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Detoxication Capacity of a Multiple (w/o/w) Emulsion for the Treatment of Drug Overdose. II.[1] Detoxication of Quinine Sulfate with the Emulsion in the Gastro-intestinal Tract of Rabbits[2]

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The detoxication of quinine sulfate as a model drug by a multiple (water-in-oil-in-water, w/o/w) emulsion was evaluated *in vitro* and *in vivo*. *In vitro* drug extraction into the emulsion was determined using a dialysis system. Drug extraction into the emulsion increased with increasing volume fraction of water-in-oil (w/o) emulsion in the w/o/w system ($F_{w/o/w}$). However, when 0.01 N HCl, which has the same pH as the stomach, was selected as a continuous aqueous solution of the emulsion, quinine was hardly extracted into the emulsion regardless of the $F_{w/o/w}$ values. Thus, we attempted the simultaneous use of the emulsions and an antacid ($NaHCO_3$) in order to increase the pH in the continuous phase of the emulsion, but combined use of the antacid increased the viscosity of the system. From the viscosity data of the emulsions and further *in vitro* extraction data, *in vivo* experiments were carried out with a suitable emulsion ($F_{w/o/w}=0.2$). The blood concentration of quinine co-administered with the multiple emulsion containing the antacid in rabbits was significantly lower than that in the control. These results indicate that the use of multiple emulsions may be a promising new approach to the emergency treatment of drug overdose.

Keywords——w/o/w emulsion; drug overdose; emergency treatment; detoxication; drug extraction; quinine sulfate; antacid; rabbit

Acute poisoning due to drug overdose is a major public health problem. However, the present modes of emergency treatment, *e.g.* peritoneal dialysis, ingestion of adsorbents such as activated charcoal and administration of emetics, have limitations. Recently, Frankenfeld *et al.*[3,4] reported the *in vitro* removal of salicylates or barbiturates by multiple (water in oil in water, w/o/w) emulsion, and they suggested that the emulsion was capable of rapid uptake of the drug *in vitro*. In addition, we have evaluated the detoxication of salicylic acid, selected as an acidic model drug, by w/o/w emulsion *in vitro* and *in vivo*.[1] The results can be summarized as follows. *In vitro* drug extraction into the emulsion was significantly higher than that of the control (without the emulsion), and the blood concentration of the drug co-administered with multiple emulsion in rabbits was significantly lower than that in the control.

The present study was undertaken to investigate the detoxication capacity of w/o/w emulsion for the treatment of drug overdose of quinine, selected as a basic model drug.

Experimental

Materials——Quinine sulfate was purchased from Wako Pure Chemical Industries, Osaka. Liquid paraffin was obtained from Kanto Chemical Co., Tokyo and was used as an oil phase for the emulsion. Two nonionic surfactants, Arlacel C (Tokyo Kasei Kogyo Co., Tokyo) and Tween 20 (Wako Pure Chemical Industries) were selected as a stabilizing agent and dispersing agent, respectively. Sodium bicarbonate (Wako Pure Chemical Industries) was used as an antacid. All other chemicals were commercial products of reagent grade.

Preparation of the Multiple Emulsion——The multiple emulsions were prepared by a modification of the method given in the preceding paper.[1] The compositions of the emulsions used in this study are shown in Table I. The procedures are as follows. Twenty %(v/v) Arlacel C in liquid paraffin as an oil phase and 0.1 N HCl as a central aqueous phase (1 : 1) were mixed and stirred with an Ultra-Turrax (Janke and Kunkel, Germany) for 5 min to prepare the w/o emulsion (1st emulsification). Fifty, seventy or ninety ml of 1/20 M

Table I. Compositions of Emulsion Formations Employed in this Investigation

			A0	A1	A2	A4
Type A	W_1	0.1 N HCl	0 ml	5 ml	10 ml	20 ml
	O	20% Arlacel C in liquid paraffin	0	5	10	20
	W_2	0.5% Tween 20 in pH 11 buffer	90	80	70	50
	Drug	Quinine sulfate suspension (5 mg/ml)	10	10	10	10
				B1	B2	B4
Type B	W_1	0.1 N HCl		5 ml	10 ml	20 ml
	O	20% Arlael C in liquid paraffin		5	10	20
	W_2	0.5% Tween 20 in pH 11 buffer		90	80	60
			C0	C1	C2	
Type C	W_1	0.1 N HCl	0 ml	5 ml	10 ml	
	O	20% Arlacel C in liquid paraffin	0	5	10	
	W_2	0.5% Tween 20 in 0.01 N HCl	90	80	70	
	Drug	Quinine sulfate suspension (5 mg/ml)	10	10	10	
					D2	
Type D	W_1	0.1 N HCl			10 ml	
	O	20% Arlacel C in liquid paraffin			10	
	W_2	0.5% Tween 20 aqueous solution			80	

sodium carbonate–sodium tetraborate buffer (pH 11) (type A, B; see Table I), 0.01 N HCl (type C) or distilled water (type D) containing 0.5% (v/v) Tween 20 as a continuous aqueous phase was added to the resulting w/o emulsion and mechanical agitation at 500 rpm with a magnetic stirrer for 10 min completed the formation of the w/o/w dispersion (2nd emulsification).

***In Vitro* Drug Extraction**——*In vitro* drug extraction into the emulsion was determined by means of dynamic dialysis through a Visking cellulose membrane (available area=3.80 cm^2) positioned between two diffusion glass cells (drug donor and acceptor phases, respectively.)[1] The whole system was maintained at 37°C in a water bath. One hundred ml of type A or C emulsion or 100 ml of type C emulsion containing 3 g of sodium bicarbonate as an antacid was placed in the donor glass cell, and the same volume of pH 11 buffer or 0.01 N HCl containing 3 g of sodium bicarbonate was placed in the acceptor glass cell. Each control experiment was carried out with the continuous aqueous phase in place of the emulsion. The experiment started immediately after the drug suspension (50 mg/ml) was added. At appropriate intervals, aliquots of 0.1 ml were withdrawn from the acceptor phase and the drug concentration was determined by fluorescence intensity measurement at 350 nm (excitation) and 450 nm (emission) in a Hitachi 650-10s fluorescence spectrophotometer after addition of 5 ml of 0.1 N H_2SO_4.

Measurement of Viscosity——The viscosity at 37°C of three multiple emulsions (type B) containing 3g of sodium bicarbonate were measured using a viscometer (Rotovisco, RV 100/CV 100, Haake, Germany) as a functions of shear rate from 0 to 30 s^{-1}.

***In Vivo* Experiments**——In order to monitor the blood level for a sufficiently long period, unanesthetized male albino rabbits weighing 1.8 to 2.2 kg were used. Food was withheld for 48 h before each experiment. Quinine sulfate suspended in 0.5% Tween 20 aqueous solution at a concentration of 50 mg/ml was given through a catheter leading into the stomach, or distilled water (9 volumes) was administered as a control in the same way. At appropriate times, venous blood samples (0.1 ml each) were collected from an ear vein with a one ml heparinized syringe. Quinine concentration in the plasma was determined using a fluorescence spectrophotometer by the method of Udenfriend.[5] As the usual dose size of quinine sulfate in man is 0.2 g according to J.P. IX, the dose size given to rabbits in these experiments (100 mg/kg) is an extreme overdose.

Results and Discussion

Twenty % (v/v) Arlacel C in liquid paraffin was selected as an oil phase in the w/o/w emulsion, as in the preceding paper,[1] but 0.5% (v/v) Tween 20 solution was used as a continuous aqueous phase instead of a cationic surfactant, cetyltrimethylammonium bromide solution, since ionic surfactants are generally irritating to biological membranes[6] and Matsumoto *et al.*[7] suggested that the yield of the w/o/w emulsion could be increased by addition of Tween 20 to the continuous phase. As quinine (used as a model drug in these experiments) is a weak base (pK_{a1}=4.13, pK_{a2}=8.52),[8] 0.1 N HCl was selected as a trapping agent for a central aqueous phase.[3] The emulsion prepared as described above was stable during slow stirring

and contained spherical oil drops (w/o drops) independently dispersed in the continuous aqueous phase. The average diameter of the oil drops was 19 μm and each oil drop contained a number of drops of central aqueous phase having an average diameter of about 1 μm.

First, the effect of the volume fraction of w/o emulsion in the w/o/w system ($F_{w/o/w}$) on the *in vitro* drug extraction into the emulsions was examined. The values of $F_{w/o/w}$ used in this study were 0.1, 0.2 and 0.4. Fig. 1 shows the amount of quinine transferred from the donor cell to the acceptor cell through a cellulose membrane without trapping by the emulsion. As is evident from the figure, the drug amount in the acceptor cell after treatment with the emulsions (A1, A2 or A3; Table I) was significantly lower than that in the control (A0). The drug amount transferred into the acceptor cell over 2 h was 74, 88 or 96% less for type A1, A2 or A4 emulsion, respectively, than in the control. These results suggest that the drug was largely removed by the emulsion in the donor cell and the amount transferred into the acceptor cell decreased. These results indicate that multiple emulsions may be useful for the extraction of drugs.

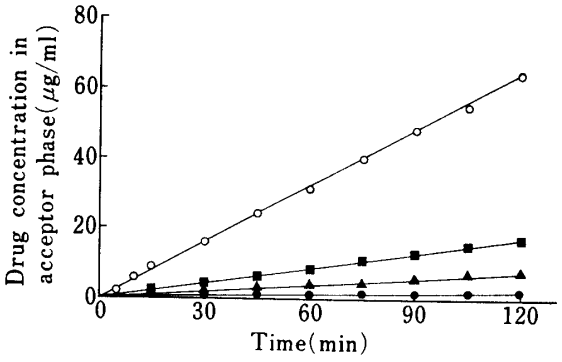

Fig. 1. Effect of Volume Fraction of w/o Emulsion in the Whole Multiple Emulsions on the Extraction of Quinine

A1 (■), A2 (▲) and A4 (●) emulsions, control (A0) (○).
Refer to Table I for symbol and composition of each emulsion.
Each point represents the mean value of three experiments.

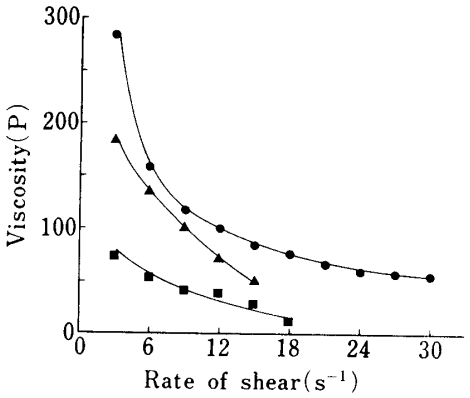

Fig. 2. Viscosities of Various Mutiple Emulsions at 37 °C

B1 (■), B2 (▲) and B4 (●) emulsions.
Refer to Table I for symbols and composition of each emulsion.
Each point represents the mean value of two experiments.

Emergency treatment of drug overdose involves attempts to reduce drug absorption from the gastro-intestinal tract. In the case of ingestion of a basic drug, however, the drug in the stomach may be mainly present in ionic form, and it is difficult to extract the drug effectively into the emulsion. One of the methods for overcoming this shortcoming is to employ an antacid, which increases the pH in the stomach and thereby increases the ratio of the concentration of the drug present in the undissociated moiety to the total concentration.

The viscosity of the multiple emulsion, however, increased on adding the antacid. This is a difficult problem. Fig. 2 shows the rheological properties of the multiple emulsions (type B) containing sodium bicarbonate (3 g per 100 ml of emulsion). As shown in the figure, these emulsions show non-Newtonian flow and thixotropy. The viscosity increased with increasing $F_{w/o/w}$ value and the effect was striking at low rate of shear. Therefore it is expected that the emulsion will show high viscosity during peristalsis in the gastro-intestinal tract. Type B4 emulsion ($F_{w/o/w}=0.4$) which showed the highest viscosity seems to overload the stomach, so the concentrated emulsion was excluded from the experiments described below.

We further examined the *in vitro* drug extraction in order to evaluate the extraction ability of two emulsions (type C; 0.1 and 0.2 in $F_{w/o/w}$) containing the antacid. In this experiment, 0.01 N HCl was selected as a continuous aqueous phase so as to adjust the pH of the phase to the pH in the stomach. Fig. 3 shows the effect of addition of Tween 20 on the

drug transfer through a membrane. In this experiment, 50 mg of quinine sulfate, 3 g of antacid and 100 ml of continuous phase were contained in the donor phase and the emulsion was not present. The pH of the continuous phase rose to over 9 after addition of the antacid. When Tween 20 was contained in the continuous phase, the drug concentration in the acceptor phase at 2 h was 20% less than that when Tween 20 was not contained. Micelles formed at such a high concentration of Tween 20 in the continuous phase (c.m.c$=1.2\times10^{-3}$ g/100 ml).[9] Therefore, some quinine sulfate molecules might be taken up into the micelles, and this might account for the 20% decrease in the amount of drug transferred through a Visking membrane. Fig. 4 shows the effect of two types of emulsions (C1, C2) and the antacid on the *in vitro* drug extraction. Two groups without the antacid did not have extraction ability under these conditions. This was a result of low partition of quinine sulfate into the oil phase in the emulsion, since drug molecules are mostly ionized in the continuous phase. The addition of the antacid to the type C1 or C2 emulsion resulted in good extraction. The drug levels in the acceptor phase after antacid treatment were lower than that in the control (Fig. 3) or groups without the antacid. The effect of $F_{w/o/w}$ on the drug extraction with type C1 or C2 emulsion was similar to that with Type A1, A2 or A4 emulsion, as shown in Fig. 1. From these results, it was suggested that combined use of antacid should be effective for the extraction of basic drugs although some of the drug molecules would be taken up into the micelles of Tween 20.

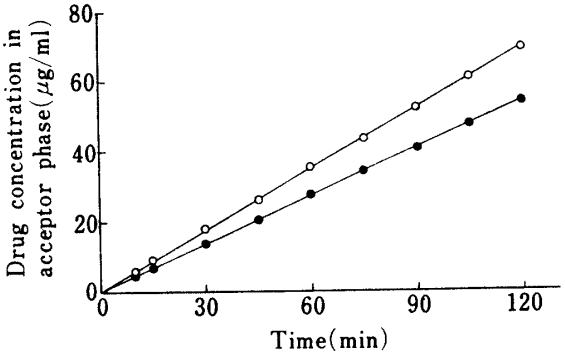

Fig. 3. Effect of Tween 20 Micelle Formation on Drug Transfer through a Membrane

0.1 N HCl containing antacid (○), 0.5% Tween 20 in 0.1 N HCl containing antacid (●).
Each point represents the mean value of three experiments.

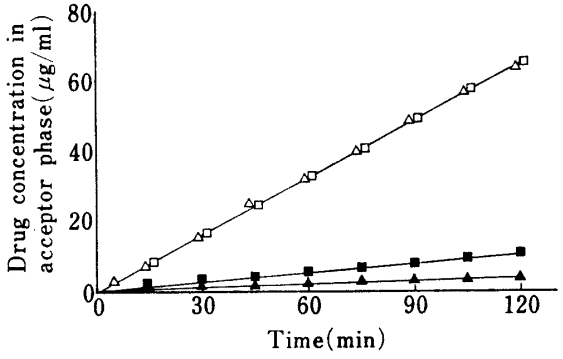

Fig. 4. Effect of Antacid on the Extracion of Quinine into Multiple Emulsions

C1 (■) and C2 (▲) emulsions containing antacid, C1 (□) and C2 (△) emulsion without antacid.
Refer to Table I for symbols and composition of each emulsion.
Each point represents the mean value of three experiments.

Next, the rabbit was selected as a model animal and the detoxication capacity of the multiple emulsion for the treatment of overdose of quinine sulfate and the effectiveness of the antacid in the gastro-intestinal tract were studied *in vivo*. The $F_{w/o/w}$ value of the emulsion was 0.2 (type D2, Table I) from the *in vitro* extraction data and vicosity data. The results of the preliminary study in a rabbit are shown in Fig. 5. We compared the blood concentrations of quine after administration of only quinine salfate (control), co-administration of quinine sulfate with the emulsion or simultaneous administration of the drug, emulsion and antacid. Combined use of the emulsion gave lower blood concentration and area under the blood concentration-time curve from 0 to 9 h (AUC_{0-9}) as compared with the control. AUC_{0-9} after co-administration of quinine sulfate with the emulsion was 25% of the control, and AUC_{0-9} after treatment with the antacid was about 10% of the control. From these decreases in blood level, the pH in the stomach can be presumed to have increased after ingestion of the antacid so that quinine molecules would be mostly extracted into the emulsion and not absorbed into the vascular system. The decrease in blood level might result not only

from the extraction of drug molecules into the emulsion but also from barrier formation by the emulsion above the mucosal membrane in the stomach or delaying of gastric emptying of the drug. Further work is necessary on these problems.

Fig. 6 shows the blood concentration of quinine in four rabbits after administration of quinine sulfate with or without the emulsion and antacid (emulsion or control group, respectively). The peak concentrations, times of peak concentration and $AUCs_{0-9}$ are summarized in Table II. The times of peak concentration of both groups were the same, but the peak concentration, which is the most important factor in the onest of poisoning, in the emulsion group was significantly lower than that in the control group. AUC_{0-9} in the emulsion group was markedly lower than in the control. From these results, it was clear that drug absorption from the gastro-intestinal tract was inhibited by the administration of the multiple emulsion and acute poisoning due to drug overdose should be diminished in severity.

In conclusion, the multiple emulsion may be useful for the emergency treatment of overdose of an orally igested drug. Although the examples described in this and preceding papers are restricted to one method of drug extraction (pH control in the central aqueous

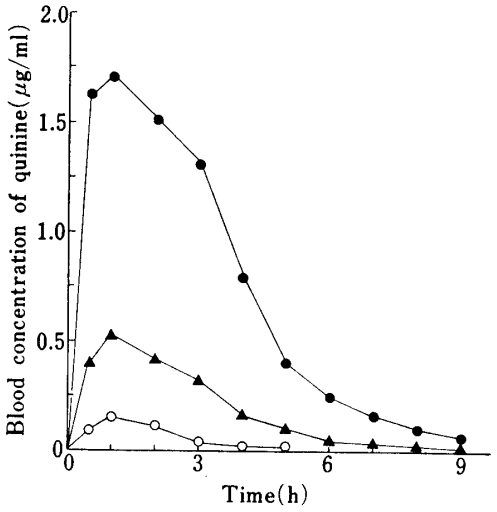

Fig. 5. Effect of Antacid on the Extraction into Multiple Emulsions after Administration of Quinine to a Rabbit

Treatment with emulsion and antacid (○), emulsion only (▲) control (●)

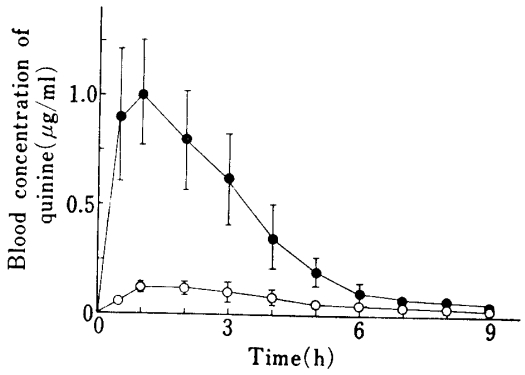

Fig. 6. Time Course of Blood Concentration of Quinine after Oral Administration

Treatment with emulsion and antacid (○), control (●).
Each point represents the mean value of four experiments.
Vertical bars indicate ± S.E.M.

Table II. Parameters for Evaluating the Extraction of Quinine Sulfate into Multiple Emulsions *in Vivo*

		Peak height concentration (μg/ml)	Time of peak concentration (h)	AUC_{0-9} (μg h/ml)
Control	1	1.70	1	7.45
	2	0.97	1	2.78
	3	0.67	2	2.82
	4	1.10	0.5	2.74
Mean ± S.E.M.		1.11 ± 0.22		3.95 ± 1.17
Emulsion	1	0.14	1	0.84
	2	0.10	0.5	0.33
	3	0.20	2	1.37
	4	0.18	1	0.50
Mean ± S.E.M.		0.16 ± 0.02		0.75 ± 0.23
Significance		$p<0.05$		No significance

phase), a variety of other trapping agents are possible. Alternate trapping agents include plasma protein, activated charcoal and specific drug antibodies. By manipulating the characteristics of the multiple emulsions, it should be possible to use emulsions for the therapy of poisoning by overdose of many drugs. The multiple emulsions used in this experiment can be readily prepared using simple equipment, such as an ordinary kichen blender. Therefore emulsions may be useful for the emergency treatment of drug overdose at home or in schools. Further studies should widen the range of applicability of such emulsions.

References and Notes

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