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Bioavailability of 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878) in beagle dogs

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Summary

The fundamental pharmacokinetic properties of ADD-3878 were evaluated and the bioavailability of the drug after oral administration was determined in beagle dogs.

A pharmacokinetic analysis after intravenous injection of the drug revealed biphasic elimination of the plasma ADD-3878 concentration following a biexponential model with a $t_{1/2,\alpha}$ of 0.27 h and a $t_{1/2,\beta}$ of 17.11 h. The gastrointestinal absorption of ADD-3878 in solution was significantly faster than those in suspensions. The particle size of ADD-3878 crystals affected both the extent and rate of bioavailability. Furthermore, a significant effect of food on the bioavailability of the drug was observed; the absorption in tablets after food ingestion was approximately double of that in the fasting state. The maximum plasma levels and the areas under the curve at various doses of tablets were proportional to the doses. In a multiple-dose study, there was no unusual accumulation of plasma ADD-3878 concentration.

Introduction

5-[4-(1-Methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione, ADD-3878, was found to have significant anti-diabetic and anti-lipidemic activities in laboratory animals (Sohda et al., 1982). The drug is currently under extensive animal and clinical studies.

The purpose of the present study was to investigate the fundamental pharmaco-

kinetic properties of ADD-3878 and the effects of particle size and food ingestion on the oral bioavailability of the drug in beagle dogs. A study was also designed to determine the effect of multiple administration of the drug on its steady-state plasma level.

Experimental

Materials

ADD-3878 and its sodium salt were synthesized in Takeda Chemical Industries. Gall powder(ox bile) was supplied from Wako Pure Chemical Industries. All other solvents and reagents were of analytical grade.

Preparation of dosage forms

Solutions of ADD-3878, 100 mg/5 ml in 20% PEG-200 saline solution, were prepared using the sodium salt for parenteral and oral use. As an oral suspension, ADD-3878 crystals of small particle sizes (particle size about 20 μm and about 2 μm) were prepared by milling the original crystals (particle size about 100 μm) using a hammer mill [1] or a mixer mill [2]. Then 100 mg of the original crystals and of the milled crystals were suspended in 5 ml of water containing 2.5% hydroxypropyl cellulose [3] and dispersed under ultrasonication: these were designated as sample 1 (100 μm), sample 2 (20 μm), and sample 3 (2 μm). Tablets containing 30 mg (sample 4), 100 mg (sample 5), and 125 mg (sample 6) of ADD-3878 were prepared using the milled crystals (20 μm). The characteristics of these tablets are listed in Table 1.

Dog study

To 4 male beagle dogs weighing 10–12 kg after an overnight fast, ADD-3878 was administered intravenously through the cubital vein and orally at one-week intervals. For oral administration, the solution, suspensions, or whole tablets were administered with 50 ml of water.

To determine the effect of food on the gastrointestinal absorption of ADD-3878, the tablets were administered immediately after taking a high lipid meal (150 g of dog food [4], 20 g of margarine, and 10 g of dry milk), a normal meal (180 g of dog food), or bread only (130 g of bread).

For multiple oral administration of ADD-3878, a tablet (sample 5) was given immediately after a normal meal, once a day for 8 days.

After administration of each dosage form of ADD-3878, 1.5 ml of blood samples were withdrawn from the cubital vein periodically. The plasma samples were frozen and stored at $-18°C$ until they were analyzed.

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[1] Atomizer, Fuji Powdal, Tokyo, Japan.
[2] Spex Mill, Spex Ind., Tokyo, Japan.
[3] HPC-L (mol. wt. 55,000–70,000), Nippon Soda, Tokyo, Japan.
[4] CD-5, Japan Clea, Tokyo, Japan.

TABLE I

CHARACTERISTICS OF ADD-3878 TABLETS

Formulation code	ADD-3878 potency (mg/tablet)	Dissolution rate [a] (%)		
		10 min	20 min	30 min
Sample 4	30	59.9	93.8	95.9
Sample 5	100	59.5	93.1	99.8
Sample 6	125	52.7	85.1	99.8

[a] Dissolution studies were performed by a modification of the USP XX paddle method using 900 ml of pH 10.7 Menzel buffer (37°C, 100 rpm).

Analytical procedure for determination of unchanged ADD-3878 in plasma

The plasma ADD-3878 concentration was determined by high-performance liquid-chromatography (HPLC). To a tube containing 0.5 ml of a plasma sample were added 1.5 ml of 0.1 N HCl and 6 ml of ethyl ether. After this solution was shaken for 10 min and centrifuged for 5 min at 2500 rpm, the aqueous layer was separated and frozen in dry-ice–acetone. The ethyl ether layer was transferred into a clean tube to evaporate at 40°C to dryness under a stream of nitrogen gas. After the residue was dissolved in 100 μl of the mobile phase, 20 μl of the solution was injected into a liquid chromatograph.

The HPLC system consisted of a Shimadzu Model LC-3A pump [5], a μBondapak C_{18} (particle size 10 μm) reversed-phase column [6] (15 × 0.4 cm i.d.) with a 1 × 0.4 cm i.d. pre-column of 10 μm Wakogel LC ODS-10H [7], a Shimadzu Model SIL-1A injector [5] and a Shimadzu Model SPD-2A detector [5] operated at 230 nm. The mobile phase, acetonitrile–0.1 M sodium acetate (50 : 50 v/v), was employed at a flow rate of 0.6 ml/min. Quantitative determination was carried out by measuring the area of the drug peak and comparing this value with a standard curve constructed after the analysis of plasma samples containing known amounts of the drug.

Solubility study

Excess amounts of ADD-3878 were added in 50 ml of 0–5% aqueous solution of gall powder which was then shaken at 25°C. After equilibration, an aliquot was filtered. The concentration of the drug in the filtrate was determined according to the HPLC method described above.

Data analysis

Pharmacokinetic parameters after the intravenous administration of ADD-3878 were calculated using a personal computer [8] and the MULTI computer program (Yamaoka et al., 1981), and applying a biexponential model. To assess the bioavaila-

[5] Shimadzu Seisakusho, Kyoto, Japan.
[6] Waters Associates, Mississauga, Ont., Canada.
[7] Wako Pure Chemical Ind., Osaka, Japan.
[8] PC-8001, Nippon Electric, Japan.

bility after oral administration, an analysis was carried out on the parameters: maximum plasma concentration (C_{max}), time-to-peak concentration (T_{max}), and area under the plasma concentration–time curve (AUC) calculated by the trapezoidal rule and extrapolation from the last 3 points of data.

The steady-state plasma levels after the multiple oral administration were simulated by a biexponential model with first-order absorption using the parameters derived from the first dosing.

Results

Determination of ADD-3878 in dog plasma

Fig. 1 shows the liquid chromatograms on the standard solution of ADD-3878, blank plasma and blank plasma to which ADD-3878 was spiked. As shown in Fig. 1B, no interfering peaks were observed near the drug peak with the specimen of a blank plasma. Retention time for ADD-3878 was 8.0 min under the described conditions of chromatography.

The results obtained through the analysis on the blank plasma spiked with various amounts of the drug are summarized in Table 2. In the 0.25–2 μg range of ADD-3878, the mean value of overall recovery of the drug in this procedure was 90.5 ± 3.0% and a linearity was observed between the amount of ADD-3878 spiked and the peak area. The minimum detectable amount of the drug was 0.01 μg in 20 μl of the mobile phase.

Chromatograms obtained from the plasma samples after administration of ADD-3878 are similar to that of the spiked plasma and metabolites with interfering peaks are not present under this condition.

Pharmacokinetics in dog

After intravenous administration, the plasma levels of ADD-3878 in dogs de-

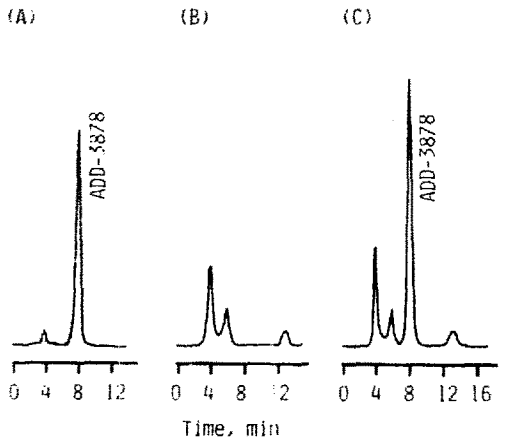


Fig. 1. Liquid chromatograms of ADD-3878 in plasma. A: standard solution (15 μg/ml in methanol). B: blank plasma. C: spiked plasma (2 μg to 0.5 ml plasma).

TABLE 2

RECOVERY OF ADD-3878 FROM DOG PLASMA

Added (μg)	Found (μg)	Recovery (%)
0.25	0.222 ± 0.013	88.8 ± 5.2
0.5	0.455 ± 0.009	91.0 ± 1.8
1	0.925 ± 0.027	92.5 ± 2.7
1.5	1.350 ± 0.046	90.0 ± 3.1
2	1.805 ± 0.053	90.3 ± 2.7
mean		90.5 ± 3.0

All values represent mean ± S.E. (n = 5).

creased biexponentially (Fig. 2). The pharmacokinetic parameters are shown in Table 3; the mean half-lifes of the drug in the α- and β-phases were 0.27 and 17.11 h, respectively.

Effect of particle size of ADD-3878 crystals on the oral bioavailability

Fig. 3 shows the mean plasma levels of ADD-3878 after oral administration of the solution and suspensions (samples 1, 2 and 3) at a dose of 100 mg ADD-3878. The bioavailability parameters (C_{max}, T_{max}, and AUC) and absolute bioavailability (B.A.) are summarized in Table 4. In a solution, the absorption of ADD-3878 through the gastrointestinal tract is rapid; the time to peak is 1 h after administration. However, in all of the suspensions (samples 1, 2 and 3), lower and delayed peak plasma concentrations were observed than those after administration of the same dose of the drug in solution. Reduction in particle size led to an increase in the rate and extent of the bioavailability of ADD-3878. The absolute bioavailbility of the

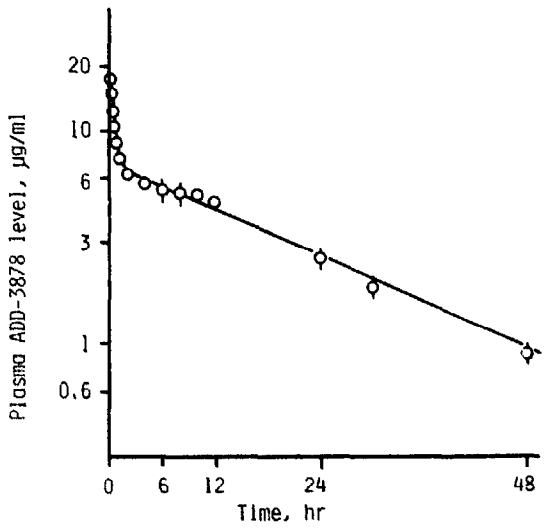


Fig. 2. Mean plasma level of ADD-3878 in beagle dogs after intravenous administration of ADD-3878 solution at a dose of 100 mg/dog after overnight fasting. The data are expressed as mean ± S.E. (n = 4).

TABLE 3

PHARMACOKINETIC PARAMETERS OBTAINED FROM A TWO-COMPARTMENT OPEN MODEL AFTER INTRAVENOUS ADMINISTRATION OF ADD-3878 (100 mg) IN BEAGLE DOGS

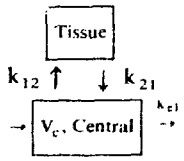
Parameter(unit)	Dog A	Dog B	Dog C	Dog D	mean	S.E.
Body weight(kg)	10	12	11	11	11.0	0.41
A	13.2	14.4	12.9	14.2	13.7	0.37
B	6.68	5.18	7.79	7.13	6.69	0.55
α (h^{-1})	2.27	1.75	3.24	3.78	2.76	0.46
β (h^{-1})	0.042	0.033	0.041	0.045	0.041	0.002
$t_{1/2,\alpha}$ (h)	0.30	0.40	0.21	0.18	0.27	0.05
$t_{1/2,\beta}$ (h)	16.1	20.5	16.5	15.2	17.1	1.1
k_{12} (h^{-1})	1.39	1.17	1.93	2.40	1.72	0.27
k_{21} (h^{-1})	0.792	0.486	1.24	1.29	0.955	0.193
k_{el} (h^{-1})	0.123	0.119	0.108	0.133	0.120	0.005
V_c (ml) [a]	5020	5090	4820	4680	4900	93
V_β (ml) [b]	14400	18100	12500	13700	14700	1200
Clearance Cl (ml/h)	619	610	524	625	594	23

[a] The volume of distribution of the central compartment (V_c = dose/(A + B)).

[b] The volume of distribution during the β-phase ($V_\beta = Cl/\beta$).

Two-compartment open model:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$



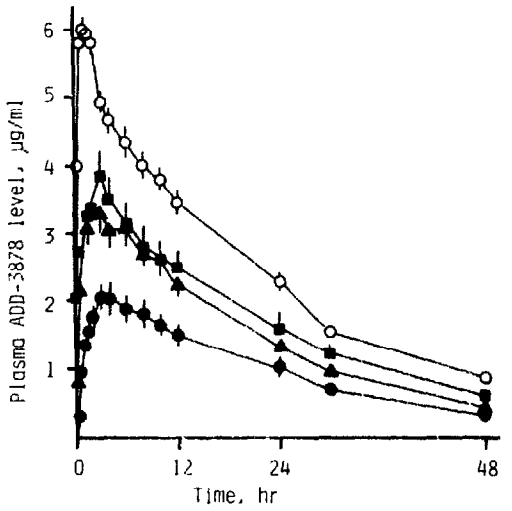


Fig. 3. Mean plasma levels of ADD-3878 in beagle dogs after oral administration of solution (○) and suspensions (sample 1 (100 μm), ●; sample 2 (20 μ), ▲; sample 3 (2 μm), ■) at a dose of 100 mg/dog after overnight fasting. The data are expressed as mean ± S.E. (n = 4).

TABLE 4

BIOAVAILABILITY PARAMETERS AFTER INTRAVENOUS AND ORAL ADMINISTRATION OF SOLUTION AND SUSPENSIONS OF ADD-3878 IN OVERNIGHT FASTED BEAGLE DOGS AT A DOSE OF 100 mg/dog

Route, Dosage Form	C_{max} (μg/ml)	T_{max} (h)	AUC(0-∞) (μg·h/ml)	B.A. [a] (%)
Intravenous	-	-	170.1 ± 7.9	100
Oral				
Solution	6.29 ± 0.40	1.0 ± 0.2	144.4 ± 7.0	85.2 ± 3.4
Suspension				
Sample 1 (100 μm)	2.11 ± 0.45	3.5 ± 0.3	59.5 ± 11.5	34.8 ± 6.5
Sample 2 (20 μm)	3.84 ± 0.33	1.6 ± 0.6	84.7 ± 11.6	49.3 ± 5.1
Sample 3 (2 μm)	4.27 ± 0.58	1.6 ± 0.6	102.4 ± 12.4	59.7 ± 9.8

[a] Absolute bioavailability = $\frac{AUC(p.o.)}{AUC(i.v.)} \times 100$.

All values represent mean ± S.E. (n = 4).

drug in the solution and the suspensions (samples 1, 2 and 3) are 85.2 ± 3.4, 34.8 ± 6.5, 49.3 ± 5.1 and 59.7 ± 9.8% (mean ± S.E.), respectively.

These results indicate that particle size of the crystals is an important factor for the gastrointestinal absorption of ADD-3878 in dogs.

Effect of food on the bioavailability

Fig. 4 shows the mean plasma levels of ADD-3878 after oral administration of a tablet (sample 5) immediately after each of 3 types of meal or in the fasting state. Bioavailability parameters (C_{max}, T_{max} and AUC) and the absolute bioavailability are shown in Table 5. The plasma concentrations after food ingestion are approxi-

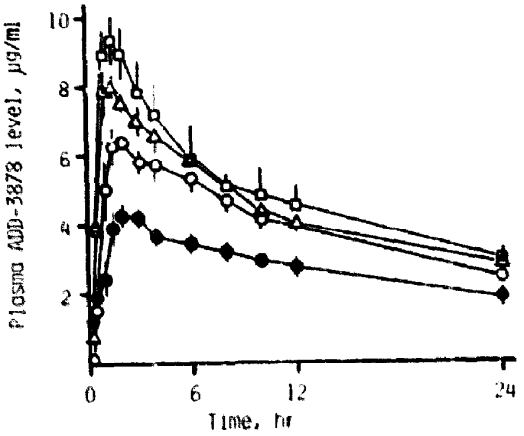


Fig. 4. Mean plasma levels of ADD-3878 in beagle dogs after oral administration of ADD-3878 tablet, 100 mg (sample 5) after overnight fasting (●), high lipid meal (□), normal meal (△), and only bread (○). The data are expressed as mean ± S.E. (n = 4).

TABLE 5

EFFECT OF FOOD ON BIOAVAILABILITY OF ADD-3878 TABLET (100 mg) IN BEAGLE DOGS IN FASTING OR POSTPRANDIAL STATE

Diet	C_{max} (μg/ml)	T_{max} (h)	AUC(0–∞) (μg·h/ml)	B.A. [a] (%)
Fasted	4.41 ± 0.24	2.1 ± 0.3	106.8 ± 8.2	62.7 ± 3.6
Fed [b]				
High lipid [c]	9.49 ± 0.76	1.6 ± 0.2	183.8 ± 19.2	107.8 ± 9.2
Normal [d]	8.14 ± 0.48	1.4 ± 0.1	180.7 ± 6.4	106.5 ± 2.4
Snack [e]	7.01 ± 0.29	2.4 ± 0.6	170.2 ± 7.3	100.2 ± 2.7

[a] Absolute bioavailability $= \frac{AUC(p.o.)}{AUC(i.v.)} \times 100$.

[b] Tablets were given immediately after feeding.

[c] 150 g of dog food, 20 g of margarine, and 10 g of dry milk.

[d] 180 g of dog food.

[e] 130 g of bread.

All values represent mean ± S.E. (n = 4).

mately double those in the fasting state. The absolute bioavailability in postprandial state is about 100%, whereas that in the fasting state is 62.7 ± 3.6 (mean ± S.E.). These results indicate that the bioavailability of ADD-3878 is enhanced markedly by food ingestion. Furthermore, it can be found that there are no significant differences in AUC and T_{max} after different types of meal, while C_{max} is significantly different ($P < 0.05$) between the high lipid meal and the bread only meal.

Fig. 5 shows the mean plasma levels of ADD-3878 after oral administration of 30 mg (sample 4), 100 mg (sample 5), and 250 mg (sample 6, 2 tablets) of the drug to dogs after a normal meal. Table 6 summarizes the bioavailability parameters at

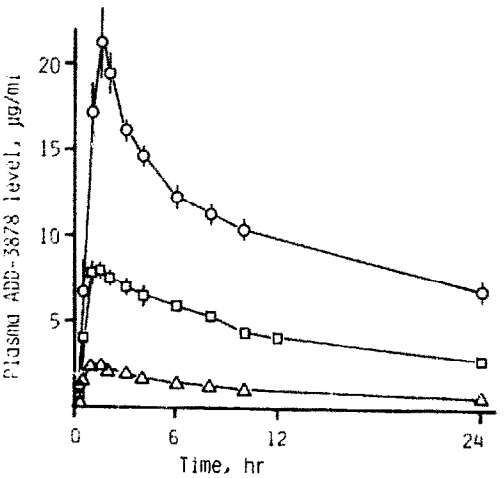


Fig. 5. Mean plasma levels of ADD-3878 in beagle dogs after oral administration of tablets (30 mg, △; 100 mg, □; 250 mg, ○) after normal meal. The data are expressed as mean ± S.E. (n = 4).

TABLE 6

BIOAVAILABILITY OF ADD-3878 TABLETS IN BEAGLE DOGS IN POSTPRANDIAL STATE

Dose	C_{max} (μg/ml)	T_{max} (h)	AUC (0–∞) (μg·h/ml)	B.A. [a] %
30 mg [b]	2.54±0.20	1.1±0.1	41.2± 4.5	80.1±5.4
100 mg [c]	8.14±0.48	1.4±0.1	180.7± 6.4	106.5±2.4
250 mg [d]	21.37±2.28	1.5±0.0	468.3±43.6	110.0±9.0

[a] Absolute bioavailability = $\frac{\text{AUC(p.o.)}\cdot\text{Dose (i.v.)}}{\text{AUC(i.v.)}\cdot\text{Dose(p.o.)}} \times 100$.

[b] Sample 4.

[c] Sample 5.

[d] Sample 6, 2 tablets.

All values represent mean ± S.E. (n = 4).

various doses. The AUC and the maximum plasma concentration are directly proportional to the dose given and the absolute bioavailability at 30 mg, 100 mg, and 250 mg doses are 80.1 ± 5.4, 106.5 ± 2.4 and 110.0 ± 9.0 (mean ± S.E.), respectively.

Multiple-dose study

The mean plasma levels of ADD-3878 after multiple administration of the tablet (sample 5) are shown in Fig. 6, compared with the predicted plasma level by a biexponential model using the parameters derived from the first dosing. The experimental plasma concentrations coincide well with those predicted. This indicates that multiple administration of ADD-3878 at a 100 mg dose does not cause any change in pharmacokinetic parameters and does not result in the phenomena such as accumulation of ADD-3878 in the plasma compartment, saturation of the drug in the tissue compartment, and inhibition or induction of its own metabolism.

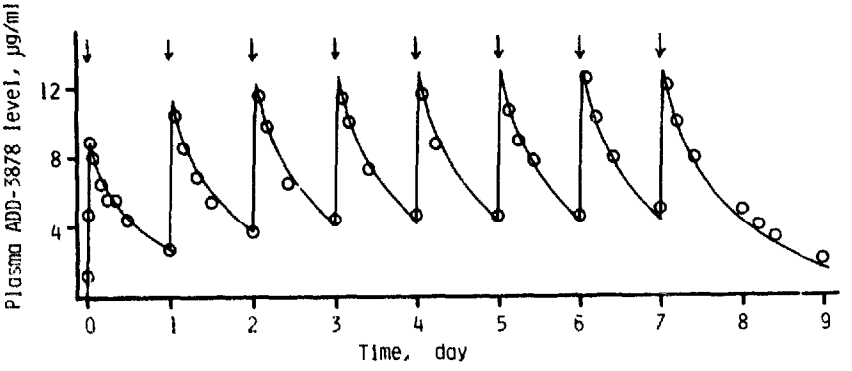


Fig. 6. Mean plasma level of ADD-3878 in beagle dogs after multiple administration of ADD-3878 tablet (sample 5). Each tablet was given immediately after a nomal meal, once a day for 8 days. Experimental, ○; predicted, ———; administration of a tablet, ↓. The experimental data are expressed as mean of 4 animals.

Discussion

Before an orally administered drug can be absorbed through the gastrointestinal membrane, it must be dissolved into solution. As the rate of dissolution is proportional to the surface area of the drug crystals, poorly water-soluble drugs are dissolved more rapidly and absorbed more efficiently from microcrystals than from macrocrystals (Shaw and Carless, 1974; Atkinson et al., 1962; Männistö, 1978).

Our results in the dog show that the gastrointestinal absorption of ADD-3878 in suspensions, even one containing micronized crystals (2 μm), are slow and incomplete when compared with that in solution. The low bioavailability is probably due to the low solubility (about 1 μg/ml at 25°C) and slow dissolution rate of ADD-3878 crystals.

Dietary effects on drug absorption and on the bioavailability of drugs have been reported by many investigators. The absorption of penicillin (McCracken et al., 1978; Watanakunakorn, 1977), aspirin (Koch et al., 1978), antipyrine and theophylline (Kappas et al., 1978) are reduced by food ingestion, whereas the absorption of griseofulvin (Crounse, 1961), nitrofurantoin (Bates et al., 1974), riboflavin (Houston and Levy, 1975), dicumarol (Melander and Wahlin, 1978) and diazepam (Greenblatt et al., 1978) are significantly improved when they are administered with food. Generally, food appears to increase the absorption of poorly water soluble lipophilic drugs.

The results of our study in dogs indicate that the bioavailability of ADD-3878 is enhanced significantly by food ingestion and the absolute bioavailability of the drug in tablet form is almost 100%. Major factors to increase the absorption may be, first, the effect of food on splanchnic blood flow and gastrointestinal motility and,

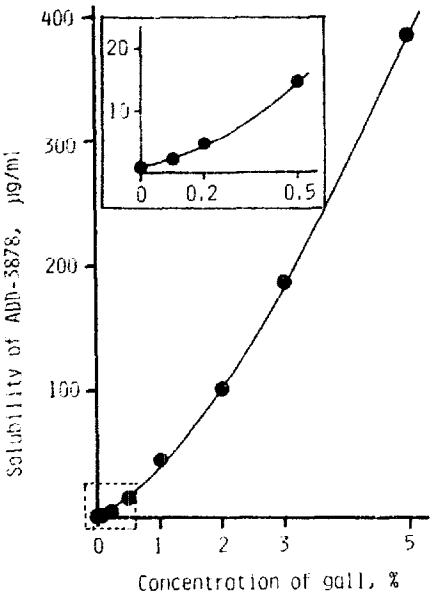


Fig. 7. Effect of gall powder on the solubility of ADD-3878 in water.

second, the induction of bile secretion for the drug dissolution; the latter factor is based on the observation that the solubility of ADD-3878 increased remarkably with the addition of gall powder (Fig. 7).

From these findings in dog studies, it was pointed out that the high bioavailability, and effective therapeutic activity of ADD-3878 could be obtained in human beings if the drug is administered immediately after food intake.

References

Atkinson, R.M., Bedford, C., Child, K.J. and Tomich, E.G., Effect of particle size on blood griseofulvin levels in man. Nature (London), 193(1962) 588–589.

Bates, T.R., Sequeira, J.A. and Tembo, A.V., Effect of food on nitrofurantoin absorption. Clin. Pharmacol. Ther., 16(1974) 63–68.

Crounse, R., Human pharmacology of griseofulvin: the effect of fat intake on gastrointestinal absorption. J. Invest. Dermatol., 37(1961) 529–533.

Greenblatt, D.J., Allen, M.D., MacLaughlin, D.S., Harmatz, J.S. and Shader, R.I., Diazepam absorption: effect of antacids and food. Clin. Pharmacol. Ther., 24(1978) 600–609.

Houston, J.B. and Levy, G., Effect of carbonated beverages and of an antiemetic containing carbohydrate and phosphoric acid on riboflavin bioavailability and salicylamide biotransformation in human. J. Pharm. Sci., 64(1975) 1504–1507.

Kappas, A., Alvares, A.P., Anderson, K.E., Pantuck, E.J., Pantuck, C.B., Chang, R. and Conney, A.H., Effect of charcoal-broiled beef on antipyrine and theophylline metabolism. Clin. Pharmacol. Ther., 23(1978) 445–450.

Koch, P.A., Schultz, C.A., Wills, R.J., Hallquist, S.L. and Welling, P.G., Influence of food and fluid ingestion on aspirin bioavailability. J. Pharm. Sci., 67(1978) 1533–1535.

Männistö, P., The effect of crystal size, gastric content and emptying rate on the absorption of nitrofurantoin in healthy human volunteers. Int. J. Clin. Pharmacol., 16(1978) 223–228.

McCracken, G.H.Jr., Ginsburg, C.M., Clahsen, J.C., Thomas, M.L., Pharmacologic evaluation of orally administered antibiotics in infants and children: Effect of feeding on bioavailability. Pediatrics, 62(1978) 738–743.

Melander, A. and Wahlin, E., Enhancement of dicoumarol bioavailability by concomitant food intake. Eur. J. Clin. Pharmacol., 14(1978) 441–444.

Shaw, T.R.D. and Carless, J.E., The effect of particle size on the absorption of digoxin. Eur. J. Clin. Pharmacol., 7(1974) 269–273.

Sohda, T., Mizuno, K., Imamiya, E., Sugiyama, Y., Fujita, T. and Kawamatsu, Y., Studies on antidiabetic agents II. Synthesis of 5-[4-(1-methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione(ADD-3878) and its derivatives. Chem. Pharm. Bull., 30(1982) 3580–3600.

Watanakunakorn, C., Absorption of orally administered nafcillin in normal healthy volunteers. Antimicrob. Agents Chemother., 11(1977) 1007–1009.

Yamaoka, K., Tanigawara, Y., Nakagawa, T. and Uno, T., A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharm. Dyn., 4(1981) 879–885.