Enhancement of Oral Bioavailability and Pharmacological Effect of 1-(3,4-Dimethoxyphenyl)-2,3-bis(methoxycarbonyl)-4-hydroxy-6,7,8-trimethoxynaphthalene (TA-7552), a New Hypocholesterolemic Agent, by Micronization in Co-ground Mixture with D-Mannitol

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To improve the bioavailability of the sparingly water-soluble drug, 1-(3,4-dimethoxyphenyl)-2,3-bis(methoxycarbonyl)-4-hydroxy-6,7,8-trimethoxynaphthalene (TA-7552), the usefulness of the co-grinding method with D-mannitol was investigated. The co-grinding was performed at various weight ratios of TA-7552 and D-mannitol using a ball mill. The particle size was markedly reduced with increasing amount of D-mannitol. A mixture ratio greater than or equal to 1:3 of the drug and D-mannitol produced submicron-sized particles. In dogs, bioavailability increased with increasing amount of D-mannitol. The 1:9 coground mixture gave complete absorption, as did a lecithin solution of the drug. Even co-ground powders with lower amounts of D-mannitol provided relatively high bioavailability in comparison with ground drug powder alone of a similar particle size. Further, pharmacological examination using rats indicated that the inhibition of cholesterol absorption was intensified with the reduction of particle size. These findings suggest that the co-grinding method with D-mannitol is useful for enhancing the bioavailability and pharmacological effectiveness of this sparingly water-soluble drug.

Key words sparingly water-soluble drug; hypocholesterolemic agent; particle size; co-ground mixture; D-mannitol; bioavailability

TA-7552, (1-(3,4-dimethoxyphenyl)-2,3-bis(methoxycarbonyl)-4-hydroxy-6,7,8-trimethoxynaphthalene), was recently developed as a hypocholesterolemic agent which competitively inhibits intestinal absorption of cholesterol.¹⁾ However, TA-7552 exhibits very poor solubility in water (less than 1 µg/ml), and the oral bioavailability of TA-7552 in dogs was extremely low (less than 1%) when the drug was administered in raw powder form. This implies that it would be difficult to formulate peroral preparations affording sufficient bioavailability and pharmacological effect.

Through a series of preformulation studies,²⁾ we found that TA-7552 essentially showed good intestinal absorption due to its lipophilic property. In fact, the absolute bioavailability increased to 36% when the drug was administered as a lecithin solution. This indicates that the bioavailability could be improved by appropriate modification of the dissolution characteristics of the drug.

Various pharmaceutical techniques to improve the dissolution properties of sparingly water-soluble drugs, such as micronization, solid dispersion, complex formation, and emulsification, have been studied. However, from the practical point of view, micronization is the method of choice, because of ease of manufacture and/or assuring the physicochemical stability of the drug. Griseofulvin,³⁾ phenytoin,⁴⁾ spironolactone,⁵⁾ and norfloxacin,⁶⁾ are typical examples of drugs whose bioavailability can be increased by reduction of the particle size.

In pharmaceutical manufacturing, various dry milling devices, including the ball mill, jet mill and hammer mill, are widely applied to improve the dissolution characteristics of drugs. However, Jimbo suggested that the reduction of particle size by such micronization processes is limited to 3 µm or so, due to the secondary coaggregation oc-

curing between particles as a result of Van der Waals' interaction and electrostatic force.⁷⁾ Recently, Takahata *et al.* developed a new micronization technique involving the co-grinding of a drug with a hydrophilic low molecule additive, such as D-mannitol, by which the particle size of drugs can be reduced to a submicron level.⁸⁾

To improve the oral bioavailability of TA-7552 by micronization, several micronization techniques were evaluated for the reduction of particle size, and the influence of particle size on the dissolution properties, bioavailability and pharmacological effectiveness was examined.

MATERIALS AND METHODS

Materials TA-7552 was supplied by the Research Laboratory of Applied Biochemistry of Tanabe Seiyaku Co., (Osaka, Japan). β-Glucuronidase was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sodium lauryl sulfate (SLS), sodium pentobarbital, cholesterol and sodium cholate were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). HCO-60 was obtained from Nikko Chemicals Co., Ltd. (Tokyo, Japan). Egg lecithin PL-100H and D-mannitol were purchased from Kewpie Co., Ltd. (Tokyo, Japan) and Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan), respectively. All other materials and solvents were of analytical reagent grade, and deionized-distilled water was used throughout the study.

Preparations (a) Solution: Lecithin solution was prepared in the same way as described previously.²⁾

(b) Micronized Powders: A jet mill (PJM-100NP, Nippon Pneumatic Mfg Co., Ltd., Mie, Japan) was used in the dry milling method. To obtain finer particles, an Eiger mill (M-25 OVSE EXP, Kurimoto Ltd., Osaka,

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Japan) was used for wet milling. The drug was suspended in water (10%, w/v) and the diameter of glass beads used was 1 mm.

(c) Co-ground Mixture with D-Mannitol: D-Mannitol was used as a carrier of the co-ground powder system. For preparing the co-ground mixture, the drug and carrier at various ratios (1 : 1, 1 : 3 and 1 : 9 in weight, respectively) were weighed and placed in a ball mill (AN-6S, Nitto Kagaku Co., Ltd., Tokyo, Japan), to be ground in a dry process for 24 h. The capacity of the mill was 62.8 l and the balls were 4.5 cm in diameter.

Measurement of Mean Particle Size The mean particle diameter (above or equal to 1 μm) was determined by using a laser granulometer (model 715, Cilas Alcatel, France) and (below 1 μm) by using a centrifugal particle size analyzer (SA-CP2, Shimadzu Co., Kyoto, Japan). A 0.01% (w/v) aqueous HCO-60 solution was used as the suspension medium.

X-Ray Diffractometry TA-7552 powder and micronized samples were evaluated for crystallinity by using an X-ray diffractometer (MXP3VA, MAC Science Co., Tokyo, Japan). The operating conditions were as follows: radiation, Ni-filtered Cu- K_{α} ; voltage/current, 40 kV/35 mA; scanning speed, 4°/min; divergence/receiving/scattering slits, 0.5°/0.3 mm/0.5°.

Differential Thermal Analysis (DTA) The melting points and heat of fusion of TA-7552 and micronized samples were determined by use of a differential thermal analyzer (Thermoflex, Rigaku Denki Ltd., Tokyo, Japan). The operating conditions were as follows: heating rate, 10°C/min; heating range, 50–350°C; sample weight, 3 mg; DTA range, 100 μV .

Dissolution Study The dissolution experiments were carried out according to the JPXI paddle method using a dissolution testing system (NTR-VS6, Toyama Sangyo Co., Ltd., Osaka, Japan). Water (900 ml) containing 0.6% SLS was used as the dissolution medium at 37°C, and the paddle rotation speed was 50 rpm. Fifty mg of the drug was added to the medium and the amount dissolved was determined by absorbance measurement with an ultraviolet spectrophotometer, UV-160 (Shimadzu Co., Kyoto, Japan) at 270 nm.

Bioavailability Study (a) Animals: Three or four male beagle dogs, weighing 10 to 13 kg (Yoshiki Farm Inc., Gifu, Japan) were used. Approximately 300 g of food (Oriental Yeast Co., Ltd., Tokyo, Japan) was given to the dogs at 1 p.m. on the day before dosing and 150 g of food at 8:30 a.m., 30 min before dosing, on the following day (the drug was administered at 9 a.m.). The animals were allowed free access to water throughout the experiment, except for the 2 h after drug administration.

(b) Oral Administration: A lecithin solution of TA-7552 was administered in the same way as described previously. Pulverized powder in hard gelatin capsules was given orally with 30 ml of water at a dose of 50 mg. Venous blood samples were taken at 1, 3, 5 and 7 h after each administration in heparinized tubes. The samples were centrifuged immediately at 3000 rpm for 5 min, and the plasma fraction was frozen for storage until assay.

Analytical Method The plasma concentrations of TA-7552 were determined according to the high-performance

liquid chromatographic (HPLC) method described previously.²⁾ TA-7552 glucuronide in the plasma was determined as follows: 1.0 ml plasma was mixed with 0.5 ml of pH 6.0 McIlvaine phosphate buffer and 5 ml of cyclohexane, and the mixture was shaken for 5 min. After centrifugation, the organic phase was removed and a portion (1.0 ml) of the aqueous layer was mixed with 0.5 ml of 1 N hydrochloric acid. To the combined solution, 5 ml of ethyl acetate was added and the mixture was shaken for 5 min. After centrifugation, a portion (4 ml) of the organic phase was passed through a Bondelute SI cartridge (Analytichem International, Harbor City, CA, U.S.A.) and glucuronide was selectively eluted with 1.0 ml of methanol. The eluate was evaporated to dryness under a nitrogen gas stream at 40°C. The residue was taken up in 1 ml of 0.2 M sodium acetate buffer (pH 5.0, β -glucuronidase 800 unit/ml) and the mixture was incubated for 16 h at 37°C. Then 5 ml of cyclohexane was added and the mixture was shaken for 5 min. After centrifugation, a portion (4 ml) of the organic phase was taken and evaporated to dryness under a nitrogen gas stream at 40°C. The residue was dissolved in 1.0 ml of the HPLC mobile phase described above, containing the internal standard (acenaphthene: 5 $\mu\text{g}/\text{ml}$), and 0.2 ml of the solution was injected into the HPLC system for analysis.

Determination of Serum and Liver Cholesterol Male Sprague-Dawley rats (4 weeks old, Clea Inc., Tokyo, Japan) were maintained on commercial laboratory food (CE-2 pellets or powder, Clea Japan Co., Ltd., Tokyo, Japan) supplemented with 0.2% cholesterol and 0.5% sodium cholate for 1 week before the experiment. For efficacy evaluation, the rats were divided into two groups of 6 rats. The experimental group was treated daily with pulverized powder of TA-7552 (mean particle sizes: 21.2–0.2 μm , 0.1% in CE-2 powder), which had been prepared by using a jet mill and ball mill, by feeding the diet *ad libitum* for 1 week, and the control group was fed CE-2 powder. After 1 week, the animals were bled from the abdominal aorta and the liver was excised under light ether anesthesia. Total cholesterol in serum was measured by the enzymatic method⁹⁾ using a commercial kit (Cholestezyme-Eiken, Eiken Chemical Co., Tokyo, Japan). For determination of total hepatic cholesterol, a portion of liver (200 mg) was refluxed in 2 ml of 10% KOH in 90% ethanol at 90°C for 90 min. Thereafter, the mixture was diluted with 2 ml of water and extracted twice with 4 ml of petroleum ether. Aliquots of the petroleum ether extracts were used for determination by the method of Zak *et al.*¹⁰⁾

Recovery of TA-7552 from Gastrointestinal Tract Male Sprague-Dawley rats (6 weeks of age) were fasted for 12 h prior to the experiment, but water was given *ad libitum*. Micronized powder (mean particle sizes: 0.6, 3.0 μm) equivalent to 10 mg/kg as TA-7552 suspended in 0.1% HCO-60 was orally intubated into the rats. At 1 h after administration of TA-7552, rats were anesthetized with sodium pentobarbital (30 mg/kg) and bled from the abdominal aorta, and the stomach, duodenum, jejunum and ileum were removed. The gastrointestinal lumen was washed gently with 30 ml of saline solution. The recovered washing solution was diluted 30-fold with HPLC mobile phase. The concentration of TA-7552 in the diluted

solution was determined by the HPLC method. The recovery (%) of the drug was calculated based on the value of the amount of drug recovered from each gastrointestinal tract divided by the administered drug.

Pharmacokinetic Analysis In this study, the concentration of TA-7552 glucuronide was represented as that of TA-7552 and the plasma concentrations were calculated by adding the concentration of TA-7552 to that of its glucuronide. Pharmacokinetic parameters were calculated by the same procedure as described previously.²⁾ Statistical analysis was carried out by using Student's *t* test. To establish a significant difference between two regression lines, the analysis of covariance was used after no significant difference had been found between the slopes of the two regression lines. A *p* value of 0.05 or less was considered to be significant.

RESULTS AND DISCUSSION

Glucuronide is one of the major metabolites of TA-7552. The glucuronide concentration was always three or four times higher than that of TA-7552 for 7 h, as with TA-7552 after oral administration. The absorption of TA-7552 might be more precisely assessed in terms of the area under the plasma concentration–time curve (*AUC*) of the glucuronide, especially in the evaluation of TA-7552 preparations with poor absorption. On this basis, all the *AUC*s of TA-7552 and glucuronide obtained in the present study are plotted in Fig. 1. A good linear relationship ($r=0.948$) was found between the *AUC* of TA-7552 and the total *AUC* of TA-7552 and its glucuronide. This suggests that the total *AUC* of both compounds can be used for evaluation of the absorption of TA-7552.

Effect of Particle Size Reduction on Bioavailability A jet mill or an Eiger mill is often used to modify the dissolution characteristics of poorly water-soluble drugs.^{11–13)} Thus, these conventional milling methods were firstly applied to see if the bioavailability of TA-7552 could be increased to a sufficient level. The size of the original

TA-7552 crystals (*ca.* 39 μm) was reduced by the jet mill and the Eiger mill to about 3 and 1 μm , respectively.

Figure 2 shows the change of total plasma concentrations of TA-7552 and its glucuronide with time after oral administration of the micronized TA-7552 powder and the lecithin solution in dogs. The more highly micronized powder preparations obtained with the Eiger mill gave a higher plasma concentration than the powder produced by the jet mill, but gave a lower plasma concentration than the lecithin solution. The relative bioavailability with respect to the lecithin solution was estimated to be about 14% for the jet-milled powder and 50% for the Eiger-milled powder. Although the attained bioavailability was still insufficient in both cases, it was anticipated that further size reduction of drug particles would improve the bioavailability.

Application of Co-grinding Technique The co-grinding technique was next applied for the pulverization of TA-7552. As an additive, D-mannitol, which was reported to be the most effective substance for the size reduction of oxolinic acid crystals,⁸⁾ was used.

Table 1 summarizes the particle size attained under various operating conditions, the amount of D-mannitol and grinding time. The mean particle size of the raw powder was 38.7 μm . When the drug was ground without any additive, the particle size was reduced only to half even after 24 h. In contrast, the co-grinding method remarkably reduced the particle size. The amount of D-mannitol had a greater effect on the micronization than

Fig. 1. Relationship between *AUC* of TA-7552 and Total *AUC* of TA-7552 and Its Glucuronide

Fig. 2. Plasma Concentration Profiles of TA-7552 and Its Glucuronide after Oral Administration of Micronized Powder and Lecithin Solution of TA-7552 at a Dose of 50 mg in Beagle Dogs

—●—, lecithin solution; —○—, micronized powder (Eiger mill); —■—, micronized powder (jet mill). Each point represents the mean $\pm$ S.E. of three or four dogs.

Table 1. Correlation between Co-grinding Time and Mean Particle Size

Weight ratio (w/w) TA-7552 : D-mannitol	Mean particle size (μm)					
	0 h	2 h	6 h	12 h	18 h	24 h
1 : 0	38.7	19.0	16.0	17.6	—	21.2
1 : 1	38.7	—	2.1	—	—	2.4
1 : 3	38.7	1.3	0.7	0.8	0.8	0.6
1 : 9	38.7	—	0.5	0.5	—	0.3

Fig. 3. Cumulative Particle Size Distribution of TA-7552 of Raw Powder and Co-ground Powder with D-Mannitol

—●—, raw powder; —○—, co-ground powder with D-mannitol at a weight ratio of 1:3.

Fig. 4. X-Ray Diffraction Patterns of Mixtures of TA-7552 with D-Mannitol

a) D-Mannitol; b) physical mixture of TA-7552 with D-mannitol at a weight ratio of 1:3; c) co-ground powder of TA-7552 with D-mannitol at a weight ratio of 1:3; d) raw powder.

the co-grinding time. The particle size of raw powder was reduced to nano-order level within 6 h when a 3-fold excess of D-mannitol was used. Figure 3 shows the cumulative particle size distributions before and after grinding. The mean diameter of co-ground powder was around $0.6\ \mu\text{m}$ and 80% of the particles was at the submicron level.

Figure 4 shows the X-ray diffraction patterns of D-mannitol alone, TA-7552 after having been co-ground with D-mannitol (1:3, w/w), its physical mixture and TA-7552 raw powder. Since the diffraction lines that originated from D-mannitol and TA-7552 crystals overlapped, it seemed difficult to determine the crystallinity of the drug. However, the diffraction pattern of the co-ground mixture (c) was coincident with that of the physical mixture (b).

Figure 5 shows the differential thermoanalysis (DTA) curves of the same samples used in X-ray diffractometry. The endothermic peaks at 168°C (a) and 183°C (d) were attributed to the melting of D-mannitol and TA-7552 crystals, respectively. The physical (b) and co-ground (c)

Fig. 5. DTA Curves of Mixtures of TA-7552 with D-Mannitol

a) D-Mannitol; b) physical mixture of TA-7552 with D-mannitol at a weight ratio of 1:3; c) co-ground powder of TA-7552 with D-mannitol at a weight ratio of 1:3; d) raw powder.

mixtures exhibited similar overlapping endothermic peaks at 168 and 183°C . The DTA results implied that there was no difference in crystallinity between the physical and co-ground powders.

As expected, the solubility of TA-7552 in the co-ground mixture was as low as less than $1\ \mu\text{g}/\text{ml}$. These results indicate that TA-7552 exists in the microcrystalline state with little change in crystallinity after co-grinding. This is quite advantageous for formulation aiming at pharmaceutical production, because, in many cases, amorphous products exhibit marked hygroscopicity and deterioration of dissolution properties during storage under humid conditions.

The dissolution rates of TA-7552 in the co-ground powders were compared with that of the raw powder by the JP paddle dissolution test (Fig. 6). In this experiment, an aqueous solution of 0.6% SLS was used as the test fluid (sink condition). The co-ground powders at weight ratios of 1:3 and 1:9 were rapidly dissolved to the extent of about 80% and 100% within 10 min, respectively, whereas the raw powder was only 40% dissolved after 90 min. This indicates that the co-ground powder dissolved faster with increasing amount of D-mannitol used in co-grinding, because of the surface area increase due to the particle size reduction.

Thus, application of the co-grinding method was ascertained to be useful and effective for the micronization of TA-7552. Poorly soluble, high-melting-point substances may be empirically preferable for micronization, as reported by Kondo *et al.*¹⁴⁾

Enhancement of Bioavailability by Co-grinding Method
Bioavailability of TA-7552 micronized by the co-grinding method was evaluated using beagle dogs. Figure 7 shows the plasma concentrations of TA-7552 and its glucuronide after administration of the co-ground powders and the

Fig. 6. Dissolution Profiles of Raw Powder and Co-ground Powder with D-Mannitol

—●—, co-ground powder with D-mannitol at a weight ratio of 1:9; —○—, co-ground powder with D-mannitol at a weight ratio of 1:3; —■—, raw powder. The dissolution of TA-7552 was measured in 900 ml of 0.6% SLS solution at 50 rpm and 37°C. Each point represents the mean of duplicate experiments.

Fig. 7. Plasma Concentration Profiles of TA-7552 and Its Glucuronide after Oral Administration of Co-ground Powders and Lecithin Solution of TA-7552 at a Dose of 50 mg in Beagle Dogs

—●—, lecithin solution; —○—, co-ground powder with D-mannitol at a weight ratio of 1:9; —■—, co-ground powder with D-mannitol at a weight ratio of 1:3; —□—, co-ground powder with equal amount of D-mannitol by weight. Each point represents the mean $\pm$ S.E. of three or four dogs.

lecithin solution. The co-ground powders showed higher plasma concentrations with increase in weight ratios of D-mannitol, and near equality to that of the lecithin solution was achieved with D-mannitol at the weight ratio of 1:9.

The relationship between particle size and relative bioavailability is shown in Fig. 8. A fairly high bioavailability compared with micronized powder obtained in the jet mill was obtained even when the particle size in the ground powder was similar to that of the single-ground powder. The relative values of bioavailability of co-ground powders with respect to the lecithin solution were 54% and 83%, at the particle size of 2.4 and 0.6 μ m, respectively, whereas with the 0.3 μ m co-ground powder, the relative value was about 100% (Table 2).

All the findings obtained from the dog studies on the powder formulations prepared by the conventional

Fig. 8. Relationship between Particle Sizes and Bioavailabilities in Comparing Co-ground Powder with Lecithin Solution and Single-ground Powder

The figures over the bars represent the ratio of drug and D-mannitol (w/w).

Table 2. Bioavailability Parameters after Oral Administration of Lecithin Solution and Co-ground Powders with D-Mannitol in Beagle Dogs at a Dose of 50 mg

Preparation	T_{max} (h)	C_{max} (ng/ml)	AUC_{7h} (ng·h/ml)	Relative ^{a)} bioavailability (%)
Lecithin solution	2.0 $\pm$ 1.0	147.1 $\pm$ 36.7	623.3 $\pm$ 89.5	100.0
TA-7552:D-mannitol (w/w)				
1:1	1.0 $\pm$ 0.0	91.3 $\pm$ 3.4 ^{d)}	364.5 $\pm$ 40.4 ^{b)}	53.6 $\pm$ 2.4 ^{b-d)}
1:3	2.0 $\pm$ 0.6	122.9 $\pm$ 14.9	515.1 $\pm$ 80.0	83.0 $\pm$ 5.0 ^{b)}
1:9	1.5 $\pm$ 0.5	165.3 $\pm$ 13.7	619.4 $\pm$ 101.9	101.7 $\pm$ 14.1

Each value represents the mean $\pm$ S.E. of three or four dogs. a) Relative bioavailability was calculated as AUC of co-ground powder/ AUC of lecithin solution; b) Significantly different ($p < 0.05$) from lecithin solution; c) Significantly different ($p < 0.05$) from co-ground powder with D-mannitol at a weight ratio of 1:3; d) Significantly different ($p < 0.05$) from co-ground powder with D-mannitol at a weight ratio of 1:9.

micronized method (single grinding) and the co-grinding method were plotted together (Fig. 9). The bioavailability increased with reduction of particle size. However, two different regression lines were obtained for the co-ground powders and single-ground powders. These regression lines were significantly different, suggesting that the co-ground powder provided significantly higher bioavailability than the single-ground powder. This implies that, even when the particle size is the same, the co-ground powder with D-mannitol can afford a higher bioavailability than the powder obtained by the conventional single-grinding method.

Van der Waals' interactions and electrostatic attraction between the particles should increase in the micronized powder, causing the particles to aggregate.¹⁵⁾ D-Mannitol may prevent such aggregation and agglomeration of the drug particles, and may help to wet the drug in the biological fluid, speeding up the dissolution of TA-7552

Fig. 9. Relationship between Particle Size and Bioavailability of Co-ground Powder and Single-ground Powder

—●—, co-ground powder with D-mannitol; —○—, single-ground powder. Each point represents the mean $\pm$ S.E. of three to four dogs. The regression lines (a) and (b) were obtained for the co-ground powder and single-ground powder data, respectively. * Significant different ($p < 0.05$) from the regression line (b).

and improving the bioavailability. The present findings suggest that the co-grinding method is effective for enhancing bioavailability and applicable for actual pharmaceutical manufacturing, because fairly high bioavailability was achieved using the co-ground mixture with D-mannitol, and preparations made with co-ground mixtures are expected to be stable on account of the micro-crystalline state of the drug.

Previously, we pointed out the possibility of active absorption of the drug administered in lecithin solution. However, the fact that the bioavailability obtained with the 1:9 co-ground powder was equivalent to that of the lecithin solution shows that lecithin has no special function to enhance the intestinal absorption of TA-7552.

Influence of Particle Size on Pharmacological Effect

The hypocholesterolemic action of TA-7552 is based on competitive inhibition of the intestinal absorption of both cholesterol and bile acid.¹⁾ This suggests that it is not necessary for the drug to be absorbed from the gastrointestinal tract to exhibit hypocholesterolemic activity. Therefore, we examined the correlation between bioavailability and hypocholesterolemic activity. The inhibition of cholesterol absorption was evaluated after administration of TA-7552 in various particle size powders in rats. As shown in Fig. 10, the extent of the decrease of cholesterol from the control value in both liver and serum was clearly related to the reduction of particle size.

Because the increment of bioavailability was also proportional to the reduction of particle size, bioavailability should be indicative of hypocholesterolemic activity, even though TA-7552 existing in the systemic circulation might not be effective pharmacologically.

Table 3 shows the recovery (%) of TA-7552 from the stomach, duodenum, jejunum and ileum at 1 h after administration of submicron powder and 3 μ m powder in rats. Approximately 46% of TA-7552 administered as submicron powder was recovered in total, of which about 30% was from the ileum, 16% from the stomach, and less than 1% from other parts of the intestine. In contrast with

Fig. 10. Relationship between Particle Size and Hypocholesterolemic Effect in Diet-Induced Hypocholesterolemic Rats

—●—, liver cholesterol; —○—, serum cholesterol. TA-7552 was administered at 0.1% in the diet for seven days. Each point represents the mean $\pm$ S.E. of six rats.

Table 3. Recovery (%) of TA-7552 from the Gastrointestinal Tract at 1 h after Oral Administration of Micronized Powder in Rats

	Mean particle size (μ m)	
	0.6 (Co-ground powder)	3.0 (Single-ground powder)
Stomach	15.56 $\pm$ 4.03	7.72 $\pm$ 1.02
Duodenum	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01
Jejunum	0.10 $\pm$ 0.09	0.04 $\pm$ 0.02
Ileum	30.40 $\pm$ 3.94 ^{a)}	2.49 $\pm$ 1.19

Each powder was given at a dose of 10 mg/kg in 0.1% HCO-60 suspension. Each value represents the mean $\pm$ S.E. of four or five rats. ^{a)} Significantly different ($p < 0.05$) from the value of the recovery from ileum at the particle size of 3.0 μ m.

submicron powder, 3 μ m powder showed only nearly 10% recovery in total. The recovery from the ileum showed a significant difference between these two micronized powders. It appears that submicron particles are accumulated in the gut, especially in the ileum.

Kreuter *et al.*¹⁶⁾ found that polyhexylcyanoacrylate nanoparticles distinctly accumulated in the section before the ileo-cecal junction, that is, the ileum, after oral administration to mouse. Couvreur *et al.*¹⁷⁾ also showed an association of nanoparticles with the gut wall and their accumulation in the ileum by macroautoradiographic observations in mice. These findings are in agreement with our result that much greater accumulation was observed with submicron particles of TA-7552. Consequently, reduction to submicron particles may not only raise the dissolution rate, but also cause more TA-7552 to remain in the ileum. The accumulation in the ileum and raised dissolution rate should enhance absorption, and intensify the hypocholesterolemic effect of TA-7552, because cholesterol is actively absorbed from the ileum.

In conclusion, the co-ground mixture with D-mannitol was found to be effective for the sparingly water-soluble drug TA-7552 to improve bioavailability and intensify the pharmacological effect. The application of this method

in actual pharmaceutical manufacturing and for the enhancement of bioavailability of other water-insoluble drugs will be reported separately.

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