Effect of cisplatin on mitochondrial protein, glutathione, and succinate dehydrogenase in Dalton lymphoma-bearing mice

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Abstract

Cisplatin treatment of tumor-bearing mice resulted a significant decrease of protein in the tissues studied (liver, kidney, and Dalton lymphoma) and also in their mitochondrial fractions. As compared to respective tissues, the protein decrease was noted to be more conspicuous in their mitochondrial fractions. Similarly, mitochondrial glutathione also decreased significantly in the tissues. However, succinate dehydrogenase activity was selectively decreased in the kidney and Dalton lymphoma cells, whereas in liver it remained almost unchanged. An increase in serum urea concentration and kidney mitochondrial lipid peroxidation was also observed after cisplatin treatment. It is suggested that the cisplatin-induced biochemical changes in mitochondria involving mitochondrial protein, glutathione, and succinate dehydrogenase could be the important potent cellular sites contributing to toxicity/cytotoxicity after cisplatin treatment.

Abbreviations: DCIP, dichlorophenolindophenol; DL, Dalton lymphoma; GSH, reduced glutathione; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; NPSH, nonprotein sulfhydryl; PBS, phosphate-buffered saline; PMS, phenazine methosulfate; SDH, succinate dehydrogenase; TSH, total sulfhydryl

Introduction

cis-Dichlorodiamineplatinum(II) or cisplatin is a frequently used and very effective chemotherapeutic drug for treatment of various malignancies (Rosenberg, 1985; Prasad and Giri, 1994; Lebwohl and Canetta, 1998; Go and Adjei, 1999). The ability of cisplatin to react with DNA and the formation of cisplatin–DNA adducts are thought to be the main mechanisms underlying its cytotoxic effect (Pinto and Lippard, 1985; Zamble and Lippard, 1995). In addition to its interaction with cellular DNA, observations of changes in various biochemical/enzymatic parameters, immune response, cell surface, etc. have led to the proposal of the involvement of multistep and multilevel effects of cisplatin in the tumor cells/host in cisplatin-mediated chemotherapy against cancers (Giri, 1995; Prasad et al., 1999).

Early studies have shown a diminished mitochondrially mediated oxidative phosphorylation in cancer cells, with glycolysis being the

main source of energy production (Warburg, 1956). Electron-microscopic studies revealing fewer and structurally altered mitochondria also support the respiratory impairment in cancer cells (White et al., 1974). Various recent reports have indicated that mitochondria may be involved in mutagenesis, maintenance of the malignant phenotype, and control of apoptosis (Cavalli and Liang, 1998; Murphy and Smith, 2000). Different chemical carcinogens including polycyclic aromatic compounds (Allen and Coombs, 1980), methylnitrosourea (Le Doux et al., 1992), aflatoxin B1 (Niranjan et al., 1982), bleomycin (Lim and Neims, 1987), nucleoside analogues (Lewis et al., 1992), and also cisplatin (Olivero et al., 1995, 1997) have been shown to bind preferentially to mito-chondrial DNA (mtDNA) as compared to nuclear DNA (nDNA). As compared to nDNA, cisplatin adduction in mtDNA is reported to be 4-fold to 8-fold higher (Olivero et al., 1995, 1997). This preferential binding of cisplatin could be explained by the naked structure of mtDNA, which makes it highly accessible to damaging agents (Salazar et al., 1982). Alterations in mitochondrial structure and function, including reduction in the mito-chondrial transmembrane potential, occur early during apoptosis, before nuclear or chromatin structures are affected (Petit et al., 1995; Zamzani et al., 1995), suggesting that mitochondria may play a pivotal role in the process. Mitochondria play a central role in cellular homeostasis and platinum(II) complexes interact with energy-dependent functions in the cells (Beltrame et al., 1984).

Glutathione, a tripeptide (L-γ-glutamyl-L-cysteinylglycine) is the most abundant cellular thiol and is an important intracellular anti-oxidant. Reduced glutathione (GSH) performs a variety of important physiological and metabolic functions in the cells. It inhibits free radical-mediated injury by eliminating toxic peroxides and protects protein sulfhydryl groups from oxidation (Meister, 1988). GSH

is also involved in the detoxification of cispla-tin (Li et al., 1997). Cancer cells can generate large amounts of hydrogen peroxide that *in vivo* may also contribute to their ability to damage normal tissues and facilitate tumor growth and invasion (Szatrowski and Nathan, 1991).

The biochemical events in mitochondria that may accompany cisplatin-induced cyto-toxicity/toxicity in the hosts are largely unknown. Thus, considering the importance of mitochondria in malignancy as well as in apoptosis, the present studies were undertaken to investigate the changes in GSH and protein in tissues and their mitochondrial fractions, along with succinate dehydrogenase (SDH, EC 1.3.99.1), in relation to tumorous condition and cisplatin treatment. As cisplatin is known to develop a major side-effect of nephrotoxicity (Kim et al., 1995), kidneys were also used along with liver (the major site of metabolism) and tumor cells for the study. Blood urea concentrations were determined to ascertain the development of nephrotoxicity after cisplatin treatment. Lipid peroxidation was also investigated in kidney mitochondria following cisplatin treatment for analysis of the correlation of mitochondria with nephro-toxicity after cisplatin treatment.

Materials and methods

Chemicals

All biochemicals were purchased from the Sigma Chemical Company, St. Louis, MO, USA. Other chemicals used in the experiments were of analytical grade. Cisplatin solution (1 mg/ml of 0.9% NaCl) was obtained from Biochem Pharmaceutical Industries, Mumbai, India. Glass double-distilled water was always used to prepare the solutions.

Tumor maintenance and cisplatin treatment

Inbred Swiss albino mice were maintained in the laboratory under conventional conditions with free access to food pellets and water. Ascites Dalton lymphoma tumor is being maintained *in vivo* in 10–12-week-old mice by serial intraperitoneal (i.p.) transplantations of 1×10^7 tumor cells per animal (0.25 ml in phosphate-buffered saline (PBS) 0.15 mol/L NaCl, 0.01 mol/L sodium phosphate buffer, pH 7.4). Tumor-transplanted hosts usually survived for 18–20 days.

The therapeutic dose of cisplatin against malignant tumors has been established to be 8–10 mg/kg body weight (Rosenberg, 1985). A single dose of cisplatin (8 mg/kg body wt, i.p.) was administered to tumor-bearing mice on the10th day after tumor transplantation when the tumor was at the logarithmic phase of growth. With the regression of Dalton lymphoma (DL) tumor, very little ascites is recoverable from the hosts after 6–7 days of cisplatin treatment. The treatment schedule for 24, 48, 72, and 96 h has been used previously by us (Prasad and Giri, 1994; Prasad et al., 1998) and the same treatment schedule was followed. Liver and kidney were dissected out from the normal, tumor-bearing and cisplatin-treated tumorous mice. Ascites Dalton lymphoma collected from mice was centrifuged (2000g at 4°C, 10 min) and the pellet was used as the DL cells.

Mitochondrial isolation

The tissues were homogenized (10% homogenate) in ice-cold 0.25 mol/L sucrose in a Teflon pestle tissue homogenizer (Remi instruments, Mumbai, India). The homogenate was then centrifuged (800g at 4°C, 10 min). The supernatant was decanted and centrifuged (14 000g at 4°C, 30 min). The sedimented mitochondrial pellet was collected and washed once in cold sucrose solution. The mitochondrial pellet was weighed, resuspended in sucrose solution, sonicated (Soniprep 150), and used as a source of SDH and for other determinations. Three or four different preparations were made in each experiment.

Protein contents were estimated in the tissues and its mitochondrial fraction using Folin phenol reagent and bovine serum albumin as a standard (Lowry et al., 1951).

Assay of SDH

SDH activity was assayed in the mitochondrial fraction using the method described by King (1967). Mitochondrial samples were sonicated on ice to prepare 5% mitochondrial homogenate in 0.25 mol/L sucrose solution. The reaction mixture contained 0.2 mol/L phosphate buffer, pH 7.8 (0.375 ml), 0.045 mol/L KCN (0.05 ml), 0.6 mol/L succinate (0.1 ml), 0.0015 mol/L dichlorophenolindophenol (DCIP, 0.05 ml), 0.009 mol/L phenazine methosulfate (PMS, 0.15 ml) in a 3 ml spectrophotometer glass cuvette. The volume was made up to 2.95 ml with distilled water. The reaction was started by the addition of 0.05 ml of mitochondrial homogenate. The change in absorbance at 600 nm (Δ600 nm) was recorded at one-minute intervals in a Beckman DU-640 spectrophotometer. The Δ600 nm is converted to μmols succinate oxidized by multiplying Δ600 nm by 0.0476.

The unit of enzyme activity was expressed as nmol succinate oxidized/min and the specific activity as units/mg mitochondrial protein.

Glutathione estimation

Glutathione was determined as nonprotein (NPSH) and total (TSH) sulfhydryl contents in the mitochondrial fraction and respective tissues of mice under different experimental conditions (untreated normal mice, tumorous mice, tumorous mice treated with cisplatin) using the method of Sedlak and Lindsay

(1968). Briefly, tissues and mitochondria were homogenized in 0.02 mol/L EDTA, pH 4.7. For determination of NPSH, the homogenate (500 μl) was precipitated with 10% trichloroacetic acid (500 μl) and centrifuged, and 800 μl of the supernatant was taken. To the supernatant 1.6 ml of Tris-EDTA buffer (0.4 mol/L, pH 8.9) was added before the addition of Ellman's reagent (10 mmol/L 5,5′-dithiobis-2-nitrobenzoic acid in methanol, 25 μl). TSH was determined by adding the homogenate or pure reduced form of glutathione (100 μl) to 0.2 mol/L Tris-EDTA buffer (1 ml, pH 8.2) and 0.02 mol/L EDTA, pH 4.7 (0.9 ml), followed by 20 μl of Ellman's reagent. After 30 min of incubation at room temperature, the absorbance was read at 412 nm in a Beckman DU-640 spectrophotometer. Samples for TSH determinations were centrifuged before absorbances of supernatants were measured.

Serum urea concentration

In another set of experiments, a group of tumor-bearing mice ($n = 10$) were administered with cisplatin (8 mg/kg body wt) on the 10th day following tumor transplantation. One mouse was sacrificed on every second day starting from the first day of cisplatin treatment (i.e., on days 1, 3, 5, 7, 9, and 11 following treatment); blood was collected promptly and allowed to clot and the serum was separated. Tumor-bearing mice serving as control were injected with 0.89% NaCl only. The concentration of urea in sera collected at various time treatments was determined using the method of Brown and Cohen (1959).

Lipid peroxidation

The concentration of thiobarbituric acid-reacting substances, mainly malondialdehyde, was determined in the mitochondria of kidneys collected from the normal mice, tumor-bearing mice, and cisplatin-treated tumor-bearing mice

using the method of Buege and Aust (1978). The mitochondrial homogenate (5%) was prepared in 0.15 mol/L NaCl by sonication. To 1 ml of mitochondrial homogenate, 2 ml of TCA (trichloroacetic acid)–TBA (thiobarbituric acid)–HCl reagent (15% TCA and 0.375% TBA dissolved in 0.25 mol/L HCl) was added and mixed thoroughly. The solution was heated in a boiling water bath for 15 min. After cooling at room temperature, the precipitate was removed by centrifugation ($1000g$, 10 min). The absorbance of the supernatant was read at 535 nm. The malondialdehyde concentration in the samples was calculated using an extinction coefficient of 1.56×10^5 L/mol and is expressed as nmol/mg protein.

Results

As compared to those in normal mice, protein contents of liver and kidney decreased to about 16% and 38%, respectively, in tumor-bearing mice. In the three tissues studied, protein contents were greater in liver and kidney than in DL cells. Cisplatin treatment caused a significant decrease of protein in the tissues as well as in their mitochondrial fractions (Table 1). Average protein decrease was noted to be greater (about 30%) in DL cells than that in liver (about 7%) and kidney (about 15%) after 24 to 96 h of cisplatin treatment. However, as compared to that in tissues, the decrease was considerably larger in the respective mitochondrial fraction and the maximum decrease was noted in Dalton lymphoma (DL) cells (Figure 1).

GSH contents showed wide variation in the tissues, being greater in liver (11.91 μmol/g) and less in the DL cells (4.43 μmol/g) (Table 2). Cisplatin treatment resulted in a very significant decrease of GSH contents in liver mitochondria but not in liver tissue (Figure 2). TSH contents decreased in kidney as well as in kidney mitochondria after cisplatin treatment

Table 1. Total protein content (mg/g wet weight) in the tissues and their mitochondrial fractions (mean ± SD)

Tissues	Treatment	Tissue protein	mt-Protein
Liver	Normal	228.56 ± 33.04	138.02 ± 7.73
	Tumor-bearing (control)	197.69 ± 13.57	107.42 ± 9.13
	Cisplatin (24 h)	182.10 ± 4.45	76.73 ± 7.20[b]
	Cisplatin (48 h)	190.06 ± 19.95	76.06 ± 4.72[c]
	Cisplatin (72 h)	184.40 ± 8.50	77.12 ± 7.00[b]
	Cisplatin (96 h)	179.80 ± 11.50	74.60 ± 6.87[b]
Kidney	Normal	232.19 ± 29.53	77.86 ± 1.16
	Tumor-bearing (control)	168.33 ± 6.09	82.73 ± 5.84
	Cisplatin (24 h)	139.67 ± 17.86[a]	68.16 ± 2.80[a]
	Cisplatin (48 h)	141.34 ± 13.78[a]	62.90 ± 3.42[c]
	Cisplatin (72 h)	144.15 ± 16.83	57.26 ± 3.11[c]
	Cisplatin (96 h)	147.60 ± 12.34	51.34 ± 4.57[c]
DL cells	Tumor-bearing (control)	153.84 ± 12.06	59.26 ± 5.53
	Cisplatin (24 h)	118.55 ± 7.51[b]	38.84 ± 4.87[b]
	Cisplatin (48 h)	109.90 ± 8.65[c]	38.62 ± 5.2[b]
	Cisplatin (72 h)	102.76 ± 6.04[c]	33.14 ± 1.64[c]
	Cisplatin (96 h)	98.65 ± 9.75[c]	29.86 ± 3.93[c]

Cisplatin (8 mg/kg body wt) was administered to tumor-bearing mice.
Student's *t*-test, $n = 4$; as compared to respective untreated tumor-bearing control: [a]$p \leqslant 0.05$; [b]$p \leqslant 0.02$; [c]$p \leqslant 0.01$.
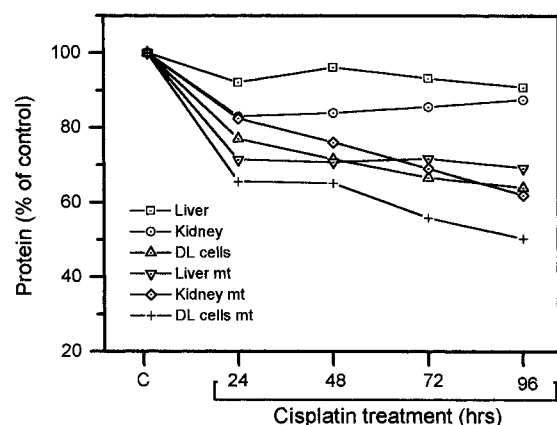

Figure 1. Comparative changes in protein concentration in the tissues and their mitochondrial (mt) fractions after cisplatin treatment of tumor-bearing mice. Compared to the respective tissues, larger decreases of protein concentration are notable in the mitochondrial fractions after the treatment. C = control (untreated tumor-bearing mice).

(Table 2; Figure 3). In contrast to the liver and kidney, in the case of DL only tissue TSH was decreased, but elevated concentrations of mt-TSH and mt-NPSH were noticed after cisplatin treatment (Table 2; Figure 4).

Succinate dehydrogenase (SDH) acitivity was assayed in the mitochondrial fraction of liver, kidney, and DL cells. Cisplatin treatment resulted in a significant decrease in the enzyme activity in kidney and DL cells, while in liver it remained almost unchanged (Table 3). The change/increase in the specific activity of the enzyme was not siginificant ($p < 0.05$) in any of the three tissues after cisplatin treatment (Table 3).

Serum urea measurement showed that cisplatin treatment of the hosts increased the serum urea concentration very significantly; this reached a maximum at 5–6 days, when it was about 2.5–3 times the control value (Figure 5).

Table 2. Total (TSH) and nonprotein (NPSH) glutathione concentration (μmol/g wet weight) in the the mitochondrial fractions and tissues of mice under different experimental conditions (mean ± SD)

Tissues	Treatment	TSH	NPSH	mt-TSH	mt-NPSH
Liver	Normal	11.72 ± 1.50	4.20 ± 0.80	2.65 ± 0.26	0.071 ± 0.002
	Tumor-bearing (control)	11.91 ± 1.06	4.50 ± 0.16	3.80 ± 0.27	0.160 ± 0.012
	Cisplatin (24 h)	11.64 ± 1.88	3.85 ± 0.20^b	2.61 ± 0.18^c	0.050 ± 0.003^d
	Cisplatin (48 h)	11.68 ± 0.42	4.01 ± 0.22^a	1.35 ± 0.11^d	0.041 ± 0.002^d
	Cisplatin (72 h)	11.75 ± 0.13	4.08 ± 0.18^a	1.32 ± 0.24^d	0.058 ± 0.003^d
	Cisplatin (96 h)	11.84 ± 0.15	4.12 ± 0.31	1.41 ± 0.31^c	0.069 ± 0.005^d
Kidney	Normal	8.03 ± 1.58	2.70 ± 0.37	2.28 ± 0.37	0.210 ± 0.031
	Tumor-bearing (control)	8.50 ± 1.22	3.02 ± 0.55	2.20 ± 0.40	0.130 ± 0.010
	Cisplatin (24 h)	6.04 ± 0.42^a	2.20 ± 0.28	1.97 ± 0.12	0.120 ± 0.004^a
	Cisplatin (48 h)	6.52 ± 0.37	2.44 ± 0.34	1.45 ± 0.28	0.110 ± 0.011
	Cisplatin (72 h)	6.94 ± 0.22	2.71 ± 0.32	1.47 ± 0.06^a	0.166 ± 0.013
	Cisplatin (96 h)	7.68 ± 0.33	2.83 ± 0.33	1.61 ± 0.09	0.173 ± 0.014^c
DL cells	Tumor-bearing (control)	4.43 ± 0.26	0.67 ± 0.11	2.27 ± 0.18	0.084 ± 0.005
	Cisplatin (24 h)	3.51 ± 0.32^a	0.61 ± 0.03	3.00 ± 0.15^a	0.140 ± 0.011^d
	Cisplatin (48 h)	3.82 ± 0.21^a	0.62 ± 0.04	2.57 ± 0.16	0.160 ± 0.023^d
	Cisplatin (72 h)	4.02 ± 0.32	0.62 ± 0.03	2.85 ± 0.05^c	0.140 ± 0.020^c
	Cisplatin (96 h)	4.21 ± 0.45	0.63 ± 0.03	2.97 ± 0.07^c	0.133 ± 0.012^d

mt = mitochondrial.

Cisplatin (8 mg/kg body wt) was administered to tumor-bearing mice.

Student's *t*-test, $n = 4$, as compared to untreated tumor-bearing control: $^a p \leqslant 0.05$; $^b p \leqslant 0.02$; $^c p \leqslant 0.01$; $^d p \leqslant 0.001$.
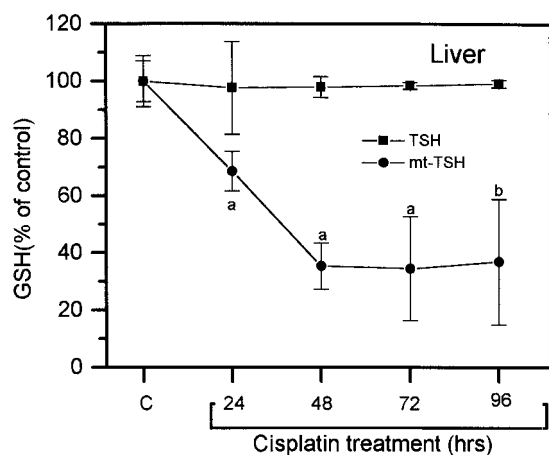

Figure 2. Changes in total glutathione in the liver and its mitochondria (mt) after cisplatin treatment of tumor-bearing mice. A very significant decrease of TSH may be noted in the mitochondria but not in liver after the treatment. C = control (untreated tumor-bearing mice). Statistical analysis as compared to the respective control, Student's *t*-test, $n = 4$ independent experimental determinations: $^a p \leqslant 0.01$; $^b p \leqslant 0.02$.
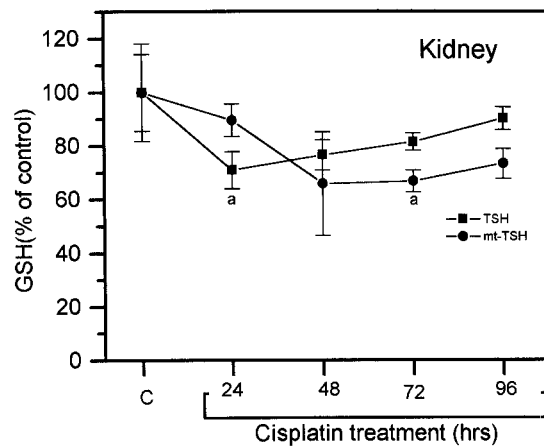
Figure 3. Changes in total glutathione in the kidney and its mitochondria (mt) after cisplatin treatment. The treatment caused greater decrease of TSH in the mitochondria (mt-TSH) than that in kidney. C = control (untreated tumor-bearing mice). Statistical analysis as compared to the respective control, Student's *t*-test, $n = 4$ separate experimental determinations: $^a p \leqslant 0.05$.
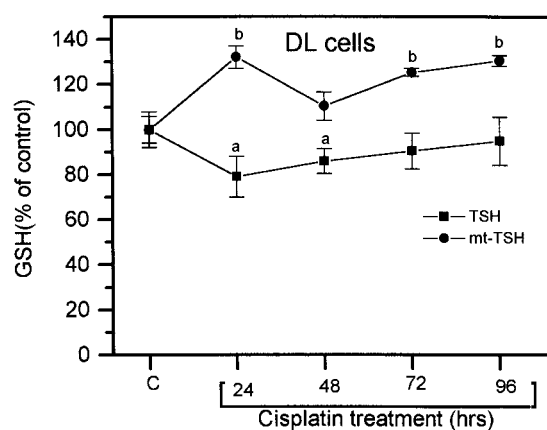
Figure 4. Changes in total glutathione in Dalton lymphoma (DL) and its mitochondria (mt) after cisplatin treatment. Mitochondrial TSH (mt-TSH) was slightly increased but DL-TSH decreased after the treatment. C = control (untreated tumor-bearing mice). Statistical analysis as compared to respective control, Student's *t*-test, $n = 4$ independent experimental determinations: [a]$p \leqslant 0.05$; [b]$p \leqslant 0.01$.
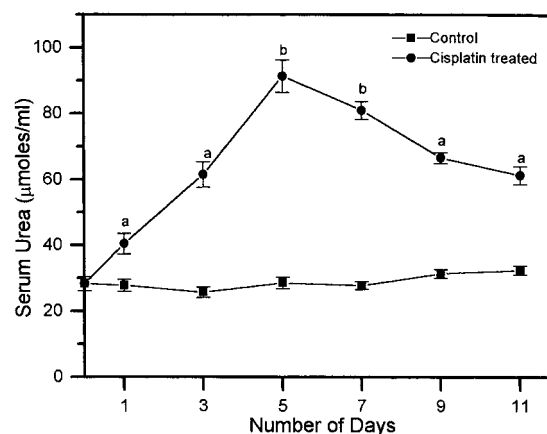
Figure 5. Changes in the serum urea concentration in the tumorous mice following cisplatin treatment. A significant increase in the urea concentration may be noted after cisplatin treatment, being maximum at 5–7 days of treatment. Control: untreated, tumor-bearing animals. Means $\pm$ SD, $n = 3$, Student's *t*-test, as compared to the control: [a]$p \leqslant 0.02$; [b]$p \leqslant 0.01$.

Table 3. Units and specific activity (mean $\pm$ SD) of succinate dehydrogenase in the mitochondrial fraction of different tissues

Treatment	Liver		Kidney		Dalton lymphoma	
	Units	Specific activity	Units	Specific activity	Units	Specific activity
Normal	8.93 ± 0.64	25.89 ± 1.86	9.28 ± 0.72	47.66 ± 3.69	–	–
TB (control)	7.16 ± 2.18	26.70 ± 8.14	11.26 ± 1.66	54.66 ± 8.05	10.49 ± 2.38	70.88 ± 16.10
Cisplatin (24 h)	7.52 ± 3.24	39.38 ± 16.96	6.89 ± 0.29[b]	41.43 ± 3.01	7.42 ± 2.01[a]	76.36 ± 24.77
Cisplatin (48 h)	9.75 ± 1.36	51.33 ± 7.17	9.38 ± 0.71[a]	59.73 ± 5.81	7.01 ± 0.21[a]	72.98 ± 2.87
Cisplatin (72 h)	9.54 ± 1.41	49.47 ± 7.31	9.43 ± 0.45	63.66 ± 3.76	8.65 ± 0.03	104.47 ± 7.56
Cisplatin (96 h)	8.92 ± 2.32	47.83 ± 5.63	8.28 ± 0.60	64.65 ± 7.46	5.90 ± 2.12[b]	79.77 ± 8.86

Unit of enzyme activity is defined as the nanomoles of succinate oxidized per minute. The specific activity is expressed as units/mg mitochondrial protein.

Student's *t*-test, $n = 3–4$, as compared to untreated tumor-bearing (TB) control: [a]$p \leqslant 0.05$; [b]$p \leqslant 0.02$.

As compared to normal mice, the lipid peroxidation in kidney mitochondria was noted to be slightly (about 8%) decreased in tumor-bearing mice. However, it increased very significantly during 48–96 h of cisplatin treatment (Figure 6).

Discussion

Cellular DNA has been indicated to be the primary target in the anticancer activity of cisplatin (Zamble and Lippard, 1995), but it has also been suggested that, because cisplatin
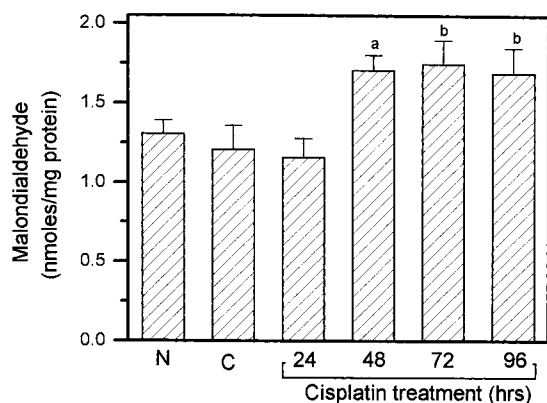
Figure 6. Lipid peroxidation (malondialdehyde levels) increased significantly in the mitochondria of kidneys following cisplatin treatment. N= untreated, nontumorous normal mice; C = control, untreated tumor-bearing mice. Means ± SD, *n* = 4, Student's *t*-test, as compared to the control: $^a p \leqslant 0.02$; $^b p \leqslant 0.05$.

is chemically very reactive, other biochemical targets besides DNA may exist in the cells and contribute to cell growth inhibition and cytotoxicity (Just and Holler, 1991). Present findings, while supporting the multilevel/multistep effects of cisplatin in tumorous hosts (Prasad et al., 1999), provide a definite hint that mitochondria might be one of the targets of potency in cisplatin-mediated cancer chemotherapy and toxicity. Cisplatin has been shown to bind preferentially to mitochondrial DNA (mtDNA): 4-fold to 8-fold higher than to nuclear DNA (nDNA) (Olivero et al., 1995, 1997). Many mitochondrial-related events (e.g., mtDNA oxidation, increased mitochondrial peroxide production, early oxidation of the mitochondrial and cytosolic GSSG/GSH couple, and decreased mitochondrial membrane potential) have been suggested to be involved during the apoptotic process (Zamzami et al., 1995; Esteve et al., 1999). Alterations in mitochondrial structure and function, including reduction in the mitochondrial transmembrane potential, also occur early during apoptosis, before nuclear or

chromatin structures are affected (Petit et al., 1995; Zamzami et al., 1995), suggesting that mitochondria may play a pivotal role in the process. Recently it has also been reported that mitochondrial dysfunction could be a major mechanism of drug-induced liver diseases such as nonalcoholic steatohepatitis, cytolytic hepatitis (Pessayre et al., 2000), and other disorders (Murphy and Smith, 2000).

The early subcellular events in cisplatin toxicity are not well established. It has been reported that mitochondrial injury is an important event during the early stages of cisplatin toxicity to renal proximal tubules (Brady et al. 1990). The present studies may suggest that cisplatin treatment causes definite mitochondrial biochemical injury, which could be involved in the resulting toxicity/cytotoxicity. Significant decrease of protein in the tissues as well as in the mitochondrial fractions was noted after cisplatin treatment (Table 1). However, more conspicuous protein decrease was observed in the mitochondrial fraction than in the respective tissues (Figure 1), which may suggest that mitochondria could be one of the most susceptible subcellular sites involved in toxicity in kidney and liver, or cytotoxicity in DL cells. It may involve inhibited protein transport from the cytosol to mitochondria and/or decreased mitochondrial protein synthesis. In carbon tetrachloride-exposed rats, decreased protein synthesis along with depressed oxidative phosphorylation and disruption in mitochondrial structure have been reported (De Wit and Brabec, 1985). Heminger et al. (1997) have reported that cisplatin inhibited protein synthesis by causing an inhibition of elongation and also suggested that this may contribute to the cytotoxic/toxic effects of cisplatin during therapy.

Serum urea content has been shown to closely parallel the glomerular function (Provoost and Molenaar, 1980). We, therefore, used the serum urea concentration as the nephrotoxic parameter after cisplatin treat-

ment. There was significant increase in the serum urea in mice following cisplatin treatment, this being maximum at 5–6 days of treatment (Figure 5), which clearly indicates the toxic effect of cisplatin in the kidneys. The observed nephrotoxic effect of cisplatin may also be related to changes in the kidney mt-GSH. Cell compartmentation of GSH is important because many of the free radical species generated in the cell are highly reactive and will attack preferentially those cell components that are close to the organelle in which the radicals are generated. This is especially important for mitochondria. GSH is critical to numerous mitochondrial functions, including membrane structure and integrity (Le-Quoc and Le-Quoc, 1989), ion homeostasis (Beatrice et al., 1984), and intramitochondrial redox status (Yagi and Hatefi, 1984). GSH decrease was more notable in the mitochondrial fractions of liver and kidney than that in respective tissues after cisplatin treatment (Table 2; Figures 2 and 3). It has been suggested that most chemical exposures that are associated with GSH depletion require depletion of mitochondrial, rather than cytosolic, GSH to elicit cellular injury (Shan et al., 1993). The decrease in mt-GSH should be related to oxidative damage to mitochondria. To substantiate this suggestion, lipid peroxidation was determined in the mitochondrial fractions of kidneys and it was observed that the mitochondrial lipid peroxidation increased significantly after 48–96 h of cisplatin treatment (Figure 6). It is thus suggested that depletion of mt-GSH and the concomitant increase in mt-lipid peroxidation could be an early and critical event during cisplatin-induced toxicity. Zglinicki et al. (1991) reported that Fe^{2+}/ascorbate-induced peroxidation of isolated rat liver mitochondria leads to initial volume changes and damage characterized by gross swelling and loss of cristae and matrix material. However, cisplatin treatment caused a more distinct decrease of GSH in DL cells than in their mitochondrial

fractions where, in fact, a slight increase in GSH was noticeable (Table 2; Figure 4). This could be due to there being fewer and/or structurally altered mitochondria and the protective mechanism in DL cells against chemotherapeutic drugs, and may indicate that the change/decrease in cytosolic GSH is more important for cisplatin-mediated cytotoxicity in DL cells than in their mitochondrial fraction. In fact, diminished mitochondrially mediated oxidative phosphorylation has been suggested in cancer cells, with glycolysis being the main source of energy production (Warburg, 1956). Our earlier work had shown that cisplatin increases the concentration of calcium in kidney and DL cells (Prasad and Giri, 1999), and the change/rise in calcium could also be related to mitochondrial injury because mitochondria play an important role in maintaining Ca^{2+} homoeostasis (Nicotera et al., 1992, Smaili et. al., 2000; Murphy and Smith, 2000). At the same time, perturbation of intracellular Ca^{2+} homoeostasis by the increased cytosolic Ca^{2+} is also believed to be related to depletion of GSH (Zhang and Lindup, 1996), which can lead to oxidative stress and cell injury (Olafsdottir et al., 1988). We also suggest a similar mechanism for cisplatin-mediated nephrotoxic effect and cytotoxic effect in DL cells with the decrease in mt-protein and GSH.

Succinate dehydrogenase is an important mitochondrial enzyme which links the Krebs cycle with the electron transport chain and oxidative phosphorylation. Thus, assay of SDH should give an indirect idea of the oxygen consumption by mitochondria. Cisplatin treatment caused a decrease in the enzyme activity in kidney and DL cells but not in liver (Table 3). The decrease of SDH activity, especially in kidney and DL cells, may therefore aid the development of some anoxic condition or mitochondrial dysfunction that, along with decrease in mt-GSH, may contribute to lysis of cells. This could be supported by our earlier

reported observation of a fall in oxygen consumption rate by DL cells and selective increase of Ca^{2+} observed in particular in kidney and DL cells after cisplatin treatment (Prasad and Giri, 1999). It should also be mentioned that the increase in specific activity of SDH was not significant ($p \leqslant 0.05$) after cisplatin treatment (Table 3). The slight increase in the specific activity ($p \leqslant 0.1$) after cisplatin treatment could be due to a larger decrease of other proteins rather than the SDH. However, the study of other complexes of respiratory chain would be very helpful in providing further insight.

These findings while supporting the multi-level/multistep effects of cisplatin in the host (Prasad et al., 1999), suggest that mitochondria are one of the important targets in cells during cisplatin-mediated chemotherapy. Although the exact mechanism of cisplatin-induced changes in mt-protein, mt-GSH, and SDH is not clearly understood at present, our findings suggest that biochemical changes in mitochondria could be important in affecting the overall metabolism in the cells, and the resulting toxicity/cytotoxicity after cisplatin treatment.

Acknowledgments

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