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Changes in glutathione-related enzymes in tumor-bearing mice after cisplatin treatment

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Abstract

The effect of cisplatin on five glutathione-related enzymes was studied in liver, kidney, and Dalton lymphoma cells of tumor-bearing mice. In liver, the activities of glutathione *S*-transferase, glutathione peroxidase, catalase, and superoxide dismutase decreased approximately 30–40%, 60–67%, 35–50% and 70–80% respectively, while glutathione reductase increased about 36-45% after cisplatin treatment. In kidney, catalase activity decreased by 47–82% at all time points (24–96 h) of cisplatin treatment, while glutathione *S*-transferase activity decreased significantly (∼24%) mainly at 72 h of treatment. An increase in glutathione reductase (∼1.5–2.5 times), glutathione peroxidase (significant at 24 h, 47%), and superoxide dismutase (∼15–60%) was noted in kidney after the treatment. In Dalton lymphoma cells, the activities of glutathione *S*-transferase, glutathione peroxidase, and catalase decreased very distinctly (∼2–5, 2–5 and 5–11 times, respectively) at all time points, but glutathione reductase decreased significantly only at 72 h of cisplatin treatment. Interestingly, the superoxide dismutase activity in Dalton lymphoma cells increased initially at 24–48 h and then decreased (∼60%) during later periods (72–96 h) of treatment. Cisplatin treatment caused a decrease in glutathione level in Dalton lymphoma cells (∼14–20%) and kidney (∼18–28%) but no change in liver. In view of the results, a definite correlation with the changes in glutathione concentrations and enzymatic activities in a tissue could not be firmly derived. It is suggested that the changes in various glutathione-related enzymes and glutathione levels in the tissues of the host during cisplatin-mediated chemotherapy could affect cellular antioxidant defense potential, which may play an important contributory role in cisplatin-mediated toxicity, particularly nephrotoxicity, and anticancer activity in the host.

Abbreviations: DL, Dalton lymphoma; GSH, reduced glutathione; GST, glutathione *S*-transferase; GR, glutathione reductase; GPx, glutathione peroxidase; CAT, catalase; SOD, superoxide dismutase; GSSG, oxidized glutathione or glutathione disulfide; CDNB, 1-chloro-2,4-dinitrobenzene; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; NADPH, nicotinamide–adenine dinucleotide phosphate, reduced

Introduction

cis-Diamminedichloroplatinum(II), commonly known as cisplatin, is established to be an effective chemotherapeutic drug against various malignancies (Prasad and Giri, 1994; Go and Adjei, 1999). Besides its ability to interact with cellular DNA (Zwelling et al., 1979; Coste et al., 1999), its effects on the host immune response (Collins and Kao, 1989), cell surface (Prasad and Sodhi, 1981), tissue calcium and potassium concentrations (Prasad and Giri, 1999), various enzymes such as 5′-nucleotidase, arginase, cathepsins and lactate dehydrogenase (Prasad et al., 1999), and mitochondria (Kharbangar et al., 2000) have also been observed, and it has been suggested that these changes are also involved as additional components in the mechanism of cisplatin's anticancer activity. However, full clinical therapeutic efficacy of cisplatin is limited by its major side-effects of nephrotoxicity (Krakoff, 1979) and also hematotoxicity (Khynriam and Prasad, 2001).

Glutathione (a tripeptide; L-γ-glutamyl-L-cysteinylglycine), usually the most prevalent intracellular thiol, functions directly or indirectly in a variety of cellular processes (Wang and Ballatori, 1998). Reduced glutathione (GSH) plays an important role in defense mechanisms by acting as an antioxidant or by reacting with electrophiles (Deleve and Kaplowitz, 1991) and toxic agents to form conjugates that are eliminated from the cell (Meister, 1991). Elevation of GSH in cellular resistance to platinum agents has been reported in several human and murine tumor cell lines (Kartalou and Essigmann, 2001) and the enzymes of glutathione metabolism play a role in cellular resistance to anticancer drugs (Black and Wolf, 1991). GSH participates in the detoxification of xenobiotics that cause cellular injury by generating free radicals (Slater, 1984; Sies, 1999). Intracellular defense mechanisms to detoxify the reactive free radicals include the GSH-related enzymes, viz., glutathione *S*-transferase (EC 2.5.1.18), glutathione reductase (EC 1.6.4.2), glutathione peroxidase (EC 1.11.1.9), superoxide dismutase (EC 1.15.1.1), and catalase (EC 1.11.1.6) (Tew, 1994; Ohkuwa et al., 1997; Teramoto et al., 1999). We have reported that cisplatin-mediated decrease in GSH concentrations could play an important role in the development of mutagenicity (Giri et al., 1998a), nephrotoxicity (Giri et al., 1998b), and hematotoxicity (Khynriam and Prasad, 2001) in the host and proposed that the GSH-related enzymes may be affected in the mechanism of cisplatin-mediated toxicity and anticancer activity (Khynriam and Prasad, 2001).

The details of the changes and significance of GSH-related enzymes in cisplatin-mediated anticancer activity or toxicity and changes in GSH concentrations is not clearly understood. As cisplatin is known to develop major side-effects of nephrotoxicity, kidneys were used along with liver (the major site of GSH metabolism) and tumor cells for the study. The present investigations were undertaken to elucidate the changes in the activity of some GSH-related enzymes in liver, kidney, and Dalton lymphoma (DL) cells of tumor-bearing mice treated with cisplatin for different periods of time.

Materials and methods

Chemicals

Reduced glutathione, oxidized glutathione (GSSG), glutathione reductase, and 5,5′-dithiobis-2-nitrobenzoic acid were purchased from Sigma Chemical Co., St. Louis, MO, USA. Cisplatin was obtained from Biochem Pharmaceutical Industries, Mumbai, India. 1-Chloro-2,4-dinitrobenzene (CDNB), nicotinamide–adenine dinucleotide phosphate reduced (NADPH), hydrogen peroxide, sodium azide,

Triton X-100, mercaptoethanol, ethylenediaminetetraacetic acid (EDTA), and Folin Ciocalteau reagent were purchased from Sisco Research Laboratories, Mumbai, India. Glass-double-distilled water was always used to prepare buffers and solutions.

Tumor maintenance and cisplatin treatment

Inbred Swiss albino mice were maintained under conventional laboratory conditions with free access to commercially available food pellets and water. Mice of both sexes, 10–12 weeks old and weighing about 25–30 g were used for the experiments. Ascites Dalton lymphoma tumor was maintained *in vivo* by intraperitoneal (i.p.) transplantations of 1×10^7 tumor cells per animal (0.25 ml vol., in phosphate-buffered saline (PBS); 0.15 mol/L NaCl in 0.01 mol/L sodium phosphate buffer, pH 7.4). Tumor-transplanted hosts usually survived for 20–21 days.

The therapeutic dose of cisplatin against malignant tumor has been established to be 8–10 mg/kg body weight (Rosenberg, 1985). A single dose of cisplatin (8 mg/kg body weight, i.p.) was administered to tumor-bearing mice on the 10th day after tumor transplantation. The time schedule of cisplatin treatment for 24, 48, 72, and 96 h has been used previously by us (Kharbangar et al., 2000; Khynriam and Prasad, 2001) and the same was followed here.

Tissue collection and preparation of cytosolic fractions

After cisplatin treatment of tumor-bearing mice DL cells, kidney and liver were collected, washed in ice-cold physiological saline, and stored at –70°C until analysis. Control tumorous mice were treated with the same volume of normal saline solution (0.89% NaCl).

Frozen tissues were brought to 0°C and a 10% homogenate was prepared in a motor-driven Teflon-pestle homogenizer at 0 ± 2°C in the specific buffer solutions for specific enzyme assays: GR assay, 0.2 mol/L potassium phosphate–1 mmol/L Na_2EDTA buffer (pH 7.5); GPx assay, 0.1 mol/L potassium phosphate–1 mmol/L Na_2EDTA buffer (pH 7.0); GST assay, 0.1 mol/L sodium phosphate–1 mmol/L Na_2EDTA buffer (pH 6.5); CAT assay, 1% Triton X-100; SOD assay, 100 mmol/L tri-diethanolamine-HCl buffer (pH 7.4). The tissue homogenates were centrifuged at 27 000*g* for 20 min at 4°C and the respective supernatants were collected as the particular enzyme source.

Glutathione S-*transferase (GST; EC 2.5.1.18) assay*

GST activity was assayed according to the method of Habig et al. (1974). The reaction volume of 1.0 ml contained 850 µl 0.1 mol/L sodium phosphate–1 mmol/L Na_2EDTA buffer (pH 6.5), 50 µl 20 mmol/L reduced glutathione in deionized water, and 50 µl 20 mmol/L CDNB in 95% ethanol. The reaction mixture (maintained at 30°C) was started by adding 50 µl enzyme and the increase in absorbance at 340 nm was monitored for 3 min in a Beckman DU-640 spectrophotometer. The enzyme activity was calculated using the extinction coefficient (E_{340} = 9.6 L $mmol^{-1}$ cm^{-1}). One unit of enzyme activity is defined as the amount of enzyme that catalyzes the conjugation of 1 µmole of CDNB per min.

Glutathione reductase (GR; EC 1.6.4.2) assay

GR activity was assayed according to Smith et al. (1988). Total assay volume (1.05 ml) contained 500 µl 0.2 mol/L potassium phosphate–1 mmol/L Na_2EDTA buffer (pH 7.5), 250 µl 3 mmol/L DTNB in 0.01 mol/L phosphate buffer, 175 µl water, 50 µl 2 mmol/L NADPH in 10 mmol/L Tris-HCl buffer (pH 7.0), and 25 µl of tissue supernatant. In the assay mixture (maintained at 24°C) the

reaction was initiated by adding 50 µl 20 mmol/L GSSG and the increase in absorbance at 412 nm was monitored for about 3 min in a Beckman DU-640 spectrophotometer. The enzyme activity was calculated using the extinction coefficient (E_{412} = 13.6 L $mmol^{-1}$ cm^{-1}). One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reduction of 1 µmole of NADPH per minute.

Glutathione peroxidase (GPx; EC 1.11.1.9) assay

GPx activity was assayed according to the method of Flohe and Gunzler (1984). The assay volume (1.10 ml) contained 500 µl 0.1 mol/L potassium phosphate–1 mmol/L Na_2EDTA buffer (pH 7.0), 100 µl 1 mmol/L NaN_3, 100 µl tissue supernatant, 100 µl glutathione reductase (0.24 U), and 100 µl 10 mmol/L reduced glutathione. After 10 min of incubation at 37°C, 100 µl 1.5 mmol/L NADPH in 0.1% $NaHCO_3$ was added. The overall reaction was started by adding 100 µl of prewarmed (37°C) H_2O_2 (1.5 mmol/L) and the decrease in absorbance at 340 nm was monitored for about 5 min in a Beckman DU-640 spectrophotometer. The enzyme activity was calculated using the extinction coefficient of 6.2 L $mmol^{-1}$ cm^{-1}. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the oxidation of 1 µmole NADPH per minute.

Catalase (CAT; EC 1.11.1.6) assay

CAT activity was assayed following the method of Aebi (1984). The assay volume (3.0 ml) contained 20 µl tissue supernatant and 1.98 ml 50 mmol/L phosphate buffer (pH 7.0). The reaction, maintained at 20°C, was started by adding 1.0 ml 30 mmol/L H_2O_2. The decrease in absorbance at 240 nm was monitored for 60 s in a Beckman DU-640 spectrophotometer. The enzyme activity was calculated using the extinction coefficient of 0.00394 L $mmol^{-1}$ mm^{-1} and the unit of enzyme activity was expressed as mmoles H_2O_2 decomposed per minute.

Superoxide dismutase (SOD; EC 1.15.1.1) assay

The method described by Paoletti and Mocali (1990) was used for the assay of SOD activity. The assay volume (1.065 ml) contained 800 µl 100 mmol/L tri-diethanolamine-HCl buffer (pH 7.4), 40 µl 7.5 mmol/L NADPH, 25 µl 100 mmol/L EDTA–$MnCl_2$ solution (pH 7.0), 100 µl 10 mmol/L mercaptoethanol, and 100 µl of the respective supernatant. The decrease in absorbance at 340 nm was monitored for 20 min over an 8 min interval at 25°C in a Beckman DU-640 spectrophotometer. The enzyme activity was calculated using the relation (sample rate/blank rate) × 100 = % inhibition. The percentage of inhibition was converted to units of SOD activity from the conversion table. One unit of enzyme activity is defined as the amount of SOD capable of inhibiting by 50% the rate of NADPH oxidation.

In all the enzyme assays, the respective blank reaction mixture was prepared by adding the same volume of specific buffer and omitting the particular tissue supernatant.

Protein estimation and enzyme specific activity

The same supernatant used for various enzyme assays was also used for protein estimation following the method of Lowry et al. (1951) using bovine serum albumin as standard. The particular enzyme specific activity was calculated by dividing the number of units of that enzyme by the number of milligrams of protein present in that assay tissue supernatant.

Glutathione estimation

Total GSH was determined using the method of Sedlak and Lindsay (1968). Briefly, 5% homogenates of tissues were made in 0.02

mol/L EDTA, pH 4.7. The homogenate or pure reduced form of glutathione (100 µl) was added to 0.2 mol/L Tris-EDTA buffer, pH 8.2 (1.0 ml) and 0.02 mol/L EDTA, pH 4.7 (0.9 ml) followed by 20 µl of Ellman's reagent (10 mmol/L DTNB in methanol). After 30 min of incubation at room temperature, the absorbance was read at 412 nm in a Beckman DU-640 spectrophotometer. Samples were centrifuged before the absorbances of supernatants were measured.

Statistical analysis

The significance of changes in enzyme activities after cisplatin treatment was compared with the respective control using paired Student's *t*-test with $p \leqslant 0.05$ considered as significant.

Results

The changes in the activities of different GSH-related enzymes in liver, kidney, and DL cells after cisplatin treatment of tumor-bearing mice are shown in Table 1.

GST. A significant decrease (35–42%) in GST activity in liver was noted at 24–48 h of cisplatin treatment. In kidney, the enzyme activity increased slightly (~9%) at 24 h of cisplatin treatment but decreased at later periods of treatment, showing significant decrease (~24%) at 72 h. However, the GST activity in DL cells decreased significantly (~60–80%) at all the four time points of cisplatin treatment.

GR. Compared to control, GR activity in liver decreased (~22%) initially at 24 h but increased gradually at later time points, showing significant increase at 96 h of cisplatin treatment. The cisplatin treatment caused a twofold increase in enzyme activity in kidney but a decrease (significant at 72 h) in DL cells.

GPx. GPx specific activity in DL cells was noted to be about 2.5 times and 8 times the activity observed in liver and kidney, respec-

Table 1. Specific activity (units/mg protein) of GSH-related enzymes in tumor-bearing mice after cisplatin treatment

Tissues	Treatment	GST	GR	GPx	Catalase	SOD
Liver	Control	2.10 ± 0.41	0.41 ± 0.08	1.93 ± 0.34	11.01 ± 0.04	2.61 ± 0.01
	Cisplatin (24 h)	1.20 ± 0.16*	0.32 ± 0.07	0.76 ± 0.12*	6.15 ± 0.21*	0.65 ± 0.01*
	Cisplatin (48 h)	1.41 ± 0.11*	0.44 ± 0.06	0.78 ± 0.05*	6.83 ± 0.01*	0.49 ± 0.03*
	Cisplatin (72 h)	1.67 ± 0.31	0.56 ± 0.13	0.71 ± 0.11*	5.52 ± 0.06*	0.65 ± 0.02*
	Cisplatin (96 h)	1.83 ± 0.40	0.61 ± 0.10*	0.64 ± 0.08*	9.60 ± 0.21*	0.66 ± 0.06*
Kidney	Control	1.10 ± 0.19	0.27 ± 0.08	0.53 ± 0.08	4.51 ± 0.02	1.32 ± 0.03
	Cisplatin (24 h)	1.20 ± 0.18	0.46 ± 0.04*	0.78 ± 0.04*	1.46 ± 0.08*	2.17 ± 0.02*
	Cisplatin (48 h)	0.95 ± 0.11	0.68 ± 0.10*	0.65 ± 0.11	1.63 ± 0.03*	1.32 ± 0.01
	Cisplatin (72 h)	0.84 ± 0.11*	0.61 ± 0.13*	0.47 ± 0.04	0.80 ± 0.01*	1.51 ± 0.04*
	Cisplatin (96 h)	0.98 ± 0.23	0.64 ± 0.14*	0.56 ± 0.08	2.37 ± 0.04*	1.62 ± 0.02*
DL cells	Control	1.28 ± 0.19	0.37 ± 0.08	5.06 ± 1.08	5.87 ± 0.31	2.82 ± 0.12
	Cisplatin (24 h)	0.43 ± 0.05*	0.29 ± 0.03	1.80 ± 0.24*	1.13 ± 0.06*	3.10 ± 0.05*
	Cisplatin (48 h)	0.55 ± 0.04*	0.37 ± 0.05	2.06 ± 0.23*	0.85 ± 0.06*	4.49 ± 0.05*
	Cisplatin (72 h)	0.25 ± 0.03*	0.25 ± 0.03*	0.91 ± 0.13*	0.73 ± 0.04*	1.04 ± 0.03*
	Cisplatin (96 h)	0.26 ± 0.04*	0.27 ± 0.02	0.94 ± 0.14*	0.52 ± 0.04*	1.03 ± 0.07*

Values represent the mean ± SD
Student's *t*-test, n = 3–5 separate determinations, as compared to respective control (untreated tumor-bearing mice): $*p \leqslant 0.05$.

tively. GPx activity in liver and DL cells decreased by approximately 60–67% and 60–80%, respectively, after cisplatin treatment. However, in kidney, GPx activity was observed to increase initially at 24 h and then slightly decrease during the later periods (48–96 h) of cisplatin treatment.

CAT. Catalase activity in liver was about twice the activity observed in kidney and DL cells. A significant decrease in CAT activity was observed from 24 to 96 h in all the three tissues (i.e., liver, kidney, and DL cells) after cisplatin treatment of mice.

SOD. It was observed that SOD activity in kidney was about half of the activity noted in liver and DL cells. After cisplatin treatment, SOD activity decreased significantly in liver ($\sim$75%) but increased in kidney at 24, 72, and 96 h of treatment. In DL cells, enzymatic activity increased initially at 24–48 h but later decreased sharply at 72–96 h of cisplatin treatment.

GSH. Total GSH concentration remained almost unchanged in liver after the cisplatin treatment. An overall decrease of GSH was observed in kidney and DL cells that was statistically significant ($p \leqslant 0.05$) at 24–48 h of cisplatin treatment (Figure 1).

Discussion

In the present study the comparative changes in five GSH-related enzymes in liver, kidney, and DL cells of tumor-bearing mice after cisplatin treatment have been evaluated and the possible significance for the anticancer activity and/or toxicity in the host has been discussed for each enzyme.

Glutathione *S*-transferases (GSTs) function as detoxification enzymes that catalyze the conjugation of electrophilic chemicals with glutathione in cells (Chien et al., 1994; Hayes and Pulford, 1995). GST is one of the several factors that are proposed to affect tumor sensitivity to anticancer drugs, including cisplatin (Kodera et al., 1994). Cisplatin treatment caused a significant decrease of GST activity (Table 1) as well as GSH concentration (Figure 1) in DL cells. This may involve a lesser conjugation of GSH with cisplatin since it is known that cisplatin–GSH conjugates can be formed directly (Jones and Basinger, 1989) or catalyzed by GST (Ishikawa and Ali-Osman, 1993). Both GSH depletion by buthionine sulfoximine and GST inhibition have been shown to increase the tumoricidal activity of melphalan, a proteolytic alkylating drug (Canada et al., 1993) and support the present view. Inhibition of GST by either ethacrynic acid or piriprost enhanced alkylator cytotoxicity to both rat and human cancer cell lines (Tew et al., 1988; Hansson et al., 1991). Although GSH concentration in DL cells recovers slightly at 72–96 h of treatment, any damage initiated in cells during the early stage of treatment (24–48 h) may be partially repaired or retained, which may lead to development of the drug's cytotoxic effects. In liver and kidney the definite pattern of changes in GST and GSH after cisplatin treatment could not be correlated, because in liver at 24–48 h of treatment GST activity decreased significantly but GSH level remained almost unchanged after the treatment, while in kidney at 72 h only GST activity was noted to decrease but GSH level decreased significantly at 24–72 h (Table 1, Figure 1). It has been reported previously by us that cisplatin treatment causes nephrotoxicity and is associated with a decrease of GSH level in kidney (Giri et al., 1998b; Kharbangar et al., 2000) and the present observation of concomitant decrease in GST activity may also be connected with the development of this side-effect.

GR catalyzes the NADPH-dependent reduction of glutathione disulfide (GSSG) to

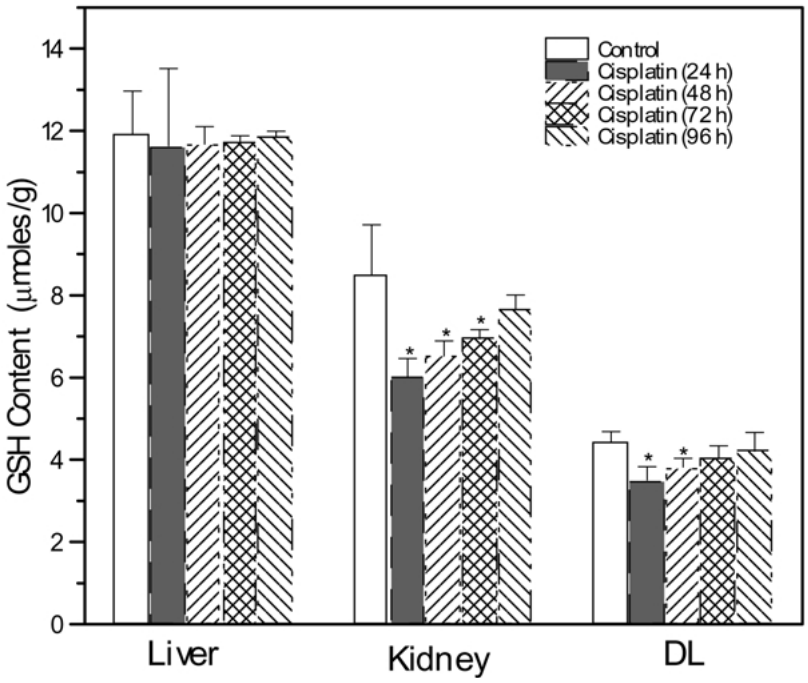

Figure 1. Quantitative changes in total reduced glutathione (GSH) in liver, kidney, and DL cells after cisplatin treatment of tumor-bearing mice. Statistical analysis: Student's *t*-test; $n = 4$ independent experimental determinations as compared to respective control, $^{*}p \leqslant 0.05$.

GSH (Sweet and Blanchard, 1991) and maintains more than 98% of intracellular glutathione in reduced form (Wang and Ballatori, 1998). The observed increase in GR activity, particularly in kidney (Table 1) may be an attempt to convert more GSSG to GSH in order to raise GSH levels and should ordinarily indicate an increase in GSH concentration; here, instead of an increase, we observed a decrease in GSH level after cisplatin treatment (Figure 1). This shows that the decrease in kidney GSH level may involve mechanisms other than this, probably by inhibiting GSH synthesis itself or by involving GPx activity, as the GPx activity was observed to increase at 24 h after cisplatin treatment (Table 1). This concept may be supported by the observation of an increase in GR and GPx activity in kidney after combined treatments of mice with buthionine sulfoximine, a specific inhibitor of γ-glutamylcysteine synthetase, to block GSH synthesis, and cisplatin (data not shown). GPx is a scavenger enzyme that catalyzes the oxidation of GSH to GSSG and the concentrations of GSH and GSSG are generally maintained by the coordinated activity of GR and GPx (Wang and Ballatori, 1998). High GPx activity has been shown in breast cancer patients (Gromadzinska et al., 1997). In contrast to kidney, in DL cells GR activity remained almost unchanged or decreased (significant at 72 h), while GPx activity decreased very appreciably at all time points (24–96 h) of treatment (Table 1). Decreased GPx activity may account for more accumulation of GSH, but in DL cells, as in kidney, GSH level was decreased after cisplatin treatment (Figure 1). This observation of changes in GR, GPx, and GSH

in DL cells suggests the possibility of involvement of mechanism(s) other than changes in GR/GPx. The changes in GSH, GR and GPx in liver also, where after cisplatin treatment, GSH level remained almost unchanged, GPx activity decreased but GR activity increased, particularly at 96 h of treatment (Table 1, Figure 1), may hint at similar reasoning.

Catalase (CAT) catalyzes the decomposition of H_2O_2 to H_2O and O_2 and thus protects cells from oxidative damage (Ueda et al., 1990). Reduction in CAT activity has been reported in experimental carcinogenesis (Corrocher et al., 1986; Arruda et al., 1996). Cisplatin treatment has been reported to increase the production of lipid peroxides in kidney cortical slices (Nakano and Gemba, 1989) and in the mitochondria of kidney (Kharbangar et al., 2000), and here the treatment caused an inhibition in CAT activity in all the three tissues studied (Table 1). This inhibition of CAT activity by cisplatin may indirectly help to increase the intracellular accumulation of hydroperoxides.

SOD plays an important role in catalyzing dismutation of superoxide radicals ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2) and molecular oxygen (Flohe and Otting, 1984), thereby preventing the dangerous Haber–Weiss reaction that generates $^{\cdot}OH$ (Fridovich, 1995). A decrease in SOD activity has been reported in cancer cells (Arruda et al., 1996; Navarro et al., 1999). After cisplatin treatment, SOD activity in DL cells increased initially at 24–48 h but decreased later at 72–96 h, while in kidney the enzyme activity was noted to increase except at 48 h of treatment (Table 1). Cancer cells have also been reported to generate a large amount of H_2O_2 (Szatrowski and Nathan, 1991). Increase in SOD activity in kidney and DL cells could be one way to protect cells against the generation of $O_2^{\cdot-}$ after cisplatin treatment, because in these tissues the GSH level was also decreased (Figure 1). On the other hand, in liver, SOD activity decreased significantly after cisplatin treatment (Table 1) but GSH levels remained unchanged (Figure 1). Thus, in contrast to DL cells and kidney, the higher and unchanged level of GSH in liver could be directly involved in the protective function against cisplatin-induced free radicals. A number of chemotherapeutic agents, including cisplatin, have been shown to stimulate the production of reactive oxygen radicals (Sadzuka et al., 1991) and it may be promoted further in the condition of decreased GSH.

Thus, it is concluded that cisplatin-mediated chemotherapy in tumor-bearing mice brings about differential but specific changes in various GSH-related enzymes and GSH concentrations in distinct tissues. The activities of GST, GPx, and catalase, along with GSH levels, were decreased very precisely in DL cells after cisplatin treatment, which should affect the cellular antioxidant defense potential and may play a contributory role in the mechanism(s) of cisplatin-mediated anticancer activity. The substantial decrease of catalase along with changes in the activity of other enzymes in liver as well as kidney may also be associated with the development of toxicity, particularly nephrotoxicity, in the host.

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