EVALUATION OF LIPID-CONTAINING ORAL DOSAGE FORMS IN RATS

YOSHIYA YAMAHIRA,* TETSUO NOGUCHI, TAKESHI NOGUCHI, HIROSHI TAKENAKA, AND TADAO MAEDA

Formulation Research Department, Pharmaceuticals Division, Sumitomo Chemical Co., Ltd., Kurakakiuchi 1-3-45, Ibaraki-City, Osaka 567, Japan

(Received September 25, 1978)

A novel method for evaluation of oral lipid formulation in rats, which enabled us to reduce the dose level to 2 mcl/rat with satisfactory accuracy, was presented. The dose level was fairly comparable to that of clinical unit dose such as a soft capsule on mcl/kg (lipid dose/body weight) basis. By using this method, lipid-containing oral dosage forms were evaluated.

A new antiinflammatory agent 1-cyclopropyl-4-phenyl-6-chlor-2(1H)-quinazolinone (SL-512) was selected as a model of poorly water soluble drug. A medium chain triglyceride was mostly used as a lipid vehicle.

It was demonstrated that the characteristics of the lipid formulation could be estimated by measuring the gastric emptying rate of the drug or sometime combined with the intestinal remaining of the drug in rats. These results basically consisted of those obtained from 20 mcl/rat dosing experiments previously reported. It was newly found that by reducing the dose level to 2 mcl/rat, the drug absorption was less affected by the dosage form factors such as the drug concentration in the preparation or the digestibility of lipid vehicle.

It was also shown by this method that compared with an aqueous suspension, the drug absorption of the lipid formulation was less variable and less affected by the concomitant food intake.

Keywords—evaluation method; rat; lipid formulation; oral dosage form; effect of food; micro gastric syringe; variation of absorption rate

INTRODUCTION

Recently, bioequivalence of drug preparations has received much attention.¹⁾ In this respect, problems were frequently found in drug products containing poorly water soluble active ingredient, and their evaluation methods are much in demand. To examine oral dosage forms containing poorly water soluble drugs, *in vitro* dissolution test has been applied. However, *in vitro* test method is of limited value from biopharmaceutical standpoint, so that an *in vivo* estimation method using an experimental animal is essential for an adequate

evaluation.

In animal experiments performed to estimate the bioavailability of drugs, the test substance must be administered as close as possible in the form that is applied to human subject at clinical conditions such as a tablet, a capsule or a unit of fluid. For this purpose, medium size animals such as dogs or rabbits are generally used. Due to the difficulty in dosing an intact solid dosage form for human, small animals such as rats are rarely used in spite of their low cost and ease in handling.

Lipid formulations have an advantage, that is,

* To whom inquiries should be directed.

being a liquid, it can be administered to small animals in the same form used in clinic. However, its characteristics has not been well investigated. It has been reported recently that intestinal absorption of poorly water soluble drugs was affected by the coexistence of lipids.^{2,3)} In these experiments, however, dose levels of lipid vehicle on rats were so large (600—3000 mcl/rat) that they showed pharmacological actions on the gastrointestinal tract.⁴⁾ In fact, little work has been reported in which rats were used to estimate the lipid formulation.

Concerning these points, we have already reported that when a small amount of lipid preparations (20 mcl) was administered into the rat stomach, the pharmacological action of the lipid was negligible, and the viscosity of lipids was inversely proportional to its gastric emptying rate (GER), and that the stage of gastric emptying was the rate limiting step of drug absorption.⁵⁾

In this paper, a new method of administering 2 mcl of lipid formulations into the rat stomach, which is fairly close to the clinical unit dosing on mcl/kg (lipid dose/body weight) basis, was introduced and the usefulness of this evaluation method was presented. Some characteristics of lipid formulations were also investigated by using this method.

EXPERIMENTAL

Materials—1-Cyclopropyl-4-phenyl-6-chlor-2-(1H)-quinazolinone (SL-512) was employed as a model of poorly water soluble drugs. Easily absorbable lipids, medium chain triglyceride (MCT) and medium chain monoglyceride (MCM) were used as lipid vehicle. A hardly digestible lipid N- α -methylbenzylinoamide (MBLA) whose pharmacological properties were already reported⁶⁻¹⁰⁾ was also employed as a reference vehicle. SL-512 was synthesized in the Research Laboratory of Sumitomo Chemical Co., Ltd. and its chemical aspects and pharmacological properties were previously published.¹¹⁾ MCT, whose fatty acid component essentially comprized 75% of octanoic acid and 25% of decanoic acid, was commercially available (ODO-1[®], Toshin Chemical Co., Japan).

FIG. 1. Structure of Micro Gastric Syringe

MCM was derived from MCT in the laboratory of Nikko Chemical Co. (Japan). Other materials used in this study were of reagent grade.

Dosage forms of SL-512 employed were lipid preparations using MCT, MCM or MBLA as the vehicle and an aqueous suspension wherein micronized SL-512 of average particle size of 1 micron was suspended in 1.0% (w/v) arabic gum aqueous solution. The concentration of SL-512 in these preparations was 0.2—20% (w/v). The dose volume of these preparations varied from 2 to 20 mcl. In the experiment of estimating the movement of lipids in the gastrointestinal tract, 1.0% (w/v) of Sudan Blue was added to the lipid preparation.

Micro Gastric Syringe—In order to administer 2 mcl of fluid orally to a rat, a micro gastric syringe shown in Fig. 1 was designed. Disposable polyethylene tubings (Intramedic[®], Clay Adams) were attached to the top of the metallic gastric probe. The polyethylene tubing No. 7425 (i.d. 0.86 mm, o.d. 1.5 mm, length 60 mm) was used as the connector for the smaller tubing No. 7400 (i.d. 0.28 mm, o.d. 0.61 mm, length 150 mm) and the metallic probe. The junction of the tubing No. 7400 and No. 7425 was sealed with an epoxy-resin glue. Upon use, liquid preparations were sucked into the polyethylene tubing No. 7400 up to the marked level, the syringe containing 0.3 ml of air was attached and the liquid preparation was administered by a blow of 0.3 ml of the air. The dose level of 2 mcl was marked on No. 7425 with a blade by checking the amount of the sucked fluid measured by weight. The variation of the administered volume was examined with MCT and the result was 2.00 ± 0.05 mcl (mean of ten experiments $\pm$ S.D.). In this case the amount of MCT was determined gas-chromatographically as

described below.

Animal Experiment—Male Wistar rats weighing 180–220 g were fasted for 20 hrs prior to experiments but with free access to water. After administration of various preparations of SL-512, blood specimen was drawn periodically from *plexus veineux ophthalmique* and serum level of SL-512 was determined. The gastrointestinal remaining of SL-512 was also determined by removing the stomach and the whole small intestine at the designated time. To investigate the effect of the food ingestion, 1.5 ml of starch sol was ingested into the stomach as soon as SL-512 was administered. The starch sol was made by boiling an aqueous suspension of 10% (w/w) corn starch for 3 min and then left standing to cool to the room temperature.

In order to carry out studies in the rats with ligated pylorus, a surgical operation was performed under ether anesthesia and the incision was closed using a commercially available instant adhesive (Cyanobond®, Sumitomo Chemical Co.). The operated rats were used as soon as they awoke.

Assay Procedure for SL-512—SL-512 was removed from the serum or the gastrointestinal specimen by ether extraction and analyzed by gaschromatography as that described in our previous report.⁵⁾ Sudan Blue was also determined simultaneously with SL-512 under the same gaschromatographic condition.

Assay Procedure for MCT—Trioctanoin, the predominant component of ODO-1, was determined gas-chromatographically. A gas-chromatograph (GC-5A, Shimadzu Seisakusho, Japan) equipped with a flame ionization detector was used. A glass column of 1 m×0.3 mm was packed with 1% SE-30 on Chromosorb W 80/100 AW-DMCS. Tetratriacontane was added to the sample solution as an internal standard. The temperature was maintained at 260° for the column and 300° for the injection port and the detector block. The flow rate of the carrier gas (99.999% N₂) was adjusted to 40 ml/min.

RESULTS AND DISCUSSION

As previously reported⁵⁾ the pharmacological effect of lipids on the gastrointestinal tract was

FIG. 2. Gastric Emptying of SL-512 and Sudan Blue after Oral Administration as MCT Preparation

○ SL-512 (2.0% (w/v)), ● Sudan Blue (1.0% (w/v)).

Dose level: 2 mcl/rat.

Data are expressed as the mean±S.E. of at least 4 animals.

negligible with the oral dose of 20 mcl/rat. In this report, a special device was designed for quantitative administration of a small amount of liquid preparation orally to rats and the dose level was reduced to 2 mcl/rat, which was fairly proportional to the human unit dose such as a soft capsule on a mcl/kg (lipid dose/body weight) basis. Using this procedure, the evaluation method of lipid formulations in rats was studied by examining the gastrointestinal movement of model drug. To determine the rate of absorption of the model drug, its disappearance rate from the gastrointestinal tract was measured instead of measuring its blood level since the pharmacokinetic treatment of the data of SL-512 absorption in rats indicated a negligible metabolic loss in the gut.⁵⁾

Gastric Emptying and the Intestinal Retention of SL-512 after Oral Administration

It was observed in our previous experiment⁵⁾ that in a case of 20 mcl/rat SL-512 was emptied from the stomach according to an apparent first-order kinetics. When SL-512 and Sudan Blue were co-administered with 2 mcl of MCT into the rat stomach, the time course of the gastric emptying of these marker substances also showed an apparent

TABLE I. *Intestinal Retention of SL-512 after Oral Administration as MCT or MCM Preparation*

Vehicle	Conc. of SL-512 (% (w/v))	% of dose remained in the small intestine at	
		1 hr	3 hr
MCT	10	1.24 ± 0.64	0.77 ± 0.20
	20	0.63 ± 0.43	0.84 ± 0.38
MCM	4	0.17 ± 0.06	0.35 ± 0.05
	10	0.83 ± 0.17	0.81 ± 0.27
	20	0.94 ± 0.30	1.30 ± 0.51

Dose level: 2 mcl/rat.

Data are expressed as the mean $\pm$ S.E. of at least 4 animals.

FIG. 3. *Effect of the Dose Volume on the GER of SL-512 Administered as MCT or MCM Preparation*

○ MCT (SL-512 2.0% (w/v)), ● MCM (SL-512 4.0% (w/v)).

The GER was determined at 60 min after oral administration.

Data are expressed as the mean $\pm$ S.E. of at least 4 animals.

first-order kinetics (Fig. 2). The residual amount of Sudan Blue was in good agreement with that of SL-512. Since Sudan Blue was practically unabsorbable from the stomach loop at the dose examined ($4.0 \pm 2.5\%$ of dose/2 hrs) and was practically insoluble in water, it was considered that the dye would be suitable as a marker of lipids in the stomach. From the results shown in Fig. 2, it seemed reasonable under this condition to use the gastric emptying rate of SL-512 during the first hour as the index of the GER of the preparation.

The intestinal retention of SL-512 at 1 and 3 hr after oral administration of 2 mcl of the MCT or

MCM preparation was as little as the case of 20 mcl dosing reported in our previous paper⁵⁾ (Table I). Therefore, it was considered that SL-512 was absorbed from the small intestine at the rate apparently equal to its GER at the dose of either 2 mcl/rat or 20 mcl/rat.

Effect of the Dose Volume on the GER of SL-512

It has already been reported that an extremely large volume of lipids was administered to rats before our previous report.⁵⁾ The larger volume of lipids (>100 mcl/rat) delayed the absorption of SL-512 due to the larger retention of SL-512 both in the stomach and in the intestine. The GER was compared between the 2 mcl/rat dose and 20 mcl/rat dose used in our previous studies. As shown in the Fig. 3, in cases of 2% MCT and 4% MCM preparations, no significant difference was observed between 2 mcl and 20 mcl/rat. However, the GER decreased at the increased dose level of 50 mcl/rat in both preparations.

Effect of Drug Concentration on the GER

In the case of lipid solution, the GER of SL-512 was proportional to the reciprocal of its viscosity.⁵⁾ From the standpoint of designing a dosage form for a clinical use, it is inevitable in some case to employ a lipid suspension depending on the dose level of the drug. Since the viscosity of the lipid suspension of SL-512 is highly dependent on the drug concentration in the preparation (Fig. 4), the relationship between the GER of SL-512 and the concentration of the drug in the preparations was examined.

When 20 mcl of MCT preparations were administered to the rat orally, the GER decreased as

FIG. 4. Relationship between the Concentration of SL-512 and the Viscosity of the Lipid Preparations

○ MCT, ● MCM.

Viscosity was measured with Brookfield synchroelectric viscometer at 24°. Data are expressed as the mean of three experiments. The solubility of SL-512 in MCT and MCM was 0.5 and 4.0% (w/v) at 37°, respectively.

the drug concentration (the viscosity of the preparation) increased (Fig. 5-(a)). On the contrary, the concentration dependency of the GER was not observed over a wide range of solution and suspension when similar investigation was made at the dose level of 2 mcl/rat in both MCT and MCM preparations (Fig. 5-(b)).

Thus, it was shown that with reduced dose volume, the effect of SL-512 concentration in the preparation, which is one of the major dosage form factors, became less influential in the animal experiments.

Effect of the Digestibility of Vehicle Lipids on the Absorption of SL-512

The effect of dose level was also studied from the standpoint of the intestinal absorption of the lipid vehicle. It has already been reported that at the dose level of 20 mcl/rat, the absorption of SL-512 from the small intestine was partly dependent on the digestibility of the lipid vehicle in the small in-

FIG. 5. Effect of the Drug Concentration of the GER of SL-512 administered as MCT or MCM Preparation

(a) 20 mcl/rat. (b) 2 mcl/rat.

○ MCT, ● MCM.

The GER was determined at 60 min after oral administration.

Data are expressed as the mean $\pm$ S.E. of at least 5 animals.

The solubility of SL-512 in MCT and MCM was 0.5 and 4.0% (w/v) at 37°, respectively.

testine.⁵⁾ However, this dependency was rather reduced at the dose level of 2 mcl/rat. As shown in Table II, the rate of serum appearance of SL-512 was greater at the dose level of 2 mcl/rat even from the MBLA preparation, whose lipid vehicle was hardly absorbed from the rat small intestine.^{9, 10)}

Therefore, it was newly observed at the dose level of 2 mcl/rat that the absorption of SL-512 was less dependent on dosage form factors of the lipid formulation such as its drug concentration or the intestinal digestibility of the lipid vehicle. These results suggested that the effect of physiological factors on the drug absorption in the test animal such

TABLE II. *Gastric Emptying Extent and Absorption of SL-512 in 1 Hr after Oral Administration of SL-512 as MBLA Solution*

	Dose level/rat	
	2% (w/v) 2 mcl	0.5% (w/v) 20 mcl
% of dose emptied from the stomach	28.2±7.5	29.3±2.1
Serum level (ng/ml)	17.2±4.0	20.4±5.0
% of dose appeared in the serum ^{a)}	18.4±3.9	8.8±2.2

Data are expressed as the mean±S.E. of at least 5 animals.

a) Calculated from the serum level by Wagner-Nelson method using data of i.v. experiment.⁵⁾

TABLE III. *Comparison of MCT Preparation with an Aqueous Suspension at 1 Hr after Oral Administration*

		% of dose remained	
Preparation		Stomach	Small intestine
Fasted	MCT	65.8±0.8	0.20±0.08
	Aq susp.	64.2±5.6 ^{a)}	0.92±0.45
Postprandial	MCT	65.7±1.8	0.18±0.10
	Aq susp.	71.4±6.9 ^{b)}	7.23±3.0

Dose level: SL-512 5.0% (w/v), 2 mcl/rat.

Data are expressed as the mean±S.E. of 6 animals.

a) $F = 44.0$, $p < 0.01$; b) $F = 6.86$, $p < 0.05$.

as gastrointestinal motility and gastric secretion might relatively increase at a low dose level such as 2 mcl/rat.

Comparison of MCT Preparation with an Aqueous Suspension

In this study, a lipid formulation was compared with an aqueous suspension, a model of the solid formulation, by using the same evaluation method in rats mentioned above. Although the stomach retention of SL-512 was almost the same for both preparations, the variation of stomach remaining of the drug differed very much in fasted rats (Table III). A statistically significant difference was found between the variation of the MCT preparation and that of the aqueous suspension. As the remaining of SL-512 in the small intestine was small in both preparations, this difference in the GER seemed to influence the absorption of SL-512 resulting in a small variation in the MCT preparation and a relatively large variation in the aqueous prepa-

ration. It was also suggested from this result that the absorption of SL-512 from MCT preparation was less affected by physiological factors such as gastrointestinal motility and gastric secretion than that from the solid formulation.

It has been reviewed by Welling about the influence of food on the gastrointestinal drug absorption¹²⁾ and two physiological functions that were of primary importance, the blood flow rate and the regulation of gastric motility and/or emptying, have been pointed out. Other physiological factors affecting the bioavailability of oral dosage forms have been reviewed by Bates and Gibaldi¹³⁾ and by Rowland.¹⁴⁾ The effect of food ingestion was then studied as one of the major physiologically variable factors using the same evaluation method. Starch sol was employed as a model food because it could be ingested quantitatively.

As shown in Table III, MCT preparation was less affected by the food ingestion both in the

TABLE IV. *Gastric Absorption of SL-512 in Rats with Ligated Pylorus*

Preparation	Serum level at 1 hr (ng/ml)	% of dose appeared in the serum ^{a)}
MCT	46.2 ± 7.7	16.2 ± 2.7
Aq. susp.	133.4 ± 20.1	34.9 ± 5.3

Dose level: SL-512 5.0% (w/v), 2 mcl/rat.

Data are expressed as the mean ± S.E. of at least 5 animals.

a) Calculated from the serum level by Wagner-Nelson method using data of i.v. experiment.⁵⁾

gastric emptying and the intestinal absorption. On the contrary, the tendency of delayed gastric emptying and decreased absorption in the small intestine were observed after the administration of the aqueous suspension. This was in good agreement with the report of Lindenbaum¹⁶⁾ that even administered postprandially, digoxin soft capsule showed the higher bioavailability than its tablet.

This characteristics of MCT preparation might be attributed to the strong affinity of SL-512 to the lipid and to the movement of the lipid in the gastrointestinal tract. Next, the pylorus of rats was ligated and the gastric absorption of SL-512 during 1 hr period was estimated. Although the gastric absorption of SL-512 seemed to be negligible under the normal condition (Fig. 2), it was rather high under the abnormal condition of the pylorus ligation (Table IV) probably due to the dissolution of SL-512 into the secreted fluids. It was noteworthy that the gastric absorption of SL-512 was lower from MCT preparation than that from the aqueous suspension. This result indicated that MCT preparation reduced the distribution of the drug into the gastric contents and that SL-512 was transported into the duodenum being kept in high drug concentration in the lipid.

As shown in Table III, SL-512 was absorbed from the rat small intestine with little disturbance by the concomitant food intake when administered as MCT preparation. Although the precise reason for this observation is not clear, it seems probable that this is attributable to the localization of SL-512 in the gastrointestinal tract, being kept in MCT, and to the rapid absorption of MCT itself from the rat small intestine.¹⁷⁾

It was then suggested, from above results, that the lipid formulation, though the influence of whose dosage form factors was reduced along with reduction in the dose level of the lipid, was characterized from solid formulations in some case to be less variable in its drug absorption rate, and this would contribute to the uniformity of the bioavailability.

REFERENCES

- 1) FDA: Bioequivalence requirements and *in vivo* bioavailability procedures, *Fed. Reg.*, **42**, 1624—1563 (1977).
- 2) P.Carrigan and T.Bates: Biopharmaceutics of drugs administered in lipid-containing dosage forms I: GI absorption of griseofulvin from an oil-in-water emulsion in the rat, *J. Pharm. Sci.*, **62**, 1476—1479 (1973).
- 3) D.Bloedow and W.Hayton: Effects of lipids on bioavailability of sulfisoxazole acetyl, dicumarol, and griseofulvin in rats, *J. Pharm. Sci.*, **65**, 328—334 (1976).
- 4) T.Bates, H.Pieniaszek, Jr., J.Sequeira, and J.Rasmussen: Gastrointestinal absorption of griseofulvin from corn oil-in-water emulsions, *Arch. Dermatol.*, **113**, 302—306 (1977).
- 5) Y.Yamahira, T.Noguchi, H.Takenaka, and T.Maeda: Biopharmaceutical studies of lipid-containing oral dosage forms: Relationship between drug absorption and gastric emptying of lipid formulations, *J. Pharm. Dyn.*, **1**, 160—167 (1978).
- 6) H.Fukushima, K.Toki, and H.Nakatani: The effect of N-(α -methylbenzyl) linoleamide on experimental atherosclerosis in rabbits, *J. Atheroscler. Res.*, **9**, 57—64 (1969).
- 7) H.Fukushima, S.Aono, and H.Nakatani: Effect of N-(α -methylbenzyl) linoleamide on lipid levels of plasma and liver in cholesterol fed rats, *J. Nutr.*, **96**, 15—20 (1968).
- 8) H.Fukushima, S.Aono, Y.Nakamura, M.Endo, and T.Imai: The effect of N-(α -methylbenzyl)linoleamide on cholesterol metabolism in rats, *J. Atheroscler. Res.*, **10**, 403—414 (1969).

- 9) H.Fukushima: Absorption, tissue distribution and excretion of N-(α -methylbenzyl) linoleamide, *Pharmacometrics*, **3**, 135—140 (1969).
- 10) A.Nagata, H.Miyawaki, M.Endo, and H.Nakatani: Enzymatic hydrolysis of N-(α -methylbenzyl)linoleamide. *Chem. Pharm. Bull.* (Tokyo), **19**, 1276—1277 (1971).
- 11) H.Yamamoto, C.Saito, S.Inaba, H.Awata, M.Yamamoto, Y.Sakai, and T.Komatsu: Novel quinazoline derivatives II. A new antiinflammatory agent, SL-512, *Arzneim.-Forsch.*, **23**, 1266—1271 (1973).
- 12) P.Welling: Influence of food on gastrointestinal drug absorption: A review, *J. Pharmacokin. Biopharm.*, **5**, 291—334 (1977).
- 13) T.Bates and M.Gibaldi: Gastrointestinal absorption of drugs. "Current Concepts in the Pharmaceutical Sciences: Biopharmaceutics," ed. by J.Swarbrick, Lea and Febiger, Philadelphia, 1970, pp. 57—99.
- 14) M.Rowland: Effect of some physiologic factors on bioavailability of oral dosage forms, "Current Concepts in the Pharmaceutical Sciences: Dosage form design and bioavailability," ed. by J.Swarbrick, Lea and Febiger, Philadelphia, 1973, pp.181—222.
- 15) G.Levy and W.Jusko: Effect of Viscosity on Drug Absorption, *J. Pharm. Sci.*, **54**, 219—225 (1965).
- 16) J.Lindenbaum: Greater bioavailability of digoxin solution in capsules. Studies in the postprandial state, *Clin. Pharmacol. Ther.*, **21**, 278—282 (1977).
- 17) M.Playoust and K.Isselbacher: Studies on the intestinal absorption and intramucosal lipolysis of a medium chain triglyceride, *J. Clin. Invest.*, **43**, 878—885 (1964).