Effects of the Physicochemical Properties of the Emulsion Formulation on the Bioavailability of Ethyl 2-Chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate in Rats

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The effect of the physicochemical properties of the emulsion formulation on the absorption of ethyl 2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate (AL-294) in rats and dogs was studied.

When emulsions of different particle sizes were administered to rats, the higher the ratio of Tween-80 to the drug was, the smaller was the particle size and the higher was the absorption. When the emulsions of similar particle size (2 μm) with different Tween-80 ratios were administered to rats, no significant difference was observed in the extent of absorption. The absorption of AL-294 was correlated with the dissolution rate from the oil phase to the aqueous phase but not correlated with the amount of AL-294 solubilized by Tween-80. These results indicate that the absorption of AL-294 from emulsions depends mainly on the particle size in the gastro-intestinal fluid and that Tween-80 serves only to reduce the particle size in the emulsion.

Keywords gastro-intestinal absorption; emulsion; particle size; oily drug; AL-294

There are reports that the bioavailability of drugs were enhanced by an emulsion form.[1] The mechanism of this absorption enhancement of drugs from an emulsion form is not simple. The emulsified griseofulvin is mainly absorbed from both the aqueous phase and the micellar phase but not from the oil phase.[2] Cyclosporine[3a] and oleic acid[3b] in emulsion form were absorbed directly from oil droplets in rats. Vitamin A acetate was absorbed predominantly from the micellar phase.[4] In the previous paper,[5] we showed that the gastro-intestinal absorption of ethyl 2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate (AL-294), which is an oily and poorly water soluble drug, was enhanced significantly in rats and beagle dogs when it was administered as an emulsion. The purpose of the present study was to elucidate the effect of the physicochemical properties of the emulsion formulations on the absorption enhancement of AL-294 in rats.

Experimental

Materials AL-294, AL-294 acid (2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionic acid), Tween-80, and testosterone were the same as described previously.[5] All other chemicals were of reagent grade.

Animal Experiments, Analytical Methods for AL-294 and AL-294 Acid, and Particle Size Measurement The techniques employed were essentially the same as described in the previous paper.[5]

Determination of Tween-80 Tween-80 was measured by the colorimetric method of E. G. Brown *et al.*[6]

Preparation of Emulsion Emulsions were prepared by an ultrasonic or a shaking method. The ultrasonic method was the same as that described previously,[5] and the shaking method is as follows: The oil phase was a mixture of 500 mg AL-294 and 500 mg Tween-80, and the aqueous phase was 10 ml of distilled water. After heating both phases to about 50 °C, the aqueous phase was poured into the oil phase under mixing with a magnetic stirrer to make an oil-in-water (o/w) emulsion. After cooling to room temperature, the emulsion was vibrated with a shaker (model VS by Iwaki Co., Ltd.) for 5 min. The formulas and the average particle sizes of the oil droplets are summarized in Table I. As shown in the table, the particle sizes in the emulsions prepared by the ultrasonic method were smaller than those by the shaking method, and the particle size of the emulsions prepared by the latter decreased with an increasing concentration of Tween-80.

TABLE I. Average Diameter of Emulsion Particles in Various Formulations

	No. of formula					
	1	2	3	4	5	6
AL-294 (mg)	500	500	500	500	500	500
Tween-80 (mg)	500	250	50	500	250	50
Distilled water (ml)	10	10	10	10	10	10
Preparation	U	U	U	S	S	S
Average diameter (μm)	2.2	2.2	2.2	3.8	8.0	11.0

U, prepared by ultrasonic method; S, prepared by shaking method.

Determination of Dissolution Rate of AL-294 from the Emulsion AL-294 which passed through a Visking membrane was assayed to determine the dissolution rate. A 300 ml solution containing equal volumes of water and methanol was poured into a glass bath and was maintained at 25 °C. Two ml of a sample emulsion in a glass cylinder covered with a Visking membrane on one end was placed in this solution. The outer solution and the sample emulsion were stirred continuously with a magnetic stirrer and a two-bladed paddle mixer, respectively. A 5 ml aliquot was collected periodically, and AL-294 was extracted into chloroform to be assayed by gas-liquid chromatography. The solubility of AL-294 in the solution was 30 μg/ml.

Determination of Solubilized AL-294 To detemine the amount of AL-294 solubilized by Tween-80 in the aqueous phase of the emulsion, an ultrafiltration cell, model No. 50, with an XM-100 membrane (Amicon Co.) was used.[7] All the plastic portions of the cell were replaced with glass to avoid the sorption of AL-294 by the plastic. First, an appropriate volume of the emulsion was centrifuged at 15000 rpm for 60 min with a Sarvall RC-2 (Sarvall Co.). About 20 ml of extremely thin emulsion was separated. This extremely thin emulsion was then ultrafiltered by the cell at an air pressure of 1 kg/cm^2. The filtrate was collected in 5 ml portions successively. The whole process was carried out at 25 °C. The AL-294 in the filtrate, the solubilized AL-294, was assayed.

Results and Discussion

Relationship between Average Particle Size in the Emulsion, Concentration of Tween-80 and Absorption of AL-294 in Rats Five formulations of AL-294, four emulsions (formulas 1, 4, 5, and 6) and a corn oil solution (formula 7), were orally administered to rats each at a dose of 50 mg/kg, and the plasma levels of AL-294 acid were assayed. As shown in Fig. 1, the oral absorption of AL-294 increased as the average diameter of the particles in the emulsion decreased or as the concentration of Tween-80 increased. The absorption from the corn oil solution was the smallest. The correlation among the average diameter, the concentration of Tween-80 and the area under the plasma concentration–time curve within 8 h ($AUC(0—8)$) is shown in Fig. 2. $AUC(0—8)$ decreased linearly with increasing particle size and with decreasing Tween-80

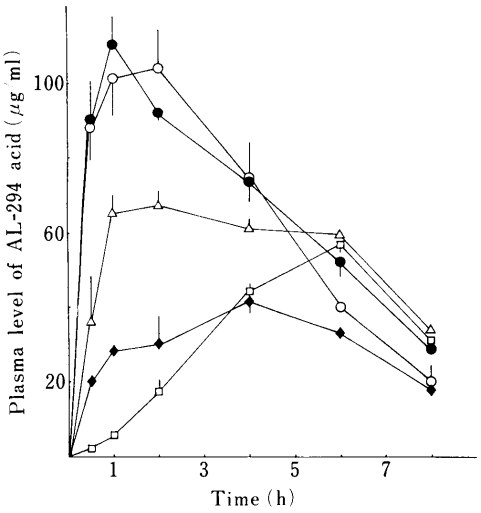

Fig. 1. Plasma Levels of AL-294 Acid in Rats after Oral Administration of AL-294 as Emulsions with Different Average Particle Sizes at a Dose of 50 mg/kg

●, formula 1 (2.2 μm); ○, formula 4 (3.8 μm); △, formula 5 (8.0 μm); ■, formula 6 (11.0 μm); □, corn oil solution. Each value represents the mean with a standard error of three rats.

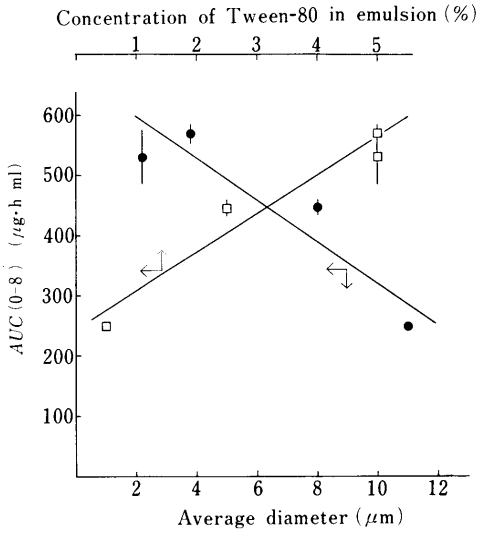

Fig. 2. Relationship between Average Diameter of Emulsion, Concentration of Tween-80 and *AUC* after Oral Administration of AL-294 as Emulsion to Rats at a Dose of 50 mg/kg

●, average diameter to *AUC*; □, concentration of Tween-80 to *AUC*. Each value represents the mean with a standard error of three rats.

concentration.

The results were similar to that of cyclosporine by Tarr *et al.*[3a] They supposed the enhancement of the drug absorption to be as follows: The smaller-droplet-size emulsion containing cyclosporine has the greater surface. With the greater surface area, the partitioning of the drug into the aqueous environment and lipase activity are increased, causing the formation of more fatty acid and monoglyceride. The greater amount of monoglyceride leads to micelles with bile acids. Cyclosporine is easily solubilized in micelles, and the cyclosporine in micelles is easily partitioned into the unstirred water layer of the intestinal surface. As a result, the absorption of cyclosporine increases.

Absorption Rate in Rats by Deconvolution Method The plasma levels in the rats after an intravenous injection of

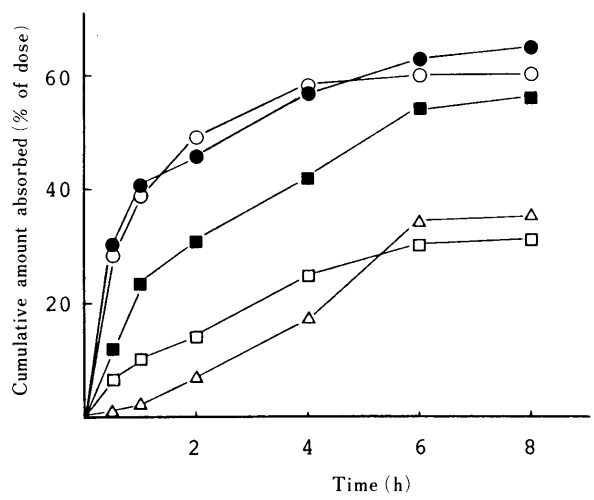

Fig. 3. The Absorption Rate of AL-294 from the Emulsions in Rats by Deconvolution Methods

●, formula 1; ○, formula 4; ■, formula 5; □, formula 6; △, corn oil solution.

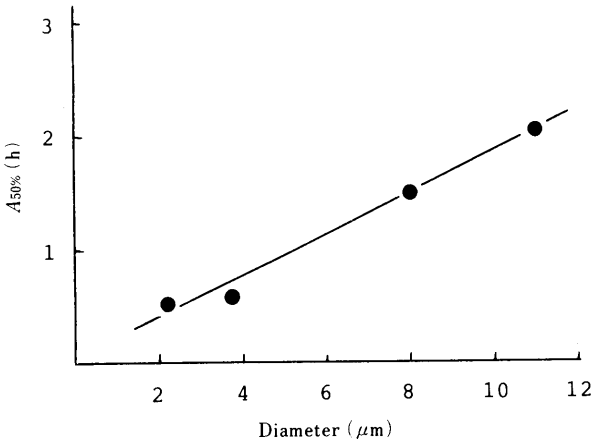

Fig. 4. Relationship between the Average Diameter of Emulsion and the Time of Absorbing Half the Amount

sodium salt of AL-294 acid in the previous paper[5] were fitted to the biexponential function by a MULTI program,[10] and the Eq. 1 was estimated.

$$C_p = 335.055\exp(-0.348T) + 0.015\exp(-0.120T) \quad (1)$$

where C_p and T represents a plasma level and time, respectively. The absorption rate can be obtained from the data in Fig. 1 and the Eq. 1 by the deconvolution method.[11] Cumulative amount absorbed–time curves of formulas 1, 4, 5, 6, and the corn oil solution are shown in Fig. 3. The absorption rate increased with a decrease in the average diameter of the emulsion. AL-294 was scarcely absorbed after 6 h, so that the times ($A_{50\%}$) when half of the cumulative amount at 8 h was absorbed were calculated from the curves. The relation of $A_{50\%}$ to the average diameter of emulsions was plotted in Fig. 4. The relation of the absorption-rate to the particle size in Fig. 4 was probably closer than that of the absorption-extent to the particle size in Fig. 2. This means that the effect of the droplet size of the emulsion on the absorption of AL-294 was larger in the rate than in the extent. This suggested that the rate of absorption took part in the mechanism of the absorption enhancement of the drug in the emulsion

TABLE II. The Percent of AL-294 Solubilized and Loaded in Various Formulas, and *AUC*(0—8) of AL-294 Acid after Oral Administration of the Same Formula to Rats

	No. of formula			
	1	4	5	6
Solution before filtration[a]	1.00	1.00	1.00	1.00
Solubilized content[a]	0.020	0.080	0.044	0.017
AUC(0—8)[b] (S.E.)	529.0 (46.5)	568.0 (16.0)	444.9 (10.3)	248.1 (1.7)

a) Each value represents the percent in the formula. *b*) Each value represents the mean of three rats. Dimension is μg·h/ml.

formulation.

Effect of Solubilized Amount of AL-294 on the Intestinal Absorption of AL-294 in Rats Since the effect of solubilization of certain drugs by surfactant micelles on intestinal absorption has been reported as not negligible,[8] the effect of the solubilized content by Tween-80 on the absorption of AL-294 was studied. As a sufficient amount of Tween-80 was used in the formulations, AL-294 could be in the Tween-80 micelles. To determine the amount of the drug solubilized by an ultrafiltration technique, a membrane which could separate the oil phase AL-294 from the micelle phase should be used. An aqueous solution with various concentrations of Tween-80 above the critical micelle concentration was filtered with two membranes, Diafro PM-30 and XM-100. The concentrations of Tween-80 in the aqueous solution before and after ultrafiltration were determined. Tween-80 micelles could pass through the XM-100 membrane freely but some Tween-80 micelles were retained by the PM-30 membrane. Oil droplets could pass through neither the XM-100 nor the PM-30 membrane. Based on these results, the XM-100 membrane was adopted to separate micelles and oil droplets.

The thin emulsions obtained by the ultracentrifugation of the preparations of formulas 1, 4, 5, and 6 were filtered with an XM-100 membrane to determine the amount of solubilized AL-294. As shown in Tables I and II, the solubilized amount and *AUC*(0—8) increased as the concentration of Tween-80 increased. However, formula 1, whose Tween-80 concentration was highest, had a low content of the solubilized drug. The extent of absorption fromformula 1 was comparable to formula 4. As the emulsion in formula 1 had the smallest particle size and the largest interface, it was assumed that most of the Tween-80 was localized on the interface of the emulsion and accordingly the amount of Tween-80 available to form micelles was less than that in the other formulas. The solubilized amount of AL-294, moreover, was less than 8% of the loaded one. Therefore, it was concluded that the concentration of the AL-294 solubilized by Tween-80 did not influence the absorption of AL-294 in the intestine.

Effect of the Concentration of Tween-80 on the Oral Absorption of AL-294 in Rats The absorption of AL-294 from formulas 1, 4, 5, and 6 in the rats indicated that the absorption of AL-294 increased with an increasing Tween-80 concentration or decreasing particle sizes. To differentiate the effect of these two factors, three emulsions

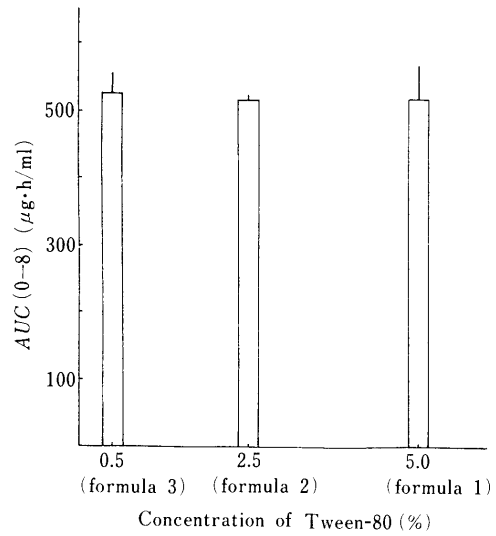

Fig. 5. *AUC*(0—8) of AL-294 Acid in Rats after Oral Administration of AL-294 as Sonicated Emulsion with Various Concentrations of Tween-80 at a Dose of 50 mg/kg

Each column represents the mean with a standard error of three rats.

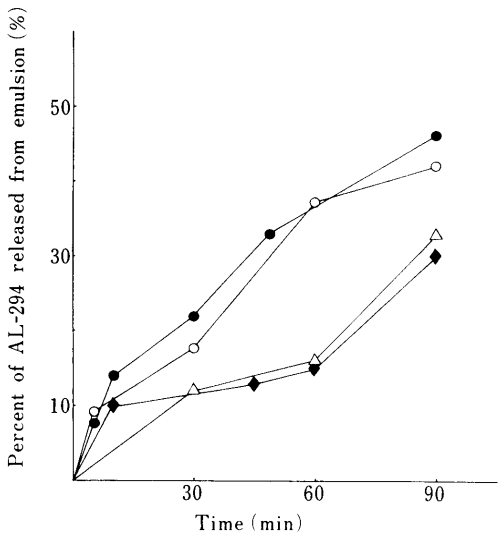

Fig. 6. Dissolution of AL-294 from the Emulsions with Different Average Particle Sizes across a Visking Membrane

●, formula 1; ○, formula 4; △, formula 5; ◆, formula 6.

having the same average particle size were prepared ultrasonically with different amounts of Tween-80. Each emulsion was orally administered to the rats at a dose of 50 mg/kg. As shown in Fig. 5, the absorption of AL-294 from these emulsions was almost the same. These results together indicated that the absorption of the drug was not influenced by the Tween-80 concentration or by the amount of solubilized drug, but by the particle size of the emulsion. In the previous paper,[5] after the oral administration of AL-294 in the physical mixture with Tween-80, the absorption in dogs increased with an increasing concentration of Tween-80; the higher amount of Tween-80 forming smaller droplets from the mixture by the motion of the gastro-intestinal tract which enabled higher absorption. This indicates that the physical mixture was easily emulsified to form small particles by simple shaking when the amount

of Tween-80 was high enough.

Relationship between the Dissolution Rate of AL-294 from the Emulsion and the Particle Size Several of the authors discussed the relationship between particle size and absorbability of drugs in suspension, and concluded that when the dissolution rate was the rate-limiting process, its absorption increased as the particle size decreased.[9] Shimamoto *et al.* reported that in certain drugs the dissolution rate to the aqueous phase from the oil phase of an emulsion was the rate-limiting process.[7] Each 5 ml of emulsion (formulas 1, 4, 5, and 6) was diluted with distilled water to a concentration of (0.5 mg AL-294)/ml and the rate of dissolution of AL-294 was determined. As shown in Fig. 6, the dissolution rate was faster when the particle size was smaller. The dissolution curves in Fig. 6 were also comparable to curves of a cumulative amount absorbed in Fig. 3. These indicated that the higher dissolution rate from the smaller emulsion was responsible for the higher absorption.

Conclusion

On the basis of the evidence presented here, absorption of an AL-294 emulsion in the gastro-intestinal tract after oral administration is influenced only by the droplet size of the formulations. The smaller droplet size of the emulsion causes a greater absorption-rate of AL-294 in rats, and as a result, causes greater absorption. From the previous results[5] that the absorption of AL-294 in a physical mixture of Tween-80 in the dogs increased with an increasing concentration of Tween-80, the oral absorption of AL-294 in dogs also probably increases with a decrease in the droplet size of the emulsion. The reason why only the droplet size is effective on the absorption-rate is not clear from the physicochemical properties of the emulsion, but the dissolution rate is comparable to the absorption-rate. We conclude that to obtain a great bioavailability of AL-294, a small particle formulation from which AL-294 is easily released is reasonable.

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