Application of Fibroin in Controlled Release Tablets Containing Theophylline

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The applicability of fibroin, a major silk protein, to controlled release type dosage tablets was investigated *in vitro* and *in vivo*. Fibroin tablets containing theophylline were easily prepared by a direct compression method without additives. Five types of fibroin tablets with the same surface area and different amounts of theophylline were prepared. In an *in vitro* drug study, the drug release from the tablets was not affected by the pH of the release medium. The greater the fibroin content in the tablets, the lower the percentage released at time *t*. The Higuchi plots of the release data showed a linear release profile, indicating that the drug release from the fibroin tablets was diffusion-controlled through the matrix. Theophylline powder or a TF-41 tablet (theophylline: fibroin=4:1), or a commercial tablet, Uniphyll (once-a-day type) was administered to 5 healthy volunteers. The areas under the saliva theophylline concentration–time curve (*AUC*) of Uniphyll and TF-41 to that of powder were 85% and 70%, respectively (fasted). Conversely, the mean residence time and mean absorption time of TF-41 were long compared to Uniphyll (fasted). Therefore, the reduction of bioavailability in TF-41 was due to the delayed release from the tablets *in vivo*. Taken after a meal, the *AUC*s of TF-41 and Uniphyll increased and the absorption was completed. This suggested that the drug release from TF-41 may increase due to the stimulation of food on TF-41 itself and due to movement of the gastro-intestinal tract. In conclusion, fibroin could be used as the matrix in controlled-release tablets.

Key words fibroin; controlled-release; healthy volunteer; theophylline; tablet; bioavailability

Recently, many controlled-release preparations have been developed. There are many matrices, including not only natural macromolecules but also synthetic ones consisting of preparations. In particular, natural macromolecules are interesting because of their safety and the reuse of natural mass *etc.* Polysaccharides such as cellulose and its derivatives, chitin, and chitosan have been studied as matrices for oral controlled-release tablets, but proteins are less applicable to that dosage form because of degradation due to gastro-intestinal enzymes. We reported a hydrophobic corn protein, zein, was applicable for oral controlled-release preparations.¹⁾

Fibroin (>400000 Da) is a major silk protein, mainly consisting of alanine, glycine, and serine. The protein has unique characteristics being water-insoluble and protease-resistant. Silk fibroin is used as a surgical suture because of its biocompatibility and harmlessness to humans. Recently, water-soluble silk powder prepared by acid hydrolysis has been used as a food additive²⁾ and in cosmetics.³⁾ In addition, it increased the adhesive force of medical fibroin paste,⁴⁾ and water soluble silk jelly was developed for the matrix of preparations for elderly patients.⁵⁾ There are no studies on the utilization of fibroin itself (macromolecule). In this study, fibroin tablets were prepared and fibroin's applicability for controlled-release type dosage forms was evaluated *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials Fibroin and pancreatin were purchased from Wako Pure Chemicals, Co., Ltd., and theophylline was obtained from Nacalai Tesque Inc. Pepsin isolated from porcine stomach mucosa was obtained from Sigma Chemical Co. Fibroin and theophylline were used after being passed through 50 mesh (300 μ m) sieves. All other chemicals and solvents

were of analytical reagent grade and were used without further purification. Uniphyll tablets containing 200 mg of theophylline were purchased from Otsuka Pharmaceutical Co., Ltd.

Tablet Preparation The theophylline/fibroin mixtures were directly compressed using a single punch tabulating machine (AR400, ERWEKA) or a briquetting press (Type: M-30-BRP-E, Maekawa, Tokyo). The latter was only used to study the effect of the compressing force (5–30 kN for 3 min). Then, 250 mg tablets with the same surface area (diameter: 1.0 cm, thickness 2.7 mm) were prepared by the former method (Table 1). 250 mg tablets with the same diameter (1.0 cm) and a different thickness were prepared by the latter method. Theophylline tablets (200 mg) were also prepared from theophylline powder without additives. A Monsanto hardness tester was used for the measurement of the hardness of tablets.

In Vitro Release Studies The release of theophylline from various tablets was determined using a JP XIII dissolution test apparatus with a paddle stirrer at 100 rpm. The dissolution media used were 900 ml of JP XIII disintegration test medium No. 1 (pH 1.2) and No. 2 (pH 6.8). Media were kept at $37 \pm 0.5^\circ\text{C}$ during measurements. Five minutes before the tablets were put into the media, 0.9 g of pepsin or pancreatin was added to the JP XIII disintegration test medium No. 1 (pH 1.2) or No. 2 (pH 6.8), respectively. Aliquots (0.2 ml) of the sample solutions were withdrawn at appropriate time intervals and filtered through a 0.45 μ m membrane filter. Ten μ l of the filtrate was injected into an ODS 120-T column (4.6 mm $\times$ 15 cm, Tosoh, Japan). A high performance liquid chromatograph (Shimadzu, Japan) equipped with a UV-8011 spectrophotometric detector (Tosoh, Japan, at 271 nm) was used. The mobile phase was an acetonitrile/0.01 M acetate buffer (pH 4.0; 10/90, v/v) and the flow rate was 1.0 ml/min. The sample solutions were analyzed spectrophotometrically

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for theophylline at 270 nm, when the media contained no enzymes. The water content in the tablet was obtained by subtracting the initial fibroin amount in the tablet and the remaining theophylline amount in the tablet after 24 h from the tablet weight after 24 h.

In Vivo Studies Five healthy volunteers (3 males, 2 females) from whom informed consent was obtained were enrolled in this study (Table 2). They were non-smokers. None of the subjects consumed any caffeine- or alcohol-containing food or beverages for 2 d prior to, and up until the end of, the study. Before each dosage, the subjects fasted overnight, and after each dosage they took a light breakfast (one slice of toast and a glass of sports drink) as soon as possible. Thereafter, they had lunch and supper different from the breakfast menu. They all also had the same food the next day. In another study, they had breakfast (hamburger, rice and vegetables *etc.*) and about 30 min later, were administered each dosage. A cross-over schedule was designed and a minimum interval of one week was allowed between each dosing. Saliva samples were collected using a citric acid-stimulating method. Blank saliva samples were collected a few minutes before the administration of the drug. A single dose of 100 mg theophylline powder, or one tablet containing 200 mg of theophylline was administered with 200 ml of water. Saliva samples were collected at appropriate time intervals up to 48 h. Theophylline concentrations in saliva were determined by the reported procedure.⁶⁾

Data Analysis The area under the saliva theophylline concentration-time curve from time zero to t (AUC_{0-t}) was calculated using the trapezoidal method. The area from time t to infinity was estimated by C_t/k , where C_t is the theophylline concentration at time t , and k is the first-order constant obtained from the slope of the log-linear portion of the curve by least regression analysis.

The mean residence times (MRT) for theophylline powder or tablets were calculated using statistical moment analysis.⁷⁾ Since the mean absorption time of powder is very small, the apparent mean absorption time for tablets (MAT_{app}) was obtained by subtracting MRT_{powder} from MRT_{tablet} . Student's t test was used for comparison between fibroin tablets and Uniphyl, or fasted and non-fasted, with $p=0.05$ as the minimal level of significance. Mean values were reported with standard deviations.

RESULTS AND DISCUSSION

In Vitro Drug Release Studies Tablets could be easily prepared by a direct compression method with fibroin powder and theophylline powder without any additives. Tablets having the same dimensions (10.0 mm diameter $\times$ 2.7 mm thickness) were prepared to reduce the compression force as the fibroin content decreased (Table 1). The hardness of the tablets reduced as the fibroin content decreased.

Release studies were carried out in three types of medium (pH 1.2, pH 6.8 and pH 1.2/pH 6.8). As shown in Fig. 1, theophylline release from the fibroin tablets (TF-41) was not affected by the medium pH. It was reported that the gastrointestinal transit time of one tablet in 6 healthy volunteers was 164 ± 92 min.⁸⁾ In this study, we performed release studies not only on the pH 1.2 medium alone, or the pH 6.8 medium alone, but also the pH 1.2 medium (0–3 h) followed by the pH 6.8 medium (3–24 h) simulating the tablets' transit in the gastro-intestinal system. The tablets did not disintegrate in the medium. Therefore, the following Higuchi's equation was used to evaluate the drug release from the homogeneous polymer matrix.⁹⁾ Assuming a sink condition;

$$f = (k \cdot t)^{1/2} \quad (1)$$

$$k = 2D \cdot C_s \cdot \epsilon / (A \cdot \tau)$$

where f is the fraction of drug released at time t , k is a constant, D is the diffusion coefficient of the drug in the medium, C_s is the solubility of the drug in the medium, ϵ is the porosity of the matrix, A is the total amount of drug per unit volume of the matrix, and τ is the tortuosity of the capillary system. Equation 1 indicates that the percent drug released is proportional to the square root of time. The Higuchi

Table 1. Formula of Fibroin Tablets Containing Theophylline

Tablet	Theophylline (mg)	Weight ratio (Theophylline : Fibroin)	Hardness (kg)
TF-11	125	1 : 1	14.4
TF-21	167	2 : 1	11.2
TF-41	200	4 : 1	7.1
TF-81	222	8 : 1	5.0
TF-91	225	9 : 1	4.1

Fig. 1. Effect of Medium pH on the Theophylline Release from Fibroin Tablets (TF-41)

A, normal plots (percent released vs. time); B, Higuchi plots (percent released vs. square root of time plots). $\circ$, pH 1.2 (0–3 h) then pH 6.8 (3–24 h); $\triangle$, pH 1.2 (0–24 h); $\square$, pH 6.8 (0–24 h). $n=3$.

plots shown in Fig. 1-B show a linear release profile, indicating that drug release from the fibroin tablets was diffusion-controlled through the matrix. Additionally, the compression force (5–30 kN) did not affect not only drug release from the fibroin tablets but also tablets' thickness (data not shown).

Five types of fibroin tablets with the same surface area and different amounts of theophylline were prepared (Table 1). The drug release from these tablets is shown in Fig. 2. The weight, diameter, and thickness of the tablets at the end of the study (24 h) are summarized in Table 3. The release profiles and physical characteristics of the TF-91 tablets are not shown, as these tablets disintegrated in the medium within 5 min of starting the release study. The drug release of the theophylline tablets consisting of theophylline powder alone was rapid (within 15 min). The fibroin tablets consisting of 10% of fibroin, TF-81, maintained their shape in the medium during the study, and the drug release from the tablets was retarded compared to the tablets prepared from the theophylline powder alone. The greater the fibroin content in the tablets, the lower the percent released at time t . Higuchi plots of each set of release data were linear in TF-11, TF-21, TF-41 and TF-81. The drug release from these fibroin tablets was diffusion-controlled through the matrix. The slope of a linear portion of the plots increased as the fibroin content decreased (*i.e.* theophylline content increased). Among the factors affecting the slope (k), drug amount per unit volume (A) and matrix porosity(ϵ)/tortuosity(τ) were different among the tablets. The constant, k is inversely proportional to A , but k is proportional to ϵ/τ (Eq. 1). When the theophylline amount in

the tablets increased (" A " increased), k should decrease. However, k increased in this study, so that the increase in ϵ/τ may have exceeded that of A . Each tablet swelled a little without inter-tablet differences (Table 3). The tablets absorbed the medium (expressed as "water content" in Table 3) to some extent. The water content per tablet was almost the same, but the water content per mg of fibroin increased as the fibroin content decreased, indicating TF-81 tablets could absorb the medium more and had a larger porosity.

It was assumed that silk fibroin molecules in the amorphous region of a membrane surface were digested by proteases and that a crystalline region or highly dense region remained.¹⁰⁾ As shown in Fig. 3, the effect of proteases on drug release from the fibroin tablets was evaluated. Typical proteases in the gastro-intestinal tract, pepsin, a gastric protease, and pancreatin, a digestive enzyme secreted from the pancreas, were added to the release medium. The release profiles were not affected by the proteases (Fig. 3). The weight of the tablets reduced a little (14%) compared to those in the protease-free medium, suggesting protease digestion (Table 3).

Theophylline is readily absorbed even from the lower re-

Table 2. Subject Characteristics

Subject No.	Sex	Age (years)	BW (kg)	Height (cm)
1	F	23	48	159
2	F	23	44	154
3	M	22	56	161
4	M	22	64	181
5	M	41	83	180

Table 3. Physical Characteristics of the Fibroin Tablets in *In Vitro* Release Studies (after 24 h)

	Weight (mg)	Diameter (mm)	Thickness (mm)	Water content ^{b)}		$T_{50\%}$ ^{c)} (h)
				(mg/tablet)	(mg/mg fibroin)	
TF-11	340.2±1.5	10.5	3.4	165.7±2.8	1.3	15.0
TF-21	309.9±0.7	10.2	3.5	178.8±3.6	2.2	8.0
TF-41	281.0±2.5	10.2	3.1	185.7±5.1	3.7	7.5
TF-41 ^{a)}	241.4±16.2	10.0	3.0	156.7±11.1	3.1	7.0
TF-81	218.2±48.1	8.0	2.4	190.5±48.1	6.9	3.0

The release medium was changed after 3 h from a pH1.2 medium (0–3 h) to a pH6.8 medium (3–24 h). The initial weight, diameter and thickness of a tablet were 250 mg, 10.0 mm and 2.7 mm, respectively. $n=3$. a) A medium containing pepsin or pancreatin. b) The value was obtained by subtracting the initial fibroin amount in the tablet and the remaining theophylline amount in the tablet after 24 h from the tablet weight after 24 h. c) Time for required 50% release of theophylline from the tablets.

Fig. 2. Theophylline Release from the Fibroin Tablets with Different Drug Contents

The release medium was changed after 3 h from a pH 1.2 medium (0–3 h) to a pH 6.8 medium (3–24 h). A, normal plots (percent released vs. time); B, Higuchi plots (percent released vs. square root of time plots). Δ , TF-11 (theophylline 125 mg); $\blacktriangle$, TF-21 (167 mg); $\circ$, TF-41 (200 mg); $\bullet$, TF-81 (222 mg); $\square$, tablets prepared from theophylline powder alone (200 mg). $n=3$.

Fig. 3. Effect of Proteases on Theophylline Release from Fibroin Tablets (TF-41)

The release medium was changed after 3 h from a pH 1.2 medium (0–3 h) to a pH 6.8 medium (3–24 h). ○, without proteases; ●, 0.9 g pepsin and 0.9 g pancreatin were added to the pH 1.2 medium and the pH 6.8 medium, respectively. $n=3$.

Fig. 4. Release Profiles of Theophylline from a Commercially Available Sustained Release Tablet (Uniphyl) and Fibroin Tablets

The release medium was changed after 3 h from a pH 1.2 medium (0–3 h) to a pH 6.8 medium (3–24 h). △, TF-11 (theophylline 125 mg); ▲, TF-21 (167 mg); ○, TF-41 (200 mg); ■, Uniphyl (200 mg). $n=3$.

gion of the colon, and is, therefore, suitable for once daily oral dosage. In order to decide which tablet was applicable for *in vivo* study, the fibroin tablets (TF-11, TF-21 and TF-41) were compared to a commercial tablet, Uniphyl (once daily type). The initial drug release from Uniphyl was slower than the fibroin tablets, but the release rate was almost the same after 3 h except for TF-11 (Fig. 4). Higuchi plots of Uniphyl were linear after a 5 min lag time, indicating the drug release from Uniphyl was diffusion-controlled (data not shown). The released percentage for TF-11 after 24 h was about 70% that of Uniphyl (Fig. 4), and the $T_{50\%}$ was 1.5 times that of the tablets (Table 3). The release profiles of TF-21 and TF-41 were almost the same as Uniphyl and simply because TF-41 contained the same theophylline content (200 mg) as Uniphyl, it was chosen for the *in vivo* study below.

In Vivo Studies in Healthy Volunteers Theophylline was absorbed almost completely following oral administration, and rapidly distributed throughout the body. The bioavailability of theophylline sustained-type preparations was almost 100%, assuming that the release was completed while the preparations existed in the absorption site. Theophylline was transferred into the saliva, and the plasma to

Fig. 5. Saliva Concentrations of Theophylline Following Oral Administration of Theophylline Powder, a Fibroin Tablet (TF-41), or a Uniphyl Tablet in 5 Healthy Volunteers

□, theophylline powder (100 mg); ○, TF-41 (theophylline 200 mg); ■, Uniphyl (theophylline 200 mg). * $p < 0.05$ TF-41 vs. Uniphyl.

saliva drug concentration ratio showed a good correlation.^{6,11–13} In this study, TF-41 and a commercial tablet, Uniphyl (once-a-day type) were administered to 5 healthy volunteers to evaluate the bioequivalency of the two tablets. Theophylline powder was also administered to the subjects instead of intravenous administration to assess the extent of bioavailability. The powder dose (100 mg) was half that of the tablets (200 mg) because of the risk of the side effects. The saliva concentration profiles under fasted-administration are shown in Fig. 5 and the pharmacokinetic parameters are summarized in Table 4. With the powder administration, absorption of theophylline was rapid and the time required to reach a maximum concentration (T_{max}) was about 1 h. The saliva theophylline decreased according to first-order kinetics with half-life of 8.8 h. T_{max} and the maximum concentration (C_{max}) of Uniphyl and TF-41 were 9 h and 2.0 $\mu\text{g/ml}$, and 7 h and 1.2 $\mu\text{g/ml}$, respectively. These were not consistent with the *in vitro* data showing that the initial release of TF-41 was faster than Uniphyl, and that the released drug amount at that time was greater in TF-41. Since the slope after C_{max} was smaller in TF-41, it was considered that TF-41 had a more sustained-release than Uniphyl. The AUC s of Uniphyl and TF-41 to normalized AUC of powder dose were 85% and 70%, respectively. On the other hand, the MRT of TF-41 was 1.3-fold, and the MAT_{app} of TF-41 was 2-fold compared to Uniphyl. Therefore, the reduction in bioavailability in TF-41 was due to the delayed release from the tablets *in vivo*. The sensitivity to the movement of gastro-intestinal tract, bile and digestive enzymes may be responsible for the delayed release.

It is known that food affects the gastric emptying time of preparations and the intestinal transit time,¹⁴ as well as degradation and drug release. Some preparations may lose sustained-release characteristics because the mechanical forces deriving from the movement of the gastro-intestinal tract can destroy them. Food increases the movement of the gastro-intestinal tract, and the degradation of the preparations is stimulated, perhaps causing dose damping. Therefore, the effect of food on the bioavailability of a controlled release dosage form should be evaluated.^{15,16} There have been reports on the effect of food on the bioavailability of theophylline sustained-release dosage forms, but the effect was

Table 4. Pharmacokinetic Parameters Following Oral Administration of Theophylline Powder, a Fibroin tablet (TF-41), or a Uniphyl Tablet in 5 Healthy Volunteers

	Fasted			Non-fasted	
	Powder	TF-41	Uniphyl	TF-41	Uniphyl
$AUC_{0-\infty}$ (mg/ml·h)	27.8±3.9 ^{a)}	39.7±12.2	47.6±9.4	52.8±14.3	53.7±16.5
MRT (h)	12.7±1.9	30.8±7.9	22.2±6.9	22.0±5.0	20.8±3.1
MAT_{app} (h)	—	18.1±6.4	9.5±5.8	9.3±4.1	8.6±2.6
C_{max} (μg/ml)	2.6±1.2 ^{a)}	1.1±0.3	2.0±0.5	2.0±0.5	2.3±0.4
T_{max} (h)	1.3±1.1	6.8±1.8	9.2±2.3	10.8±2.3	10.5±3.0

a) Each value was compared twice for powder containing a half amount of theophylline (100 mg) compared to the others (200 mg). Student's *t* test was used for the fasted group (powder vs. TF-41, powder vs. Uniphyl, TF-41 vs. Uniphyl). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Student's *t* test was also used for the fasted group vs. the corresponding non-fasted group. † $p < 0.05$, ‡ $p < 0.01$.

Fig. 6. Saliva Concentrations of Theophylline Following Oral Administration of a Fibroin Tablet (TF-41) or a Uniphyl Tablet in 5 Healthy Volunteers

○, TF-41 (theophylline 200 mg); ■, Uniphyl (theophylline 200 mg).

not consistent (increase, decrease or constant).^{17–20)} It is unclear whether food affects the bioavailability of preparations. The saliva concentrations of theophylline under fasted or non-fasted conditions are shown in Figs. 5 and 6. Dose damping was not observed with TF-41 administration. Although the T_{max} and C_{max} of TF-41 changed significantly, those of Uniphyl did not. The AUC of TF-41 in non-fasted cases was also 1.4-fold higher than in fasted, and almost the same as the normalized AUC powder dose, indicating complete absorption (Table 4). The MAT_{app} in non-fasted cases was shorter than in fasted cases. This suggested that the drug release from TF-41 was increased due to the stimulation of food on TF-41 itself and on the movement of the gastro-intestinal tract. TF-41 kept its shape, absorbed the release medium and showed softness during the *in vitro* drug release study. Fibroin tablets may have sponge-like characteristics, and release theophylline *via* mechanical stimulation.

The saliva concentration profiles of non-fasted cases were almost the same between TF-41 and Uniphyl (Fig. 6). Although the profiles were different in fasted cases between the two preparations, the AUC of the preparations of non-fasted

cases increased to almost the same extent compared to fasted cases (38% for TF-41 and 24% for Uniphyl). It was reported that the AUC , T_{max} and C_{max} of theophylline (Uniphyl) increased 25%, 46% and 25%, respectively.²¹⁾ The increase in absorption was believed not to be derived from theophylline itself, but from the structure of the preparations (wax-matrix type). The addition of a surfactant to the release medium and an increase in the stirring speed increased drug release. Therefore, it was considered that the increased absorption was due to the stimulated secretion of a bile acid based on the intake of food and mechanical mixing with food.²¹⁾ Food affected the bioavailability of TF-41, and the effect was less for Uniphyl in this study. Therefore, TF-41 should be taken after meals. In conclusion, fibroin is applicable as the matrix in controlled-release tablets.

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