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Effects of Prochlorperazine on Experimental Nephrotoxicity

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Summary. *In early studies of the antitumor drug 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1nitrosourea (methyl-CCNU), animal models consistently predicted that the compound would be nephrotoxic in humans. Nephrotoxicity in cancer patients who had received methyl-CCNU was not confirmed until about 6 years after clinical trials began. We have investigated the possibility that prochlorperazine, a commonly used antiemetic, might affect the development of neprhotoxicity. Prochlorperazine (1, 2, 5, and 8 mg/kg IP on days 1–3) produced a dose-related reduction in the concentrations of plasma urea nitrogen in mice that received nephrotoxic doses of methyl-CCNU (42, 52, or 63 mg/kg IP on day 1). The frequency and severity of renal lesions evaluated histopathologically were reduced significantly as the prochlorperazine dose increased. To study further this apparent protective activity of prochlorperazine, we chose a second nephrotoxin, mercuric chloride ($HgCl_2$, 1 mg/kg IP on day 1) and a rodent species used more commonly as a model for nephrotoxicity, the rat. Prochlorperazine (2.5 or 10 mg/kg IP on days 1–5) inhibited $HgCl_2$-induced urinary excretion of N-acetylglucosaminidase and leucine aminopeptidase. Urinary excretion of these enzymes on day 1 reflected proximal tubular epithelial degeneration and necrosis in rats that received $HgCl_2$ alone. The severity of $HgCl_2$-induced renal lesions evaluated histopathologically on day 16 was significantly reduced by combination treatment with prochlorperazine. Phenothiazines have numerous pharmacologic properties that might account for this observation, and additional studies will be required to establish the mechanism of this protective effect of prochlorperazine against acute nephrotoxicity in rodents.*

Introduction

In 1976, our initial studies of methyl-CCNU indicated it was a nephrotoxin in mice [2]. Other data available at that time and reviewed recently [6] indicated that methyl-CCNU was nephrotoxic in dogs and monkeys as well. Because nephrotoxicity in cancer patients who received methyl-CCNU was not reported until later [5], it seemed at the time that animal models historically predictive for drug toxicity in humans had overpredicted methyl-CCNU nephrotoxicity. After the clinical report by Harmon et al. in 1979 [5], the question of the sensitivity of the animal models became a question of the apparent insensitivity of humans. Whatever its cause, species-specific insensitivity to drug toxicity is one factor that often contributes to delays in detecting drug toxicity in humans [15, 23]. For methyl-CCNU, the question of the cause for delay in detecting clinical nephrotoxicity remains open. We present here evidence that prochlorperazine, an antiemetic used frequently to control nausea and vomiting in cancer patients receiving chemotherapy, exerts a protective effect against acute nephrotoxicity in two rodent species. A similar effect, if it occurred in humans, may have prevented, masked, or delayed nephrotoxicity in earlier clinical studies of methyl-CCNU.

Material and Methods

Mouse Studies. Young, adult male CDF_1 mice were obtained from Simonsen Laboratories, Gilroy, CA, USA. Each mouse weighed 18–23 g at the time it was treated, and all the mice were treated on the basis of their individual body weights. Husbandry during these studies has been described [7]. The mice were caged individually and were fed Wayne Lab-Blox F6 (Allied Mills, Inc., Chicago, IL, USA) and tap water ad libitum. Mice were randomly distributed into the following groups of 10–20 mice each except where noted otherwise: three groups (5/group) received prochlorperazine 1, 5, or 10 mg/kg/day for 3 days; three groups received methyl-CCNU 42, 52, or 63 mg/kg; 15 groups received prochlorperazine 1, 2, 5, 8, or 10 mg/kg followed 30 min later by methyl-CCNU, 42, 52, or 63 mg/kg. The mice that received the combination also received two subsequent daily doses of prochlorperazine. Control mice received drug diluent only. Methyl-CCNU was supplied by the Developmental Therapeutics Program, DCT, NCI (Bethesda, MD, USA). It was suspended in aqueous NaCl (0.9 g/100 ml) that contained Tween 80 (0.05 g/100 ml). Each suspension was injected within 15 min after preparation. Prochlorperazine was obtained commercially (Compazine, Smith Kline & French Laboratories Philadelphia, USA). Drug concentrations were chosen so that IP injection of 0.1 ml/10 g body wt delivered the desired dosages. All drugs were administered as single daily doses. The doses of methyl-CCNU (42, 52, and 63 mg/kg) approximated the respective LD_{10}, LD_{50}, and LD_{100}. Mice were killed on day 6 (day of first treatment = day 1). Blood was collected for determination of urea nitrogen, and the kidneys were collected for histopathologic evaluation. Procedures for euthanasia, blood collection, sample preparation, necropsy, histotechnology, semiquantita-

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The methyl-CCNU studies presented here were described briefly in an abstract in Pharmacologist 19: 236, 1977

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tive histopathologic evaluation, and plasma urea nitrogen determination have been described [7, 8].

Rat Studies. Young, adult male Sprague-Dawley rats were obtained from Harlan Industries, Inc., Indianapolis, IN, USA. Each rat weighted 100–150 g at the time it was treated, and all the rats were treated on the basis of their individual body weights. The rats were caged individually in polycarbonate cages with hardwood bedding (Ab-sorb-dri, Garfield, NJ, USA), and they were fed Purina rodent chow (no. 5001) and tap water ad libitum. In duplicate experiments, rats were randomly distributed into groups of four each that received either single IP doses of mercuric chloride ($HgCl_2$, 1 mg/kg, Sigma Chemical Co., St. Louis, USA) or a combination of $HgCl_2$ (1 mg/kg) and prochlorperazine (2.5 or 10 mg/kg IP). Prochlorperazine or diluent was administered as a single dose 1 h before $HgCl_2$ on the first day of treatment (day 1) and daily thereafter for an additional 4 days. Prochlorperazine edisylate was the generous gift of Dr Barry Berkowitz, Smith Kline & French Laboratories, Philadelphia, USA, $HgCl_2$ and prochlorperazine were each dissolved in aqueous NaCl (0.9 g/100 ml) and diluted to permit injection of 0.3 ml/100 g body wt. Control rats received no treatment.

Rats were weighed on days 1–7, 15, and 16. On days 1, 3, 6, and 15, rats were placed in polycarbonate metabolism cages for 24 h for the collection of urine. The volume of each 24-h urine sample was recorded, and each sample was centrifuged at 1,000 *g* to remove sediment. Samples were refrigerated at 5° C until determinations of creatinine, *N*-acetylglucosaminidase (NAG), and leucine aminopeptidase (LAP) were performed. Creatinine concentration was determined using an alkaline picrate procedure (Sigma no. 555, Sigma Chemical Co. St. Louis, USA). NAG activity was determined by hydrolysis of *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Sigma Chemical Co. St. Louis, USA) according to the method of Lockwood and Bosmann [12]. LAP activity was determined by hydrolysis of L-leucyl-β-naphthylamide (Sigma no. 251, Sigma Chemical Co. St. Louis, USA). To correct for individual variability in urine production in the rats, urinary NAG and LAP activities were expressed as units per milligram of creatinine excreted. Units of enzyme activity were defined as described in the methods indicated [12; Sigma no. 251]. On day 16, rats were euthanized by exsanguination under chloroform anesthesia. Kidneys were removed quickly, trimmed of fat, weighed as pairs, and fixed in buffered formalin (10 g formaldehyde/100 ml phosphate buffer, pH 7). Kidney samples were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin [14]. Slides were coded, randomized, and read blind. Lesion severity was scored as described previously [8] using the scores indicated in the footnote to Table 3.

Statistical Analyses. Data on the frequency of renal lesions in mice were tested for a linear trend in proportions [19]. Lesion severity scores from rats that received $HgCl_2$ plus prochlorperazine were compared with scores from rats that received only $HgCl_2$ by Wilcoxon's rank sum test for unpaired samples [22]. Enzyme activity data were assumed to conform to a log-normal distribution. Student's *t*-test [22] was applied to these data after a log transformation [9]. Kidney : body wt ratios were also subjected to Student's *t*-test [22].

Results

Effects of Prochlorperazine on Methyl-CCNU-Induced Nephrotoxicity in Mice

Production of proximal renal tubular necrosis in mice by single doses of methyl-CCNU was reproducible and consistent throughout many experiments. We had observed that the microscopic appearance of these lesions depended upon the time permitted for recovery after treatment [2]. Lesions were well developed and azotemia was maximal 6 days after a single IP injection. Kidneys evaluated 30 days posttreatment exhibited varying degrees of fibrosis and evidence of attempted tubular repair [2]. In the present experiments, the mice were euthanized on day 6 to avoid early deaths. Figure 1A indicates that plasma urea nitrogen concentrations on day 6 increased in response to higher methyl-CCNU doses. This dose-response was reflected in the severity and frequency of renal lesions

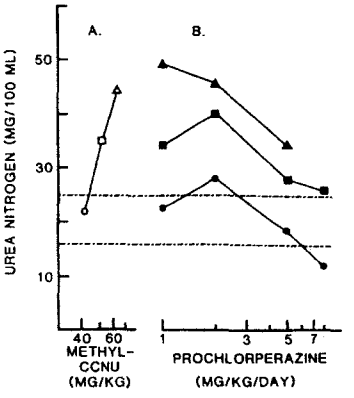

Fig. 1 A and **B.** Effect of prochlorperazine on methyl-CCNU-induced azotemia in mice. **A** Plasma urea nitrogen concentrations of day 6 after a single IP dose of methyl-CCNU: (0) 42 mg/kg; (□) 52 mg/kg; (△) 63 mg/kg. **B** Plasma urea nitrogen concentrations on day 6 in mice that received 1, 2, 5, or 8 mg prochlorperazine/kg IP on days 1–3. The first prochlorperazine dose was followed in 30 min by a single IP dose of methyl-CCNU: (●) 42 mg/kg; (■) 52 mg kg; (▲) 63 mg/kg. Each *point* represents the mean of at least 10 mice. The *horizontal broken lines* define the reference range for urea nitrogen concentration determined for untreated mice

Table 1. Effect of prochlorperazine on the severity of renal lesions in mice treated with Methyl-CCNU

Methyl-CCNU (mg/kg)	Prochlorperazine (mg/kg/day)					
	0	1	2	5	8	10
0	0	0	–	0	–	0
42	0.98 ± 0.08[a]	0.80 ± 0.13	1.10 ± 0.10	0.63 ± 0.11	0.40 ± 0.10	0
52	1.13 ± 0.08	1.11 ± 0.16	1.00 ± 0.01	0.97 ± 0.12	0.46 ± 0.11	0
63	2.12 ± 0.06	1.75 ± 0.25	1.90 ± 0.16	1.17 ± 0.09	[b]	[b]

[a] Mean (± SEM) degree of lesion severity, using 0 = no lesion, 1 = mild, 2 = moderate, and 3 = severe
[b] No survivors

Table 2. Effect of prochlorperazine on the frequency of renal lesions of severity ≥ 1 in mice treated with methyl-CCNU[a]

Prochlorperazine (mg/kg/day)	Methyl-CCNU (mg/kg)		
	42	52	63
0	16/20[b]	18/19	13/13
1	8/10	7/9	1/2
2	10/10	10/10	8/10
5	12/20	14/17	1/12
8	3/15	1/13	[c]
10	0/4	0/1	[c]

[a] Data for 63 mg/kg methyl-CCNU are the frequencies of lesions of severity ≥ 2

[b] The numerator is the number of mice with renal lesions of the severity indicated, and the denominator is the number of survivors available for study on day 6

[c] No survivors

(Tables 1 and 2) in mice that received methyl-CCNU alone. Figure 1B indicates the effects on plasma urea nitrogen when prochlorperazine was administered in combination with methyl-CCNU. Although the differences in urea nitrogen concentrations were not statistically significant, the data suggest that as the dose of prochlorperazine was increased, less renal damage developed. This is confirmed by the data presented in Table 1. The severity of renal damage reflected the methyl-CCNU dose, and as the combined dose of prochlorperazine increased, the severity of the renal lesions decreased. Table 2 presents data on the frequency of lesions of specified severity. The proportion of mice with renal lesions decreased as the dose of prochlorperazine increased. The trends in these data are statistically significant ($P < 0.001$) for each of the three methyl-CCNU doses studied (Table 2).

Effects of Prochlorperazine on $HgCl_2$ Nephrotoxicity in Rats

Rats were used for these experiments because most of the available data on mechanisms of nephrotoxicity have been obtained with the rat model [10]. We verified that 1 mg $HgCl_2$/kg IP was an optimal, sublethal, nephrotoxic dose [10]. Renal lesions induced by $HgCl_2$ were maximally severe in microscopic appearance on day 6. They were nevertheless readily evaluable on day 16, and we chose to kill the rats on day 16 to permit adequate evaluation of the time-course of enzymuria. Figure 2 indicates that urinary excretion of NAG in

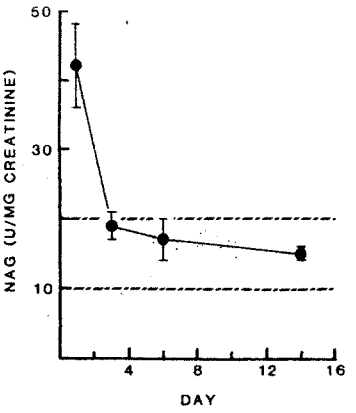

Fig. 2. Urinary excretion of NAG by rats treated with $HgCl_2$. Each rat received a single IP dose of 1 mg $HgCl_2$/kg on day 1, and urine was collected for 24 h on days 1, 3, 6, and 15. Urine was analyzed for NAG activity and creatinine concentration. Each *point* represents the mean ± SEM of 27 rats. The *horizontal broken lines* define the reference range for urinary NAG activity determined for untreated rats

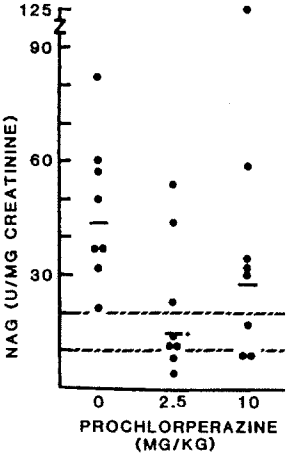

Fig. 3. Effect of prochlorperazine on $HgCl_2$-induced urinary excretion of NAG in rats. Rats were treated with prochlorperazine at the doses indicated plus 1 mg $HgCl_2$/kg. Each *point* represents the urinary NAG activity of an individual rat; data are for the first 24 h after $HgCl_2$ treatment. The *horizontal broken lines* define the reference range for urinary NAG activity determined for untreated rats. The *horizontal bars* represent the mean log of each group. *The means of the $HgCl_2$-only group and the 2.5 mg prochlorperazine/kg group are significantly different ($P < 0.001$)

Table 3. Effects of prochlorperazine treatment on indicators of nephrotoxicity in rats that received 1 mg/kg of $HgCl_2$

Prochlorperazine (mg/kg/day)	Urinary NAG activity (U/mg creatinine)[a]	Urinary LAP activity (U/mg creatinine)[a]	Microscopic evidence of renal tubular degeneration on day 16	
			Frequency[b]	Severity[c]
0	44 ± 2	27 ± 3	8/8	3.1 ± 0.3
2.5	15 ± 2[d]	8 ± 6[d]	8/8	1.7 ± 0.3[d]
10.0	28 ± 2[d]	13 ± 5	6/8	1.9 ± 0.6

[a] Day 1, mean (± SD)

[b] The numerator is the number of rats with microscopically evident lesions, and the denominator is the number of rats studied

[c] Mean (± SEM) degree of lesion severity using 0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate, and 4 = moderately severe

[d] Significantly lower ($P < 0.05$) than value for group that received $HgCl_2$ only

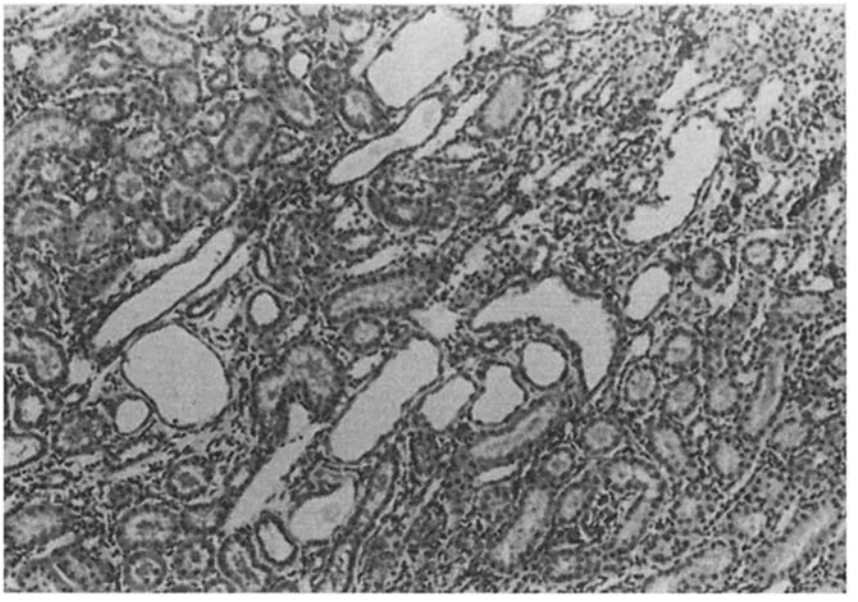

Fig. 4. Effect of $HgCl_2$ on rat kidney. This rat received a single IP dose of 1 mg $HgCl_2$/kg on day 1 and was killed on day 16. Dilated tubules are evident in the inner cortex and in the outer stripe of the medulla. (H & E; × 100)

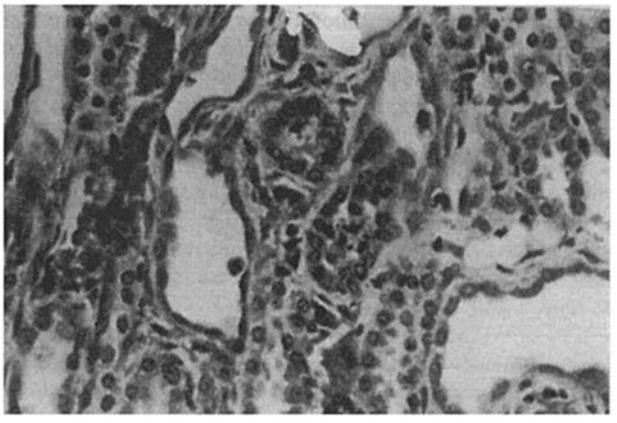

Fig. 5. Effect of $HgCl_2$ on rat kidney. Higher magnification of the section described in Fig. 4 reveals dilated tubules lined by flattened, elongated (low-lying) epithelial cells. Degenerating cells and cellular debris are evident in the lumen of the adjacent tubule. (H & E; × 400)

rats treated with 1 mg $HgCl_2$/kg was maximal during the first 24 h posttreatment. NAG activity then remained within the reference range throughout the remainder of the observation period. The time course of urinary LAP excretion was virtually identical with this (data not shown).

Urinary excretion of NAG during the first 24 h after $HgCl_2$ or $HgCl_2$ plus prochlorperazine is presented in Fig. 3. The data reveal individual variability similar to that observed in humans who received nephrotoxic doses of *cis*-diamminedichloroplatinum II [11]. Individual LAP data (not shown) were similar to the data for NAG (Fig. 3). NAG and LAP excretion is summarized in Table 3. The differences in urinary NAG and LAP between rats treated with $HgCl_2$ only and rats treated with $HgCl_2$ plus prochlorperazine (2.5 mg/kg/day) were statistically significant. The biological significance of this apparent protection is evident from the histopathologic evaluation summarized in Table 3 and illustrated in Figs. 4–7. On day 16, proximal tubules distributed focally within the inner cortex and outer stripe of the medulla were dilated and lined by flattened, elongated (low-lying) epithelial cells (Fig. 4). Many of these

Table 4. Effect of prochlorperazine on kidney: body weight ratios in rats that received 1 mg $HgCl_2$/kg

Prochlorperazine (mg/kg/day)	Kidney wt (g) / Body wt (g) (%)[a]
0	1.22 ± 0.06
2.5	0.97 ± 0.03[b]
10.0	1.08 ± 0.03[b]

[a] Mean ± SEM; kidneys were paired
[b] Significantly different ($P < 0.05$) from value for rats that received $HgCl_2$ only

epithelial cells contained large atypical regenerating hyperchromatic nuclei. Degenerating cells and cellular debris were present in the lumen of isolated tubules (Fig. 5). Epithelial cells lining some of the affected tubules were heavily mineralized. These changes (Figs. 4–7) were scored semiquantitatively (see footnote, Table 3). Kidneys from two rats that received the highest dose of prochlorperazine exhibited no lesions. Rats that received 2.5 mg/kg/day developed renal lesions that were significantly milder overall ($P < 0.05$) than lesions that resulted from $HgCl_2$ alone (Table 3). These histopathologic results were also reflected in kidney: body wt ratios presented in Table 4.

Discussion

Although animal models consistently predicted the nephrotoxicity of methyl-CCNU [6], reports of methyl-CCNU-induced nephrotoxicity in human cancer patients did not begin to appear until the compound had received relatively extensive clinical evaluation [5]. Vesell [23] and Melmon and Nierenberg [15] have discussed a number of reasons for such delays. In cancer patients, observations of drug toxicity are additionally complicated by the extent of drug treatment prior to or simultaneous with the use of a new drug. Multidrug therapy obscures the identity of a causative toxicant and enhances the probability of drug interactions [20]. An interaction by prochlorperazine was suggested by a report that promethazine

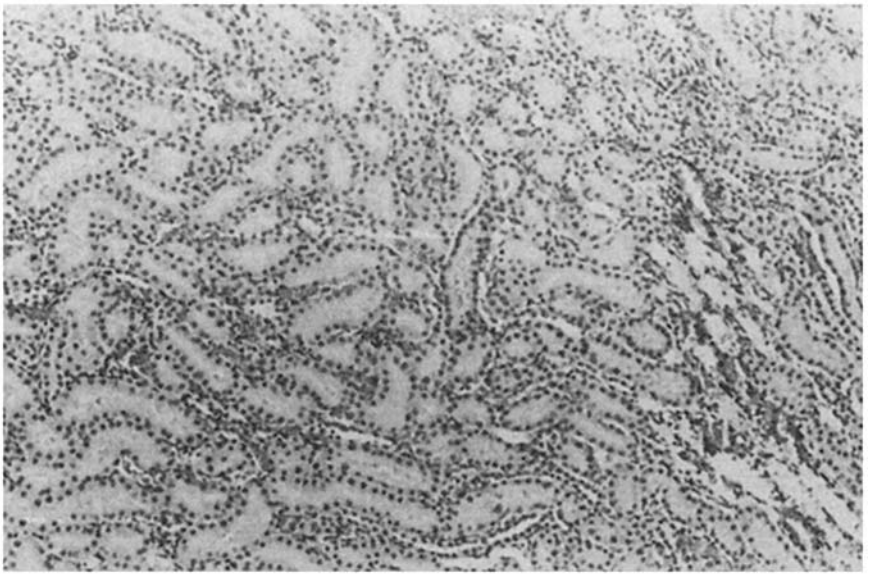

Fig. 6. Effect of prochlorperazine on the $HgCl_2$-induced renal lesion. This rat received 2.5 mg prochlorperazine/kg on days 1–5, combined with $HgCl_2$ 1 mg/kg on day 1. The inner cortex and the outer stripe of the medulla exhibit little evidence of $HgCl_2$ nephrotoxicity and resemble the kidneys of rats that received no treatment (not shown). (H & E; × 100)

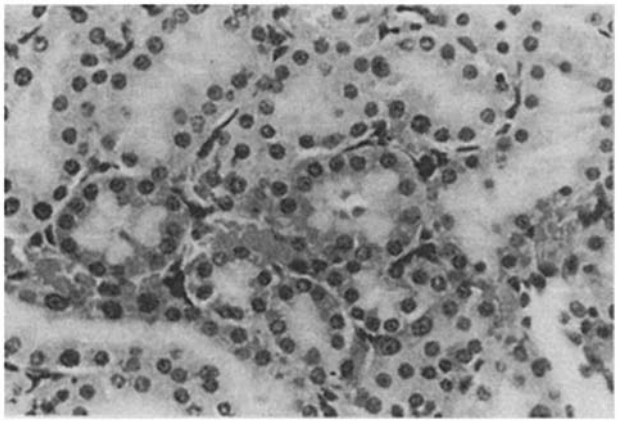

Fig. 7. Effect of prochlorperazine on the $HgCl_2$-induced renal lesion. Higher magnification of the section described in Fig. 6 reveals proximal tubules unaffected by $HgCl_2$ treatment. (H & E; × 400)

modified the pathologic progression of carbon tetrachloride toxicity in rats [17]. Promethazine protected rats against the development of hepatic necrosis. Patients treated with methyl-CCNU commonly received prochlorperazine (Compazine) or other phenothiazines to control the nausea and vomiting induced by this nitrosourea [4, 25]. An effect of prochlorperazine on the kidney similar to the reported effect of promethazine on the liver might have affected the onset of nephrotoxicity in humans. The results of our studies indicate that prochlorperazine protects the kidney against the nephrotoxic effects of methyl-CCNU in mice. This raised the question as to whether this effect was limited to methyl-CCNU, nitrosoureas as a class, or alkylating agents in general, or whether it extended to other nephrotoxins that may act by different mechanisms. The ultrastructural and biochemical effects of mercuric ions have been described extensively [10], and $HgCl_2$ is a classic nephrotoxin [18] well suited for testing the effect of prochlorperazine.

The choice of urinary enzyme activity as an indicator of nephrotoxicity in rats was based on the growing usefulness of this technic [3, 13, 21]. NAG and LAP are localized in the renal tubular epithelium, and activity in the urine is diagnostic for proximal tubular injury [11]. Moreover, enzymuria usually precedes other indications of tubular damage [13]. In the present study, prochlorperazine (2.5 mg/kg/day) prevented an $HgCl_2$-induced increase in enzymuria and reduced by half the severity assessed for the residual renal lesions on day 16. This apparent protective effect was not as clearly evident with a prochlorperazine dose of 10 mg/kg/day, although two individual rats that received this dose plus $HgCl_2$ developed no lesions. The more extensive dose-response data from our mouse studies suggest that at 10 mg/kg/day, prochlorperazine itself may have contributed to the toxicity. We made no attempt to optimize the prochlorperazine dosage regimen, which was based on the observations of Rees and Spector [17].

Phenothiazines exert a number of pharmacologic effects that might account for our observations [1]. These include varying degrees of diuresis and a tendency to increase renal blood flow. Although the relative ability of prochlorperazine to affect renal physiology directly is not clear, studies in progress in our laboratory indicate that cytoprotection by phenothiazines may not be limited to prochlorperazine. Structure-activity correlations [16] may help to clarify whether cytoprotection is related to effects on renal dynamics. Alternative explanations for cytoprotection must consider biologic activities common to phenothiazines as a class, for example, the effect of phenothiazines on intracellular calcium regulation [24]. Additional studies will be required to determine how prochlorperazine produces the apparant cytoprotection observed here.

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