Bull. Environ. Contam. Toxicol. (1991) 46:120–127

Bulletin of Environmental Contamination and Toxicology

Effects of Surfactants on the Contents of Metallothionein, Heme and Hemoproteins and on the Activities of Heme Oxygenase and Drug-Metabolizing Enzymes in Rats Pretreated with Phenobarbital or β-Naphthoflavone

Toshihiko Ariyoshi, Hiroyuki Hasegawa, Hideki Matsumoto, and Koji Arizono

Department of Hygienic Chemistry and Toxicology, Faculty of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-cho, Nagasaki, 852, Japan

Synthetic surfactants as major constituent of detergent products are widely used in consumer and industrial fields, and hence environmental and toxicological investigations of surfactants are numerous. In the previous study, we observed that intraperitoneal administration of surfactants such as sodium dodecyl sulfate(SDS), sodium n-dodecylbenzenesulfonate(LAS) and polyoxyethyleneglycol nonylphenyl ether (Emulgen 913) to rats depressed the content of microsomal cytochrome P-450, while they enhanced markedly the activity of heme oxygenase, the first and rate-limiting enzyme in heme degradation (Ariyoshi et al. 1990). In addition, we noted an increase of metallothionein content in the liver of rats treated with LAS.

In this study, we investigated the effects of surfactants on the contents of metallothionein, heme and hemoproteins and on the activities of heme oxygenase and drug-metabolizing enzymes in the liver of rats pretreated with phenobarbital or β-naphthoflavone.

MATERIALS AND METHODS

Enzymes, cofactor and chemical sources were as follows:D-glucose -6-phosphate, D-glucose-6-phosphate dehydrogenase and NADPH from Boehringer-Mannheim-Yamanouchi Co.,Ltd.,(Tokyo, Japan);NADP from Oriental Yeast Co.,Ltd.,(Tokyo,Japan); cytochrome c and bovine serum albumin from Sigma Chemical Co.,(St.Louis, USA); β-naphthoflavone(β-NF) from Aldrich Chemical Co.,Inc.,(Milwaukee, USA); Emulgen 913 from Kao Co.,Ltd.,(Tokyo,Japan); SDS,LAS,sodium phenobarbital(PB) and other chemicals 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 rat chow and water ad libitum. Rats were generally given a single intraperitoneal injection of SDS, LAS and Emulgen 913 dissolved in distilled water at 2 ml per dose per kg of body weight, respectively. In the case of cytochrome P-450 inducer pretreatment, animals were intraperitoneally administered PB at the dose of 80 mg per kg of body weight, dissolved in distilled water at 2 ml, once a day for 5 days followed by Emulgen 913.

Send reprint requests to T. Ariyoshi at the above address.

β-NF treated animals received 80 mg β-NF per kg of body weight, dissolved in corn oil at 2 ml, intraperitoneally for 3 consecutive days followed by surfactant. Control animals received intraperitoneal distilled water or corn oil injections at the same time. Animals were sacrificed 18 hr after the administration of surfactants. The livers were perfused in situ with a cold 0.9% saline and then removed. The microsomes and cytosol were prepared as described previously(Ariyoshi et al. 1990).

Methods for determination were as follows-------Protein content: Lowry et al.(1951); metallothionein(MT) content: Onosaka et al.(1978); microsomal total heme:Schenkman et al.(1973); cytochrome P-450 and b_5: Omura and Sato(1964); heme oxygenase activity:Maines and Kappas(1976); DT-diaphorase activity: Ernster (1967); NADPH-cytochrome c reductase: Omura and Takesue(1970); aniline hydroxylase : Imai et al.(1966); Aminopyrine demethylase: Cochin and Axelrod(1959); 7-ethoxycoumarin O-deethylase :Ullrich and Weber(1972).

RESULTS AND DISCUSSION

Table 1 shows the effects of surfactants used at a single dose of 50 or 100 mg/kg on the contents of cytosolic zinc, MT, microsomal total heme and hemoproteins and on the activities of heme oxygenase, NADPH-cytochrome c reductase and 7-ethoxycoumarin O-deethylase. MT content was remarkably increased after the injection of surfactants in the doses used, and that of zinc also augmented except LAS treatment when compared with that of respective control. The results in other measured parameters were similar in magnitude to that obtained from previous experiment(Ariyoshi et al. 1990). These results partly agree with observation of Miura(1987), who demonstrated that intraperitoneal injection of Emulgen 913(50 or 100 mg/kg) or SDS(25 or 50 mg/kg) significantly decreased the cytochrome P-450 content and aminopyrine N- and p-nitroanisole O-demethylation activities in the liver of rats, despite of 3 consecutive injections.

Effects of Emulgen 913 on the contents of zinc and MT and on the activities of DT-diaphorase and NADPH-cytochrome c reductase in the liver of rats pretreated with PB or β-NF are shown in Table 2. PB alone increased the NADPH-cytochrome c reductase activity, confirming previous reports(Orrenius et al. 1965; Kato 1966), but had no effect on the other parameters. Pretreatment of rats with PB decreased the Emulgen 913-induced elevation in the contents of zinc or MT, but did not exert any significant effects on the activities of NADPH-cytchrome c reductase or DT-diaphorase in comparison with PB alone. These results suggest that PB enhanced Emulgen 913 metabolism in vivo, and consequently might reduce the contents of surfactant or its metabolites in the liver.

In contrast, β-NF alone markedly increased the activity of DT-diaphorase, confirm previous finding (Kumaki et al. 1977), and the content of MT, whereas decreased the activity of NADPH-cytochrome c reductase. The increased MT content or DT-diaphorase activity caused by β-NF were further enhanced by Emulgen 913, while NADPH-

Table 1. Effects of the surfactants on the contents of zinc, metallothionein, heme and hemoproteins and on the activities of heme oxygenase and drug-metabolizing enzymes in the liver of rats

	Control	SDS 50mg/kg	LAS 50mg/kg	Emulgen 913 100mg/kg
Cytosol				
Zinc(μg/mg protein)	4.81 ± 0.11	7.95 ± 0.63*	5.53 ± 0.51	8.06 ± 1.30*
Metallothionein(μg/mg protein)	0.12 ± 0.01	1.08 ± 0.25**	0.59 ± 0.04**	1.39 ± 0.09***
Microsome				
Total heme(nmole/mg protein)	1.80 ± 0.06	1.58 ± 0.10	1.44 ± 0.04***	1.35 ± 0.10***
Cytochrome P-450(nmole/mg protein)	0.74 ± 0.02	0.61 ± 0.04	0.52 ± 0.04***	0.38 ± 0.04***
Cytochrome b_5(nmole/mg protein)	0.21 ± 0.01	0.20 ± 0.02	0.19 ± 0.01	0.13 ± 0.03*
Heme oxygenase#	1.34 ± 0.07	2.30 ± 0.23*	2.35 ± 0.13**	3.48 ± 0.22***
NADPH-cytochrome c reductase##	46.0 ± 0.4	36.6 ± 1.6*	38.3 ± 1.1**	33.4 ± 2.2**
7-Ethoxycoumarin O-deethylase##	0.77 ± 0.01	0.63 ± 0.06	0.58 ± 0.05*	0.54 ± 0.02***

Rats were intraperitoneally injected with surfactants(50 or 100mg/kg) at 18hr before sacrifice. Values are the mean ± S.E. of 4 rats. # nmole/mg protein/hr; ## nmole/mg protein/min. Significantly different from corresponding mean of control(*P<0.05; **P<0.02; ***P<0.01).

cytochrome c reductase was depressed as shown in Table 2. These observations suggest that the alteration of Emulgen 913 metabolism by β-NF may have some effects on the enhancement of MT content or DT-diaphorase activity.

Effects of pretreatment with PB or β-NF on the content of cytochrome P-450 and on the activities of heme oxygenase and drug-metabolizing enzymes by Emulgen 913 are shown in Table 3. Heme oxygenase activity remained unaltered by PB injection alone, although that activity was significantly depressed by β-NF injection alone. PB pretreatment did not prevent the increase in the heme oxygenase activity caused by Emulgen 913, but that activity induced by surfactant was slightly inhibited by β-NF pretreatment.

On the other hand, the contents of cytochrome P-450 were markedly increased by treatment with PB or β-NF. However, the increased content of cytochrome P-450 caused by PB was remarkably depressed by subsequent injection of surfactant, whereas that of enhanced cytochrome P-450 by β-NF was not affected by surfactant administration. This suggests that Emulgen 913 may have a distinct effect on the cytochrome P-450 isozymes.

PB or β-NF induced the activities of aniline hydroxylase(114 or 96%), aminopyrine demethylase(135 or 68%) and 7-ethoxycoumarin O-deetylase(316 or 727%), respectively, when compared with those of respective control. Combined administration of the PB plus surfactant caused a further decrease in the activities of aniline hydroxylase or aminopyrine demethylase than the values obtained by PB treatment alone. However, the combination of β-NF and surfactant did not cause any notable changes in these activities in comparison with β-NF alone. In contrast, no effect was observed in the 7-ethoxycoumarin O-deethylase activity by combined injection of the PB plus Emulgen 913 as compared with PB injection alone. However, the combination of β-NF and surfactant treatment produced a further decrease in that activity than the values obtained by β-NF alone. These results suggest that Emulgen 913 has an effect on the affinity of the cytochrome P-450 isozymes for their respective substrates.

In the previous time-course experiment, Emulgen 913 treatment in rats revealed that a maximum effects on the contents of heme and hemoproteins and on the activities of heme oxygenase and drug-metabolizing enzymes were noted at 18 hr after injection(Ariyoshi et al. 1990). In the case of dose-relate experiment, animals were administered Emulgen 913 at a single dose of 25, 50 and 100 mg/kg, and we observed a maximum effects on the above parameters at the dose of 100 mg/kg(data not shown). In this study, therefore, all animals used were sacrificed 18 hr after treatment with surfactants, and Emulgen 913 was given to animals at a dose of 100 mg/kg. From this study we observed the increased MT levels by surfactants treatment as shown in Table 1. However, this elevation mechanism in MT content by surfactants is unclear. Our previous study indicated the enhanced apoenzyme activity of tryptophan pyrrolase by Emulgen 913 treatment(Ariyoshi et al. 1990). Schimke et al.(1965) reported that glucocorticoid induction of the

Table 2. Effects of Emulgen 913 on the contents of zinc and metallothionein and on the enzymes activities in the liver of rats pretreated with phenobarbital or β-naphthoflavone

A)	Control (Saline)	Emulgen 913	Phenobarbital	Phenobarbital + Emulgen 913
Cytosolic zinc (μg/mg protein)	6.13 ± 0.15	9.94 ± 0.04*	6.47 ± 0.60	6.68 ± 0.81
Metallothionein (μg/ mg protein)	0.09 ± 0.01	2.44 ± 0.05***	0.22 ± 0.08	2.07 ± 0.39*
DT-Diaphorase(nmole/mg protein/min)	157.0 ± 14.7	156.7 ± 23.2	195.2 ± 40.1	294.8 ± 36.3
NADPH-cytochrome c reductase#	37.8 ± 1.0	28.0 ± 0.40*	59.7 ± 2.2***	60.4 ± 1.9***

B)	Control (Corn oil)	β-Naphthoflavone	β-Naphthoflavone + Emulgen 913
Cytosolic zinc (μg/mg protein)	4.59 ± 0.05	4.75 ± 0.07	5.13 ± 0.31
Metallothionein (μg/ mg protein)	0.36 ± 0.08	1.07 ± 0.14**	2.08 ± 0.17***
DT-Diaphorase(nmole/mg protein/min)	107.3 ± 8.4	523.5 ± 30.0***	1029.7 ± 265.0*
NADPH-cytochrome c reductase#	40.8 ± 1.4	33.1 ± 2.0*	26.4 ± 1.7**

Values are the mean ± S.E. for each group of 3 to 4 rats. # nmole/mg protein/min. Significantly different from corresponding mean of saline or corn oil injection group(*P<0.05; **P<0.02; ***P<0.01).

Table 3. Effects of Emulgen 913 on the content of cytochrome P-450 and on the activities of heme oxygenase and drug-metabolizing enzymes in the liver of rats pretreated with phenobarbital or β-naphthoflavone

A)	Control (Saline)	Emulgen 913	Phenobarbital	Phenobarbital + Emulgen 913
Cytochrome P-450(nmole/mg protein)	0.95 ± 0.05	0.66 ± 0.03***	1.86 ± 0.13***	1.05 ± 0.04
Heme oxygenase(nmole/mg protein/hr)	0.85 ± 0.13	3.56 ± 0.29***	1.10 ± 0.34	4.10 ± 0.30***
Aniline hydroxylase#	0.97 ± 0.04	0.62 ± 0.03**	1.09 ± 0.07	0.62 ± 0.02***
Aminopyrine demethylase#	5.31 ± 0.33	2.90 ± 0.64*	7.18 ± 0.40*	4.57 ± 0.65
7-Ethoxycoumarin O-deethylase#	0.62 ± 0.03	0.32 ± 0.02***	1.96 ± 0.28***	1.95 ± 0.26***

B)	Control (Corn oil)	β-Naphthoflavone	β-Naphthoflavone + Emulgen 913
Cytochrome P-450(nmole/mg protein)	0.68 ± 0.04	1.02 ± 0.05***	0.93 ± 0.09*
Heme oxygenase(nmole/mg protein/hr)	1.41 ± 0.12	0.96 ± 0.01***	2.74 ± 0.10***
Aniline hydroxylase#	0.76 ± 0.08	0.73 ± 0.05	0.53 ± 0.05
Aminopyrine demethylase#	4.04 ± 0.46	2.75 ± 0.42	2.50 ± 0.24
7-Ethoxycoumarin O-deethylase#	0.56 ± 0.08	4.07 ± 0.35***	3.14 ± 0.29***

Values are the mean ± S.E. for each group of 3 to 4 rats. # nmole/mg protein/min. Significantly different from corresponding mean of saline or corn oil injection group(*P<0.05; **P<0.02; ***P<0.01).

tryptophan pyrrolase activity is associated with an enhanced synthesis of the apoenzyme. Judging from above report and our findings, MT induction by surfactants seems to be based upon the hormone-mediated-type.

Denner et al.(1969) reported that alkyl sulfates administered to rats were mainly accumulated in the liver and extensively metabolized to butyric acid 4-sulfate as major end-product. In addition, there is a report in sewage sludge concerning the accumulation of toxic metabolites from nonionic surfactants(Giger et al. 1984). Therefore, several metabolites derived from Emulgen 913, SDS or LAS may be responsible for the induction of heme oxygenase activity or the loss of cytochrome P-450 in the liver of rats. Further study is required to elucidate the effects of small amounts of surfactants or their metabolites on the above measured parameters. However, we examined in vitro Emulgen 913 effects on the cytochrome P-450 and drug-metabolizing enzymes, namely we added 15-20 mg of surfactant(this amount is calculated as all accumulated in the liver after administration) to the liver homogenates of untreated rats, and prepared the microsomes. No significant difference from control value was observed(data not shown).

On the other hand, this Emulgen 913 has been extensively used for the solubilization of cytochrome P-450 and NADPH-cytochrome c reductase from various organs or tissues (Imai 1976; Guengerich 1977; Mayer and Durrant 1979). Judging from this study, it will be needed a careful use for experimental research although this surfactant is necessary, useful and important agent.

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Received February 20, 1990; accepted June 5, 1990.