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URINARY PORPHYRINS AS BIOLOGICAL INDICATORS OF OXIDATIVE STRESS IN THE KIDNEY

INTERACTION OF MERCURY AND CEPHALORIDINE

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Abstract—Reduced porphyrins (hexahydroporphyrins, porphyrinogens) are readily oxidized *in vitro* by free radicals which are known to mediate oxidative stress in tissue cells. To determine if increased urinary porphyrin concentrations may reflect oxidative stress to the kidney *in vivo*, we measured the urinary porphyrin content of rats treated with mercury as methyl mercury hydroxide (MMH) or cephaloridine, both nephrotoxic, oxidative stress-inducing agents. Rats exposed to MMH at 5 ppm in the drinking water for 4 weeks showed a 4-fold increase in 24-hr total urinary porphyrin content and a 1.3-fold increase in urinary malondialdehyde (MDA), an established measure of oxidative stress *in vivo*. Treatment with cephaloridine alone (10–500 mg/kg, i.p.) produced a dose-related increase in urinary MDA and total porphyrin levels up to 1.6 and 7 times control values, respectively. Injection of MMH-treated rats with cephaloridine (500 mg/kg) caused a synergistic (20-fold) increase in urinary porphyrin levels, but an additive (1.9-fold) increase in the MDA concentration. Studies *in vitro* demonstrated that cephaloridine stimulated the iron-catalyzed H_2O_2-dependent oxidation of porphyrinogens to porphyrins in the absence of either microsomes or mitochondria. Additionally, porphyrinogens were oxidized to porphyrins in an iron-dependent microsomal lipid peroxidation system. Moreover, porphyrinogens served as an effective antioxidant ($EC_{50} \sim 1$–$2\ \mu M$) to lipid peroxidation. These results demonstrate that MMH and cephaloridine synergistically, as well as individually, promote increased oxidation of reduced porphyrins in the kidney and that this action may be mechanistically linked to oxidative stress elicited by these chemicals. Increased urinary porphyrin levels may, therefore, represent a sensitive indicator of oxidative stress in the kidney *in vivo*.

Numerous chemicals promote oxidative stress to tissues, characterized by increased oxidation of critical biomolecules, such as lipids, proteins, and nucleic acids [1–3]. Currently, several methods are available to assess oxidative stress *in vivo*, many of which are based on the detection of metabolites of lipid peroxidation, such as lipid hydroperoxides or malondialdehyde (MDA)‡ [4, 5]. Although these metabolites may be detected in urine, assays of these constituents are often fraught with problems, such as interfering substances, special equipment needs, or poor detection sensitivity [1, 5].

Previous studies from this laboratory have demonstrated the property of mercury compounds to promote oxidative stress in the kidney [6, 7]. Based on these findings and the established porphyrinogenic potential of mercury [8–10], we have investigated the hypothesis that urinary porphyrin levels increase as a consequence of oxidative stress to the kidney *in vivo* during exposure to mercury and other chemicals. Urinary porphyrins are an attractive candidate as an indicator of oxidative stress *in vivo*, because the intracellular reduced forms of porphyrins (hexahydroporphyrins, porphyrinogens) are readily oxidized in the presence of free radical generating systems [11–17]. Also, once oxidized, the 8- through 4-carboxyl porphyrins are not known to be further metabolized or rereduced to the corresponding porphyrinogen *in vivo*, but are excreted largely into the urine [17, 18]. Additionally, urinary porphyrin content can be easily and sensitively monitored by HPLC with spectrofluorometric detection [9, 19, 20].

In the present studies, we compared changes in urinary porphyrin and MDA levels following treatment of rats with either methyl mercury hydroxide (MMH), an established renal porphyrinogen and pro-oxidant [6, 8, 10], or cephaloridine, a known oxidative stress-inducing drug [21, 22]. The findings demonstrate that urinary porphyrins increase in proportion to urinary MDA levels, suggesting that urinary porphyrin content reflects oxidative stress in the kidney *in vivo*. In addition, we describe studies *in vitro* which show that the oxidative conversion of porphyrinogens to porphyrins is promoted by iron-catalyzed lipid peroxidation. These results suggest that urinary porphyrin levels may serve as an indicator of oxidative stress *in vivo*.

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‡ Abbreviations: MDA, malondialdehyde; MMH, methyl mercury hydroxide; BHT, butylated hydroxytoluene; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid; and NTA, nitrilotriacetic acid.

MATERIALS AND METHODS

Materials. Male Fischer 344 rats (200–225 g) were

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acquired from Simonsen Laboratories (Gilroy, CA). Porphyrins and porphyrin standards (as the I isomer) were obtained from Porphyrin Products (Logan, UT). MMH was purchased from Alfa Products (Danvers, MA). Cephaloridine, butylated hydroxytoluene (BHT) and 2-thiobarbituric acid (TBA) were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade and were obtained from standard commercial sources. Distilled deionized water was used for all aqueous solutions.

Animal treatments. Immediately upon receipt from the supplier, rats were transferred to individual hanging, wire-bottom cages and permitted unlimited access to food and deionized water. Animal facilities were maintained at 22 ± 1° and on a 12-hr light/dark cycle. Following a 1-week acclimation period, rats were divided into two groups of four animals each and were given deionized water (controls) or deionized water containing 5 ppm MMH for 4 weeks. This concentration provided a dose of approximately 0.6 mg Hg/kg body weight/day, as determined from daily water consumption monitoring data. Rats were then treated with either cephaloridine (500 mg/kg) or saline by i.p. injection and placed in individual metabolism cages for 24-hr urine collections. To determine the dose–response effects of cephaloridine on urinary porphyrin and MDA content, rats without prior MMH treatment were injected with the indicated dose of cephaloridine, and urine was collected over the subsequent 24-hr period.

Urinary porphyrin analysis. Urine was collected into 50-mL polypropylene flasks containing 50 mg sodium bicarbonate and 4 mg EDTA. Porphyrin analysis was performed on a 5-mL aliquot as previously described [9, 20]. Briefly, the pH of the urine sample was adjusted to between 2 and 3 using HCl. The acidified urine was applied to C-18 solid phase extraction column (Waters Associates, Milford, MA), and washed with a 35% methanol/65% sodium phosphate buffer, pH 3.5, to remove several interfering substances in the urine [20]. Porphyrins were eluted with 100% methanol, the methanol was evaporated, and the samples were reconstituted in 0.5 mL of 1 N HCl. Porphyrins were subjected to HPLC analysis using a Waters HPLC system, equipped with an Econosphere C-18 column. Fifty millimolar sodium phosphate, pH 3.5, was used as the starting mobile phase, and individual porphyrin congeners were eluted with a linear gradient of increasing methanol concentration. Porphyrins were measured by their fluorescence intensity (excitation wavelength, 395 nm; emissions wavelength, 620 nm) using a Shimadzu RF-535 detector. Porphyrin identification and concentrations were determined by comparison to a standard curve of authentic porphyrin isomers.

Urinary MDA analysis. Urinary MDA concentrations were determined essentially as described by Draper and Hadley [4]. A 0.5-mL aliquot of the collected urine was adjusted to pH < 3 by the addition of either HCl or H_3PO_4, boiled for at least 30 min (to hydrolyze any protein bound MDA), and the protein was precipitated by the addition of 30% trichloroacetic acid (TCA). An aliquot of the supernatant was mixed with 0.3% TBA in 0.25 N HCl/15% TCA and heated at 100° for 20 min. The absorbance at 532 nm (minus the absorbance at 532 nm of the appropriate blanks, i.e. urine with no TBA added) was used to quantitate the urinary MDA content. MDA concentration was determined by comparison to a standard curve of the absorbance at 532 nm of the reaction product of 1,1,3,3-tetramethoxypropane with the TBA/TCA mixture.

Porphyrinogen oxidation assay. Coproporphyrinogen or uroporphyrinogen oxidation was measured by the increase in absorbance at 392 or 397.5 nm, respectively, at 37°. The rate of porphyrinogen oxidation was followed for up to 10 min. The amount of porphyrin formed was determined from a standard curve. Porphyrinogens were prepared by the anaerobic reduction, under N_2, of the corresponding type I porphyrin isomer using freshly ground 3% sodium amalgam, and were neutralized to pH 7.5 before use. Reduction was judged complete when no detectable fluorescence was observed. A final concentration of 2.5 μM porphyrinogen was used on all studies.

Microsomal lipid peroxidation. Rat liver or kidney microsomes were prepared by differential centrifugation, essentially as described by Pederson and Aust [23]. Microsomal lipid peroxidation was assessed by MDA formation, using a method similar to that described above, except that 0.06% BHT was included with the TBA/TCA mixture to prevent artifactual lipid peroxidation.

Statistical analyses. Statistical analyses were conducted using ANOVA followed by Bonferroni's test for significance at the $P < 0.05$ level of significance.

RESULTS

The urinary porphyrin concentrations of rats exposed to MMH or cephaloridine are presented in Table 1. The most abundant porphyrin in unexposed (control) rat urine was coproporphyrin (4-carboxyl porphyrin), comprising approximately 84% of total rat urine porphyrin content. Among MMH-exposed rats, significant increases were observed in urinary concentrations of penta- and coproporphyrins, by 2 to 4.3 times, respectively, those observed in urine of unexposed rats. More highly carboxylated porphyrins were also increased in urine of MMH-treated rats, although not to a significant extent. Treatment of rats with cephaloridine (500 mg/kg, i.p.) immediately prior to initiation of the 24-hr urine collection period also produced a significant increase in urinary porphyrin content. However, the profile in this case was distinctly different from that observed in MMH-treated rats. Injection of cephaloridine resulted in a marked increase in the concentrations of essentially all urinary porphyrins and an overall increase in total urinary porphyrin content of 6.3-fold. Injection of MMH-treated rats with cephaloridine (500 mg/kg) resulted in a dramatic increase in penta- and coproporphyrin levels with an overall increase in total urinary porphyrin content of approximately 20-fold, as compared with the control value.

Determination of urinary MDA content from the treatment groups described in Table 1 revealed

Table 1. Effects of MMH or cephaloridine on rat urinary porphyrin concentrations

Treatment	Urinary porphyrin concentration (pmol/24 hr)				
	Uro	Hepta	Hexa	Penta	Copro
Control	72 ± 26	79 ± 15	25 ± 4	75 ± 71	1,340 ± 192
MMH	131 ± 64	122 ± 81	51 ± 21	152 ± 38*	5,750 ± 1,120*
Cephaloridine	358 ± 125*	104 ± 39	68 ± 17*	453 ± 67*	8,980 ± 978*
MMH + cephaloridine	81 ± 35	47 ± 14	77 ± 7*	1,060 ± 195*	30,600 ± 3,120*

Rats were treated with MMH (5 ppm) in drinking water for 4 weeks or with cephaloridine (500 mg/kg) by i.p. injection at the start of the 24-hr urine collection period. Values are the means ± SD of quadruplicate assessments.

* Significantly different from control value ($P < 0.05$).

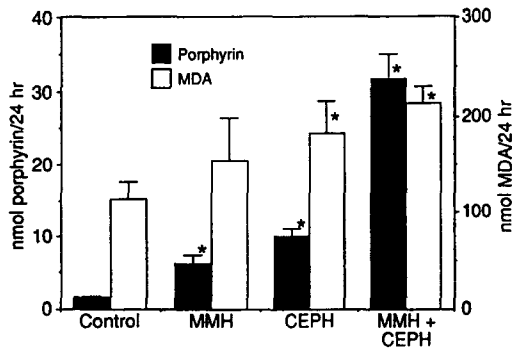

Fig. 1. Effects of methyl mercury hydroxide (MMH) and cephaloridine (CEPH) on urinary porphyrin and MDA concentrations. Rats were treated with MMH (5 ppm) or cephaloridine (500 mg/kg) as described in Materials and Methods. Porphyrin values are for total porphyrin content. Values are means ± SD of quadruplicates. Key: (*) significantly different from control value ($P < 0.05$).

significantly increased urinary MDA concentrations among cephaloridine-treated animals, with the greatest increase observed in rats receiving both MMH and cephaloridine (Fig. 1). In contrast, urinary porphyrin concentrations were elevated significantly in all treatment groups. Moreover, whereas the urinary MDA content increased maximally by only 2-fold, the urinary porphyrin content increased approximately 4-, 6-, and 20-fold in MMH, cephaloridine and combined treatment groups, respectively. Additionally, the changes in urinary MDA levels following treatment of rats with MMH, cephaloridine, or both appeared to be additive, whereas the change in urinary porphyrin content elicited by treatment with both compounds appeared to be greater than additive or synergistic.

The effect of the dose of cephaloridine on urinary porphyrin and MDA content is shown in Table 2. Total urinary porphyrins increased with the magnitude of dose of cephaloridine administered, with a 7-fold increase in total porphyrin concentration observed at the highest dose level. A significant increase of at least 2-fold in the concentrations of uro-, hepta- and hexacarboxyl porphyrins was observed in the highest dose group, whereas urinary concentrations of penta- and coproporphyrins increased by 4- to 8-fold. Total urinary porphyrin concentrations also were increased significantly in the 50 and 100 mg/kg dose groups. As shown in Fig. 2, urinary MDA levels also increased with increasing dose of cephaloridine. However, a significant change from control levels was seen at only the highest cephaloridine dose level. Moreover, as described in Fig. 2, urinary porphyrin concentrations increased to a much greater extent than urinary MDA levels in response to cephaloridine treatment at each dose level.

To investigate the potential mechanisms underlying the increase in urinary porphyrin concentrations promoted by cephaloridine treatment, studies *in vitro* on the effects of cephaloridine on reduced porphyrin (porphyrinogen) oxidation were undertaken. The oxidative conversion of porphyrinogens to the corresponding porphyrin by metal ions is well documented [11–17]. As shown in Table 3, co-incubation of coproporphyrinogen with either EDTA- or nitrilotriacetic acid (NTA)-chelated Fe(III) in the absence of H_2O_2 resulted in a modest rate of coproporphyrinogen oxidation. Inclusion of H_2O_2 in the reaction mixture dramatically increased the rate of oxidation catalyzed by either iron chelate [35-fold for EDTA:Fe(III) and 15-fold for NTA:Fe(III)]. The presence of 1 mM cephaloridine in the reaction mixture increased the rate of H_2O_2-dependent coproporphyrinogen oxidation catalyzed by either iron chelate by 34 and 26%, respectively. No porphyrinogen oxidation was observed in the presence of H_2O_2 alone.

Cephaloridine has been reported to cause lipid peroxidation *in vivo* and *in vitro* [21, 24]. One proposed mechanism of cephaloridine-induced lipid peroxidation involves a redox cycle between NADPH and cephaloridine mediated by NADPH–cytochrome P450 reductase [24]. In our investigation of microsomal redox cycling of cephaloridine and its relationship to porphyrinogen oxidation, we were unable to demonstrate consistent and reproducible redox cycling of cephaloridine in either liver or kidney microsomes; yet we could demonstrate substantial NADPH oxidation by paraquat (an

Table 2. Effects of cephaloridine dose on rat urinary porphyrin concentration

Cephaloridine dose (mg/kg)	Urinary porphyrin concentration (pmol/24 hr)				
	Uro	Hepta	Hexa	Penta	Copro
Control	80 ± 29	46 ± 19	19 ± 9	59 ± 24	860 ± 346
10	110 ± 53	61 ± 11	48 ± 14*	56 ± 18	1,230 ± 460
50	115 ± 15	63 ± 23	49 ± 11*	69 ± 19	1,404 ± 246*
100	114 ± 56	89 ± 23	91 ± 58*	121 ± 10*	1,580 ± 399*
500	157 ± 12*	104 ± 23*	53 ± 21*	247 ± 43*	6,930 ± 1,231*

Rats were treated with cephaloridine by i.p. injection at the doses indicated at the start of the 24-hr urine collection period. Values are the means ± SD of quadruplicate assessments.
* Significantly different from control value ($P < 0.05$).

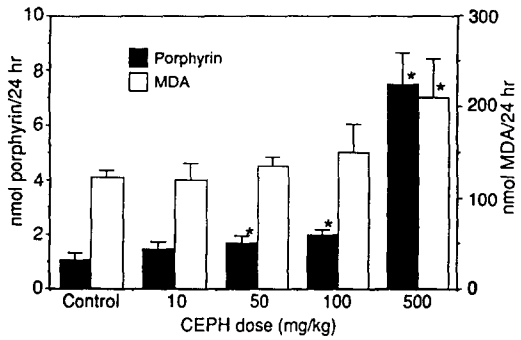

Fig. 2. Effects of cephaloridine dose on urinary porphyrin and MDA concentrations. Rats were injected with cephaloridine (CEPH) as described in Materials and Methods. Porphyrin values are for total urinary porphyrin content. Values are means ± SD of quadruplicates for control and triplicates for treated samples. Key: (*) significantly different from control value ($P < 0.05$).

Table 3. Stimulation of iron-catalyzed porphyrinogen oxidation by cephaloridine (CEPH) *in vitro*

Catalyst	Coproporphyrinogen oxidation rate (pmol/min)	
	$-H_2O_2$	$+H_2O_2$
EDTA:Fe(III)	4.2 ± 1.0	148 ± 11
+CEPH	6.3 ± 1.8	198 ± 21 (+34%)
NTA:Fe(III)	3.9 ± 2.4	59 ± 2.4
+CEPH	5.6 ± 1.2	75 ± 5.2 (+26%)

Reaction mixtures contained 2.5 μM coproporphyrinogen and where indicated, 50 μM Fe(III) chelated to either 55 μM EDTA or 100 μM NTA, or 1 mM CEPH in 50 mM HEPES buffer, pH 7.5. The rate of coproporphyrinogen oxidation was measured continuously at 392 nm for 10 min at 37°. No detectable coproporphyrinogen oxidation occurred in the absence of added iron, hydrogen peroxide, or CEPH. Values are the means ± SD of triplicate measurements.

established redox cycling agent [25]) with the same microsomal preparations (data not shown). In addition, little or no NAD(P)H consumption was observed in isolated liver or kidney mitochondria upon addition of cephaloridine, compared with rates of NAD(P)H oxidation observed in the absence of cephaloridine. Thus, to investigate the possible mechanistic relationship between oxidative stress (e.g. lipid peroxidation) and porphyrinogen oxidation *in vivo*, we chose to investigate porphyrinogen oxidation in relation to microsomal-mediated lipid peroxidation in the absence of cephaloridine.

As shown in Table 4, the addition of uroporphyrinogen to rat kidney microsomes resulted in small but measurable rates of uroporphyrinogen oxidation and lipid peroxidation (MDA formation). The addition of NAD(P)H to the reaction mixture slightly but non-significantly increased the rate of porphyrinogen oxidation, but rendered MDA formation non-discernable. The addition of ADP:Fe(III) without NAD(P)H to microsomes caused substantially more uroporphyrinogen oxidation and a detectable, although non-significant, increase in lipid peroxidation. The addition of the chain breaking antioxidant, BHT, to reaction mixtures containing microsomes and ADP:Fe(III) completely abolished the observed lipid peroxidation, but only marginally reduced the rate of uroporphyrinogen oxidation. Addition of both NADPH and ADP:Fe(III) to microsomes produced a substantial increase in the rates of both porphyrinogen oxidation and lipid peroxidation. These effects were also reduced significantly or abolished by BHT.

As is also evident from Table 4, the presence of uroporphyrinogen in a microsomal lipid peroxidation assay system resulted in significant inhibition of the rate of MDA formation compared with the rate observed in the absence of uroporphyrinogen (0.52 compared with 0.82 nmol MDA/min/mL, respectively). The effect of BHT in this system was comparable to that observed when uroporphyrinogen was present.

As shown in Fig. 3, uroporphyrinogen inhibited microsomal lipid peroxidation with an apparent IC_{50} of approximately 2 μM. Comparable results were obtained with coproporphyrinogen. As anticipated, however, studies with the more lipophilic coproporphyrinogen resulted in a somewhat lower IC_{50} value (approximately 1 μM) (data not shown).

Table 4. Stimulation of uroporphyrinogen oxidation by microsomal lipid peroxidation

Reactants	Uroporphyrinogen oxidation (pmol/min/mL)	MDA formed (nmol/min/mL)
Microsomes (control)	0.50 ± 0.33	0.01 ± 0.02
+NADPH	0.87 ± 0.33	0
+ADP:Fe(III)	2.4 ± 0.43*	0.03 ± 0.02
+BHT	2.1 ± 0.19*	0
+NADPH, ADP:Fe(III)	9.0 ± 0.26*	0.52 ± 0.04†
+BHT	0.50 ± 0.21	0
+NADPH, ADP:Fe(III) (−urogen)	NA	0.82 ± 0.07
+BHT	NA	0.01

Reaction mixtures contained 0.1 mg/mL rat liver microsomal protein, 0.25 M KCl, 50 mM HEPES buffer, pH 7.5, and where indicated, 2.5 μM uroporphyrinogen (urogen), 0.2 mM NADPH, 50 μM Fe(III) chelated to 250 μM ADP, or 0.001% BHT (in ethanol). Urogen oxidation was measured continuously by the increase in absorbance at 397.5 nm for 10 min at 37°: MDA formation was determined discontinuously over 10 min. Values are the means ± SD of triplicate measurements. NA = not applicable.

* Significantly different from control value ($P < 0.05$).

† Significantly different from system without urogen ($P < 0.05$).

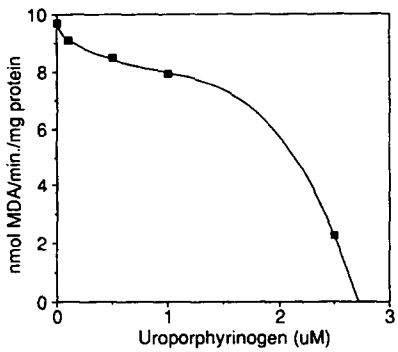

Fig. 3. Inhibition of microsomal lipid peroxidation by uroporphyrinogen. Rat liver microsomal lipid peroxidation was determined as described in Materials and Methods. The reaction mixture consisted of 50 μM Fe(III) chelated to 250 μM ADP, 0.2 mM NADPH, 0.1 mg/mL microsomal protein, 0.25 M KCl, and 50 mM HEPES buffer, pH 7.5, and the indicated concentrations of uroporphyrinogen. Reactions were conducted at 37° in a Dubnoff metabolic shaker in the absence of light. Values are the averages of duplicate samples measured 10 min after initiation of the reaction.

DISCUSSION

The present studies suggest that urinary porphyrin content may represent a novel biological indicator of oxidative stress to the kidney *in vivo*. Total urinary porphyrin content increased upon treatment of rats with the nephrotoxicants MMH, cephaloridine, or both, consistent with an increase in the urinary concentration of MDA. The sensitivity and responsiveness of urinary porphyrin content to oxidative stress, however, appears superior to that of urinary MDA, inasmuch as total urinary porphyrins increased by as much as 10-fold more than urinary MDA content under similar treatment conditions.

Many structurally diverse chemicals elicit porphyrinuria [26, 27], although altered heme biosynthetic pathway enzyme activities or depletion of cellular heme content in target tissues has not been demonstrated as the underlying cause of this effect in many such cases. The present findings raise the possibility that the porphyrinuria elicited by some classes of chemicals represents oxidative stress in tissue cells resulting in oxidation of reduced porphyrins which are normally synthesized in excess of that required for heme synthesis. Support for this possibility is provided in previous studies from this laboratory which demonstrate increased reactive oxidant production as a common mechanism underlying both porphyrinogen oxidation and oxidative stress caused by mercury compounds in kidney cells [6, 7, 15, 16, 28, 29]. Of particular interest in the present studies is the observation of the apparent synergistic interaction of MMH and cephaloridine in terms of their porphyrinogenic action as pertains specifically to penta- and coproporphyrins (Table 1). Previous studies from this laboratory [30, 31] have demonstrated that mercury specifically impairs the enzymatic sites within the renal heme biosynthetic pathway which catalyze decarboxylation of 5- and 4-carboxyl porphyrinogens, accounting for the dramatic increase in these specific porphyrins in the urine during mercury exposure. The present results suggest that the synergistic effect of MMH and cephaloridine arises from the combined pro-oxidant actions of these agents directed toward the oxidation of the excess 4- and 5-carboxyl porphyrinogens that accumulate in kidney cells because of their impaired utilization for heme biosynthesis due to mercury exposure. Further support for this hypothesis would be derived from similar observations using specific chemicals that selectively impair porphyrinogen

metabolism at different points of the heme biosynthetic pathway.

The observed synergistic interaction of mercury and cephaloridine suggests that other oxidative stress-inducing agents may interact with mercury in a synergistic fashion. Given that a finite but variable body burden of mercury exists within human tissues, it is possible that mercury may be an important risk factor to consider when evaluating the potential of oxidative stress caused by occupational or environmental exposure to chemicals or radiation. In addition, mercury may exacerbate the endogenous level of free radical oxidation in target tissues, such as kidney and brain [6, 7, 32, 33]. The present findings support the potential of mercury compounds to exacerbate the toxicity of other oxidative stress eliciting agents in biological tissues.

Urinary porphyrins are an attractive candidate as a biological indicator of oxidative stress in the kidney *in vivo* for several reasons. First, the renal cortical intracellular porphyrin pool normally contains a relatively higher proportion of 8- through 4-carboxyl porphyrinogens, as well as higher absolute porphyrinogen concentrations, than is found in the liver or other tissues [34, 35]. This relative abundance of reduced porphyrins in kidney cells enhances the likelihood that oxidative events occurring in the kidney will result in increased concentrations of oxidized porphyrins available for excretion from kidney cells into the urine. Second, unlike MDA and many other indicators of oxidative stress *in vivo*, urinary porphyrins are not known to undergo further metabolism *in vivo*. Therefore, the possibility of underestimating an oxidative stress episode because of metabolic conversion or covalent attachment of porphyrins to nucleophiles, as can occur with MDA [5], is greatly reduced. Third, the excretion of porphyrins into the urine requires that the corresponding porphyrinogen be oxidized. Since endogenous porphyrinogen oxidation is known to occur only in tissues, rather than in tubular fluid, the presence of porphyrins in the urine in excess of normally excreted concentrations is indicative of an oxidative event *in vivo*. Fourth, porphyrinogens are constitutively synthesized, providing a constant source of potential indicator of oxidative stress, in comparison with MDA, which is derived from a fixed content of polyunsaturated fatty acids (within a short period of time), and whose production from lipid peroxidation depends greatly on conditions such as dioxygen tension in tissues [5]. Lastly, urinary porphyrins are easily and sensitively detected by HPLC using fluorescence detection [9, 20]. At present, the limit of detection of any individual porphyrin isomer is 0.2 pmol, assuring detection of even small changes in urinary porphyrin content.

The observed increase in urinary porphyrins, concomitant with an increase in MDA content, is in good agreement with the concept that cephaloridine toxicity is caused by oxidative stress to kidney proximal tubule cells [21, 22, 24, 36]. Several studies [21, 22, 24] support the hypothesis that cephaloridine toxicity is related to oxidative stress, which the present data corroborate. However, in the present *in vitro* studies, we were unable to demonstrate NADPH consumption by cephaloridine in the presence of rat liver or kidney microsomes, in contrast to the report of Cojocel *et al.* [24]. Other reports have also questioned the hypothesized relationship of redox cycling to cephaloridine toxicity [36]. Thus, the precise biochemical mechanism for the oxidative stress caused by cephaloridine remains to be elucidated.

Finally, the present studies *in vitro* suggest that porphyrinogens are oxidized to porphyrins by reactions considered to contribute to or cause oxidative stress, i.e. production of reactive oxidants and lipid peroxidation. In light of the observed attenuation of microsomal lipid peroxidation by porphyrinogens (Fig. 3), it is tempting to speculate that porphyrinogens may represent another in the class of endogenous chemicals regarded as antioxidants, such as ascorbate, β-carotene, glutathione, vitamin E and the bile pigments, biliverdin and bilirubin. In addition to possessing a 6 electron reducing capacity, porphyrinogens have metal binding capacity and are synthesized in tissues in considerable excess of that required for heme biosynthesis. An additional property possessed by porphyrinogens to a greater extent than by the established antioxidants mentioned above is the amphophilic propensity of the porphyrinogens, especially coproporphyrinogen, the oxidized form of which is most prevalent in urine. This property is important with respect to antioxidant potential, because it suggests that porphyrinogens may be able to scavenge both water-soluble (e.g. $OH^{\cdot}$, $GS^{\cdot}$) and lipid-soluble (e.g. peroxyl radicals) oxidants. Thus, porphyrinogens may represent an important group of chemicals that serve to limit deleterious free radical reactions *in vivo*.

In summary, the present studies demonstrate that increased urinary porphyrin excretion is elicited by chemicals that cause oxidative stress in the kidney *in vivo*. This effect is most substantially expressed during exposure to chemicals that either singly or concomitantly cause both oxidant stress and porphyrinogen accumulation in tissue cells. These results demonstrate the potential utility of urinary porphyrin measurements as a biomarker of oxidant stress in the kidney *in vivo*.

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