Kidney International, Vol. 54 (1998), pp. 1562–1569

Role of cytochrome P-450 as a source of catalytic iron in cisplatin-induced nephrotoxicity

RADHAKRISHNA BALIGA, ZHIWEI ZHANG, MITHRA BALIGA, NORISHI UEDA, and SUDHIR V. SHAH

Department of Pediatrics, University of Mississippi Medical Center, Jackson, Mississippi; Department of Internal Medicine, Division of Nephrology, University of Arkansas for Medical Sciences, and John L. McClellan Memorial VA Hospital, Little Rock, Arkansas, USA

Role of cytochrome P-450 as a source of catalytic iron in cisplatin-induced nephrotoxicity.

Background. Iron plays a role in free radical-mediated tissue injury, including cisplatin-induced nephrotoxicity. However, the source of iron (catalyzing free radical reactions) is not known. We examined the role of cytochrome P-450 as a source of catalytic iron in cisplatin-induced nephrotoxicity both *in vivo* and *in vitro*.

Methods. Cisplatin-induced acute renal failure was produced in rats by intraperitoneal injection of cisplatin (10 mg/kg body wt). Piperonyl butoxide, a cytochrome P-450 inhibitor, was administered intraperitoneally (400 mg/kg body wt twice at 48-hr intervals) prior to cisplatin injection. The effects of cisplatin in the absence or presence of piperonyl butoxide on the belomycin-detectable iron, cytochrome P-450 content in the kidney, and renal functional and histological changes were evaluated. In an *in vitro* study, the effect of cytochrome P-450 inhibitors, cimetidine or piperonyl butoxide, on cisplatin-induced cytotoxicity and catalytic iron release from LLC-PK_1 cells was examined.

Results. In cisplatin-treated rats, there was a marked decrease in the cytochrome P-450 content specifically in the kidney, accompanied by increased bleomycin-detectable iron content in the kidney. Piperonyl butoxide prevented cisplatin-induced loss of cytochrome P-450 as well as the increase of bleomycin-detectable iron in the kidney, along with both functional and histological protection. Both cimetidine and piperonyl butoxide prevented cisplatin-induced increase in bleomycin-detectable iron and cytotoxicity in LLC-PK_1 cells. Treatment of cimetidine did not affect cellular uptake of cisplatin.

Conclusion. Cytochrome P-450, a group of heme proteins, may serve as a significant source of catalytic iron in cisplatin-induced nephrotoxicity.

A large body of evidence has implicated an important role of iron in *in vivo* and *in vitro* models of reactive oxygen metabolite-mediated tissue injury [1–10], including cisplatin-induced nephrotoxicity [10]. Iron, a transition metal, can participate in the generation of powerful oxygen metabolites such as hydroxyl radical by the metal-catalyzed Haber-Weiss reaction and/or in the generation of highly reactive iron-oxygen complexes such as ferryl or perferryl ion [1]. Indeed, iron chelators including deferoxamine and 2,3-dihydroxybenzoic acid have been shown to be protective in several forms of ischemic, toxic and immune-mediated renal injury [2–7]. The catalytic iron capable of catalyzing free radical reactions, as measured by the bleomycin assay, has been reported to be increased in ischemia/reperfusion-induced [8] and myoglobinuric acute renal failure [9].

Cisplatin is one of the most effective chemotherapeutic agents, and plays a major role in the treatment of a variety of human solid tumors [10, 11]. The most common adverse effect limiting the use of cisplatin is nephrotoxicity that develops primarily in the S3 segment of the proximal tubule [12]. Aggressive hydration and forced diuresis have been utilized to reduce cisplatin-induced nephrotoxicity [13]. The mechanism underlying this nephrotoxicity is not well understood [14], however, reactive oxygen metabolites have been implicated [15]. The role of iron in mediating tissue injury via the generation of hydroxyl radical formation in this model of renal injury has been examined in our previous study [10]. In addition, a recent study has demonstrated that the induction of heme oxygenase provided marked protection against cisplatin nephrotoxicity [16] by rapid degradation of the labile heme moiety rendering the heme iron unavailable to participate in oxidant stress.

Cytochrome P-450 as a potential source of iron was first described in reperfusion injury of rabbit lung [17] and rat kidneys [18], as well as in myoglobinuric acute renal failure [9]. However, the role of cytochrome P-450 in cisplatin-induced nephrotoxicity has not been previously examined. If cytochrome P-450 serves as a source of iron then this should result in the loss of cytochrome P-450. Indeed, relevant to the current study is the observation that the cytochrome P-450 content in the kidney was markedly decreased in cisplatin nephrotoxicity [19]. Therefore, the present study was designed to test the hypothesis that the

Key words: cimetidine, piperonyl butoxide, LLC-PK_1 cells, acute renal failure, free radical, tissue injury, chemotherapeutic agent.

Received for publication December 17, 1997
and in revised form May 21, 1998
Accepted for publication June 16, 1998

heme moiety of cytochrome P-450 may serve as a significant source of catalytic iron in cisplatin-induced nephrotoxicity.

METHODS

In vivo study

Cisplatin-induced acute renal failure. Cisplatin model of acute renal failure was induced in rats as described by Capasso et al [20] and in our previous studies [10]. Male Sprague-Dawley rats weighing 200 to 250 g were allowed free access to rat chow (Purina) and water. The animals received intraperitoneal injection of cisplatin (Bristol-Myers Squibb Co., Princeton, NJ, USA) at a dose of 10 mg/kg body wt and were sacrificed at 72 hours after cisplatin injection. Blood, kidneys, and a piece of liver were obtained for the various measurements. Renal impairment was assessed by blood urea nitrogen (BUN), plasma creatinine, and kidney histology. BUN and creatinine were measured by the use of Sigma diagnostic kits (Sigma Chemical Co., St. Louis, MO, USA). Kidney histology was studied as described below.

Kidney histology. The kidneys were sectioned and a portion fixed in 10% formalin, dehydrated and embedded in paraffin. Sections were cut at 2 μm and stained with periodic acid-Schiff reagent. The slides were coded and semiquantitative analysis of the kidney sections was performed without knowledge of the treatment protocol as previously described [10, 21]. The changes seen were limited to the tubulointerstitial areas and graded as follows: 0 = normal; I = areas of tubular epithelial cell swelling, vacuolar degeneration, necrosis, and desquamation involving $<25\%$ of cortical tubules; II = similar changes involving $>25\%$ but $<50\%$ of cortical tubules; III = similar changes involving $>50\%$ but $<75\%$ of cortical tubules; IV = similar changes involving $>75\%$ of cortical tubules.

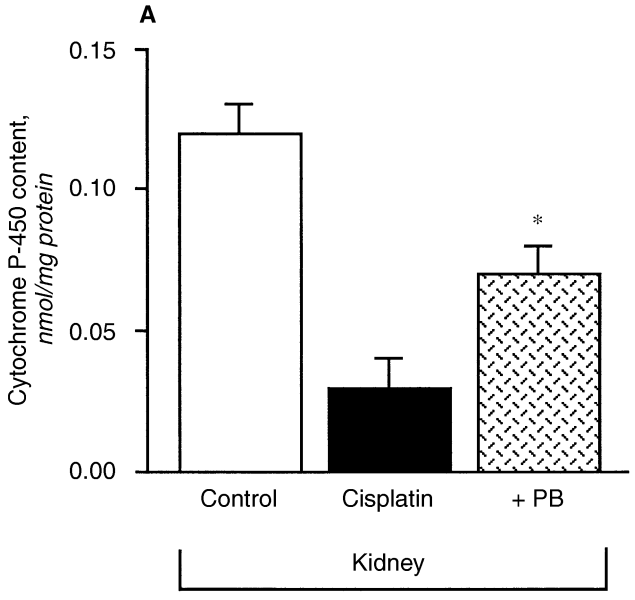
Bleomycin-detectable iron assay. Catalytic iron content in the kidney was measured by the bleomycin-detectable iron assay as detailed in our previous studies [8–10]. Briefly, the kidney tissue was homogenized in Chelex-treated pyrogen-free water, loaded into a Centriprep-30 that has a molecular cut-off value of 30 kD, and centrifuged at 1500 g for 30 minutes at 4°C. The resulting clear ultrafiltrate was collected and used for the bleomycin-detectable iron assay. All reagents except for the sample under test were made up in Chelex-treated pyrogen-free water in iron-free plastic containers and shaken with Chelex-100 to remove as much contaminating iron as possible. The reaction mixture contained in order: 0.5 ml of calf thymus DNA (1 mg/ml), 0.05 ml of bleomycin sulfate (1 mg/ml), 0.1 ml of $MgCl_2$ (50 mM), 0.1 ml of sample, either 0.05 ml of HCl (10 mM) [8–10] or 0.1 ml of imidazole [10, 22], 0.1 ml of Chelex-treated pyrogen free water and 0.1 ml of ascorbic acid (8 mM). Sample blank was identical except that bleomycin was omitted. The samples were mixed and then incubated at 37°C for two hours with shaking. The reaction was stopped by adding 0.1 ml of 0.1 M EDTA, and mixed with 1 ml thiobarbituric acid (1% wt/vol in 50 mM NaOH) and 1 ml HCl (25%, vol/vol). The reaction mixture was then heated at 100°C for 15 minutes, cooled, and the resulting chromogen measured using spectrophotometer by its absorbance at 532 nm. A standard curve was prepared using various concentrations of $FeCl_3$ (0 to 50 μM) in Chelex-treated pyrogen free water. The amount of bleomycin-detectable iron in the test sample was calculated from the standard curve and the results expressed as nmol/mg tissue protein recovered from the homogenate. Protein concentration was measured by the use of Bio-Rad reagent (Bio-Rad Laboratories, Hercules, CA, USA).

Determination of cytochrome P-450 content. Fresh kidney or liver tissue was homogenized in ice-cold isotonic KCl (0.15 M). The homogenate was then centrifuged at 15,000 g for 20 minutes at 4°C and the precipitate discarded. The resulting supernatant was further centrifuged at 105,000 g for 60 minutes at 4°C to obtain microsomes. The firmly packed pellet of microsomes was resuspended in 0.1 M potassium phosphate buffer, pH 7.4, usually at a concentration of 10 mg of protein per ml. Cytochrome P-450 content was measured by the method of Omura and Sato [23]. In brief, the suspensions of microsomes were diluted to approximately 1 mg of protein per ml with the assay buffer containing 0.1 M potassium phosphate buffer, pH 7.25, 20% glycerol, and 0.2% tergitol. After recording the baseline, the sample was reduced with a few crystals of dithionite, followed by CO bubbling for about one minute. The CO spectrum difference of reduced microsomes was measured on a Shimadzu UV-2101PC spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA).

Interventional therapy with a cytochrome P-450 inhibitor. Piperonyl butoxide (Aldrich Chemical Co., Milwaukee, WI, USA), a cytochrome P-450 inhibitor, was administered intraperitoneally in a dose of 400 mg/kg body wt twice at 48-hour intervals. The first injection was given 15 hours prior to cisplatin injection. The effect of piperonyl butoxide on the cytochrome P-450 content and bleomycin-detectable iron in the kidney as well as renal functional and histological changes subjected to cisplatin injection was evaluated.

In vitro study

Cell culture. LLC-PK_1 cells (renal proximal tubular epithelial cells) purchased from American Type Culture Collection (CRL 1392; Rockville, MD, USA) were maintained in Medium 199 supplemented with 3% fetal bovine serum and penicillin (100 U/ml) in a humidified atmosphere of 95% air—5% CO_2. The cells were maintained in 75 cm^2 tissue culture flask and the monolayer was subcultured using 0.05% trypsin, 0.53 mM EDTA in calcium- and magnesium-free Hank's balanced salt solution (HBSS). For the experiment, the cells were grown in a 6- or 12-well tissue culture plate until confluency. All experiments were



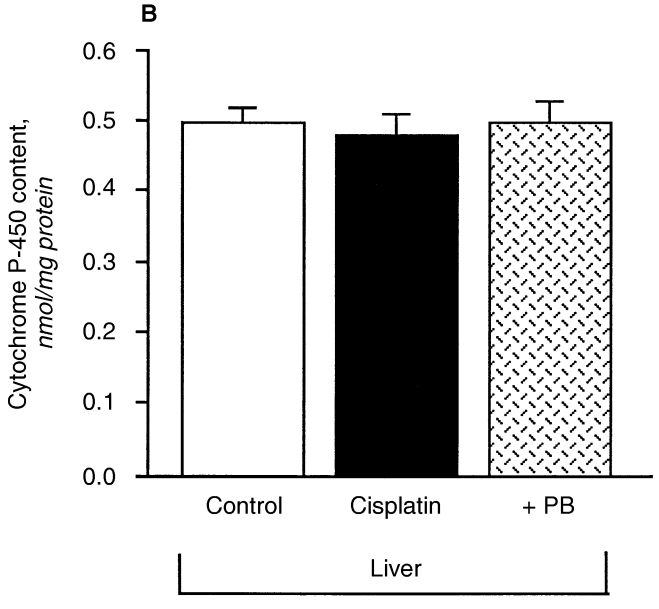


Fig. 1. Effect of cytochrome P-450 inhibitor on cytochrome P-450 content in both kidneys and liver of cisplatin-treated rats. Rats were given an intraperitoneal injection of cisplatin (10 mg/kg body wt) and were sacrificed at 72 hours after injection. A cytochrome P-450 inhibitor, piperonyl butoxide (PB), was administered intraperitoneally twice in a dose of 400 mg/kg body wt at 48-hour intervals. Values are means ± SE. The number of animals used was: control, $N = 5$; cisplatin, $N = 9$; PB, $N = 9$. $^*P < 0.01$ compared with cisplatin treatment alone.

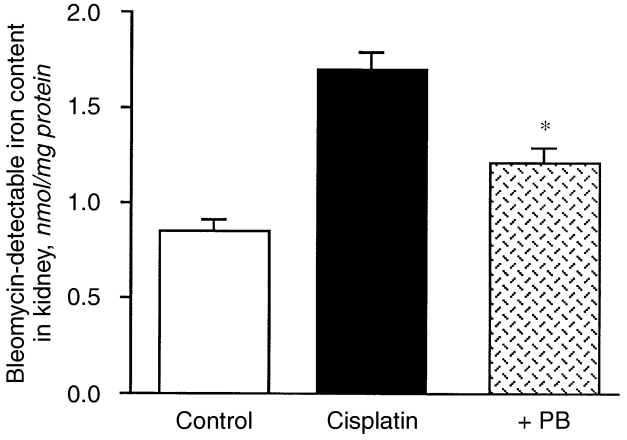


Fig. 2. Effect of cytochrome P-450 inhibitor on bleomycin-detectable iron content in kidney of cisplatin-treated rats. Rats were given intraperitoneal injection of cisplatin (10 mg/kg body wt) and were sacrificed at 72 hours after injection. A cytochrome P-450 inhibitor, piperonyl butoxide (PB), was administered intraperitoneally twice in a dose of 400 mg/kg body wt at 48-hour intervals. Values are means ± SE, $N = 5$. $^*P < 0.01$ compared with cisplatin treatment alone.

carried on confluent cell monolayer between passages 205 to 216.

Effect of cytochrome P-450 inhibitors on cisplatin-induced cytotoxicity. LLC-PK_1 cells were washed twice with HBSS and then incubated in HBSS at 37°C with cytotoxic dose of cisplatin for a period of time necessary to induce consistent cytotoxicity (200 μg/ml for 4 hr) based on our previous studies [10]. Two different inhibitors of cytochrome P-450 were used in the study. Cimetidine (2 mM; SmithKline Beecham Pharmaceuticals, Philadelphia, PA, USA) or piperonyl butoxide (1 mM) was added to the incubation buffer 30 minutes prior to the addition of cisplatin and was maintained throughout the incubation period. At the end of incubation, the incubation buffer was collected and the remaining cell monolayer was dissolved in 0.1% Triton X-100. The lactate dehydrogenase (LDH) level in the buffer and in the cellular fraction was measured by the use of assay kit purchased from Sigma [10]. The degree of cell injury was expressed as LDH release which is the percentage of LDH released into the buffer to total LDH recovered from both buffer and cellular fraction.

Effect of cytochrome P-450 inhibitors on cisplatin-induced catalytic iron release. LLC-PK_1 cells were washed twice with Chelex-treated HBSS and then incubated in Chelex-treated HBSS with a cytotoxic dose of cisplatin for a period of time before substantial cell killing occurred (200 μg/ml for 2.5 hr) [10]. Cimetidine (2 mM) or piperonyl butoxide (1 mM) was added to the incubation buffer 30 minutes prior to the addition of cisplatin. At the end of incubation, the incubation buffer was collected and the remaining cell monolayer was dissolved in 0.2 N NaOH for the measurement of cellular protein concentration by the use of Bio-Rad reagent. The catalytic iron released into the buffer was measured by the bleomycin-detectable iron assay as described and the results expressed as nmol/mg cellular protein recovered from the cell monolayer.

Effect of a cytochrome P-450 inhibitor, cimetidine, on platinum level. To exclude the possibility that cytochrome P-450 inhibitors may interfere with cellular uptake of

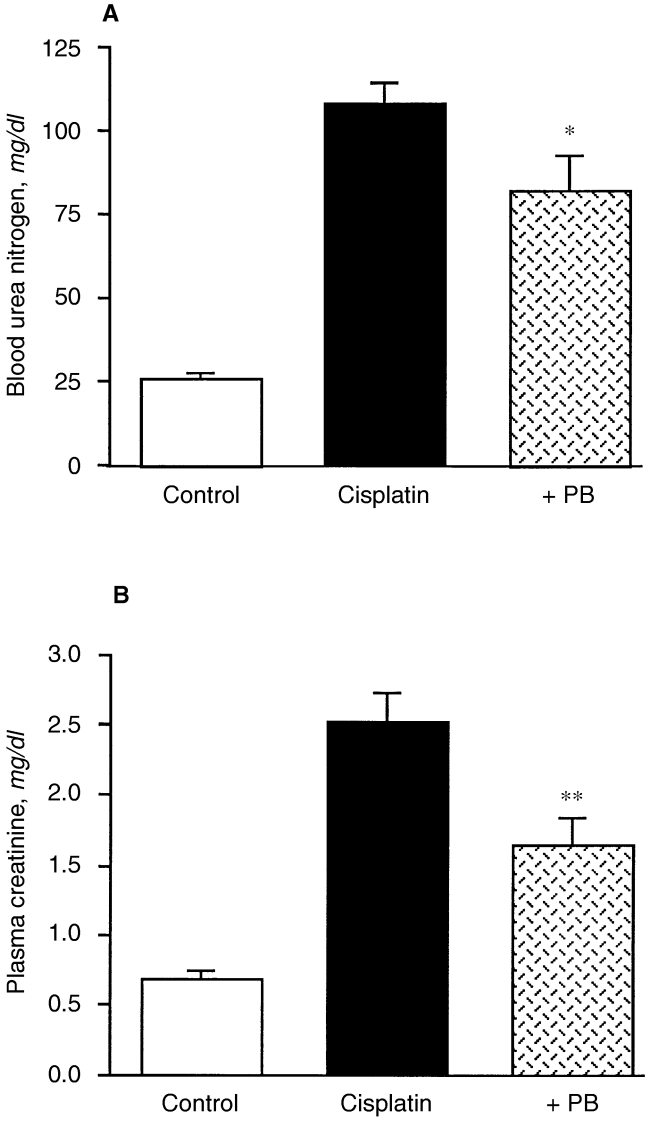


Fig. 3. Effect of cytochrome P-450 inhibitor on cisplatin-induced acute renal failure as measured by blood urea nitrogen (*A*) and plasma creatinine (*B*). Rats were given intraperitoneal injection of cisplatin (10 mg/kg body wt) and were sacrificed at 72 hours after injection. A cytochrome P-450 inhibitor, piperonyl butoxide (PB), was administered intraperitoneally twice in a dose of 400 mg/kg body wt at 48 hour intervals. Values are means ± SE. The number of animals used was: control, $N = 5$; cisplatin, $N = 9$; PB, $N = 9$. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with cisplatin treatment alone.

platinum, LLC-PK$_1$ cells were incubated with 200 μg/ml of cisplatin in HBSS for four hours at 37°C in the absence or presence of cimetidine (2 mM). At the end of experiment, the cells were scraped and washed four times with phosphate-buffered saline. Then the cells were lysed in 100 μl of water and sonicated. After centrifugation, the resulting supernatants were used to measure platinum level by atomic absorption spectroscopy with electrothermal atomization [24]. The assay was performed by Dr. Thomas Moyer in the Department of Laboratory Medicine and Pathology at Mayo Clinic in Rochester, Minnesota.

Table 1. Semiquantitative analysis of renal tubule histology in rats

Group	*N*	Histologic grade				
		0	I	II	III	IV
Control	5	5				
Cisplatin	5				2	3
+ Piperonyl butoxide	5	1	2	1	1	

Five rats in each group were used for kidney histology study. Renal function study in these subsets was not different from that in the overall experimental groups. Renal tubule histologic grading is as follows: 0, normal; I, areas of tubular epithelial cell swelling, vacuolar deneneration, necrosis, and desquamation involving < 25% of cortical tubules; II, similar changes involving > 25% but < 50%; III, similar changes involving > 50% < 75% of cortical tubules; IV, similar changes involving > 75%.

Statistical analysis

Values are expressed as mean ± standard error. Statistical analyses were performed using unpaired Student's *t*-test (for only two groups) and analysis of variance (for more than two groups). Statistical significance was considered at P value < 0.05.

RESULTS

In an *in vivo* study administration of cisplatin at a dose of 10 mg/kg body wt resulted in acute renal failure similar to our previous studies [10]. At 72 hours after cisplatin injection, BUN was significantly increased from 26 ± 1 ($N = 5$) to 109 ± 6 mg/dl ($N = 9$, $P < 0.01$) and plasma creatinine increased from 0.69 ± 0.05 ($N = 5$) to 2.58 ± 0.19 mg/dl ($N = 9$, $P < 0.01$), respectively. Catalytic iron content, as measured by the bleomycin-detectable iron assay, was significantly increased from a control value of 0.85 ± 0.06 to 1.70 ± 0.09 nmol/mg protein ($N = 5$, $P < 0.01$) in the kidney of rats treated with cisplatin. To determine whether cisplatin alters renal cytochrome P-450 content, we examined the cytochrome P-450 content in cisplatin-induced acute renal failure. As shown in Figure 1, there was a marked decrease in cytochrome P-450 content (0.03 ± 0.01 nmol/mg protein, $N = 9$, $P < 0.001$) in the kidney in cisplatin-treated rats compared to the control animals (0.12 ± 0.01 nmol/mg protein, $N = 5$). The loss of cytochrome P-450 content in the kidney was injury specific because there was no difference in the level of cytochrome P-450 content in the liver between the untreated and cisplatin-treated animals (Fig. 1).

We postulated that the heme moiety of cytochrome P-450 may serve as a significant source of catalytic iron in cisplatin-induced nephrotoxicity. To test this hypothesis, we examined the effect of a cytochrome P-450 inhibitor, piperonyl butoxide (which yields a metabolite that binds to the heme moiety of cytochrome P-450) [25] on the renal content of cytochrome p-450 and catalytic iron in cisplatin-induced acute renal failure. Piperonyl butoxide significantly prevented the loss of cytochrome P-450 content in the kidney of cisplatin-treated rats (Fig. 1). As a result of preservation of cytochrome P-450 in the kidney, piperonyl

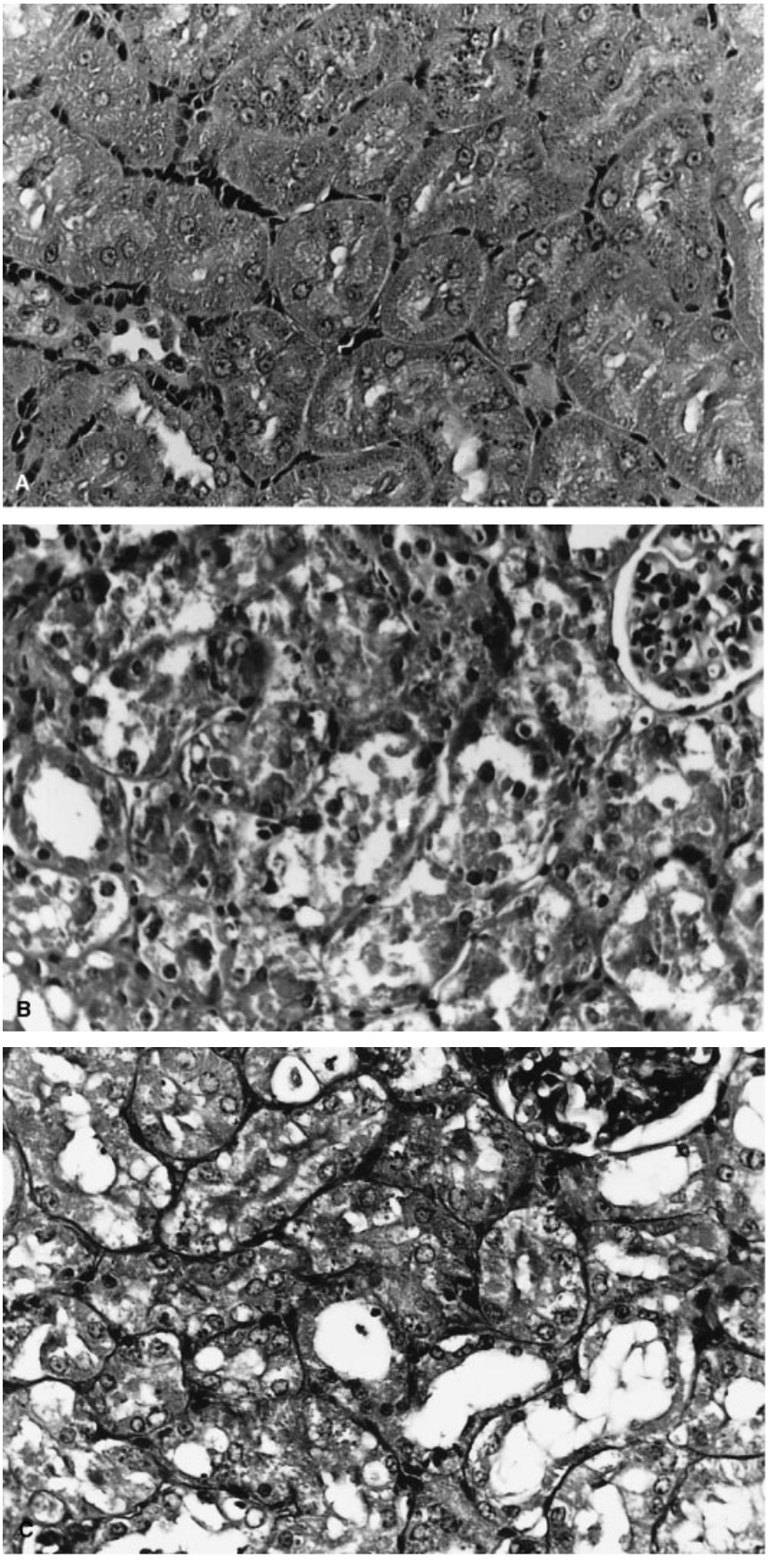


Fig. 4. Light-microscopy section of kidney. (*A*) Control rat showing no abnormalities. (*B*) Rat treated with cisplatin showing extensive epithelial cell vacuolization, swelling, desquamation and necrosis (grade IV) occurring predominantly in the proximal convoluted tubules. (*C*) Rat treated with a cytochrome P-450 inhibitor, piperonyl butoxide, showing a marked reduction in the extent of tubular damage (grade I).

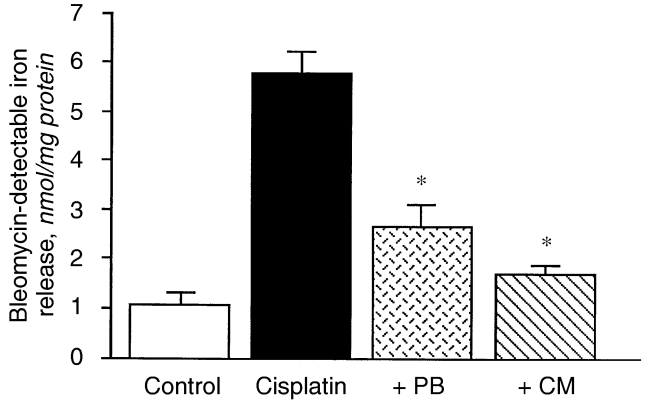


Fig. 5. Effect of cytochrome P-450 inhibitors on bleomycin-detectable iron release from LLC-PK$_1$ cells exposed to cisplatin. Cells were incubated with a cytotoxic dose of cisplatin (200 μg/ml) for a period of time before substantial cell killing occurred (2.5 hr) in the presence or absence of cytochrome P-450 inhibitors, cimetidine (2 mM) and piperonyl butoxide (1 mM). Values are means ± SE, $N = 3$. * $P < 0.01$, compared with cisplatin alone. Abbreviations are: Con, control; Cis, cisplatin; CM, cimetidine; PB, piperonyl butoxide.

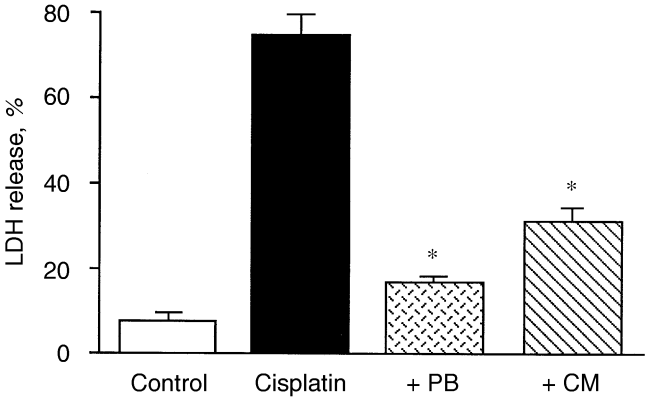


Fig. 6. Effect of cytochrome P-450 inhibitors on cisplatin-induced cytotoxicity to LLC-PK$_1$ cells as measured by LDH release. Cells were incubated with a cytotoxic dose of cisplatin (200 μg/ml) for a period of time necessary to induce consistent cytotoxicity (4 hr) in the presence or absence of cytochrome P-450 inhibitors, cimetidine (2 mM) and piperonyl butoxide (1 mM). Values are means ± SE $N = 4$. *$P < 0.01$ compared with cisplatin alone. Abbreviations are: Con, control; Cis, cisplatin; CM, cimetidine; PB, piperonyl butoxide.

butoxide also significantly blocked the increase in bleomycin-detectable iron content in the kidney of animals treated with cisplatin (Fig. 2). We then examined the effect of piperonyl butoxide on renal functional and histological change subjected to cisplatin treatment. Piperonyl butoxide provided a marked protection against cisplatin-induced acute renal failure as measured by BUN and creatinine (Fig. 3). For histology study, we obtained five rats from each group (control, cisplatin alone, cisplatin + piperonyl butoxide). Renal function studies in these subsets were not different from the overall experimental groups. The histological changes graded as described in the methods were summarized in Table 1. As compared to control rats (Fig. 4A), cisplatin-treated rats had extensive epithelial cell vacuolization, swelling, desquamation and necrosis (grade IV) occurring predominantly in the proximal convoluted tubules (Fig. 4B). There was a marked reduction in the extent of tubular damage (grade I) in those animals treated with piperonyl butoxide (Fig. 4C).

We also examined the role of cytochrome P-450 in an *in vitro* model of cisplatin-induced cytotoxicity to LLC-PK$_1$ cells. We used two different inhibitors of cytochrome P-450, cimetidine and piperonyl butoxide, to increase the specificity of our observation. Cimetidine has imidazole and cyano groups that inhibit cytochrome P-450 by interacting with the heme moiety [26]. This effect of cimetidine is specific for cytochrome P-450 as it does not interact with other heme enzymes [27]. Exposure to cisplatin led to a significant increase in catalytic iron release from LLC-PK$_1$ cells as measured by the bleomycin-detectable iron assay (Fig. 5). Both inhibitors, cimetidine and piperonyl butoxide, significantly prevented the bleomycin-detectable iron release from LLC-PK$_1$ cells subjected to cisplatin treatment (Fig. 5). Cimetidine and piperonyl butoxide also markedly reduced cisplatin-induced cytotoxicity to LLC-PK$_1$ cells as measured by LDH release (Fig. 6). The cytochrome P-450 inhibitor, cimetidine, did not affect the cellular uptake of cisplatin (data not shown). Taken together, our data strongly suggest a role for cytochrome P-450, a group of heme proteins, as a significant source of catalytic iron in cisplatin-induced nephrotoxicity.

DISCUSSION

Despite compelling evidence for the role of iron in various forms of tissue injury, the source of iron available to participate in the generation of hydroxyl radical or other iron-oxygen species remains largely unknown. *In vivo* most of the iron is bound to heme and nonheme proteins and does not directly catalyze the generation of hydroxyl radical [1]. Based on the observation that hydrogen peroxide and organic hydroperoxides can oxidatively degrade hemoglobin and promote the release of iron from the heme chelate [25], recent studies have demonstrated that cytochrome P-450 serves as a potential source of catalytic iron in several *in vivo* and *in vitro* models of myoglobinuric [9] and ischemia/reperfusion-induced acute renal failure [18].

In our recent study [10], we have demonstrated that the catalytic iron as measured by the bleomycin-detectable iron assay was significantly increased in LLC-PK$_1$ cells exposed to cisplatin and in the kidney of rats treated with cisplatin. Treatment with both iron chelators and hydroxyl radical scavengers substantially prevented cisplatin-induced cytotoxicity *in vitro* and acute renal failure *in vivo*. Taken together, these data suggest a critical role for iron in hydroxyl radical mediated cisplatin-induced nephrotoxicity.

However, the role of cytochrome P-450 as a source of catalytic iron in cisplatin-induced nephrotoxicity has not

been examined previously. In the present study, we have demonstrated that the treatment with cisplatin resulted in a significant increase of catalytic iron content accompanied by decreased cytochrome P-450 content in the kidney. The loss of cytochrome P-450 in the kidney induced by cisplatin appears to be injury specific, because there is no difference in cytochrome P-450 content in the liver between the untreated and cisplatin-treated rats. The cytochrome P-450 inhibitor, piperonyl butoxide, prevented cisplatin-induced loss of cytochrome P-450 and the increase of the bleomycin-detectable iron in the kidney. Similarly, piperonyl butoxide provided marked functional and histological protection against cisplatin-induced acute renal failure. In *in vitro* studies using LLC-PK_1 cells, the cytochrome P-450 inhibitors, cimetidine and piperonyl butoxide, also provided significant protection against cisplatin-induced increase in catalytic iron content and cytotoxicity. The cytochrome P-450 inhibitor, cimetidine, did not affect the cellular uptake of cisplatin (data not shown). Taken together, our data strongly suggest a role for cytochrome P-450, a group of heme proteins, as a significant source of catalytic iron capable of catalyzing free radical reactions in cisplatin-induced nephrotoxicity. However, our study does not exclude other roles of cytochrome P-450 in this model of renal injury.

In a model of cisplatin nephrotoxicity, reactive oxygen metabolites have been implicated [10, 15, 16, 19] and increased release of endogenous heme have been demonstrated [16]. It is very likely that oxidative stress could have directly attacked the heme moiety of the cytochrome P-450 promoting the release of heme from its binding protein. The inhibitors of cytochrome P-450 utilized in our study may have stabilized and protected the cytochrome P-450 enzymes, thereby decreasing the destruction of the heme chelate and the release of its iron. This is indicated by the marked protection against cisplatin-induced loss of cytochrome P-450 content in the kidney, and cytotoxicity afforded by the cytochrome P-450 inhibitors both *in vivo* and *in vitro* associated with a significant decrease in the bleomycin detectable iron.

The combination of cimetidine and verapamil has been tested in a limited number of patients treated with cisplatin [28]. This co-administration has been shown to exert a beneficial effect on renal hemodynamics and has been able to prevent the decrease in renal function that normally occurs with cisplatin chemotherapy. Verapamil by itself has been shown to have no significant effect in a rat model of cisplatin-induced nephrotoxicity [20]. Our results have shown that the cytochrome P-450 inhibitors afford the beneficial effect on cisplatin-induced nephrotoxicity. Thus, our observations may have obvious clinical implications in future studies with cytochrome P-450 inhibitors playing a protective role against cisplatin-induced nephrotoxicity.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health, the Department of Defense, Office of the Navy, and the Department of Veterans Affairs. We thank Ms. Xiaoman Hong for her technical assistance and Ms. Ellen Satter for her secretarial assistance.

Reprint requests to Sudhir V. Shah, M.D., Department of Medicine, Division of Nephrology, University of Arkansas for Medical Sciences, 4301 W. Markham Street, Slot 501, Little Rock, Arkansas 72205, USA.
E-mail: shahsudhirv@exchange.uams.edu

REFERENCES

1. Halliwell B, Gutteridge JMC: Role of free radicals and catalytic metal ions in human disease. An overview. *Method Enzymol* 186:1–85, 1990
2. Paller MS, Hedlund BE: Role of iron in postischemic renal injury in the rat. *Kidney Int* 34:474–480, 1988
3. Shah SV, Walker PD: Evidence suggesting a role for hydroxyl radical in glycerol-induced acute renal failure. *Am J Physiol* 255:F438–F443, 1988
4. Walker PD, Shah SV: Evidence of the role of hydroxyl radical in gentamicin-induced acute renal failure in rats. *J Clin Invest* 81:334–341, 1988
5. Shah SV: Role of reactive oxygen metabolites in experimental glomerular disease. *Kidney Int* 35:1093–1106, 1989
6. Walker PD, Shah SV: Hydrogen peroxide cytotoxicity in LLC-PK_1 cells: A role for iron. *Kidney Int* 40:891–898, 1991
7. Paller MS, Neumann TV: Reactive oxygen species and rat renal epithelial cells during hypoxia and reoxygenation. *Kidney Int* 40:1041–1049, 1991
8. Baliga R, Ueda N, Shah SV: Increase in bleomycin-detectable iron in ischaemia/reperfusion injury to rat kidneys. *Biochem J* 291:901–905, 1993
9. Baliga R, Zhang Z, Baliga M, Shah SV: Evidence for cytochrome P-450 as a source of catalytic iron in myoglobinuric acute renal failure. *Kidney Int* 49:362–369, 1996
10. Baliga R, Zhang Z, Baliga M, Ueda N, Shah SV: In vitro and in vivo evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. *Kidney Int* 53:394–401, 1998
11. Rozencweig M, von Hoff DD, Slavik M, Muggia FM: Cis-diamminedichloroplatinum (II). A new anticancer drug. *Ann Intern Med* 86:803–812, 1977
12. Blachley JD, Hill JB: Renal and electrolyte disturbances associated with cisplatin. *Ann Intern Med* 95:628–632, 1981
13. Ozols RF, Young RC: High-dose cisplatin therapy in ovarian cancer. *Semin Oncol* 12(Suppl 6):21–30, 1985
14. Safirstein R, Winston J, Moel D, Dikman S, Guttenplan J: Cisplatin nephrotoxicity: Insights into mechanism. *Int J Androl* 10: 325–346, 1987
15. Sugihara K, Gemba M: Modification of cisplatin toxicity by antioxidants. *Jpn J Pharmacol* 40:353–355, 1986
16. Agarwal A, Balla J, Alam J, Croatt AJ, Nath K: Induction of heme oxygenase in toxic renal injury: A protective role in cisplatin nephrotoxicity in the rat. *Kidney Int* 48:1298–1307, 1995
17. Bysani GK, Kennedy TP, Ky N, Rao NV, Blaze CA, Hoidal JR: Role of cytochrome P-450 in reperfusion injury of the rabbit lung. *J Clin Invest* 86:1434–1441, 1990
18. Paller MS, Jacob HS: Cytochrome P-450 mediates tissue-damaging hydroxyl radical formation during reoxygenation of the kidney. *Proc Natl Acad Sci USA* 91:7002–7006, 1994
19. Bompart G: Cisplatin-induced changes in cytochrome P-450, lipid peroxidation and drug-metabolizing enzyme activities in rat kidney cortex. *Toxicol Lett* 48:193–199, 1989
20. Capasso G, Giordano DR, de Tommaso G, De Santo NG, Massry SG: Parathyroidectomy has a beneficial effect on experimental cisplatin nephrotoxicity. *Clin Nephrol* 33:184–191, 1990
21. Cushner HM, Barnes JL, Stein JH, Reineck HJ: Role of volume depletion in the glycerol model of acute renal failure. *Am J Physiol* 250:F315–F321, 1986

22. ZAGER RA: Combined mannitol and deferoxamine therapy for myohemoglobinuric renal injury and oxidant tubular stress. Mechanistic and therapeutic implications. *J Clin Invest* 90:711–719, 1992
23. OMURA T, SATO R: The carbon monoxide-binding pigment of liver microsomes. I. Evidence of its hemoprotein nature. *J Biol Chem* 239:2370–2378, 1964
24. BALCERZAK M: Analytical methods for the determination of platinum in biological and enviromental materials. A review. *Analyst* 122:67R–74R, 1997
25. MAYS DC, HILLIARD JB, WONG DD, GERBER N: Activation of 8-methoxypsoralen by cytochrome P-450. Enzyme kinetics of covalent binding and influence of inhibitors and inducers of drug metabolism. *Biochem Pharmacol* 38:1647–1655, 1989
26. RENDIC S, KAJFEZ F, RUF HH: Characterization of cimetidine, ranitidine, and related structures' interaction with cytochrome P-450. *Drug Metab Dispos* 11:137–142, 1983
27. BAIRD MB, SFEIR GT, SLADE-PACINI CD: Lack of inhibition of mouse catalase activity by cimetidine: An argument against a relevant general effect of cimetidine upon heme metabolic pathways. *Biochem Pharmacol* 36:4366–4369, 1987
28. SLEIJFER DT, OFFERMAN JJG, MULDER NH, VERWEIJ M, VAN DER HEM GK, SCHRAFFORDT KOOPS H, MEIJER S: The protective potential of the combination of verapamil and cimetidine on cisplatin-induced nephrotoxicity in man. *Cancer* 60:2823–2828, 1987