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Profile of Hemoproteins and Heme-Metabolizing Enzymes in Rats Treated with Surfactants

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Surface-active agents including non-ionic surfactants are widely used in industrial, agricultural and household fields as well as in biological research. From this viewpoint, their toxicological properties have been investigated extensively, that is, usual acute and chronic toxicity tests (Oser and Morgareidge 1965; Buehler et al. 1971), metabolic absorption and excretion studies(Michael 1968; Merits 1975; Cresswell et al. 1978; Drotman 1980), carcinogenic and teratogenic toxicity studies(Saffiotti et al. 1962; Palmer et al. 1975), reproductive toxicity test(Buehler et al. 1971), and so on(Burke et al. 1976).

Previous studies in our laboratories demonstrated that intraperitoneal administration of anionic surfactant linear alkylbenzenesulfonate(LAS) to red carp remarkably depressed the content of cytochrome P-450 and the activity of P-450 dependent 7-ethoxycoumarin(7-EC) O-deethylase in hepatopancreas, despite no significant change in the activity of heme oxygenase, the first and rate-limiting enzyme in heme degradation. In addition, we observed also decrease of metal binding proteins, like metallothionein, which plays an important role in homeostasis of essential metals and in protection from metal toxicity, in hepatopancreas of red carp treated with LAS and non-ionic surfactant polyoxyethyleneglycol nonylphenyl ether(Emulgen 913).

Therefore, in this study, we have undertaken to clarify the detailed effects of synthetic surfactants having different chemical structures and properties on the contents of hepatic heme, hemoproteins and metallothionein and on the activities of heme- and drug-metabolizing enzymes in rats.

MATERIALS AND METHODS

D-Glucose-6-phosphate,D-glucose-6-phosphate dehydrogenase and NADPH were purchased from Boehringer Mannheim-Yamanouchi Co., Ltd.,(Tokyo, Japan), and NADP and ATP were obtained from Oriental Yeast Co.,Ltd.,(Tokyo, Japan). Emulgen 913 was obtained from Kao Co.,Ltd.,(Tokyo, Japan). Sodium dodecyl sulfate(SDS), LAS and

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other chemicals used were of highest grade quality purchased from Wako Pure Chemical Industries Ltd.,(Osaka,Japan). Male Wistar rats were used in all experiments. Animals received normal chow and water ad libitum. Rats were given a single intraperitoneal injection of Emulgen 913, SDS and LAS which dissolved in distilled water at 2ml per dose per kg, respectively. After treatment with surfactants animals were killed by decapitation, and the liver was perfused in situ with a cold 0.9% NaCl solution and then removed, washed and weighed. The livers were homogenized with 4 volumes of 0.25M sucrose in a Potter-Elvehjem homogenizer with a teflon pestle. Preparation of 105000xg soluble fraction and microsomes were carried out by procedures described Arizono et al.(1982) and Ariyoshi et al.(1970), respectively.

Methods for determination were as follows --- protein:Lowry et al. (1951); microsomal total heme:Schenkman et al.(1973); cytochrome P-450 and b_5 content:Omura and Sato(1964); δ-aminolevulinic acid (δ-ALA) synthetase:Marver et al.(1966); ferrochelatase:Rifkind (1979); heme oxygenase:Maines and Kappas(1976); tryptophan pyrrolase:Badawy and Evans(1975); NADPH-cytochrome c reductase:Omura and Takesue(1970); aniline hydroxylase:Imai et al.(1966); aminopyrine demethylase:Cochin and Axelrod(1959); 7-EC O-deethylase:Ullrich and Weber (1972); UDP-glucuronyltransferase(UDPGT):Storey (1965); phenol sulfate conjugating(PSC) activity:Roy(1956); metallothionein content: Onosaka et al.(1978).

RESULTS AND DISCUSSION

The effects of the injection of two kinds of surfactants, anionic SDS and non-ionic Emulgen 913, at a single dose of 50mg/kg on the contents of heme and hemoproteins and on the activities of heme- and drug-metabolizing enzymes are summarized in Table 1. The gain of body weight was slightly inhibited by each surfactant in the doses used, and a ratio of liver weight to 100g body weight was significantly decreased. The contents of P-450, b_5 and microsomal total heme were depressed by both SDS and Emulgen 913. On the contrary, heme oxygenase activity was highly enhanced by both SDS and Emulgen 913, whereas no significant changes were observed in the activities of δ-ALA synthetase or ferrochelatase, which is first or last and rate-limiting enzyme in heme biosythetic pathway, respectively, when compared with that of respective control. These findings suggest that the stimulation of heme oxygenase may be associated with the degradation of microsomal heme and hemoproteins.

As it was demonstrated that tryptophan pyrrolase may play a unique role in heme utilization by liver hemoprotein(Badawy 1978), we have made an attempt to clarify the heme-saturation ratio of pyrrolase by Emulgen 913 treatment. Consequently, rats were sacrificed at 6, 12, 18, 24 and 48 hr after the last injection of Emulgen 913 at a dose of 100mg/kg and the results are shown in Table 2 and 3. Heme-saturation ratio calculated for tryptophan pyrrolase activity was decreased as compared with that of 0-time as shown in Table 2. This means to limit the availability of heme by inhibiting its synthesis or enhancing its degradation by stimulating heme oxygenase activity. In fact, as shown in Table

Table 1. Effects of the synthetic detergents on the contents of heme and hemoproteins and on the activities of heme- and drug-metabolizing enzymes in the liver of rats

	Control	SDS	Emulgen 913
Body weight Initial(g)	193 ± 2	192 ± 3	191 ± 2
Final(g)	197 ± 2	185 ± 4	186 ± 3
Liver weight(g/100g body weight)	5.08 ± 0.05	4.19 ± 0.26*	3.85 ± 0.18*
Microsomal total heme(nmole/mg protein)	1.69 ± 0.07	1.26 ± 0.03***	1.39 ± 0.13
Cytochrome P-450(nmole/mg protein)	0.94 ± 0.05	0.69 ± 0.04*	0.73 ± 0.03*
Cytochrome b_5(nmole/mg protein)	0.22 ± 0.01	0.16 ± 0.01**	0.19 ± 0.01*
δ-ALA synthetase(nmole/g liver/hr)	51.7 ± 4.6	47.2 ± 2.9	49.1 ± 1.4
Ferrochelatase(nmole/mg protein/hr)	1.49 ± 0.03	1.42 ± 0.05	1.43 ± 0.08
Heme oxygenase(nmole/mg protein/hr)	1.14 ± 0.10	2.44 ± 0.22**	1.84 ± 0.24*
NADPH-cytochrome c reductase#	53.1 ± 0.3	47.0 ± 1.5*	46.5 ± 1.7*
Aniline hydroxylase#	0.83 ± 0.02	0.73 ± 0.05	0.76 ± 0.07
Aminopyrine demethylase#	4.38 ± 0.32	3.56 ± 0.50	3.59 ± 0.27
7-Ethoxycoumarin O-deethylase#	0.53 ± 0.03	0.43 ± 0.02	0.39 ± 0.03*
UDP-glucuronyltransferase(nmole/mg protein/30min)	19.5 ± 0.1	16.8 ± 0.7	20.6 ± 0.9

Animals were intraperitoneally injected with detergents(50mg/kg) at 24hr before sacrifice. Values are the mean ± S.E. of 4 to 6 rats. # nmole/mg protein/min. Significantly different from corresponding mean of control(*$P < 0.05$;**$P < 0.02$;***$P < 0.01$).

Table 2. Time-course of the effect of Emulgen 913 treatment on rat liver tryptophan pyrrolase activity and the heme-saturation ratio

Time after treatment(hr)	Tryptophan pyrrolase activity (μmole/g liver/hr)		Heme-saturation ratio
	Holoenzyme activity	Total enzyme activity	
0	0.98 ± 0.05	2.53 ± 0.16	0.63 ± 0.04
6	1.12 ± 0.12	3.30 ± 0.24	0.51 ± 0.05
12	1.43 ± 0.22	4.54 ± 0.05*	0.45 ± 0.07
18	1.45 ± 0.13	4.48 ± 0.34*	0.47 ± 0.05
24	1.86 ± 0.18*	6.43 ± 0.74*	0.41 ± 0.04**
48	0.83 ± 0.09	2.67 ± 0.11	0.47 ± 0.10

Animals were ip injected with Emulgen 913 at the dose of 100mg/kg and sacrificed at indicated time. Values are the mean ± S.E. of 4 to 6 rats. Significantly different from corresponding mean of 0-time(*$P < 0.05$;**$P < 0.02$). Heme-saturation ratio is the ratio of holoenzyme/apoenzyme activity. Apoenzyme activity was calculated by difference of holoenzyme and total pyrrolase activities.

Table 3. Time-course of the effect of Emulgen 913 treatment on the contents of heme and hemoproteins and on the activities of heme- and drug-metabolizing enzymes in rats

	Time after treatment(hr)					
	0	6	12	18	24	48
Microsomal total heme (nmole/mg protein)	1.51±0.03	102±2	90±5	85±6	72±12	97±4
Cytochrome P-450 (nmole/mg protein)	0.74±0.06	119±7	88±5	68±6*	81±11	102±6
Cytochrome b_5 (nmole/mg protein)	0.20±0.01	95±9	84±1	79±2	68±5*	89±1
δ-ALA synthetase (nmole/g liver/hr)	53.6±2.1	111±4	87±5	81±6	120±4	106±4
Heme oxygenase (nmole/mg protein/hr)	1.29±0.06	164±25	302±29**	356±42***	306±46*	98±16
NADPH-cytochrome c reductase (nmole/mg protein/min)	57.6±2.6	94±4	86±3	101±6	80±4*	100±5
Aniline hydroxylase (nmole/mg protein/min)	0.79±0.05	103±7	85±2	72±5*	92±4	98±1
Aminopyrine demethylase (nmole/mg protein/min)	5.90±0.65	83±8	64±6*	47±3**	71±6	94±8
7-Ethoxycoumarin O-deethylase (nmole/mg protein/min)	0.64±0.03	94±1	77±9	65±6**	74±8*	85±5
UDP-glucuronyltransferase (nmole/mg protein/30 min)	16.5±1.6	89±6	117±5	134±5	89±2	88±7

Conditions were as described in Table 2. Each value represents as percentage of corresponding value of 0-time(control). Significantly different from corresponding mean of 0-time(*$P < 0.05$; **$P < 0.02$;***$P < 0.01$).

Table 4. Effect of the sodium n-dodecylbenzenesulfonate(LAS) on the contents of heme, hemoprotein and metallothionein and on the activities of heme- and drug-metabolizing enzymes in rats

	Control	LAS	
		50mg/kg	100mg/kg
Body weight Initial(g)	200 ± 4	198 ± 3	197 ± 2
Final(g)	210 ± 4	196 ± 4	194 ± 4
Liver weight(g/100g body weight)	4.92 ± 0.10	4.11 ± 0.09**	3.80 ± 0.13***
Microsomal total heme(nmole/mg protein)	1.76 ± 0.07	1.50 ± 0.03*	1.20 ± 0.01***
Cytochrome P-450(nmole/mg protein)	0.69 ± 0.06	0.58 ± 0.02	0.43 ± 0.06*
Cytochrome b_5(nmole/mg protein)	0.18 ± 0.02	0.18 ± 0.01	0.09 ± 0.06*
δ-ALA synthetase(nmole/g liver/hr)	51.0 ± 7.4	44.4 ± 5.6	25.0 ± 6.6
Heme oxygenase(nmole/mg protein/hr)	1.18 ± 0.19	2.25 ± 0.11**	4.03 ± 0.82*
NADPH-cytochrome c reductase#	48.3 ± 1.2	42.8 ± 0.1	37.7 ± 2.2*
Aniline hydroxylase#	0.73 ± 0.07	0.69 ± 0.02	0.47 ± 0.08
Aminopyrine demethylase#	4.18 ± 0.44	4.16 ± 0.17	2.80 ± 0.55
7-Ethoxycoumarin O-deethylase#	0.78 ± 0.19	0.63 ± 0.11	0.27 ± 0.03*
UDP-glucuronyltransferase(nmole/mg protein/30 min)	15.6 ± 2.3	18.7 ± 1.2	16.7 ± 1.2
Phenol sulfate conjugating(nmole/mg protein/hr)	27.9 ± 1.8	19.4 ± 1.6	22.0 ± 1.3
Metallothionein(μg/mg protein)	0.14 ± 0.01	1.14 ± 0.35*	2.29 ± 0.25***

Animals were ip injected with LAS(50 or 100mg/kg) at 18hrs before sacrifice. Values are the mean ± S.E. of 4 to 6 rats. # nmole/mg protein/min. Significantly different from corresponding mean of control(*$P<0.05$;**$P<0.02$;***$P<0.01$).

3, Emulgen 913 increased heme oxygenase 3-, 3.6- or 3.1-fold at 12, 18 or 24 hr after treatment, respectively, whereas slight inhibition or no significant alteration was observed in the activity of δ-ALA synthetase when compared with that of respective 0-time. The contents of P-450, b_5 and microsomal heme were also depressed at 18 or 24 hr. However, the magnitude of decrease was fairly different for measured parameters at each time after treatment, and that decrease restored nearly to normal level in 48 hr.

Table 4 shows that the effects of LAS at a single dose of 50 or 100 mg/kg on the above measured parameters including metallothionein content and PSC activity. LAS at the dose of 100mg/kg used decreased markedly the ratio of liver weight to 100g body weight and the contents of P-450, b_5 and total heme 18 hr after treatment. In addition, NADPH-cytochrome c reductase and 7-EC O-deethylase activity were greatly depressed, and also δ-ALA synthetase, aniline hydroxylase, aminopyrine demethylase and PSC activities showed the tendency to be decreased. However, no marked alteration in UDPGT activity was noted. In contrast, heme oxygenase activity and metallothionein content were markedly enhanced by LAS in the doses used in this experiment. This observation differs apparent from previous finding which obtained from hepatopancreas of red carp treated with LAS(Ariyoshi et al. 1989). This reason is unclear, but it seems to be due to the species differences. However, it is of interest that synthetic surfactants have a potential inducibility for heme oxygenase even if a magnitude of enhancement is obviously different from each material. This suggests that hepatic heme oxygenase in mammals may be one of common active-sites against surfactants.

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