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Tin Protoporphyrin Inhibits Carbon Monoxide Production in Adult Mice

GREGORY S. MILLEVILLE, MICHAEL D. LEVITT, AND ROLF R. ENGEL

Departments of Pediatrics and Internal Medicine, Hennepin County Medical Center, Research Service, Veteran's Administration Medical Center, and the University of Minnesota School of Medicine, Minneapolis, Minnesota 55415

ABSTRACT. We studied the effect of tin protoporphyrin, a potent inhibitor of heme oxygenase (EC 1.14.99.3), on carbon monoxide (CO) production in mature mice. Measurements of CO production provide a sensitive, noninvasive means of quantitating heme catabolism. CO accumulation in the gas space of closed chambers was decreased by about 25% for mice treated with two 50 nmol/g doses of tin protoporphyrin as compared to saline-treated controls. Calculated rates of CO production were 0.28 ± 0.07 and 0.40 ± 0.05 nmol·g^{-1}·h^{-1} for mice injected with tin protoporphyrin and saline, respectively ($p < 0.01$). When hemin (125 nmol/g) was administered to simulate hemolysis, CO production increased markedly in both saline- and tin protoporphyrin-treated mice. However, the rate of CO production in tin protoporphyrin-treated mice was only 44% that of saline-treated animals ($p < 0.0001$). These studies demonstrate that tin protoporphyrin inhibits heme catabolism in both the basal- and heme-loaded states and confirm that this inhibition is at the heme oxygenase step in the heme to bilirubin pathway. (*Pediatr Res* 19: 94–96, 1985)

Abbreviation

CO, carbon monoxide

Heme oxygenase (EC 1.14.99.3) catalyzes the equimolar formation of CO and biliverdin from heme, which is the first step in the conversion of heme to bilirubin (17). Since CO production results solely from heme catabolism, CO production quantitatively reflects the rate of heme catabolism and parallels bile pigment production (3).

Recently, Drummond and Kappas (6) reported that administration of tin protoporphyrin to newborn rats prevented the rise in heme oxygenase activity and serum bilirubin concentration which normally occurs during the neonatal period. In the present report we utilized measurements of CO production to quantitate the influence of tin protoporphyrin on heme catabolism in adult mice.

MATERIALS AND METHODS

Materials. Tin protoporphyrin was obtained from Porphyrin Products (Logan, UT). Hemin (bovine) was purchased from Sigma (St. Louis, MO). Each of these components was dissolved in 0.5% $NaHCO_3$ solution. Swiss-White mice were obtained from Bio-Lab Corp (St. Paul, MN). Soda lime (Sodasorb) was obtained from W. R. Grace & Co. (Lexington, MA).

Gas assays. Gas samples were analyzed by gas chromatography for O_2 with a thermal conductivity detector (Beckman, Palo Alto, CA) and for CO with a reduction detector (Trace Analytical, Menlo Park, CA) in series. Samples were injected via gas sample valves into a 3 ft by ⅛ inch diameter stainless steel column packed with molecular sieve 5A. Column temperature was maintained at 100° C and highly purified argon was used as a carrier gas at a flow rate of 40 ml/min.

Experimental procedure. Swiss-White mice aged 6–8 wk were randomly assigned to receive tin protoporphyrin or normal saline. The treated animals received 50 nmol/g of tin protoporphyrin via intraperitoneal injection while controls received an equal volume of normal saline. The mice were then placed on a liquid diet of 5% glucose and electrolytes (Pedialyte, Ross Labs., Columbus, OH) for the duration of the experiment, as previous studies had demonstrated that mice fed commercial rodent food produced increased amounts of CO which did not reflect endogenous heme degradation (10). In addition, a pilot study for the present series demonstrated that three mice fed infant formula produced appreciably more CO than that expected from a theoretical calculation of endogenous heme turnover in the mouse as well as greater CO than that subsequently observed with Pedialyte feedings. Eighteen hours after the first injection, the same dose of tin protoporphyrin or saline was readministered and the animals were placed in individual glass chambers as previously described (9). Briefly, each mouse was placed in an airtight chamber which had a volume of 680 ml. Soda lime in the chambers absorbed CO_2. A one-way valve allowed O_2 to enter each chamber to replace that consumed by the animal. An initial gas sample was taken after a 4-h period of equilibration and a final sample was obtained 18 h later. Oxygen and CO concentrations were measured in each sample.

A second series of experiments was performed which was similar to that described above with the exception that each animal was also given an intraperitoneal injection of hemin (125 nmol/g) just prior to placement in the chamber.

Calculations. The moles of CO accumulated in the gas space for each animal over an 18-h interval was determined after correcting for ambient temperature. The rate of CO production was calculated from the change in gas space CO content and CO stores in the mouse with correction for CO removed during gas sampling and CO added via trace contamination of the O_2 supply. CO catabolism was presumed to be negligible (12) and, therefore, was not included in the calculated rate of production. Total mouse CO stores were calculated assuming a blood volume of 5.5 ml/100 g, Hb concentration of 13.5 g/dl (15), extravascular CO stores of 20% of total body CO content (12), and COHb as

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Requests for reprints should be addressed to Dr. Rolf Engel, Neonatal ICU-862, Hennepin County Medical Center, 701 Park Avenue South, Minneapolis, MN 55415.
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Table 1. *Increase of CO content of chamber gas space*

Group	Normal saline	Tin protoporphryin	Normal saline + hemin	Tin protoporphyrin + hemin
No. of mice	6	6	14	13
Wt (g)	24.1 ± 2.7	25.3 ± 3.4	28.5 ± 6.7	26.5 ± 5.1
Net CO increase in gas space over 18 h (ppm)	5.9 ± 0.9	4.6 ± 1.1*	32.4 ± 9.8	14.3 ± 4.2***
Rate of CO increase in gas space ($nmol \cdot g^{-1} \cdot h^{-1}$)	0.38 ± 0.03	0.29 ± 0.05**	1.80 ± 0.43	0.85 ± 0.17***

All data given mean ±1 SD.
* $p < 0.05$, ** $p < 0.01$ compared to normal saline group, *** $p < 0.0001$ compared to normal saline + hemin group.

calculated below by the Haldane equation (5):

$$K = \frac{COHb}{O_2Hb} \cdot \frac{P_{AO_2}}{P_{ACO}}$$

where K is the relative affinity constant of Hb for CO and O_2, and COHb and O_2Hb are the fractions of Hb saturated with CO and O_2, respectively. Under the conditions of the experiment, O_2Hb may be expressed as 1-COHb. K for mouse Hb is approximately 148 (5). Alveolar O_2 concentration was assumed to be 45 torr less than inspired O_2 and alveolar CO concentration was assumed to equal measured CO concentration in the chamber gas space. A trace amount of CO was present in the oxygen supply (1.4 ppm): correction for this amount was calculated using the reported average oxygen consumption rate of 1.59 ml/g/h (13).

The data are presented as ± 1 SD. Statistical significance was determined by the unpaired *t* test.

RESULTS

CO accumulation in the gas space was significantly decreased ($p < 0.05$) for mice that had been injected with tin protoporphyrin (Table 1). For mice receiving saline injections, CO accumulated at a rate of 0.38 $nmol \cdot g^{-1} \cdot h^{-1}$ in the chamber gas space. In contrast, for mice injected with tin protoporphyrin, the average rate of CO accumulation in the chamber gas space was 24% less, or 0.29 $nmol \cdot g^{-1} \cdot h^{-1}$.

A slightly greater difference between control and tin protoporphyrin-treated animals was observed when the rate of CO production was calculated. The calculated rate of CO production for control mice was 0.40 $nmol \cdot g^{-1} \cdot h^{-1}$ *versus* 0.28 $nmol \cdot g^{-1} \cdot h^{-1}$ for mice treated with tin protoporphyrin ($p < 0.01$) (Fig. 1).

The injection of hemin (125 nmol/g) increased the rate of CO accumulation in the chamber gas space to 1.80 $nmol \cdot g^{-1} \cdot h^{-1}$ as opposed to the mean rate of 0.38 $nmol \cdot g^{-1} \cdot h^{-1}$ for the nonheme injected control mice (Table 1) ($p < 0.0001$). The effect of hemin injections on calculated CO production was equally pronounced with a rise from 0.40 $nmol \cdot g^{-1} \cdot h^{-1}$ for control mice to 2.38 $nmol \cdot g^{-1} \cdot h^{-1}$ after hemin injection.

The rate of CO accumulation in the gas space for hemin-loaded mice was decreased from 1.80 $nmol \cdot g^{-1} \cdot h^{-1}$ for saline-treated mice to 0.85 $nmol \cdot g^{-1} \cdot h^{-1}$ for tin protoporphyrin-injected mice ($p < 0.0001$) (Table 1). The calculated rate of CO production was depressed by about 56% with controls producing 2.38 $nmol \cdot g^{-1} \cdot h^{-1}$ and tin protoporphyrin-treated mice producing 1.05 $nmol \cdot g^{-1} \cdot h^{-1}$ ($p < 0.001$) (Fig. 2). Tin protoporphyrin decreased the fraction of exogenous heme that was catabolized to CO from 28 to 11% during the 18 h of the experiment.

DISCUSSION

Tin protoporphyrin is an inhibitor of heme oxidation *in vitro* and *in vivo* (6–8, 11) Drummond and Kappas (6) reported that a single injection of this synthetic metalloporphyrin prevented the rise of serum bilirubin normally observed in neonatal rats.

In contrast to serum bilirubin concentrations which reflect the balance between heme breakdown and bilirubin excretion, CO

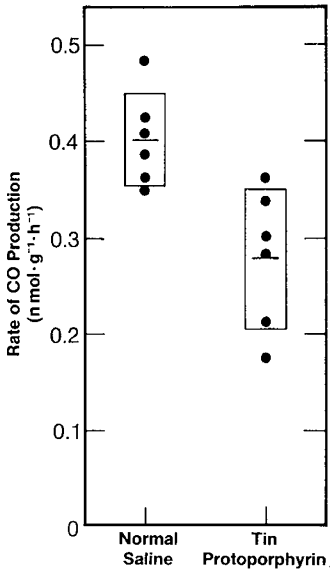

Fig. 1. Calculated rates of CO production for mice treated with tin protoporphyrin or normal saline. $p < 0.01$. *Rectangles* indicate mean ± 1 SD.

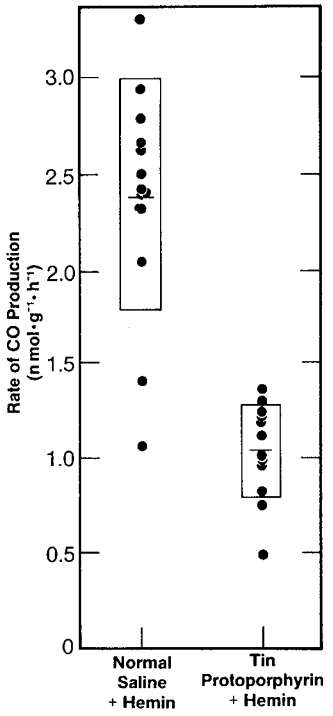

Fig. 2. Calculated rates of CO production for hemin-injected mice given tin protoporphyrin *versus* normal saline. $p < 0.0001$. *Rectangles* indicate mean ± 1 SD.

production is a quantitative measure of heme catabolism. While several assumptions concerning body stores of CO were necessary to calculate production rates, the validity of these assumptions is supported by the calculated rate of CO production for saline-treated animals of 0.40 $nmol \cdot g^{-1} \cdot h^{-1}$, a value close to the predicted rate of 0.48 $nmol \cdot g^{-1} \cdot h^{-1}$.

In mature mice, we found a 30% decrease in CO production after treatment with tin protoporphyrin. This result indicates that tin protoporphyrin decreases heme breakdown in adult animals with normal rates of heme catabolism. In addition, the reduced CO production confirms that tin protoporphyrin reduces serum bilirubin levels by inhibition of heme oxygenase rather than by inhibition of biliverdin reductase activity, decreased bilirubin binding by albumin, or accelerated bilirubin clearance by the liver (11).

Tin protoporphyrin also inhibited heme breakdown when mice were given a hemin burden approximately 10 times their normal daily heme turnover. In this situation, tin protoporphyrin decreased the rate of CO production by about 50% compared to saline-injected animals, but did not completely prevent a rise in CO production after hemin injection. This finding is similar to that of Sassa *et al.* (16), who found that tin protoporphyrin decreased, but did not normalize serum bilirubin levels in mice with severe hemolytic anemia. The greater percent inhibition of CO production following hemin administration as compared to the basal state is what would be expected in the *in vivo* situation if heme levels were normally well below the saturating levels of the enzyme. Partial inhibition of the enzyme would cause the heme levels to rise to a concentration which results in an increased rate of formation of the product (CO). In contrast, in the hemin loaded state when the enzyme is functioning at near saturation levels, further increases in heme concentration will not increase the rate of CO formation.

In contrast to the present report on adult mice, Cowan *et al.* (4) found that tin protoporphyrin injections did not affect the CO production of newborn rats. Both their experimental design and their biological model differed from ours in several respects that could conceal heme oxygenase inhibition by tin protoporphyrin. The rat pups were studied 24 h after receiving 25 nmol/g of tin protoporphyrin, whereas the mice received 50 nmol/g with a repeat dose just before the CO sampling period started. The disparity in tin protoporphyrin dose is even greater when allowance is made for the several-fold decrease in hepatic heme oxygenase activity that occurs during the first postnatal month in rats (8, 18), and the gradual decrease in hepatic weight relative to total body weight during infancy. In 7-day-old rats, the effect of tin protoporphyrin on serum bilirubin concentration has been shown to be dose dependent (8). The rat pups were allowed to suckle before the determination of CO exhalation. We eliminated milk feedings 18 h before the study because milk appeared to produce a spurious increase in CO production, which probably did not result from heme catabolism. The rat pups were fasted during the CO production study while adult mice had access to a sugar water solution. Since a fast of 3 h can increase hepatic heme oxygenase activity in newborn rats (18), this could further obscure inhibition of the enzyme by tin protoporphyrin. The attempt by Posselt *et al.* (14) to elicit a latent inhibition of heme oxygenase 100 h after giving tin protoporphyrin by relying on the gradual release of heme 55 h after forming a hematoma is probably less apt to succeed than the intraperitoneal injection of a bolus of hemin just before the CO collection period. The disparate results for newborn rats as opposed to adult mice suggest that under steady-state conditions, tin protoporphyrin will only reduce CO and bilirubin production when heme oxygenase activity is inhibited sufficiently to result in alternative routes for eliminating heme (1, 2, 8).

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