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# Effects of Sodium Metallochlorophyllins on the Activity and Components of the Microsomal Drug-Metabolizing Enzyme System in Rat Liver

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Several sodium metallochlorophyllins (Me-Chl-Na), *i.e.*, sodium copper chlorophyllin (Cu-Chl-Na), sodium cobalt chlorophyllin and sodium iron chlorophyllin, decreased the activities of aminopyrine *N*-demethylase and aniline hydroxylase and the content of cytochrome P-450 in liver microsomes when given intraperitoneally to rats. The lowest levels of these variables were observed 24 h after dosing. Me-Chl-Na administration did not modify the substrate-induced spectral changes of cytochrome P-450. Of the kinetic parameters studied in aminopyrine *N*-demethylation and aniline hydroxylation, only the $V_{max}$ values were markedly decreased by Cu-Chl-Na, indicating that the enzyme inhibition was non-competitive. Me-Chl-Na also reduced cytochrome $b_5$ and total heme contents and NADPH-cytochrome c reductase activity in microsomes. Preincubation of liver microsomes from control rats with Cu-Chl-Na did not cause any reduction in the aminopyrine- and aniline-metabolizing enzyme activities and in the cytochrome P-450 content.

These findings strongly suggest that the decreases in the activities of the hepatic microsomal drug-metabolizing enzyme system caused by Me-Chl-Na are correlated with the reduction in the contents of microsomal cytochromes P-450 and $b_5$, and are brought about by primary action(s) of Me-Chl-Na on the control mechanism of microsomal hemoprotein levels, not by direct action of Me-Chl-Na on the system.

**Keywords**——metallochlorophyllin; drug metabolism; cytochrome P-450; cytochrome $b_5$; NADPH-cytochrome c reductase; spectral change; sleeping time; heme; microsome; rat liver

## Introduction

Sodium metallochlorophyllin (Me-Chl-Na) is a derivative of chlorophyll in which the chelated metal, magnesium, is replaced by other metals such as copper, cobalt or iron. Sodium copper chlorophyllin (Cu-Chl-Na), which has copper as the chelated metal, was reported to be a mixture of copper chelates of chlorophyll derivatives,[1)] and is used as a food additive[2)] and as a gastrointestinal medicine.[3)] Sodium cobalt chlorophyllin (Co-Chl-Na) and sodium iron chlorophyllin (Fe-Chl-Na) have also been used as hematopoietics.[4)]

In our previous studies,[5)] Cu-Chl-Na or some substance(s) derived from Cu-Chl-Na was shown to be distributed in liver microsomes when Cu-Chl-Na was given intraperitoneally (i.p.) to rats. Maines and Kappas reported that several porphyrin compounds, closely related to Me-Chl-Na in chemical structure, exerted potent degradative effects *in vitro* on microsomal cytochromes P-450 and $b_5$, heme, and cytochrome P-450-mediated drug oxidation in rat liver,[6)] and that the administration of cobalt protoporphyrin IX[7)] and methemalbumin[8)] to rats caused decreases in the contents of cytochrome P-450 and heme and in the activity of cytochrome P-450-mediated drug oxidation in liver microsomes.

It was, therefore, of interest to examine the effects of Me-Chl-Na (Cu-Chl-Na, Co-Chl-

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Na and Fe-Chl-Na), mainly by using Cu-Chl-Na, on the activities and contents of components of the hepatic microsomal drug-metabolizing enzyme system in rats. In the present studies, we have demonstrated that all three Me-Chl-Na have inhibitory effects on the activities of aminopyrine *N*-demethylase, aniline hydroxylase and NADPH-cytochrome c reductase, and cause decreases in the contents of cytochromes P-450 and $b_5$ and heme in rat liver microsomes.

## Experimental

**Materials**——Cu-Chl-Na was purchased from Wako Pure Chemical Ind., Ltd., Tokyo, Japan. Co-Chl-Na and Fe-Chl-Na were kindly donated by Nampoh Yakuhin Ind., Ltd., Tokyo, Japan. Nicotinamide adenine dinucleotide phosphate (oxidized and reduced forms, $NADP^+$ and NADPH, respectively) and nicotinamide adenine dinucleotide (reduced form, NADH) were obtained from Oriental Yeast Co., Ltd., Tokyo, Japan. Glucose 6-phosphate (G6P, disodium salt) and G6P dehydrogenase (G6PDH, from yeast, 350 U/mg) were purchased from Boehringer Mannheim Yamanouchi Co., Ltd., Tokyo, Japan. Cytochrome c (from horse heart, Type III) was obtained from Sigma Chemical Co., Ltd., St. Louis, MO, U.S.A. Hexobarbital was purchased from Teikoku Chemical Ind., Ltd., Osaka, Japan. Other chemicals were of reagent grade.

**Animal Treatments**——Male Wistar rats, weighing approximately 200 g, were used throughout the experiments. They were housed in an air-conditioned room with a commercial chow and tap water *ad lib*. Each Me-Chl-Na was dissolved in 0.9% saline, and the resulting solution (1.0 ml/100 g body weight) was administered i.p. to rats. Control animals received an equivalent volume of the vehicle. All animals were fasted for about 18 h before sacrifice or administration of hexobarbital, but were allowed free access to water.

**Preparation of Microsomes**——The animals were killed by decapitation. The livers were removed after perfusion *in situ* with ice-cold 1.15% KCl and homogenized with 2 volumes of ice-cold 1.15% KCl–50 mM potassium phosphate buffer (pH 7.4) in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. The homogenate was centrifuged at $9000 \times g$ for 20 min, and the resulting supernatant fraction was centrifuged at $105000 \times g$ for 1 h. The microsomal pellet was suspended in ice-cold 100 mM potassium phosphate buffer (pH 7.4). The microsomal protein was determined by the method of Lowry *et al.*[9] Bovine serum albumin was used as a standard.

**Enzyme Assay**——Aminopyrine *N*-demethylase and aniline hydroxylase activities were assayed as follows. The reaction mixtures consisted of 3 mg of microsomal protein, 0.5 mM $NADP^+$, 5 mM G6P, 0.6 U G6PDH, 5 mM $MgCl_2$, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 90 mM KCl, 50 mM potassium phosphate buffer (pH 7.4) and substrate (2 mM aminopyrine or 1 mM aniline) in a final volume of 2.0 ml. Incubations were carried out at 37 °C for 20 min. The amounts of formaldehyde and *p*-aminophenol formed during incubation were determined by the methods of Nash[10] and Imai *et al.*,[11] respectively. NADPH–cytochrome c reductase activity was measured by the method of Phillips and Langdon.[12]

**Cytochrome Measurement**——Microsomal cytochrome P-450 was determined by the method of Omura and Sato,[13] and cytochrome $b_5$ was measured by using NADH as the reducing agent.[14]

**Sleeping Time**——Rats were injected i.p. with hexobarbital sodium (150 mg/kg). Sleeping time was determined as the time between the loss of righting reflex and its recovery.

**Measurement of Substrate-Induced Spectral Changes**——Substrate-induced spectral changes were measured by the method of Schenkman *et al.*[15]

**Determination of Heme**——Microsomal heme content was determined by the method of Matteis.[16]

## Results and Discussion

The activities of aminopyrine *N*-demethylase and aniline hydroxylase and the content of cytochrome P-450 in liver microsomes were determined at various time intervals after the i.p. administration of Cu-Chl-Na at a dose of 100 mg/kg to rats. As shown in Fig. 1, Cu-Chl-Na decreased the enzyme activities and the content of cytochrome P-450. Both enzyme activities began to decrease gradually and reached their lowest levels at 24 h after dosing. Both enzyme activities had recovered almost to their initial values by 72 h. The effect of Cu-Chl-Na on cytochrome P-450 content was similar to that on the enzyme activities.

In the following experiments, we examined the effects of various doses of Cu-Chl-Na on the activities of aminopyrine *N*-demethylase and aniline hydroxylase and on the content of cytochrome P-450. We also examined whether other Me-Chl-Na such as Co-Chl-Na and Fe-Chl-Na have similar effects on the enzyme activities and the cytochrome content. Tables I, II

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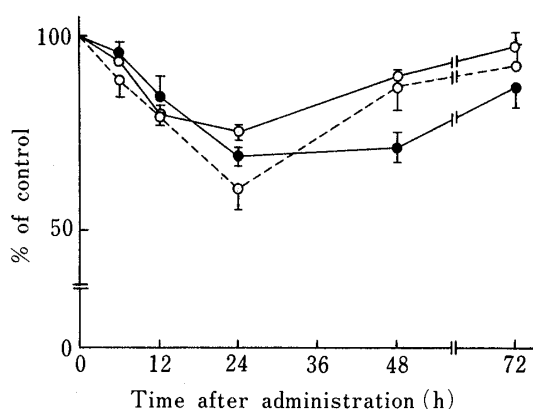


Fig. 1. Time Courses of Drug-Metabolizing Enzyme Activities and Cytochrome P-450 Content in Liver Microsomes of Rats Treated with Cu-Chl-Na

Rats were given i.p. Cu-Chl-Na (100 mg/kg). Each point is expressed as per cent of the control value at the indicated times after administration and represents the mean ± S.E. for 4 experiments.

—●—, aminopyrine *N*-demethylation; ---○---, aniline hydroxylation; —○—, cytochrome P-450 content.

Table I. Effect of Me-Chl-Na at Various Dose Levels on Microsomal Aminopyrine *N*-Demethylase Activity in Rat Liver

| Me-Chl-Na treatment (mg/kg) | Cu-Chl-Na | | Co-Chl-Na | | Fe-Chl-Na | |
|---|---|---|---|---|---|---|
| | Activity[a] | Dec. (%) | Activity[a] | Dec. (%) | Activity[a] | Dec. (%) |
| Control | 91.2±4.5 | | 97.6±1.5 | | 86.2±2.0 | |
| 10 | 89.4±0.6 | 2.0 | 92.3±3.0 | 5.4 | 77.5±5.2 | 10.0 |
| 25 | 75.7±3.1[b] | 17.0 | 68.3±2.1[b] | 30.0 | 67.6±2.8[b] | 21.6 |
| 50 | 67.9±2.8[b] | 25.5 | 60.4±2.7[b] | 38.1 | 63.6±3.0[b] | 26.2 |
| 100 | 62.7±4.1[b] | 31.3 | 63.8±3.9[b] | 34.6 | 59.4±1.2[b] | 31.3 |

Rats were given i.p. Me-Chl-Na at various doses as indicated and sacrificed 24 h after the injection. Each result is the mean ± S.E. for 5—7 rats. *a*) nmol HCHO formed/mg protein/20 min. *b*) Significantly different from the control, $p < 0.05$.

Table II. Effect of Me-Chl-Na at Various Dose Levels on Microsomal Aniline Hydroxylase Activity in Rat Liver

| Me-Chl-Na treatment (mg/kg) | Cu-Chl-Na | | Co-Chl-Na | | Fe-Chl-Na | |
|---|---|---|---|---|---|---|
| | Activity[a] | Dec. (%) | Activity[a] | Dec. (%) | Activity[a] | Dec. (%) |
| Control | 22.5±0.4 | | 22.5±0.8 | | 21.5±1.1 | |
| 10 | 21.5±0.6 | 3.6 | 20.5±0.9 | 8.9 | 20.7±0.7 | 3.7 |
| 25 | 18.2±1.0[b] | 19.1 | 12.4±0.5[b] | 44.9 | 16.2±0.9[b] | 24.7 |
| 50 | 13.8±0.4[b] | 38.7 | 10.4±0.7[b] | 53.8 | 13.4±0.5[b] | 37.7 |
| 100 | 11.8±0.4[b] | 47.6 | 9.2±0.7[b] | 59.1 | 9.8±0.5[b] | 54.4 |

Treatment of rats was carried out as described in the legend to Table I. Each result is the mean ± S.E. for 5—7 rats. *a*) nmol *p*-aminophenol formed/mg protein/20 min. *b*) Significantly different from the control, $p < 0.05$.

and III show the results. All three Me-Chl-Na at doses of 25 mg/kg or above caused a significant decrease of both aminopyrine *N*-demethylase and aniline hydroxylase activities, and significantly reduced the content of cytochrome P-450. The maximal effects were observed with the dose of 100 mg/kg. Co-Chl-Na showed a relatively high reductive activity among the three Me-Chl-Na examined.

To clarify whether the decrease of the enzyme activities by Me-Chl-Na is ascribed either to an altered affinity of cytochrome P-450 for the enzyme substrates or to the reduction in the

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Table III. Effect of Me-Chl-Na at Various Dose Levels on Microsomal Cytochrome P-450 Content in Rat Liver

| Me-Chl-Na treatment (mg/kg) | Cu-Chl-Na | | Co-Chl-Na | | Fe-Chl-Na | |
|---|---|---|---|---|---|---|
| | Content[a] | Dec. (%) | Content[a] | Dec. (%) | Content[a] | Dec. (%) |
| Control | 1.031±0.020 | | 1.090±0.021 | | 1.023±0.017 | |
| 10 | 0.995±0.011 | 3.5 | 1.012±0.034 | 7.2 | 0.946±0.046 | 7.5 |
| 25 | 0.912±0.032[b] | 11.5 | 0.803±0.017[b] | 26.3 | 0.860±0.017[b] | 15.9 |
| 50 | 0.783±0.032[b] | 24.1 | 0.713±0.015[b] | 34.6 | 0.806±0.041[b] | 21.2 |
| 100 | 0.738±0.024[b] | 28.4 | 0.710±0.023[b] | 34.9 | 0.714±0.022[b] | 30.2 |

Treatment of rats was carried out as described in the legend to Table I. Each result is the mean±S.E. for 5—7 rats. *a*) nmol/mg protein. *b*) Significantly different from the control, $p<0.05$.

Table IV. Effects of Me-Chl-Na Administration on Substrate-Induced Spectral Changes of Cytochrome P-450 in Liver Microsomes of Rats

| Me-Chl-Na treatment | Dose (mg/kg) | Spectral change | |
|---|---|---|---|
| | | Aminopyrine[a] | Aniline[b] |
| Control | | 7.8±0.2 | 15.4±0.4 |
| Cu-Chl-Na | 50 | 7.5±0.3 | 14.0±0.3[c] |
| | 100 | 7.5±0.4 | 14.1±0.3[c] |
| Co-Chl-Na | 50 | 7.6±0.4 | 14.2±0.3[c] |
| | 100 | 7.9±0.3 | 15.3±0.1 |
| Fe-Chl-Na | 50 | 7.1±0.2[c] | 14.0±0.5 |
| | 100 | 8.0±0.4 | 14.4±0.4 |

Rats were given i.p. Me-Chl-Na and sacrificed 24 h after the injection. Difference spectra induced with 2 mM aminopyrine or aniline were determined in the mixture containing 6 mg of microsomal protein, 102.7 mM KCl, and 50 mM potassium phosphate buffer (pH 7.5) in a final volume of 3.0 ml. Each result is the mean±S.E. for 5—7 rats. *a*) $\Delta A_{500-421\,nm}\times 10^3$/nmol cytochrome P-450. *b*) $\Delta A_{431-500\,nm}\times 10^3$/nmol cytochrome P-450. *c*) Significantly different from the control, $p<0.05$.

content of the cytochrome, or to both, we determined the substrate-induced spectral changes of the cytochrome P-450 and carried out a kinetic analysis of aminopyrine *N*-demethylation and aniline hydroxylation in liver microsomes. As shown in Table IV, the Me-Chl-Na tested had little or no effect on the spectral interactions between the microsomal cytochrome P-450 and its substrates, *i.e.*, aminopyrine and aniline. The Lineweaver-Burk plot of aminopyrine *N*-demethylase activity in liver microsomes from rats killed 24 h after the administration of a 100 mg/kg dose of Cu-Chl-Na is depicted in Fig. 2. The enzyme in control hepatic microsomes had two apparent $K_m$ values (0.54 and 1.63 mM) toward aminopyrine, in accordance with the findings reported by Pederson and Aust,[17] and Aust and Stevens.[18] The enzyme in microsomes from rats treated with Cu-Chl-Na also had two apparent $K_m$ values (0.51 and 1.47 mM), which were almost the same as those of the control, whereas the $V_{max}$ values were much lower in microsomes from the Cu-Chl-Na-treated rats (88 and 126 nmol HCHO formed/mg protein/20 min) than in those from control rats (125 and 184 nmol HCHO formed/mg protein/20 min). These results indicate that the inhibition of aminopyrine *N*-demethylation is non-competitive. In the case of aniline hydroxylase, we observed one $K_m$ value and one $V_{max}$ value for the aniline hydroxylation in microsomes both from control and from treated rats, in contrast to the case of aminopyrine *N*-demethylation, when the substrate was used at concentrations of 0.05 to 0.2 mM. The inhibition of aniline hydroxylase caused by

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Table V. Effects of Me-Chl-Na Administration on Content of Cytochrome $b_5$ and Activity of NADPH–Cytochrome c Reductase in Rat Liver Microsomes

| Me-Chl-Na treatment | Dose (mg/kg) | Cytochrome $b_5$ | | NADPH–cytochrome c reductase | |
|---|---|---|---|---|---|
| | | Content[a] | Dec. (%) | Activity[b] | Dec. (%) |
| Control | | 0.528±0.012 | | 79.1±2.1 | |
| Cu-Chl-Na | 50 | 0.475±0.018[c] | 10.0 | 70.1±1.7[c] | 11.4 |
| | 100 | 0.443±0.014[c] | 16.1 | 65.9±1.7[c] | 16.7 |
| Co-Chl-Na | 50 | 0.414±0.018[c] | 21.6 | 63.5±0.4[c] | 19.7 |
| | 100 | 0.414±0.018[c] | 21.6 | 63.5±2.2[c] | 19.7 |
| Fe-Chl-Na | 50 | 0.450±0.013[c] | 14.8 | 68.1±2.4[c] | 13.9 |
| | 100 | 0.427±0.019[c] | 19.1 | 67.5±1.6[c] | 14.7 |

Treatment of rats was carried out as described in the footnote to Table IV. Each result is the mean±S.E. for 5—6 rats. *a*) nmol/mg protein. *b*) nmol cytochrome c reduced/mg protein/min. *c*) Significantly different from the control, $p<0.05$.

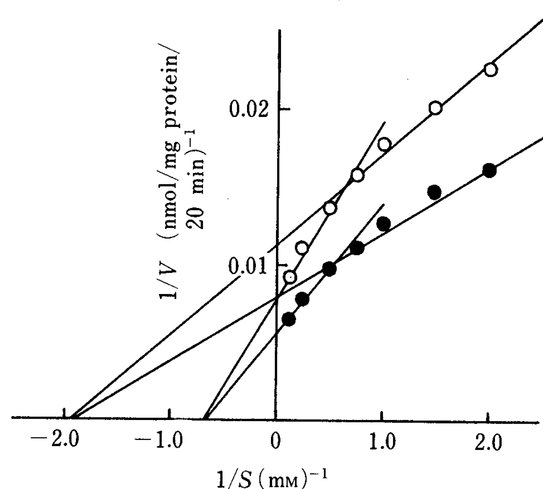


Fig. 2. Lineweaver–Burk Plot of Aminopyrine *N*-Demethylation in Liver Microsomes from Cu-Chl-Na-treated Rats

Rats were treated with Cu-Chl-Na (100 mg/kg, i.p.) and sacrificed 24 h after the injection. Each point represents the mean of 2 groups of 3 rats.
—●—, control; —○—, Cu-Chl-Na-treated.

Table VI. Effect of Cu-Chl-Na Administration on Hexobarbital Sleeping Time in Rats

| Cu-Chl-Na treatment (mg/kg) | Sleeping time (min) | % |
|---|---|---|
| Control | 30.3±1.4 | 100 |
| 50 | 40.4±0.9[a] | 133 |
| 100 | 42.7±2.1[a] | 141 |

Twenty-four hours after i.p. administration of Cu-Chl-Na, rats were given i.p. hexobarbital (sodium salt, 150 mg/kg). Each result is the mean±S.E. for 6—7 rats. *a*) Significantly different from the control, $p<0.05$.

Cu-Chl-Na treatment was also non-competitive: the $K_m$ values for the control and treated rat liver microsomes were 0.035 and 0.037 mM, respectively, and the $V_{max}$ values for the control and treated rat liver microsomes were 18.2 and 9.9 nmol *p*-aminophenol formed/mg protein/20 min, respectively. These findings indicate that the decreases in oxidative drug-metabolizing enzyme activities in liver microsomes caused by Me-Chl-Na treatment might be mainly associated with a reduction in the content of cytochrome P-450, but not with a change in the affinity of the cytochrome for its substrates. In the case of aniline hydroxylation, the specific activities, when expressed as activities per nmol cytochrome P-450, were somewhat reduced by Me-Chl-Na-treatment (*cf.* Tables II and III). This may be due to a decrease of the cytochrome P-450 species responsible for the metabolism of aniline by Me-Chl-Na treatment.

We also examined the effects of the three Me-Chl-Na on the content of another microsomal hemoprotein, cytochrome $b_5$, and on the activity of another microsomal enzyme,

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NADPH–cytochrome c reductase, of the hepatic drug-metabolizing enzyme system. As shown in Table V, all three Me-Chl-Na reduced the cytochrome $b_5$ content, though the extent of reduction was less than that of the reduction in cytochrome P-450 content (Table III), and inhibited, though to a minor extent, the reductase activity. These results indicate that Me-Chl-Na might have an inhibitory effect and a lowering effect on the activities and components, respectively, of the microsomal drug-metabolizing enzyme system in the liver when given to rats.

Table VI shows the effect of pretreatment of rats with Cu-Chl-Na on the hexobarbital-induced sleeping time. Cu-Chl-Na at doses of 50 and 100 mg/kg caused a significant prolongation of sleeping time. This is thought to be mainly a reflection of the inhibition by Cu-Chl-Na of the activities of the hepatic drug-metabolizing enzyme system by which the barbiturate is metabolized to pharmacologically inactive metabolites, not to an alteration in the action mechanism of the drug in the central nervous system. This may be further supported by the finding that neither trisodium copper chlorin $e_6$ nor disodium copper isochlorin $e_4$, constituents of Cu-Chl-Na,[19] penetrated the brain in rats when given i.p.[20]

In the following experiments, liver microsomes from rats receiving no Cu-Chl-Na were preincubated with Cu-Chl-Na to observe the *in vitro* effects of Cu-Chl-Na on the activities of aminopyrine *N*-demethylase and aniline hydroxylase and on the content of cytochrome P-450. The content of Cu-Chl-Na in liver microsomes from rats 24 h after the i.p. injection of a 100 mg/kg dose of Cu-Chl-Na was $0.514 \pm 0.017$ $\mu$g eq to Cu-Chl-Na/mg protein, as measured by the method of Sato *et al.*[5] As the content can be calculated to be approximately equivalent

TABLE VII. Effects of Preincubation of Rat Liver Microsomes with Cu-Chl-Na on Drug-Metabolizing Enzyme Activities and Content of Cytochrome P-450

| Concn. of Cu-Chl-Na (%, w/v) | Aminopyrine *N*-demethylase[a] | Aniline hydroxylase[b] | Cytochrome P-450 content[c] |
|---|---|---|---|
| 0 | 105.4±3.1 | 17.3±0.4 | 1.053±0.009 |
| $0.15\times10^{-3}$ | 106.6±3.4 | 17.5±0.4 | — |
| $0.3\times10^{-3}$ | 109.1±4.0 | 17.5±0.3 | — |
| $0.5\times10^{-3}$ | 109.4±3.2 | 17.5±0.4 | 0.999±0.009 |
| $1.0\times10^{-3}$ | 109.3±3.6 | 17.4±0.4 | 0.991±0.005 |

After the preincubation (37 °C, 30 min) of microsomes (3 mg protein/ml) with Cu-Chl-Na at various final concentrations as indicated, the enzyme activities and cytochrome P-450 content were determined. Each result is the mean±S.E. for 3 experiments. *a*) nmol HCHO formed/mg protein/20 min. *b*) nmol *p*-aminophenol formed/mg protein/20 min. *c*) nmol/mg protein.

TABLE VIII. Effect of Me-Chl-Na at Various Dose Levels on Heme Content in Rat Liver Microsomes

| Me-Chl-Na treatment (mg/kg) | Cu-Chl-Na | | Co-Chl-Na | | Fe-Chl-Na | |
|---|---|---|---|---|---|---|
| | Content[a] | Dec. (%) | Content[a] | Dec. (%) | Content[a] | Dec. (%) |
| Control | 1.704±0.026 | | 1.749±0.046 | | 1.664±0.015 | |
| 10 | 1.654±0.020 | 2.9 | 1.629±0.039 | 6.9 | 1.626±0.024 | 2.3 |
| 25 | 1.544±0.047[b] | 9.4 | 1.359±0.029[b] | 22.3 | 1.443±0.025[b] | 13.3 |
| 50 | 1.402±0.054[b] | 17.7 | 1.255±0.033[b] | 28.2 | 1.375±0.086[b] | 17.4 |
| 100 | 1.324±0.055[b] | 22.3 | 1.259±0.026[b] | 28.0 | 1.242±0.033[b] | 25.4 |

Treatment of rats was carried out as described in the footnote to Table I. Each result is the mean±S.E. for 5—7 rats. *a*) nmol/mg protein. *b*) Significantly different from the control, $p<0.05$.

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to $0.17 \times 10^{-3}\%$ (w/v) or 2.5 $\mu$M, the concentrations ranging from $0.15 \times 10^{-3}$ to $1.0 \times 10^{-3}\%$ (w/v) of Cu-Chl-Na used in the present study were approximately equal to or higher than those observed in *in vivo* experiments. As shown in Table VII, preincubation of the microsomes with various concentrations of Cu-Chl-Na did not cause any alteration in the enzyme activities and cytochrome content. This result suggests that the reduction of enzyme activities and cytochrome content in hepatic microsomal drug-metabolizing enzyme system is not brought about by degradation of cytochrome P-450 itself by Me-Chl-Na.

Considering the findings with porphyrin compounds reported by Maines and Kappas,[6-8] it can be assumed that the decrease in the content of microsomal cytochromes by Me-Chl-Na administration is associated with an alteration in the content of protoheme as their prosthetic group. Thus, we measured the total heme content in liver microsomes from rats killed 24 h after the administration of Me-Chl-Na. Table VIII shows the results. The total heme content exceeded the summed contents of the two microsomal hemoproteins, cytochromes P-450 (Table III) and $b_5$ (Table V), in control rat liver microsomes in accordance with the results reported by Maines and Kappas.[6] Significant reductions in the heme contents were observed with doses of 25 mg/kg or above of any of the three Me-Chl-Na. The maximal effect was found at the doses of 50 and 100 mg/kg of Co-Chl-Na, which caused about 28% decrease in heme content. Co-Chl-Na showed relatively high activity among the three Me-Chl-Na. These results were similar to those in the experiment to determine the effects on the cytochrome P-450 content (Table III). The reduction in total heme content seems to be associated with a reduction in the contents of the two hemoproteins but not with a reduction in the content of heme fraction except the hemoproteins in microsomes (*cf.* Tables III and V).

Our present results strongly suggest that the decreases in the activities of the hepatic microsomal drug-metabolizing enzyme system caused by Me-Chl-Na are brought about by primary actions of Me-Chl-Na on the control mechanism of microsomal hemoprotein levels. The mechanisms by which liver microsomal hemoproteins are decreased by Me-Chl-Na remain to be elucidated and are under investigation in our laboratories.

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