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Effect of Fluid Volume on Gastric Emptying and Intestinal Drug Absorption. II.[1] Dihydrocodeine and Thiopental Concentration Profiles in Plasma after Oral Administration in Different Fluid Volumes to Mice

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Dihydrocodeine was orally administered to mice and the effect of fluid volume on intestinal absorption and acute toxicity was investigated. An increase in fluid volume from 5 to 20 ml/kg resulted in a significant increase in maximum plasma dihydrocodeine concentration (C_{max}); the time required to reach C_{max}(T_{max}) was reduced from 15 to 5 min. However, fluid volume had little effect on the area under the plasma dihydrocodeine concentration–time curve ($AUC_{0\to90}$). The acute toxicity of dihydrocodeine increased significantly with increasing fluid volume and the gastric emptying rate of dihydrocodeine in the initial stage after oral administration increased in proportion to the fluid volume. These results indicate that early acceleration of the gastric emptying rate due to the increase of fluid volume increases the rate, but not the extent, of dihydrocodeine absorption, resulting in the enhancement of acute toxicity. As for thiopental, the influence of fluid volume on intestinal absorption was also examined using [^{35}S]-thiopental. The C_{max} value increased and T_{max} was reduced from 20 to 5 min by increase of the fluid volume. However, the volume did not influence $AUC_{0\to60}$ values.

Keywords——fluid volume; oral administration; plasma concentration; acute toxicity; gastric emptying; absorption; dihydrocodeine; thiopental; mouse

We previously reported[1] that the absorption rate of orally administered aminopyrine, dihydrocodeine and thiopental in the small intestine, and the pharmacological effects of these drugs, were enhanced in mice due to acceleration of the gastric emptying rate brought about by an increase in the fluid volume. In the present study, we examined the effect of fluid volume on the plasma dihydrocodeine and thiopental concentrations to elucidate the relationship between fluid volume and the pharmacological effect or toxicity.

Experimental

Materials——Dihydrocodeine, purchased from Takeda Pharmaceutical Co. Inc., Osaka, was dissolved in distilled water to obtain doses of 5, 10, 30 and 40 ml/kg. [^{35}S]-Thiopental was synthesized from [^{35}S]-thiourea and diethyl ethyl(1-methyl-butyl)malonate in the presence of sodium ethylate by the method of Kunugi *et al.*[2] and dissolved by adding an equimolar amount of Na_2CO_3. The drug solution (40 mg/50 μCi/kg) was infused orally at a rate of 0.5 ml/s.

Animals——Male ddN mice, weighing 24±1 g, were fasted for 16—20 h; water was provided *ad-libitum* up to 2 h before the experiments.

Acute Toxicity——The number of deaths within 24 h of dosing was recorded. Food and water were again provided 6 h after dosing. Each group consisted of ten mice; the method of Litchfield and Wilcoxon[3] was used to determine LD_{50} and the potency ratio of LD_{50}(P_R).

Gastric Emptying Experiments——These were performed by a slight modification of the method of Watanabe *et al.*[4] The animals were killed by a blow to the head immediately after oral drug administration; a quick abdominal incision was performed and the stomach was ligated at the esophageal and pyloric ends. The stomach was then homogenized with 5 ml of water and 1 ml of 1 N HCl, then enough water was added to make a final volume of 10 ml. After centrifugation for 10 min at 3000 rpm, 0.1 ml of the supernatant was added to 0.1 ml of CH_3OH containing chlorpheniramine (internal standard). The mixture was then analyzed by high-performance liquid chromatography (HPLC) for the determination of dihydrocodeine.

Plasma Dihydrocodeine Determination——Blood samples (0.3 ml) were collected by heart puncture at given times after dosing and centrifuged at 3000 rpm for 15 min. Plasma samples (100 μl) were added

to 600 μl of CH_3CN containing chlorpheniramine. After centrifugation of the mixture for 10 min at 4000 rpm, the supernatant was passed through a 0.45 μ Millipore filter. The filtrate was injected into the HPLC machine.

Radioactivity in Blood after [^{35}S]-Thiopental Administration——Blood samples (0.3 ml), collected by heart puncture after oral administration of [^{35}S]-thiopental, were solubilized with Protosol (New England Nuclear). The radioactivity was determined in a liquid scintillation counter (Aloka LSC-673).

HPLC Conditions——A Hitachi 633 liquid chromatograph equipped with a universal injector and Hitachi gel 3010 (particle size, 5 μm; 500×2.2 mm i.d.) were used. The mobile phase consisted of CH_3OH and H_2O (9: 1); the flow rate was 1.5 ml/min. The column eluate was monitored at 240 nm with an ultraviolet (UV) detector.

Results and Discussion

The dose–response curves of dihydrocodeine, orally administered at a fluid volume of 5 or 20 ml/kg, are shown in Fig. 1. The lines were parallel and the slope functions were in close agreement. As the fluid volume increased, so did the lethal ratio at each dose; the LD_{50} (95% confidence limits) value decreased from 500 (450.5—550.0) to 390 (440.5—327.5) mg/kg. Between 5 and 20 ml/kg fluid volume, there was a significant difference ($p < 0.05$) with respect to the potency ratio in terms of LD_{50} values (P_R 1.31 > f_{PR} 1.20).

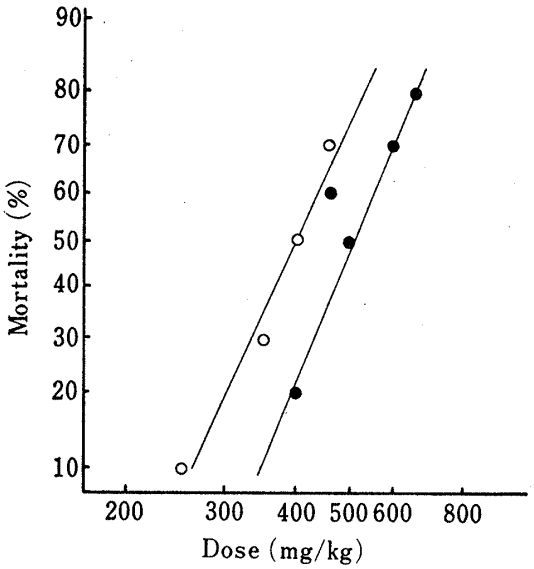

Fig. 1. Dose–response Curve of Dihydrocodeine orally administered at 5 (●) and 20 (○) ml/kg Fluid Volumes

Each point represents the value for ten mice. Slope functions (95% C.L.) for 5 and 20 ml/kg were 1.08 (1.17—1.01) and 1.05 (1.11—1.02), respectively.

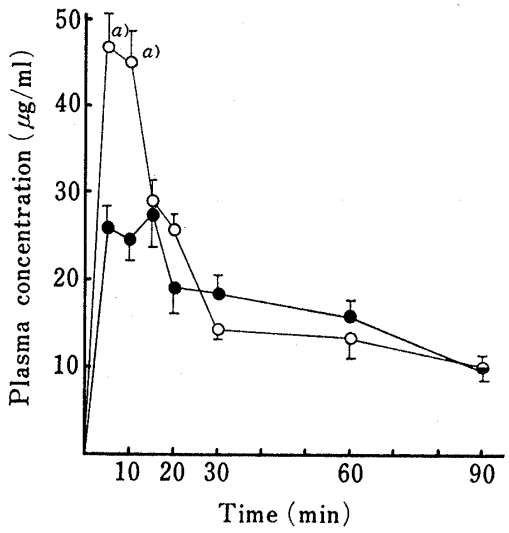

Fig. 2. Time Course of Plasma Dihydrocodeine Concentration after Oral Administration (50 mg/kg) at 5 (●) and 20 (○) ml/kg Fluid Volumes

Each point represents the mean±S.E. of six mice.
a) Significantly different from 5 ml/kg fluid volume, $p < 0.05$.

Fig. 2 shows the time course of plasma dihydrocodeine concentration after oral drug administration at fluid volumes of 5 and 20 ml/kg. At the higher fluid volume, the plasma concentration increased significantly at 5 and 10 min post-administration. In addition, the increase of fluid volume significantly altered the maximum plasma concentration (C_{max}) from 26.6±3.7 to 46.9±3.8 μg/ml, and the time to C_{max} (T_{max}) from 15 to 5 min. However, no remarkable variation was noted in the area under the curve ($AUC_{0\to90}$). Table I shows the bioavailability parameters (C_{max}, T_{max} and $AUC_{0\to90}$), after oral drug administration at fluid volumes ranging from 5 to 40 ml/kg.

According to the pH-partition hypothesis, basic drugs such as dihydrocodeine are absorbed

TABLE I. Bioavailability Parameters (C_{max}, T_{max} and AUC) and Gastric Emptying at Zero Time after Oral Administration at Various Fluid Volumes

Drug	Fluid volume (ml/kg)	Remaining in stomach ± S.E.[a] (%)	AUC[b] (μg·h/ml)	T_{max} (min)	C_{max} ± S.E. (μg/ml)
Dihydrocodeine	5	78.6±2.1	26.8	15	26.6±3.7
	10	62.7±2.4	28.3	15	34.7±5.5
	20	53.0±3.7	27.8	5	46.9±3.8
	40	49.4±1.9	29.1	10	42.8±3.6
[^{35}S]-Thiopental	5	87.2±2.5	15.4	20	18.8±1.7
	20	51.5±3.4	16.2	5	22.5±2.5

a) The experiments were performed immediately after oral drug administration.
b) AUC of dihydrocodeine and [^{35}S]-thiopental are $AUC_{0\to90}$ and $AUC_{0\to60}$, respectively.

rapidly from the small intestine, while there is little absorption from the stomach. Using the *in situ* loop method, we obtained unpublished results showing that the absorption of dihydrocodeine in the mouse intestine was much greater than that in the stomach. Alteration of gastric emptying is likely to have important effects on the rate of drug absorption. Sakiya *et al.*[5] observed that the bioavailability of quinine was markedly reduced by delay of the gastric emptying due to increase of osmotic pressure and viscosity. Gothoni *et al.*[6] reported that metoclopramide increases the absorption of tetracycline and pivampicillin by accelerating the gastric emptying. In interpreting the dihydrocodeine increase of C_{max} and the reduction of T_{max} brought about by an increase in fluid volume, the gastric emptying rate seems to be the most important factor. We previously reported[1] that, upon oral administration to mice, aminopyrine and thiopental were transferred from the stomach to the small intestine at a relatively fast rate in the initial stage. This was subsequently followed by gradual evacuation according to an exponential process. In addition, the initial fast gastric emptying rate was further accelerated in proportion to the administered fluid volume. However, the subsequent gastric emptying rate (exponential evacuation with time) was not affected by the increase of fluid volume. The present results regarding the gastric emptying rate of dihydrocodeine upon the oral administration of different fluid volumes are similar to those we obtained for aminopyrine.[1] As shown in Fig. 3, there was a good correlation between the amount of dihydrocodeine remaining in the stomach immediately after oral drug administration ($t=0$) and the coadministered fluid volume. The gastric emptying rate in the initial stage after dihydrocodeine administration increased in proportion to the fluid volume. This finding supports our conclusion that when dihydrocodeine is orally administered in the presence of large fluid volumes, the increased gastric emptying rate in the initial post-administration stage leads to an increase in the absorption rate, resulting in acute toxicity.

Our results show that an increase in the coadministered fluid volume brings about an increase in the initial plasma dihydrocodeine concentration. Similar findings were obtained when [^{35}S]-thiopental, instead of dihydrocodeine, was used as the acid drug. Fig. 4 shows the radioactivity in the blood of mice orally administered with 40 mg/kg [^{35}S]-thiopental in the presence of 5 or 20 ml/kg fluid volume. At the higher fluid volume, the radioactivity in blood increased significantly at the initial post-administration stage. Further, the T_{max} of [^{35}S]-thiopental was reduced from 20 to 5 min by an increase of fluid volume. The bioavailability parameters are shown in Table I. This result confirms the role of fluid volume in increasing the hypnotic effect of thiopental previously reported.[1]

We demonstrated that the increase of fluid volume increases not only the rate of dihydrocodeine or [^{35}S]-thiopental absorption due to gastric emptying at the initial post-administration stage, but also the acute toxicity of dihydrocodeine. Ferguson[7] reported that the acute toxicity of twelve drugs increased with the increase in fluid volume in mice and rats. Further-

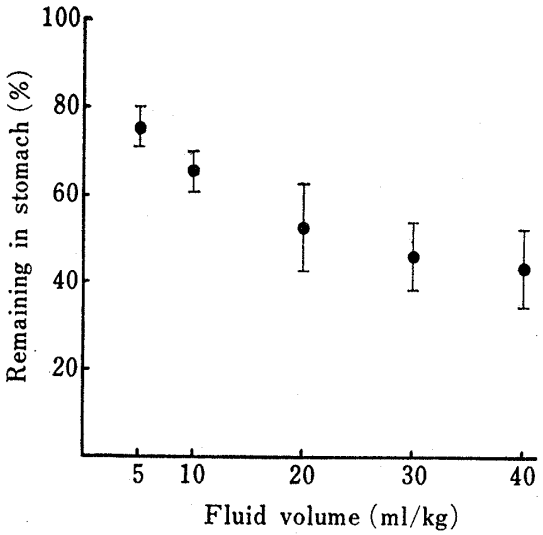

Fig. 3. Relationship between the Gastric Emptying of Dihydrocodeine at Zero Time[a] and the Coadministered Fluid Volume

Each point represents the mean ± S.E. for six mice.
a) The experiments were performed immediately after oral drug administration.

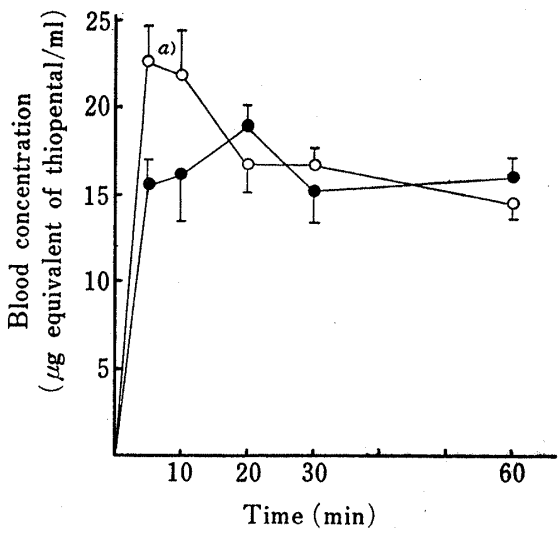

Fig. 4. Radioactivity in the Blood after Oral Administration of [^{35}S]-Thiopental (40 mg/kg) at 5 (●) and 20 (○) ml/kg Fluid Volumes

Each point represents the mean ± S.E. of six mice.
a) Significantly different from 5 ml/kg fluid volume, $p<0.05$.

more, Henderson *et al.*[8] reported that the increased fluid volume enhanced the plasma concentration of pentobarbital or quinine in rats. These observations are in accord with the results obtained in this study. Our present results indicate that the fluid volume must be taken into account in assessing the pharmacological response to drugs in small animals.

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References and Notes

1) Part I: O. Tsuzuki, M. Matsumoto, and K. Kouno, *Yakugaku Zasshi*, **101**, 548 (1981).
2) A. Kunugi, Y. Nagase, S. Baba, and Y. Aizawa, *Yakugaku Zasshi*, **89**, 1199 (1969).
3) J.T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
4) J. Watanabe, H. Okabe, T. Ichihashi, K. Mizojiri, H. Yamada, and R. Yamamoto, *Chem. Pharm. Bull.*, **25**, 2147 (1977).
5) Y. Sakiya, Y. Miyauchi, and Y. Tsuemura, *Chem. Pharm. Bull.*, **29**, 539 (1981); *idem*, *ibid.*, **29**, 1470 (1981).
6) G. Gothoní, P. Petikainen, H.J. Vapaatalo, R. Hackman, and K.A. Bjorksten, *Ann. Clin. Res.*, **4**, 228 (1972).
7) H.C. Ferguson, *Toxicol. Appl. Pharmacol.*, **4**, 759 (1962).
8) M.L. Henderson, A.L. Piechioni, and L. Chin, *J. Pharm. Sci.*, **55**, 1311 (1966).