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Selection of Metalloporphyrin Heme Oxygenase Inhibitors Based on Potency and Photoreactivity

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ABSTRACT. The heme oxygenase inhibitor, tin protoporphyrin, is being studied for the prevention of neonatal jaundice. This potential drug, however, is also a photosensitizer that could cause serious and unknown side effects when administered to newborns. Therefore, we have developed *in vitro* and *in vivo* procedures for the screening and further characterization of potentially safe heme oxygenase inhibitors. The ideal inhibitor: 1) contains a biocompatible metal, *2*) is not degraded in tissues, *3*) is a highly potent inhibitor of heme oxygenase, and *4*) does not participate in photochemical reactions. Proto- and mesoporphyrin derivatives with the tin, zinc, manganese, chromium, nickel, and magnesium were screened *in vitro* for suitability. Chromium protoporphyrin and mesoporphyrin were further studied *in vitro* and *in vivo* and were found to meet the ideal criteria. Chromium mesoporphyrin appeared to be the most potent *in vitro* inhibitor of adult Wistar rat tissue heme oxygenase. Four μmol of chromium protoporphyrin or chromium mesoporphyrin/kg body weight, administered intraperitoneally to adult male Wistar rats given a heme load through intraperitoneal administration of 30 μmol heme/kg body weight, caused significant suppression of hemolysis-induced increase in carbon monoxide production to 72 and 44% of control, respectively, 5.5 h after treatment. At t = 6 h, the tissue heme oxygenase activity, measured *in vitro*, was significantly reduced to 33 and <5% in liver and to 22 and <5% in spleen after the administration of chromium protoporphyrin and mesoporphyrin, respectively, but was not reduced in brain. The results show that there exist effective metalloporphyrin heme oxygenase inhibitors without photosensitizing properties. (*Pediatr Res* 33: 195–200, 1993)

Abbreviations

CO, carbon monoxide
VeCO, total body CO excretion
HO, heme oxygenase
CrPP, chromium protoporphyrin
CrMP, chromium mesoporphyrin
MnPP, manganese protoporphyrin
NiPP, nickel protoporphyrin
SnPP, tin protoporphyrin
SnMP, tin mesoporphyrin
PP, protoporphyrin
MP, mesoporphyrin

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Physiologic jaundice is still being considered a risk factor during the neonatal period. The phenomenon results from the abrupt cessation of bilirubin clearance by the placenta and a transient deficiency in hepatic bilirubin removal. It is exacerbated by the increased rate of bilirubin production in the neonate, which is 2- to 3-fold greater than that of the adult. Several factors and conditions further increase this rate (1). In particular, hemolytic disease such as ABO and Rh incompatibility cause significant elevation in total body bilirubin production rates, as indexed by CO excretion rates (2). Markedly elevated serum bilirubin concentrations have been associated with neurotoxicity (3).

Although the risk factor of moderately elevated serum bilirubin levels [<20 mg/dL (342 μmol/L)] in term well babies without hemolysis is being questioned (4), there is less doubt that hyperbilirubinemia in premature infants and in those with hemolytic disease may be deleterious. Traditionally, jaundice is treated through removal of accumulated bilirubin by phototherapy or blood-exchange transfusion (5). A potentially more effective and preventive treatment may be the inhibition of bilirubin production through administration of a specific inhibitor of HO (EC 1.14.99.3), the rate-limiting enzyme in the bilirubin production pathway from heme (6, 7). Synthetic analogs of heme, especially the tin and zinc derivatives of PP and MP, have been studied for this purpose (7–11). SnPP has been shown to retard the development of human neonatal hyperbilirubinemia (8). However, the tin metalloporphyrins have also been shown to be potent photosensitizers capable of producing singlet oxygen (12), which can oxidize a large number of organic molecules, including membrane lipids, energy metabolites, proteins, and nucleic acids (13, 14). The destruction of some of these compounds may be responsible for the mortality observed in neonatal rats treated with photosensitizing metalloporphyrins and subsequently exposed to visible light (15, 16).

Because the potentially serious side effects of this photoreactivity have been incompletely studied, we have developed the following criteria and procedures for identification of metalloporphyrins potentially suitable for treatment of human neonatal hyperbilirubinemia. The ideal inhibitor: *1*) contains a metal that occurs naturally in the body or is harmless in trace amounts, *2*) is not degraded in tissue to possibly harmful substances, *3*) can effectively inhibit HO at relatively low (<40 μmol/kg) doses, and *4*) does not participate in photodestructive reactions. In this study, we investigated several synthetic metalloporphyrins with manganese, chromium, nickel, and magnesium as central metals. The HO-inhibiting and metabolic characteristics of some of these compounds have been studied by others (7, 17–19). Results with heme, tin, and zinc porphyrins were included for comparison. The evaluation of these metalloporphyrins consisted of a screening phase followed by a more in-depth examination of promising compounds.

MATERIALS AND METHODS

Materials. Metalloporphyrins (Porphyrin Products, Inc., Logan, UT) were each dissolved in 500 μL of 10% (wt/vol) ethanolamine. After addition of 7 mL of distilled water and adjusting the pH to 7.4 with 20-μL aliquots of 1 N HCl, the volume was adjusted to 10 mL to obtain solutions of 650 μM concentration. Metalloporphyrin solutions for determination of *in vivo* efficacy were made up to 6 mM. The metalloporphyrins studied included proto and meso derivatives (PP and MP, respectively) of iron, tin, zinc, manganese, chromium, nickel, and magnesium porphyrin. Buffer, 0.1 M KPO_4 at pH 7.4, was prepared from KH_2PO_4, KOH and distilled water. All procedures involving metalloporphyrin and bilirubin solutions were carried out under near-dark conditions.

Animals. This protocol was approved by the research committee of the Stanford University Division of Laboratory Animal Medicine. Animal care and use were in accord with guidelines established by the National Institutes of Health. Adult male Wistar rats (Simonsen Laboratories, Inc., Gilroy, CA), weighing 275 to 325 g were used as the source of HO and as a hemolytic model for the *in vivo* efficacy testing of metalloporphyrins. These rats were fasted for 16 h before the beginning of any experiment, but had unlimited access to water and were housed in a temperature-controlled room (25 ± 1°C) with a 12-h light cycle. Neonatal Wistar rat litters (Simonsen) 12 to 24 h of age, were used for the *in vivo* phototoxicity test. The pups had unlimited access to the fed mother until the beginning of the test. All animals were killed by decapitation at the end of each experiment.

HO assay. Tissue HO activity was determined with a gas chromatographic assay (20). For the *in vitro* screening procedure, fasting adult male Wistar rat livers were collected, rinsed, and immediately homogenized with a Biohomogenizer (Biospec Products, Inc., Bartlesville, KY) in four volumes of ice-cold buffer. The homogenate was centrifuged at 13 000 × *g* for 15 min. The HO activity in the supernatant was determined by measuring the amount of CO generated by the enzyme from 50 μM/11.2 μM methemalbumin in the presence of 1.5 mM NADPH at 37°C in the dark. *In vitro* inhibition potential of metalloporphyrins was determined by addition of small volumes of metalloporphyrin to the reaction medium (40 μM for the screening procedure and 0.1–40 μM for the *in vitro* efficacy experiments). The ability of administered metalloporphyrins to inhibit organ HO activity *in vivo* was determined in the same manner. Tissues used for this purpose were harvested from the same animals used to study metalloporphyrin effects on the *in vivo* bilirubin production rates (VeCO, see below). HO activity is expressed in terms of nmol CO produced/h/mg protein.

Metalloporphyrin metabolism assay. In vitro metabolism of metalloporphyrins was determined by substituting equimolar amounts of metalloporphyrin (50 μM) for the heme in the HO assay. Traditionally, metabolism like that observed with heme is defined as the CO production by a tissue preparation from the substrates under study in the presence of NADPH minus that produced in the absence of NADPH.

Protein determination. The protein concentration of tissue preparations was determined by the method of Lowry *et al.* (21), using BSA as standard.

VeCO. Intraperitoneal heme loading of fasted adult male Wistar rats was used as an iatrogenic model of hyperbilirubinemia. The ability of metalloporphyrins to suppress this exaggerated bilirubin production was determined by measuring the decrease of VeCO (22). Each rat was weighed and then placed in a Plexiglas tube (26 × 5.5 cm inner diameter, 690 mL), supplied with a measured CO-free air flow at approximately 110 mL/min. After a 1- to 2-h equilibration period, the CO in the chamber outlet gas was determined by gas chromatography (t = 0 h). Each rat was then given an intraperitoneal injection of 30 μmol heme/kg body weight. In addition, experimental rats were given 4 μmol metalloporphyrin/kg body weight, and the control rats received an equal volume of metalloporphyrin solvent. The animals were returned to their chambers, and the VeCO was determined every half hour for 6 h. At the completion of this procedure, animals were decapitated and the tissue HO activity was determined (see above).

In vitro photoreactivity. Photoreactive compounds have been found to photooxidize organic compounds to yield CO (14). NADH, like NADPH, is one of the compounds that are particularly sensitive to degradation by this process. For this study, we determined the photoreactivity of metalloporphyrins *in vitro* by incubating 40 μM metalloporphyrin in 60 μL of 4.5 mM NADH (Sigma Chemical Co., St. Louis, MO) at 37°C in clear, septum-sealed vials (2 mL). The vials were then exposed to cool white light (20 $\mu W \cdot cm^{-2} \cdot nm^{-1}$ or 30 $W \cdot m^{-2}$, see below) for 15 min. The generated CO was quantitated by injecting the vial headspace gas into a reduction gas analyzer (Trace Analytical, Inc., Menlo Park, CA). The results, corrected for dark control reactions determined simultaneously, were expressed in terms of pmol CO generated per vial per 15-min period.

In vivo phototoxicity. In vivo phototoxicity of the chromium and manganese metalloporphyrins was assessed as described earlier (15). Metalloporphyrins at a dose of 40 μmol/kg body weight were administered to each of 12- to 24-h-old neonatal Wistar rats. Animals were placed three to a glass chamber (25 × 2.5 cm inner diameter) supplied with CO-free air at 100 ± 5 mL/min. The chambers were placed for 12 h over a bank of four cool white fluorescent light tubes (20 W each, 20 $\mu W \cdot cm^{-2} \cdot nm^{-1}$ or 30 $W \cdot m^{-2}$). The temperature was maintained at 30 ± 1°C with circulating air. The survival rate after 12 h of light exposure was taken as an index of phototoxicity. Animals treated with 30 μmol SnPP/kg were used as positive controls.

Bilirubin degradation. Photoreactive metalloporphyrins also enhance the photodegradation of bilirubin (23). Twenty-five μL of 18 mg (30.8 μmol) bilirubin dissolved in 100 mL 4% (wt/vol) human serum albumin solution in 2-mL clear reaction vials were incubated at 37°C with 40 μM metalloporphyrin for 30 min over cool white light (20 $\mu W \cdot cm^{-2} \cdot nm^{-1}$ or 30 $W \cdot m^{-2}$). After completing the reaction, 1 mL of buffer was added, and the bilirubin concentration in the solution was measured with the spectrophotometric UB analyzer, UA-1 (Arrows Co., Ltd., Osaka, Japan). Photodegradative loss of bilirubin was expressed as a percentage of dark controls and compared with bilirubin degradation in the absence of metalloporphyrin (light control).

Light measurements. Radiance ($\mu W \cdot cm^{-2} \cdot nm^{-1}$) was measured with a Mark II Bili-Meter (Olympic Medical, Seattle, WA) over the range of 425 to 475 nm. Light intensities were also determined with a model 2M thermopile detector with sapphire window and argon gas (Dexter Research Center, Dexter, MI). This detector has a flat spectral response from UV to far infrared and a linear signal output from 10^{-2} to 10^{3} $W \cdot m^{-2}$.

Statistics. The data are presented as means ± SD. The results of VeCO measurements were tested using the one-way factorial analysis of variance. All other experiments were evaluated by repeated-measures analysis of variance. The Scheffe F-test was considered significant at $p < 0.05$ as indicated by appropriate letters defined in each table and figure.

RESULTS

The results in Figure 1 show that, as expected, the reference compound, heme, served as the only effective substrate for the HO reaction. Of the other compounds tested, only the manganese porphyrins appeared to support observable but low-level CO production. However, the net CO production was due to suppression of CO production in the blank rather than an increase in CO production in the presence of NADPH, which is characteristic for HO substrates. Therefore, this CO production is not likely to be due to metabolism of the manganese porphyrins but is perhaps representative of an inhibitory effect on the as-yet-

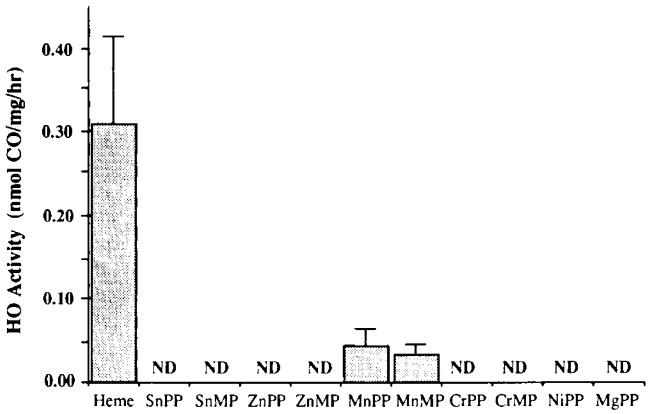

Fig. 1. Metalloporphyrins as substrates for rat liver HO. Postmitochondrial supernatants prepared from the livers of fasted adult male Wistar rats were incubated for 15 min at 37°C with 50 μM of the indicated metalloporphyrin and 1.5 mM of NADPH. CO generation was quantitated by gas chromatography. PP and MP represent the proto- and mesoporphyrin derivatives, respectively. The HO activity level for each of the synthetic metalloporphyrins was significantly different ($p < 0.05$) from the heme control but not from other metalloporphyrins ($n = 5$). *ND*, activity not detected; *Zn*, zinc; *Mg*, magnesium; *Sn*, tin; *Mn*, manganese; Cr, chromium; *Ni*, nickel.

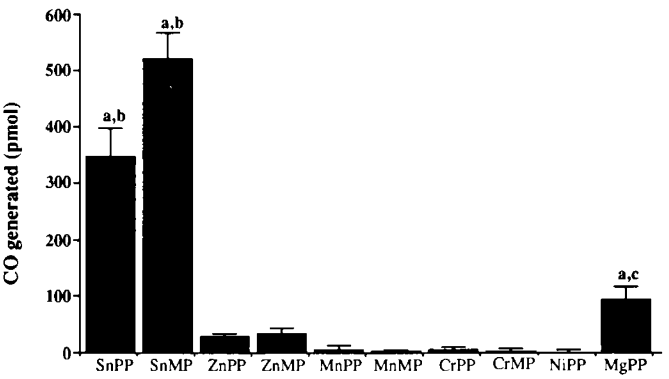

Fig. 2. Metalloporphyrin-supported photogeneration of CO from NADH. NADH (4.5 mM) was incubated with 40 μM of the indicated metalloporphyrin in sealed vials and exposed for 15 min at 37°C to cool white light (20 $\mu W \cdot cm^{-2} \cdot nm^{-1}$ or 30 $W \cdot m^{-2}$). *a*, Significantly different ($p < 0.05$) when compared with zero; *b*, when compared with all other compounds; and *c*, when compared with all other compounds except ZnPP and ZnMP ($n = 4$).

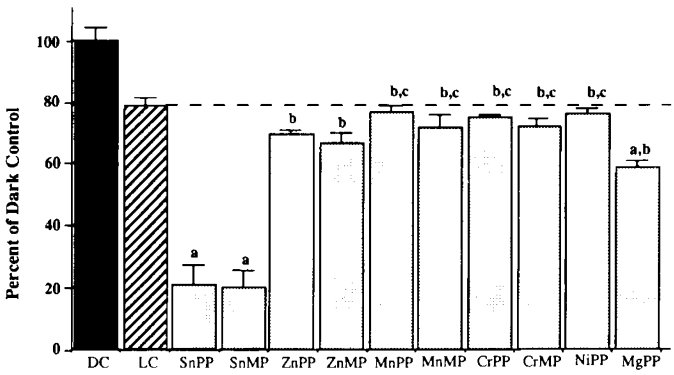

Fig. 3. Metalloporphyrin-enhanced photodegradation of bilirubin. Bilirubin/human serum albumin solutions (18 mg/100 mL) were incubated with 40 μM of the indicated metalloporphyrin at 37°C and exposed for 30 min to cool white light (20 $\mu W \cdot cm^{-2} \cdot nm^{-1}$ or 30 $W \cdot m^{-2}$). After dilution with buffer, the remaining bilirubin was measured spectrophotometrically. *DC*, dark control, *LC*, light control (also indicated by the *horizontal dashed line*). LC and all compounds were significantly different from DC ($p < 0.05$). Compounds labeled with *a* were significantly different from LC. Compounds labeled with *b* were significantly different from SnPP and SnMP, and those labeled with *c* were significantly different from MgPP.

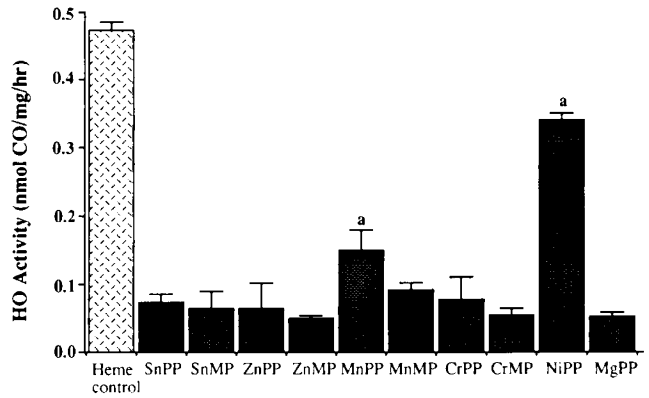

Fig. 4. Inhibition of HO with metalloporphyrins. Postmitochondrial supernatants prepared from the livers of fasted adult male Wistar rats were incubated with 40 μM of the indicated metalloporphyrin and HO assay reagents for 15 min at 37°C. The generated CO, representing HO activity, was measured by gas chromatography. All compounds significantly ($p < 0.05$) inhibited the heme control reaction. Also significantly different were compounds labeled with *a*, when compared with all other inhibitors ($n = 4$).

unidentified minor process that is responsible for CO production in the absence of NADPH.

Figure 2 presents each metalloporphyrin's ability to photooxidize NADH and generate CO. The reference tin analogs were the most powerful photooxidizers, with SnPP evolving 350 pmol of CO and SnMP over 500 pmol during the 15-min light exposure period. Magnesium porphyrin expressed only slight, but statistically significant, photoreactivity. Of the photoreactive porphyrins, the MP species appeared to be more reactive than the PP analogs, but the difference was not statistically significant. Zinc, manganese, chromium, and nickel compounds had no statistically significant photoreactivity as determined by this sensitive assay.

Figure 3 presents the results of another index of photoreactivity, the enhancement of bilirubin degradation. Light exposure alone decreased the bilirubin concentration in the bilirubin/human serum albumin to 79% of dark control (*dashed line*). The presence of either SnPP or SnMP enhanced the effect of light exposure by decreasing the bilirubin concentration to 21% of dark control. Magnesium-PP was much less potent, decreasing the bilirubin concentration to 58%. The two zinc compounds only caused a decrease to about 67% of dark control. None of the other compounds significantly enhanced the photodegradation of bilirubin. These results parallel those found with the CO production assay shown in Figure 2.

When rat neonates were treated intraperitoneally with 40 μmol each of CrPP, CrMP, MnPP, manganese mesoporphyrin, or SnPP, only the SnPP-treated animals suffered light-dependent death as has been shown previously (15, 16). These animals had symptoms as described previously and died within 4 h of the start of light exposure. None of the chromium- and manganese-porphyrin-treated animals died or showed any signs of observable adverse effects as described for SnPP (data not shown).

The ability of each metalloporphyrin to inhibit rat liver HO *in vitro* at the screening concentration of 40 μM is illustrated in Figure 4. All metalloporphyrins inhibited HO activity to a significant extent. NiPP and MnPP were the least effective inhibitors in this series.

The results of the screening procedure indicated that the chromium metalloporphyrins were particularly attractive for further study. These compounds were not metabolized, did not

cause photodestructive reactions, and potently inhibited HO. Therefore, we investigated the efficacy of CrPP and CrMP to inhibit rat liver and spleen HO *in vitro* over a range of concentrations. Furthermore, even though the manganese compounds appeared to have an effect on non-HO-related CO production, their total lack of photoreactivity made these compounds also attractive for some further *in vitro* efficacy study. Table 1 shows that there was significant, concentration-dependent inhibition at concentrations as low as 1 μM for all four compounds. In the case of the chromium compounds, the MP appeared to be more potent. Spleen HO activity is more completely inhibited by all four compounds than is liver HO activity.

The chromium analogues were additionally tested for their ability to inhibit bilirubin production and tissue HO activity *in vivo*. The results of CO excretion rate experiments with an iatrogenic hemolytic model, using adult male Wistar rats, are shown in Figure 5. After injection of heme (30 μmol/kg body weight), the VeCO of each animal began to increase significantly to reach approximately four times the control rate by t = 4.5 h. However, the steep rise in VeCO observed in control animals was less steep in the animals simultaneously given an intraperitoneal dose of 4 μmol/kg body weight of either CrPP or CrMP. As expected from the *in vitro* tests performed earlier, the CrMP was a more powerful inhibitor of CO production than CrPP (significant only at t = 6 h), decreasing the maximum rate of CO production (recorded at t = 5.5 h after heme administration) to 58 and 79% of the total excretion CO rate, respectively, or 44 and 72%, respectively, of the CO excretion rate increase due to heme administration.

After 6 h of *in vivo* monitoring, the rats were killed and the livers, spleens, and brains were excised for determination of HO activity. As seen in Table 2, the liver and spleen HO activity at t = 6 h after treatment was significantly lower in rats given either CrPP or CrMP. Again, the CrMP more potently inhibited HO than CrPP. However, the inhibitors did not appear to cross the blood-brain barrier, because no significant inhibition of brain HO was observed after the 6-h experimental time course.

DISCUSSION

The results of this investigation show that there exist effective synthetic metalloporphyrin HO inhibitors without photosensitizing properties. Thus, these compounds could be considered as alternative agents to the tin metalloporphyrins for suppressing the excessive neonatal jaundice often associated with hemolytic disease. Although phototoxicity is not likely to be the only side effect of these potential drugs, it remains one property that we believe may be significant to neonates receiving simultaneous light treatment. Several other side effects of metalloporphyrins have been reported (24), and the significance of these needs future attention.

The screening procedure also identified metalloporphyrins that were not metabolized to CO and other products that may be detrimental to the organism. Besides heme, the intended substrate for HO, only the manganese porphyrins appeared to be degraded to CO under HO assay reaction conditions. These findings were unexpected. Closer examination of the test results showed that it was the blank CO production that was suppressed by the presence of the manganese porphyrins. The CO production in the presence of NADPH was similar to that in the absence of any porphyrin or to that in the presence of non-manganese-containing porphyrins, either in the presence or absence of NADPH. The blank reaction, for which the NADPH-containing reaction is corrected, represents a minor process of CO production which has not yet been identified. This process has not been previously observed to be affected by the presence of metalloporphyrin. It could possibly be related to lipid peroxidation processes, which we have found to generate CO as well as thiobarbituric acid–reactive substances (25). Maines and Kappas (18) found that metalloporphyrins (such as Mn-, Ni-, and SnPP), which do not bind molecular oxygen, cannot be substrates for HO. Although MnPP metabolism has been reported to occur in bacteria (26), there is no evidence of a similar process in mammals (27). If such a process exists, the administration of manganese porphyrins, either as HO inhibitors or as magnetic reso-

Table 1. *In vitro efficacy of manganese and chromium metalloporphyrins**

Treatment	HO activity (nmol CO/mg protein/h)			
	Liver	Remaining activity (% of control)	Spleen	Remaining activity (% of control)
Control	0.32 ± 0.10	100	1.58 ± 0.59	100
MnPP				
0.1 μM	0.28 ± 0.09	88	1.41 ± 0.54	89
1 μM	0.12 ± 0.04†	38	0.56 ± 0.25	35
10 μM	0.07 ± 0.03†‡	22	0.03 ± 0.02†‡	2
40 μM	0.08 ± 0.04†‡	25	0.02 ± 0.03†‡	1
MnMP				
0.1 μM	0.28 ± 0.08	88	1.25 ± 0.45	79
1 μM	0.08 ± 0.03†‡	25	0.41 ± 0.13†	26
10 μM	0.06 ± 0.05†‡	19	0.01 ± 0.02†‡	1
40 μM	0.05 ± 0.01†‡	16	0.00 ± 0.00†‡	0
CrPP				
0.1 μM	0.29 ± 0.10	91	1.30 ± 0.44	82
1 μM	0.15 ± 0.07	47	0.58 ± 0.31	37
10 μM	0.05 ± 0.04†‡	16	0.00 ± 0.00†‡	0
40 μM	0.04 ± 0.02†‡	12	0.00 ± 0.00†‡	0
CrMP				
0.1 μM	0.25 ± 0.13	78	0.90 ± 0.73	57
1 μM	0.07 ± 0.05†‡	22	0.08 ± 0.14	5
10 μM	0.03 ± 0.04†‡	9	0.00 ± 0.00	0
40 μM	0.01 ± 0.00†‡	3	0.00 ± 0.00	0

* Postmitochondrial supernatants from fasted adult male Wistar rat livers and spleens were incubated with the listed concentrations of manganese and chromium metalloporphyrins and HO assay reagents ($n = 3$). The statistical comparisons are limited to control and to different concentrations of each compound for a given tissue.

† $p < 0.05$ compared with controls.

‡ $p < 0.05$ compared with 0.1 μM.

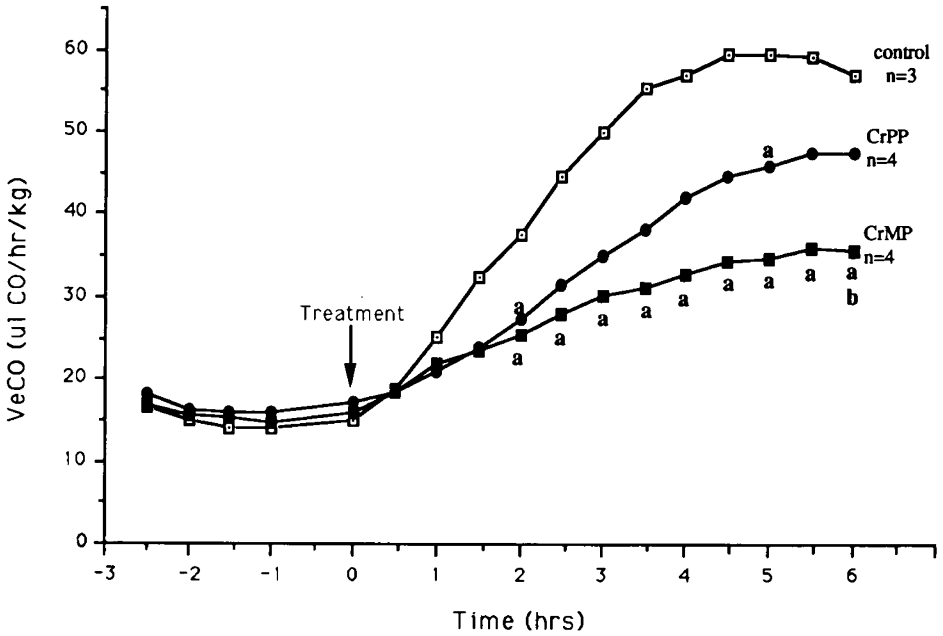

Fig. 5. Effects of CrPP and CrMP on the VeCO of iatrogenic hemolytic rats. VeCO of fasted adult male Wistar rats was monitored for 3 h before intraperitoneal administration of 30 μmol heme/kg body wt and 4 μmol CrPP or CrMP/kg body wt (*Treatment*). The VeCO was then monitored for the next 6 h as indicated. The SD for the data points ranged from 11 to 25%, 7 to 23%, and 1 to 15% of the mean for the control, CrPP-treated, and CrMP-treated animals, respectively. Significantly different ($p < 0.05$) when compared with control (*a*) and CrPP (*b*) for each time point.

Table 2. *In vivo inhibition of rat tissue HO with chromium porphyrins**

Treatment	HO activity (nmol CO/mg protein/h)		
	Liver	Spleen	Brain
Control ($n = 4$)	2.06 ± 0.25 (100%)	3.06 ± 0.77 (100%)	0.84 ± 0.22 (100%)
CrPP ($n = 4$)	0.48 ± 0.10† (23%)	1.48 ± 0.86† (48%)	0.86 ± 0.27 (102%)
CrMP ($n = 4$)	0.08 ± 0.06†‡ (4%)	0.22 ± 0.15†‡ (7%)	0.87 ± 0.32 (103%)

* The livers, spleens, and brains of the rats described in Figure 5 were tested for HO activity after death at $t = 6$ h. The numbers in parentheses denote the level of activity left as a percentage of control activity.

† $p < 0.05$ compared with control.

‡ $p < 0.05$ compared with CrPP.

nance imaging contrast agents, needs to be approached with caution, because the Mn^{++} ion has been shown to be a cardiac toxin, and the LD_{50} for MnPP was found to be 0.19 ± 0.01 mM (27). However, our *in vivo* phototoxicity test did not reveal evidence that manganese porphyrin administration had any noticeable side effects at the concentration tested (40 μM). No tests were performed to detect biochemical interferences by these compounds.

Assessment of the photoreactivity of metalloporphyrins, determined from both the photogeneration of CO from NADH and the photodegradation of bilirubin, yielded similar results. Both procedures identified the tin porphyrins as potent photosensitizers, followed by the weaker NiPP. Magnesium PP and the zinc derivatives displayed very slight, but nonsignificant, photoreactivity in these tests. The reported photochemical behavior of zinc porphyrins is not entirely consistent, and ranges from no activity to substantial *in vitro* photoreactivity (28, 29). However, none of the *in vivo* studies with zinc PP have reported signs of toxicity, chemical or otherwise (11, 15, 16). The chromium and manganese porphyrins in this study also showed no phototoxicity *in vivo*.

We have chosen to eliminate from consideration most (except possibly zinc deuteroporphyrin bis glycol) photosensitizing compounds because their risk of side effects may not outweigh the benefit of the drug. However, this property may be used in other clinical contexts, *e.g.* in the localization and photodynamic treatment of neoplastic tissues (13) and psoriasis (30). Perhaps, for these purposes, one would select metalloporphyrins with poor HO inhibition and favorable paramagnetic, pharmacokinetic, and photochemical properties.

In this study, the metalloporphyrins were administered intraperitoneally, because earlier studies have shown that most metalloporphyrins are not readily absorbed when administered orally (31, 32). However, Vallier *et al.* recently showed that the readily soluble zinc deuteroporphyrin bis glycol (33) and CrMP (34) were absorbed enterally by neonatal rats and subsequently inhibited HO activity in liver and spleen. Enteral absorptivity will be a highly desirable quality for an HO inhibitor intended to inhibit systemic as well as enteral HO (35). The results in Table 2 indicate that brain HO activity was not inhibited after intraperitoneal administration of CrPP or CrMP. This is most likely due to the impermeability of the blood brain barrier to those compounds, because HO-2, the predominant isoform in the brain, is inhibitable *in vitro* by metalloporphyrins (36). Only SnPP has been reported to cross the blood-brain barrier and inhibit HO activity *in vivo* (37).

We have chosen the hemolytic model to study the effect of metalloporphyrins, because it is the hyperbilirubinemia caused by hemolysis that needs to be reduced in human neonates (4). Furthermore, we believe that tissue HO, even though it is the rate-limiting enzyme in the heme-degrading pathway, usually occurs in excess relative to the heme available. Thus, in the present rat model most if not all HO is engaged in the degradation of heme, and it is important to study the effect of metalloporphyrins under this condition.

In summary, the results of this study show that CrPP and CrMP are potent HO inhibitors without photoreactive properties. This combination of characteristics makes these compounds attractive for further evaluation as possible agents for the prevention of excessive neonatal jaundice caused by hemolysis. Despite the advantage offered by the nonphotosensitizing metalloporphyrins that have HO inhibitory potency, more research specifically designed to address safety, including pharmacokinetics and

side effects besides death, would be required before any serious consideration could be given to human experimentation.

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