these enzymes in the heart and the lung, which showed no increase in lipid peroxide in our previous study, were not significantly decreased. Cisplatin does not directly increase lipid peroxidation *in vitro*; therefore, the increase of lipid peroxide in the kidneys of these rats treated with cisplatin can be attributed to a decrease in the activities of lipid peroxide-protecting enzymes.

Treatment of cancer with cisplatin is subject to dose-limiting nephrotoxicity [1]. Various procedures have been examined clinically to reduce this toxicity [2], and its underlying mechanism has been studied experimentally [3–5], but remains unclear. Our previous studies showed that lipid peroxide levels in the rat kidney increased after cisplatin administration [6]. In this study, we examined changes in the activity of Cu,Zn-superoxide dismutase (Cu,Zn-SOD*), Mn-SOD, glutathione peroxidase (GSHpx), glutathione *S*-transferase (GST) and catalase in rat tissues after cisplatin administration, in order to clarify whether increases in lipid peroxidation are induced by decreases in the activity of these enzymes.

Materials and Methods

Chemicals. Cisplatin injection, 10 mg/20 mL vial (Randa® inj.) was purchased from Nippon Kayaku Co. Ltd (Tokyo). All chemicals were of the highest purity available.

Measurement of enzyme activity. Male Wistar rats, 6 weeks old, were obtained from Japan SLC Co. Ltd (Hamamatsu). Cisplatin (5 mg/kg, i.p.) was injected into the rats. The animals were killed by cervical dislocation at specific times within 6 days of cisplatin administration. The lungs, hearts, livers and kidneys were rapidly dissected out. Determination of SOD, GSHpx and GST, and catalase activity in each tissue was carried out according to the method of Oyanagi [7], Hafeman *et al.* [8] and Okazaki *et al.* [9], respectively.

Results

A time course was determined for the activity of each lipid peroxidation-protecting enzyme in rat tissues following cisplatin administration. The results are shown in Figs 1–4.

Kidney. (a) SOD activity (Fig. 1): On the second day after cisplatin administration, Cu,Zn-SOD activity had decreased to 72% ($P < 0.05$) of the normal value; it then decreased to 54% ($P < 0.01$) on the fifth day. In contrast, Mn-SOD activity increased gradually after cisplatin administration. The activity of Mn-SOD was maximal on the fourth day after cisplatin administration, being 142% of its normal value.

(b) GSHpx and GST activity (Fig. 2): From the first day after cisplatin administration, the GSHpx activity decreased gradually, falling to 61% ($P < 0.05$) and 58% ($P < 0.05$) of normal levels on the fourth and fifth days, respectively. GST activity decreased significantly from the third day after cisplatin administration. On the fifth day after cisplatin administration, GST activity decreased to 13% ($P < 0.01$) of its normal level, the maximum decrease of this enzyme.

(c) Catalase activity (Fig. 1): On the third day after cisplatin treatment, this activity decreased significantly, and the maximum decrease was 52% ($P < 0.01$) on the fourth day after cisplatin administration.

Liver. (a) SOD activity (Fig. 3): Cu,Zn-SOD and Mn-SOD activities did not change from normal levels.

(b) GSHpx and GST activity (Fig. 4): On the first day after cisplatin administration, GSHpx activity fell and the maximum decrease was 23% ($P < 0.01$) on the second day after cisplatin administration. GST activity was significantly decreased, to 72% ($P < 0.01$) and 68% ($P < 0.01$) on the fourth and fifth day respectively, after cisplatin administration.

(c) Catalase activity (Fig. 3): This activity was elevated to 130% ($P < 0.01$) of the normal level on the first day after cisplatin treatment; subsequently it decreased significantly on the fourth day. The activity on the fifth day fell to 41% ($P < 0.01$) of its maximum decrease.

Discussion

McGuiness *et al.* [4] and Sugihara *et al.* [5] suggested that cisplatin-induced nephrotoxicity is related to increases in lipid peroxide levels in the kidney. In our previous paper, we reported that these levels increased after cisplatin treatment, especially in the kidney [6]. Furthermore, we found that cisplatin did not increase enzymatic or non-enzymatic system-dependent lipid peroxidation *in vitro* [6]. These results suggested that the increase in lipid peroxide levels *in vivo* was mediated by a mechanism other than the direct peroxidation of membrane lipids induced by cisplatin.

It is well known that SOD, GSHpx, GST and catalase play important roles as protective enzymes against lipid peroxidation in tissues. Regarding changes in the activity of these enzymes in rat tissue after cisplatin administration, it is important, in connection with its nephrotoxicity, to clarify the mechanism by which cisplatin induces increased lipid peroxidation in the kidney. However, so far there have been no reports addressing this point. In the present study, we examined changes in the activity of these enzymes after cisplatin treatment, in order to clarify whether the increases in lipid peroxides were induced by decreases in the activity of these lipid peroxidation protecting enzymes.

We found that the activity of catalase, GSHpx and GST in the kidney and the liver were significantly decreased

* Abbreviations: SOD, superoxide dismutase; GSHpx, glutathione peroxidase; GST, glutathione *S*-transferase.

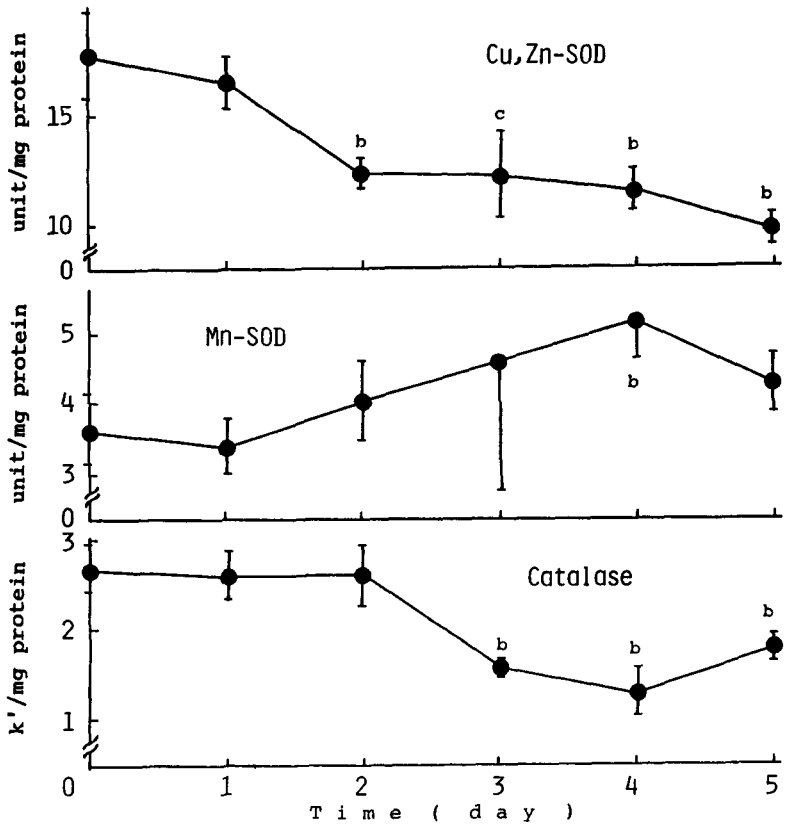

Fig. 1. Time course of renal Cu,Zn- and Mn-SOD and catalase activity after cisplatin administration to rats. Each point represents the mean ± SD of five rats. Significant differences from normal values are indicated by [a] $P < 0.001$, [b] $P < 0.01$ and [c] $P < 0.05$.

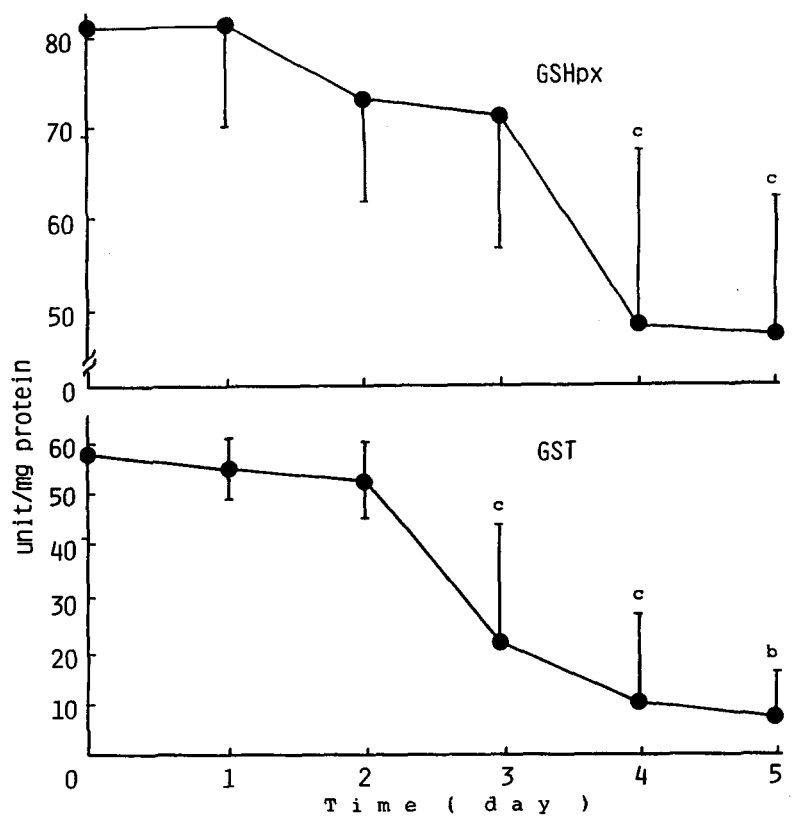

Fig. 2. Time course of renal GSHpx and GST activity after cisplatin administration to rats. Each point represents the mean ± SD of five rats. Significant differences from normal values are indicated by [a] $P < 0.001$, [b] $P < 0.01$ and [c] $P < 0.05$.

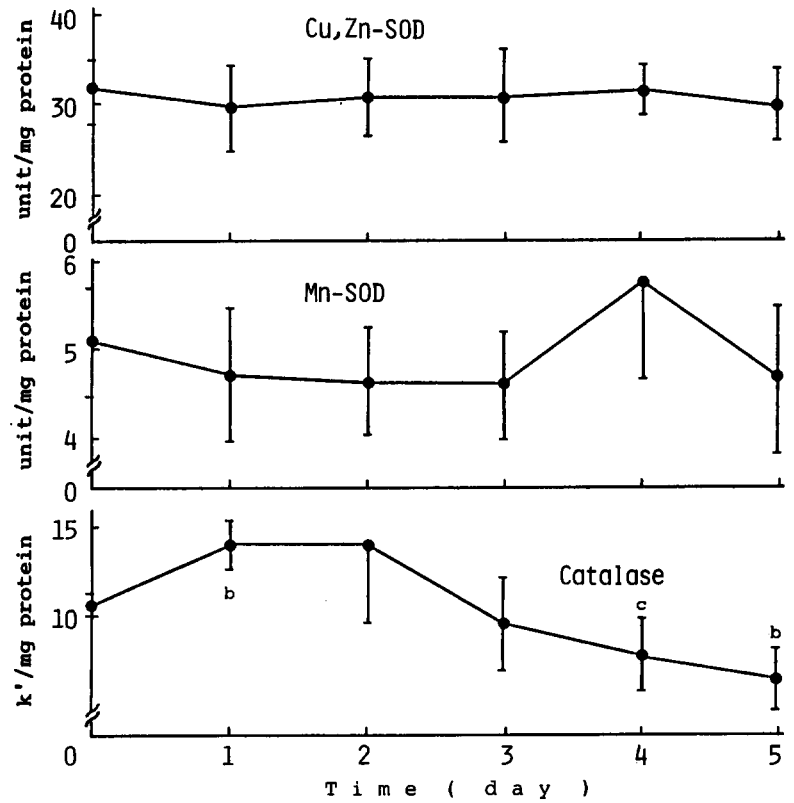

Fig. 3. Time course of hepatic Cu,Zn- and Mn-SOD and catalase activity after cisplatin administration to rats. Each point represents the mean ± SD of five rats. Significant differences from normal values are indicated by [a] $P < 0.001$, [b] $P < 0.01$ and [c] $P < 0.05$.

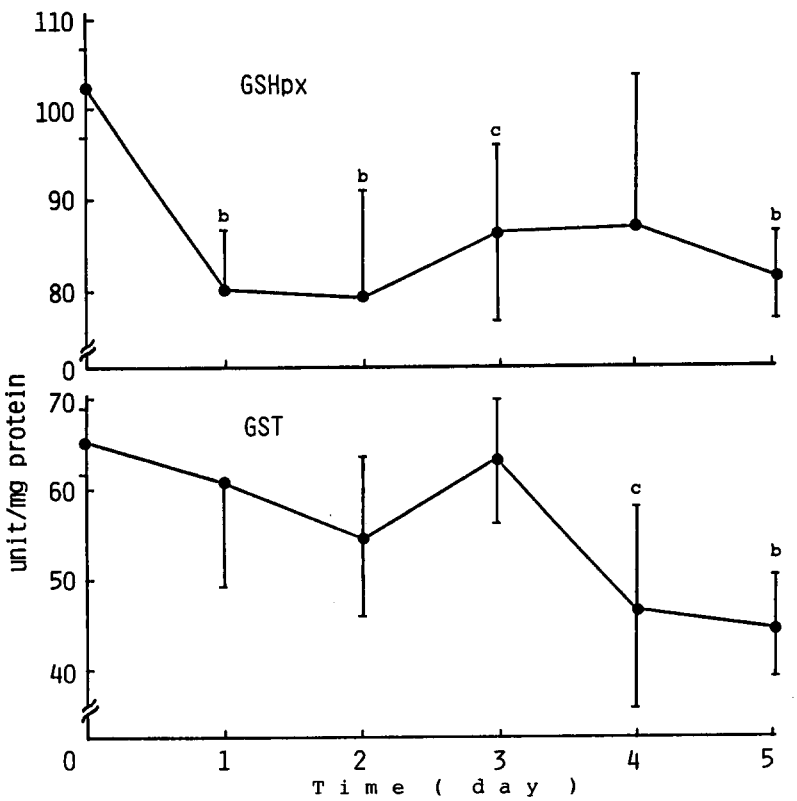

Fig. 4. Time course of hepatic GSHpx and GST activity after cisplatin administration to rats. Each point represents the mean ± SD of five rats. Significant differences from normal values are indicated by [a] $P < 0.001$, [b] $P < 0.01$ and [c] $P < 0.05$.

after cisplatin administration. The maximum decrease ratios of these enzymes in the kidney were 1.3, 1.9 and 2.7 times, respectively, those in the liver. It was shown that the action of GST supplemented that of GSHpx. However, we assumed that cisplatin-induced damage in the kidney and the liver was severe because the activity of both GSHpx and GST decreased. The percentage decrease of GST activity in the kidney, in particular, was 87.3%, this was the largest decrease shown by any of these enzymes in any of the tissues examined. These results support the contention that cisplatin-induced nephrotoxicity is serious. Furthermore, it has been reported by Feinfeld and Fuh [10] that the appearance of GST in the urine is a good marker for proximal tubular injury. Therefore, we consider that the decrease of GST activity in the kidney after cisplatin administration contributed to the increase in lipid peroxide.

It is worthy of note that Cu,Zn-SOD activity after cisplatin administration was significantly decreased only in the kidney, where it reached 54.1% ($P < 0.001$) of the normal level. The elevation of Mn-SOD activity in the kidney after cisplatin administration would be expected to make up for the decrease in Cu,Zn-SOD activity, since the normal activity of Mn-SOD was 20% that of Cu,Zn-SOD in the kidney and the increased ratio of Mn-SOD activity was 41.8% of the normal level. Mn-SOD is distributed in the mitochondrial fraction and Cu,Zn-SOD is found mainly in the cytosolic fraction. Histological studies of cisplatin-induced nephrotoxicity have shown marked vacuolation in the mitochondria of proximal tubule cells [11, 12], i.e. cisplatin-induced toxicity involves disruption of the mitochondria, which contain the intracellular respiratory chain. The increase in Mn-SOD activity is considered to be protective against this toxicity. Cu,Zn-SOD is a radical scavenger that quenches superoxide anions. The toxicity of these anions is thought to be negligible, however, hydroxyl radicals, which are extremely toxic, are produced by the reaction between superoxide anions and hydroperoxide. The specific decrease in the activity of catalase and GSHpx, which are hydroperoxide scavenging enzymes, in the kidney after cisplatin administration enhances the production of hydroperoxide. Therefore, production of hydroxyl radicals is increased by the enhanced production of hydroperoxide and superoxide anions, and this is considered to damage the kidney cells. In contrast, the activity of these enzymes in the heart and the lung, which showed no increase in lipid peroxide level in our previous study [6], did not significantly increase.

In conclusion, it is clear that the increase in lipid peroxide in the kidneys of rats treated with cisplatin is attributable to a decrease in the activity of lipid peroxide-protecting enzymes.

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