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Lack of *in vivo* evidence of a cytochrome P450 metabolite participating in aminoglycoside nephrotoxicity

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Abstract—Recent *in vitro* evidence has suggested that the cytotoxicity of aminoglycosides may be mediated by a metabolite generated by the hepatic cytochrome P450 drug-metabolizing system. This postulate has been tested by pretreating rats with cobalt protoporphyrin IX (CoP) to suppress hepatic P450 levels prior to administration of gentamicin. CoP pretreatment was observed to suppress antipyrine clearance markedly but not to alter gentamicin nephrotoxicity.

Recent *in vitro* studies have raised the possibility that the hepatic P450-dependent drug metabolism system may be involved in the cytotoxicity of aminoglycosides. Thus, when cochlear outer hair cells in short-term culture were exposed to gentamicin, no cytotoxicity was observed unless an aliquot of the "S9" hepatic fraction was included in the culture medium [1]. Further, this cytotoxicity was diminished when the cytochrome P450 cofactor, NADPH*, was omitted and when a non-selective cytochrome P450 inhibitor, ketoconazole [2], was added. To investigate the possible role of hepatic P450 in the cytotoxicity of aminoglycosides, advantage has been taken of the capacity of cobalt protoporphyrin IX (CoP), given as a single i.p. administration, to cause an extensive and long-lasting (>9 days) depression of hepatic cytochrome P450 in rats [3, 4]. This communication reports that CoP suppression of hepatic P450 activity did not result in protection of the rats from gentamicin nephrotoxicity.

Methods and Results

Animals. Forty-two male pathogen-free Sprague–Dawley rats, 6 weeks of age and weighing 218 ± 19 g, were obtained from Charles River Laboratories, Raleigh, NC. The rats were randomly assigned to five treatment groups and one negative control group, 5 rats per group (Table 1). An additional four rats were included in groups 1, 2, and 3 to determine the effect of the CoP treatment on antipyrine half-life in order to confirm that the pretreatment regimen had suppressed hepatic P450 in these experimental animals.

Antipyrine elimination. All rats in groups 2, 3, 5 and 6 received a single s.c. injection of CoP (Sigma Chemical Co., St. Louis, MO) on day zero. Forty-eight hours later (day 2), the four extra rats in each of groups 1, 2 and 3 were used to examine the effect of the CoP pretreatment regimens on antipyrine clearance. As shown in Fig. 1, CoP pretreatment caused a marked lengthening of the half-life of antipyrine in these experimental animals after both 25 and 50 μmol/kg, as compared with that of saline-treated controls. Calculation of antipyrine clearance values [3] indicated that the CoP pretreatments had caused a 55% depression after 25 μmol/kg and a 73% depression after 50 μmol/kg. Since antipyrine elimination is considered to depend solely on hepatic P450 [5], these data confirm that the rats used in this study had experienced significant suppression of their hepatic P450 levels.

Nephrotoxicity assessment. Following the single injection of CoP, rats from groups 4, 5 and 6 received 100 mg/kg gentamicin (Solo Pak, Franklin Park, IL) as a single daily s.c. injection for a total of 7 days from day 2 through day 8. Five rats in groups 1, 2 and 3 received sterile water for the same period.

(a) *Renal function.* When compared to the negative controls, analysis of 24-hr urine samples collected from all rats from all groups on day 7 through day 8 indicated significantly greater urine volume and numbers of epithelial cells and casts ($P < 0.05$) in urine of rats receiving gentamicin and gentamicin plus CoP at either dose. However, there were no significant differences in urine volume or epithelial cell numbers in rats receiving gentamicin alone when compared to rats receiving gentamicin and CoP at either dose. Casts were less obvious in the 50 μmol/kg CoP plus gentamicin group but this parameter is a less sensitive indicator of renal tubular damage than the excretion of cells [6]. CoP alone was without significant effect compared to negative controls.

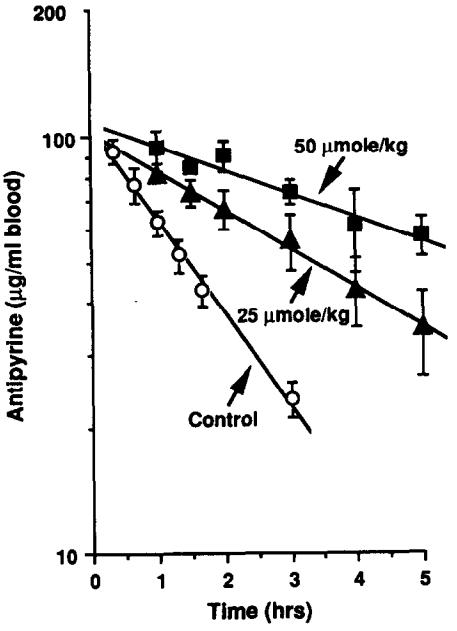
(b) *Renal morphology.* On day 8 rats were placed under deep anesthesia with pentobarbital, blood was collected from the heart, and the kidneys were removed and placed in 10% buffered formalin. Subsequently, the kidneys were sectioned and stained with hematoxylin and eosin for light microscopic evaluation. Without knowledge of treatment, microscopic nephrotoxicity was scored as previously described utilizing six separate nephrotoxic lesions [7]. The histopathologic scores of rats receiving gentamicin and gentamicin plus CoP were significantly different ($P < 0.05$) from controls (Table 1). However, there were no significant differences in nephrotoxicity scores between rats receiving gentamicin alone and rats receiving either dose of CoP plus gentamicin. A single injection of CoP was without renal effects at either dose.

Discussion

The pathogenic mechanisms responsible for either the ototoxic or nephrotoxic effects of aminoglycosides are still unresolved [1, 8]. Metabolism of these antibodies has been excluded based primarily on the almost complete recovery of the administered dose if the delayed urinary excretion is considered [9]. The initial *in vitro* demonstration that a toxic hepatic metabolite(s) may be required for cytotoxicity was determined by exposure of outer hair cells isolated from the cochlea of guinea pigs to aminoglycosides [1]. The cytotoxic results were attributed to cytochrome P450 metabolic activity. In our experiment, neither the histopathologic scores, a widely-utilized, sensitive comparative method [8], nor quantitative urine analysis, an accurate renal function test [6], indicated any decreased aminoglycoside nephrotoxicity when the cytochrome P450 system was suppressed by CoP.

Single doses of 50 μmol/kg of CoP profoundly depress hepatic cytochrome P450 content within 48 hr and the levels remain depressed for at least 7 days [3, 4]. CoP also markedly lowers cytochrome P450 levels in the kidney although this effect is somewhat slower in onset [4].

* Abbreviations: NADPH, reduced nicotinamide adenine dinucleotide phosphate; and CoP, cobalt protoporphyrin IX.



CoP μmole/kg	$T_{1/2}$ hr	Cls ml/min/kg
0	1.4 ± 0.06	6.92 ± 0.3
25	3.4 ± 0.70	3.15 ± 0.6
50	5.1 ± 0.85	1.89 ± 0.3

Fig. 1. Effect of CoP pretreatment on antipyrine elimination. Groups of four rats received CoP or saline as indicated, 48 hr prior to antipyrine (100 mg/kg, i.p.). Serial antipyrine plasma levels were obtained for each rat, and half-life and clearance were determined as previously described [3]. Values are means ± SEM, N = 4.

Table 1. Effect of cobalt protoporphyrin on gentamicin-induced nephrotoxicity

Group	Treatment	N	Urine volume* (mL)	Epithelial cells ($\times 10^4$/mL)	Casts ($\times 10^4$/mL)	Kidney scores
1	Control	5	14.2 ± 1.3	10.1 ± 6.8	0	1.0 ± 0.3
2	CoP 25	5	17.8 ± 2.1	1.7 ± 0.8	0	1.0 ± 0.3
3	CoP 50	5	21.8 ± 2.3	5.7 ± 3.3	0	0.4 ± 0.3
4	Gentamicin	5	34.4 ± 3.1†	77.2 ± 27.5†	8.5 ± 3.5†	8.6 ± 1.4†
5	CoP 25 + gentamicin	5	40.0 ± 4.8†	127.4 ± 14.4†	4.8 ± 1.4†	8.8 ± 0.9†
6	CoP 50 + gentamicin	5	41.0 ± 4.9†	88.6 ± 22.5†	1.5 ± 0.8	10.4 ± 0.5†

Cobalt protoporphyrin was given as a single s.c. injection of either 25 or 50 μmol/kg; gentamicin was given as a single daily s.c. injection (100 mg/kg/day) for a total of 7 days (day 2 through day 8). Scheffe's test was used for multiple comparisons of treated groups. All values are means ± SEM.

* Twenty-four hour collection period.

† Significantly different from control ($P < 0.05$).

Nevertheless, it is theoretically possible that a small amount of a cytotoxic metabolite could have been produced *in vivo* by some remaining active hepatic or renal P450 system in our experiment. However, no statistical difference in functional or morphologic nephrotoxicity was evident when the CoP plus gentamicin-treated rats were compared to rats that received only gentamicin. Considering that almost all of the aminoglycoside dose is recovered in the urine or retained in renal tissue as parent antibiotic [9], it is difficult to believe that a small amount of a toxic metabolite(s) remaining in the presence of CoP would be capable of producing nephrotoxicity equivalent to the "gentamicin only" treatment in which the full capacity of cytochrome P450 metabolism was available.

Very recently, the group which originally proposed the involvement of cytochrome P450 in the formation of a toxic metabolite from gentamicin extended their fractionation and identified a hepatic cytosolic fraction devoid of cytochrome P450 as responsible for the metabolism [10]. Our *in vivo* attempt to assess the involvement of a cytochrome P450-generated cytotoxic metabolite(s) in aminoglycoside nephrotoxicity in rats was unsuccessful and corroborates this latter report.

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