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Biopharmaceutical Study of the Hepato-biliary Transport of Drugs. VII.[1] Improvement of the Bioavailability of Rifampicin by Dosage Form Design

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Improvement of the bioavailability of rifampicin (RFP) was attempted by dosage form design. As lipid vehicles, sesame oil, soybean oil, oleic acid and middle chain triglyceride (MCT) such as tricapriline, were employed. When sesame oil or MCT was used as a lipid vehicle, the percentage recovery of RFP into rat bile was significantly decreased and rat serum RFP level was increased. This improvement of the bioavailability of RFP was also occurred in a dog. The mechanism by which the bioavailability of RFP was improved by lipid vehicles was studied and it has been revealed that this improvement of the bioavailability of RFP is not due to its depressed hepatic uptake from the blood stream after absorption from the gastrointestinal tract but due to the depressed active biliary excretion from the hepatocytes into the bile.

Keywords——bioavailability; rifampicin; dosage form design; hepatic first-pass effect; biliary excretion

In our previous reports,[1,3] the basic principles of the hepato-biliary transport of organic anions were investigated, and it was revealed that the transport of organic anions from the blood stream into the bile is composed of at least three processes, (1) hepatic uptake from the blood stream, (2) intracellular transport in the liver parenchymal cells, and (3) excretion from the liver parenchymal cells into the bile canaliculi. However, the last excretory process was revealed to be the rate-limiting step for the total organic anion transport from the blood stream into the bile. As the hepatic first-pass effect of an orally administered drugs, which is a very important event concerning to the bioavailability of drugs, is thought to be composed of not only the metabolic process in the liver but also this hepato-biliary transport process, it is considered to be very valuable to decrease the hepatic first-pass effect by depressing their hepato-biliary transport, especially, excretion from the hepatocytes into the bile.[4] When it becomes possible to decrease the hepatic first-pass effect of orally administered drugs, the bioavailability of these drugs will be greatly increased.

Using rifampicin (RFP) which is a very expensive and potent antibiotics for tuberculosis,[5] and of which bioavailability is very low because of its selective uptake and metabolism by the liver and of large excretion in the bile, the possibility that the hepatic uptake of RFP could be depressed by dosage form design has been investigated in this report.

1) H. Nakae, H. Okamoto, K. Takada, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **25**, 427 (1977).
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4) S. Kenwright and A.J. Levi, *Lancet*, **ii**, 1405 (1973).
5) N. Maggi, S. Furesz, R. Pallanza, and G. Pelizza, *Arzn. Forsch.*, **19**, 651 (1969).

Experimental

Materials——Rifampicin (RFP) is a gift from Dai-ichi Pharmaceutical Co., Ltd. Middle chain triglyceride (MCT), tricapryline, is a gift from Ono Pharmaceutical Co., Ltd. Lipid vehicles such as sesame oil, soy bean oil and oleic acid were purchased from Nakarai Chemicals Co., Ltd. The six different RFP dosage forms were prepared as follows; (1) RFP Suspension: RFP was suspended in 0.2% carboxy methyl cellulose (CMC) solution, (2) RFP–MCT Suspension: MCT, decuple weight times to RFP, was added to RFP suspension, (3) RFP Sesame Oil: RFP was dissolved in sesame oil, (4) RFP Emulsion: O/W emulsion, the mixture of sesame oil, RFP solution and emulsifier (0.2% w/v Polysorbate 80), was prepared by sonicating after shaken vigorously at 20 kHz, 100 W for 10 min with sonicator, Ohtake Seisakusho No. 5202, (5) RFP Soy Bean Oil: RFP was dissolved in soy bean oil, (6) RFP Oleic Acid: RFP was dissolved in oleic acid.

Animal Experiment with Rats——Male Wistar rats ranging in weight from 280 to 320 g were anesthetized by intraperitoneal injection of sodium pentobarbital. The common bile duct was cannulated with a polyethylene tubing (0.75 mm in the outside diameter). Body temperature was monitored *via* rectal probe. Each dosage form was intraduodenally administered and the bile samples were collected continuously by 24 hr. The analytical method for RFP in the bile samples is the same as described in our previous report.[3a]

With respect to the dosage forms from which the biliary recoveries of RFP were decreased as compared to the control dosage form (RFP suspension), RFP blood level was monitored. Namely, after the intraduodenal administration of each RFP dosage form, blood was collected from a cut at the end of the tail into a tube at 15 and 30 min, 1, 2, 3, 4, 5 and 24 hr. While, the liver was removed at 1, 2, 3, 4 and 5 hr after administration. Serum and liver RFP contents were measured using Sarcina lutea ATCC 9341 according to the microbiological assay of Boman,[6]

Electrophoresis——After the intraduodenal administration of three different RFP dosage forms, RFP suspension, RFP-MCT suspension, RFP sesame oil, bile samples were collected for 1 hr. Not only thus obtained bile samples but also the normal bile and the bile containing the liver plasma membrane "band III protein" which was collected by the method described precisely in our previous report, were used for electrophoresis.[3a]

Experiment with a Dog——A male dog (10 kg) who was overnight fasted before dosing and food was withheld for 24 hr after administration of RFP dosage forms. Water was available *ad libitum*. Each dosage form was administered by means of a stomach catheter followed by rinsing with 5 ml of water or lipid vehicles. A blank blood sample was taken before administration and 3 ml of blood were sampled at each sampling time. The analytical method is the same as described in the rat experimental method.

Results

The recoveries of RFP in the rat bile from five different dosage forms are shown in Fig. 1, 2 and 3. As compared with the control dosage form, RFP suspension, the recovery of

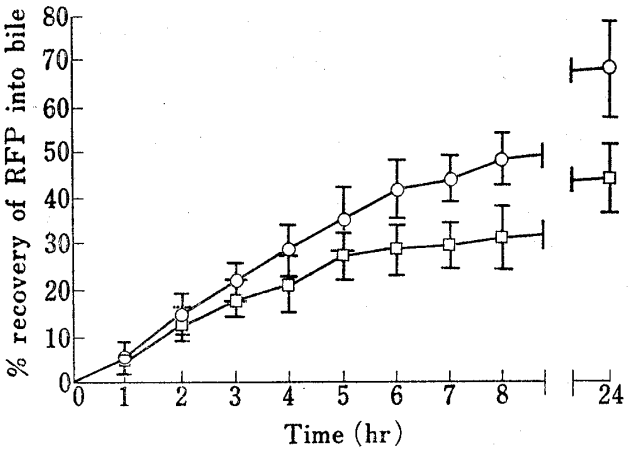

Fig. 1. Comparison of Biliary Recovery of the Intraduodenally Administered RFP Suspension (○—○) and RFP–MCT Suspension (□—□)

The rats received 8 mg of RFP/kg intraduodenally.
Each point represents the mean ± S.E. for four to six animals.

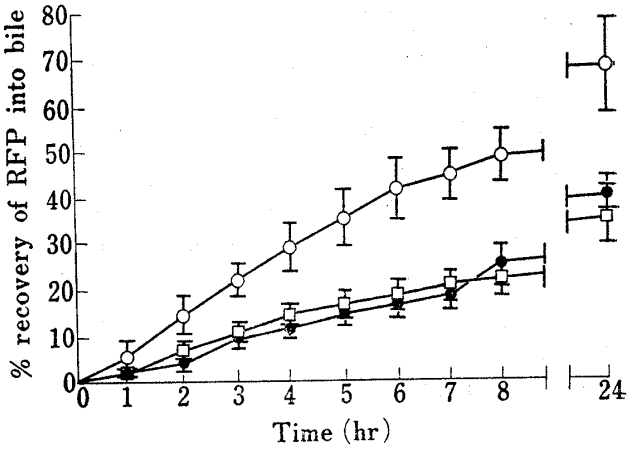

Fig. 2. Comparison of Biliary Recovery of the Intraduodenally Administered RFP Suspension (○—○), RFP in Sesame Oil (□—□) and RFP Emulsion (●—●)

The rats received 8 mg of RFP/kg intraduodenally.
Each points represents the mean ± S.E. for four to six animals.

6) G. Boman and A.S. Malmborg, *Europ. J. Clin. Pharmacol.*, 7, 51 (1974).

RFP was decreased about one-half when lipid vehicles such as sesame oil and MCT were used respectively. To clarify the question whether these results are ascribed to the depressed intestinal absorption of RFP itself, dissolved dosage form, O/W emulsion, was made and the same experiment was carried out. However, as shown in Fig. 2, the recovery of RFP into rat bile from emulsified dosage form was not increased. On the other hand, the recovery of RFP into the bile was not significantly decreased when soy bean oil was used as a lipid vehicle (Fig. 3). Therefore, the possibility that the bioavailability of RFP may be increased by dosage form design was diminished in the case of soy bean oil dosage form.

In Fig. 4 to 7, the bioavailability of RFP from six different dosage forms are revealed by measuring the serum RFP levels and by calculating the area under the curve for the time

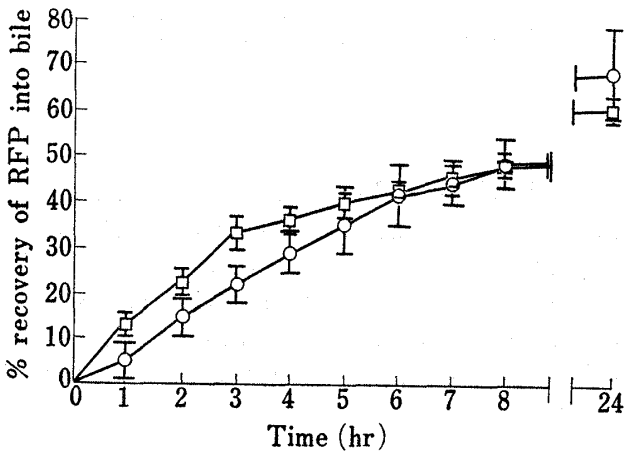

Fig. 3. Comparison of Biliary Recovery of the Intraduodenally Administered RFP Suspension (○—○) and RFP in Soy Bean Oil (□—□)

The rats received 8 mg of RFP/kg intraduodenally.
Each point represents the mean ± S.E. for four to six animals.

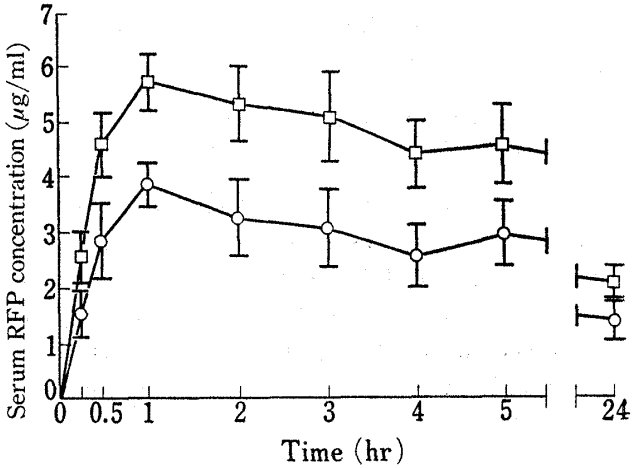

Fig. 4. Serum RFP Concentration Profiles from RFP Suspension (○—○) and RFP-MCT Suspension (□—□)

The rats received 8 mg of RFP/kg intraduodenally.
Each points represents the mean ± S.E. for four to six animals.

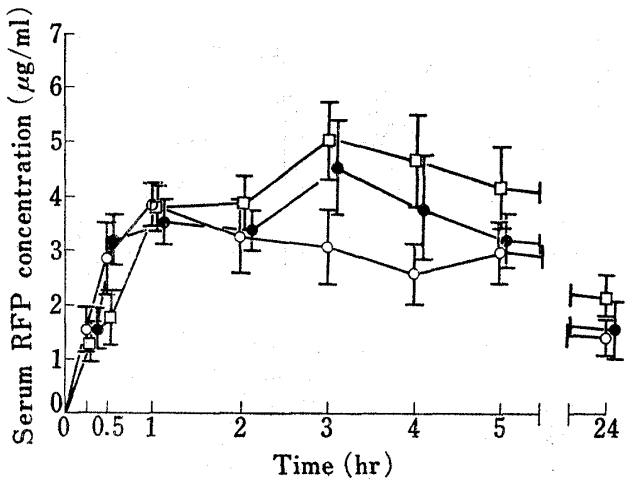

Fig. 5. Serum RFP Concentration Profiles from RFP Suspension (○—○), RFP in Sesame Oil (□—□) and RFP Emulsion (●—●)

The rats received 8 mg of RFP/kg intraduodenally.
Each point represents the mean ± S.E. for four to six animals.

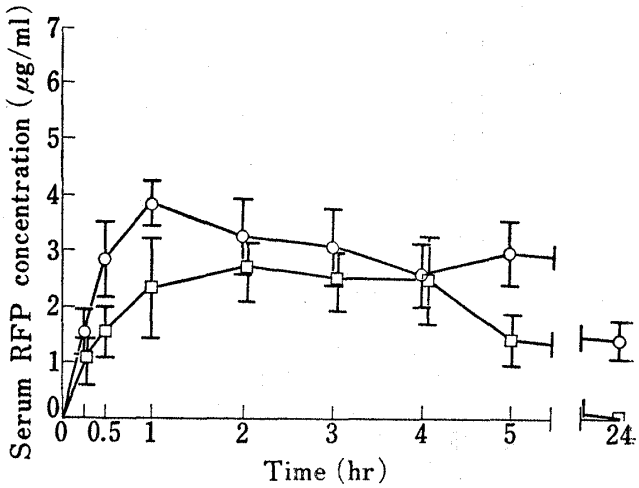

Fig. 6. Serum RFP Concentration Profiles from RFP Suspension (○—○) and RFP in Soy Bean Oil (□—□)

The rats received 8 mg of RFP/kg intraduodenally.
Each point represents the mean ± S.E. for four to six animals.

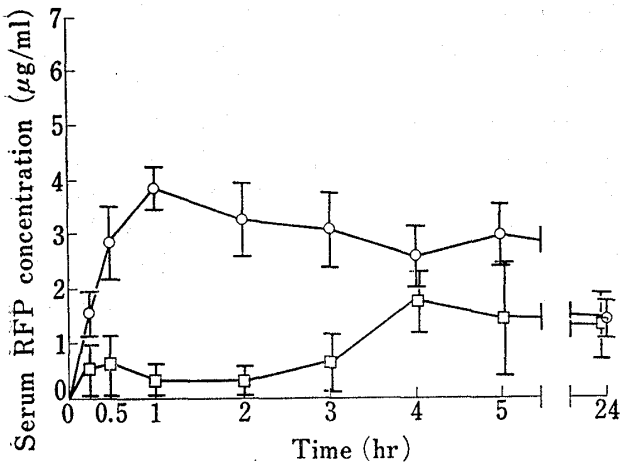

Fig. 7. Serum RFP Concentration Profiles from RFP Suspension (○—○) and RFP in Oleic Acid (□—□)

The rats received 8 mg of RFP/kg intraduodenally.
Each point represents the mean±S.E. for four to six animals.

Table I. Extent of Bioavailability of RFP from Six Different Dosage Forms

Dosage forms	$AUC^{0\to24}$ [(μg/ml)·hr]	
RFP suspension	54.7±11.8	
RFP–MCT suspension	85.0 ±19.9	(+)
RFP sesame oil	88.1 ±10.4	(+)
RFP emulsion	63.3 ± 8.53	(0)
RFP soy bean oil	19.6 ± 7.70	(−)
RFP oleic acid	5.21± 3.67	(−)

The (+) and (−) indicate a significant ($p<0.05$) increase or decrease, respectively, in $AUC^{0\to24}$ compared to the RFP suspension dosage form: the (0) indicates no significant difference.

0 to 24 hr, $AUC^{0\to24}$ [(μg/ml)·hr] using the trapezoidal rule.[7] The result is represented in Table I. As suggested in Figs. 4, 5 and Table I, the bioavailability of RFP from RFP–MCT suspension and RFP sesame oil dosage forms is greatly improved as compared with that from the control dosage form, RFP suspension. However, in the case of RFP emulsion, its bioavailability almost equals to that of the control dosage form. On the other hand, both the RFP soy bean oil dosage form and RFP oleic acid dosage form have small bioavailabilities.

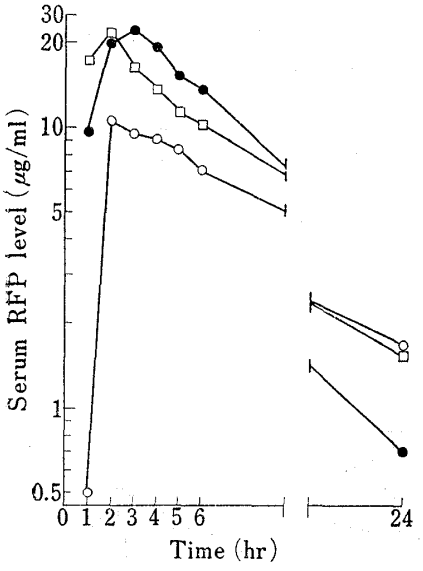

Fig. 8. Dog Serum RFP Concentration Profiles from RFP Suspension (○—○), RFP–MCT Suspension (●—●) and RFP in Sesame Oil (□—□)

A male dog (10 kg) received 8 mg of RFP/kg orally.
Each circle represents the average of triplicate assays of RFP in the dog.

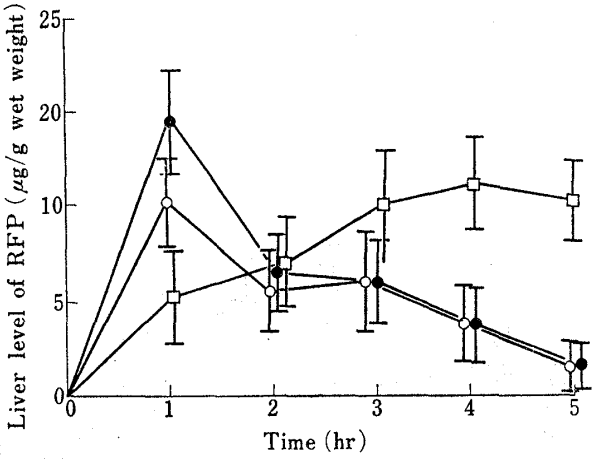

Fig. 9. Time Course of the RFP Liver Level after the Intraduodenal Administration of RFP Suspension (○—○), RFP–MCT Suspension (●—●) and RFP in Sesame Oil (□—□)

The rats received 8 mg of RFP/kg intraduodenally.
Each point represents the mean±S.E for four to six animals.

7) M. Gibaldi and D. Perrier, "Pharmacokinetics," Marcel Dekker, Inc., New York, 1975.

To ascertain the possibility to improve the bioavailability of RFP by dosage form design in another species, three different dosage forms were orally administered to a dog and serum RFP concentration was monitored for 24 hr. As shown in Fig. 8, higher serum RFP level was observed in both dosage forms, RFP–MCT suspension and RFP sesame oil, than in the control dosage form.

To clarify whether this improvement of the bioavailability of RFP depends on its depressed hepatic uptake, the liver RFP level was monitored by 5 hr after intraduodenal administration of the three different RFP dosage forms and the result is represented in Fig. 9. If the hepatic uptake of RFP was inhibited by MCT or some component which is contained in sesame oil, the liver RFP level might be decreased. However, as liver RFP level was not decreased, improvement of the bioavailability of RFP is thought to be due to the other factors. Accordingly, the effect of dosage form on the active biliary excretory system for organic anions was investigated by electrophoresis which was developed by the author[3e] and the result is represented in Fig. 10. In the cases of normal rat bile and the rat bile to whom RFP suspension was intraduodenally administered, there detects a few protein bands of which concentration is very low. While, there detects a further concentrated protein band which is suggested by the arrow in this figure when electrophoresis was carried out using the rat bile to whom RFP–MCT suspension or RFP sesame oil were administered intraduodenally. By comparing with the electrophoretical pattern of the rat bile to whom the intrabiliary retrograde infusion with 50 μl aliquot of 4% triton X-100 isotonic solution, pH 7.4, was carried out, it is revealed that this band is identical with "band III protein". As this protein, which was isolated by the author,[3e] is thought to be the major component of the active biliary excretory system for organic anions, the effect of the lipid vehicles such as sesame oil and MCT is thought to have their origin to this system.

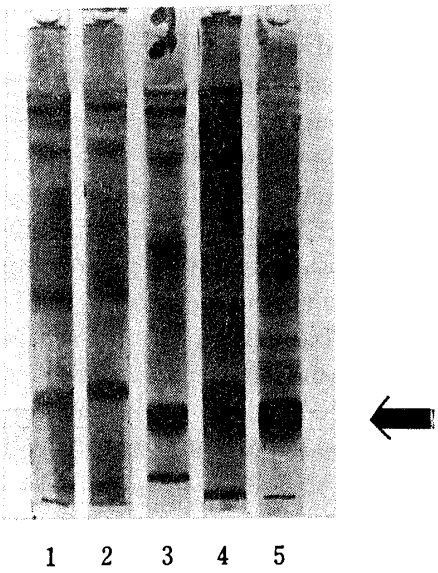

Fig. 10. Polyacrylamide Electrophoretical Pattern of Rat Bile Samples

After bile samples were applied to the gels (3.75%: upper gel, 6.0%: lower gel), electrophoresis was carried out at 5 mA/gel for 5 hr. Staining was performed for protein with coomassie blue.
Samples from left to right are as follows:
1: normal bile,
2: RFP suspension,
3: RFP sesame oil,
4: RFP–MCT suspension,
5: rat bile obtained by intrabiliary retrograde infusion with 4% triton X-100 solution.

Discussion

According to the present results, it seems that lipid vehicles such as sesame oil and triglyceride, MCT, are able to improve the bioavailability of an organic anion such as RFP by depressing its hepato-biliary transport.

Though it was reported that lipid vehicles raise the bioavailability of drugs, arachis oil against α-tocopherol[8] and cholesteryl *n*-decylate against salicylate,[9] these authors did not mention the mechanism or reason why these phenomena occurred. In this report, it was attempted to reveal this important point.

8) J. Kelleher, T. Davis, C.L. Smith, B.E. Walker, and M.S. Losowsky, *Intn. J. Vit. Nutr. Res.*, **42**, 394 (1972).
9) S.P. Patel and C.I. Jarowsky, *Drug Develop. Commun.*, **2**, 465 (1976).

When sesame oil or MCT was used as lipid vehicle, the bioavailability of RFP was considerably increased. However, lipid vehicles such as soy bean oil and oleic acid did not enhance the bioavailability of RFP. In addition, when solubilized dosage form, O/W emulsion, was used, the bioavailability of RFP was not enhanced either. Taking these points into the consideration, the improved bioavailability of RFP is not thought to be due to the enhanced absorption from the gastrointestinal tract. Although the liver RFP level was monitored to examine whether this enhanced bioavailability of RFP is due to its depressed hepatic uptake, significant difference has not been detected between the three dosage forms, RFP suspension, RFP sesame oil and RFP–MCT suspension. Therefore, it may be mentioned that the enhanced bioavailability of RFP is not caused by the depression of its hepatic uptake. When electrophoresis was carried out with respect to the rat bile to whom different RFP dosage forms were administered intraduodenally, there detects a significant difference between their electrophoretical patterns. Especially, there detects a considerable amount of the "band III protein" in the rat bile to whom RFP–MCT suspension or RFP sesame oil was administered as compared with the control rat bile sample. As this "band III protein" is revealed to have an important role on the active biliary excretory process for organic anions from the hepatocytes into the bile,[3e] the enhanced bioavailability of RFP is thought to be due to the solubilization of this transport system by the lipid vehicles.

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