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Biopharmaceutical Evaluation of Gelatin Microcapsules of Several Oral Antibiotics[1)]

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Microencapsulation of three orally administered antibiotics (ampicillin, amoxicillin and cefalexin) was studied. Good reproducibility in microcapsule preparation was obtained, as in the case of the sulfonamides reported previously. In comparison with the release profile of ampicillin powder, sustained ampicillin release was observed from the microcapsules in the neutral medium. Gastric-emptying-controlled rabbits were used for the *in vivo* evaluation of microcapsules. No sustained release was observed from microcapsules of amoxicillin and cefalexin. However, the gelatin microcapsules containing ampicillin showed significant sustained release of ampicillin.

Keywords——microencapsulation; orally administered antibiotic; sustained release formula; gastric-emptying-controlled rabbit; gelatin microcapsule of ampicillin

There have been a number of reports concerning encapsulation[2-6)] of various pharmaceuticals but few concerning the biopharmaceutical properties of microcapsules containing drugs. In the previous paper,[1)] a simple microencapsulation process using gelatin as the wall material was studied and significant biopharmaceutical efficacy, including sustained release was found for gelatin-sulfamethizole microcapsules. The blood concentration of unchanged sulfamethizole after oral administration of microcapsules to gastric-emptying-controlled rabbits decreased more gradually than that after administration of powder.

This study was undertaken to evaluate the biopharmaceutical properties of gelatin microcapsules containing pharmaceuticals. For this purpose, ampicillin (AP), amoxicillin (AO) and cefalexin (CE) were studied. The main aim of this investigation was to obtain standards and find suitable conditions for microencapsulating pharmaceuticals by the gelatin-microencapsulation method to produce sustained release formulations.

Experimental

Materials——Three oral antibiotics (ampicillin, amoxicillin and cefalexin) were kindly provided by Takeda Pharmaceuticals and Fujisawa Pharmaceuticals Co. All chemicals and solvents used were of analytical reagent grade.

Microencapsulation——The procedure was similar to that described previously[7)] except that (1) the weight scale was 1/5 that of the previous method[7)] (2) the reaction vessel was maintained under reduced pressure to exclude air from the microcapsules. The flow chart for microencapsulation in this study is shown in Chart 1. This procedure was considered to form diffusion-controlled microcapsules consisting of dispersed solid drug particles in a gelatin matrix.

Gastric-emptying-controlled Rabbit——The procedure for the gastric-emptying-controlled rabbits was the same as in the previous study.[1)]

Design of Absorption Experiment——Single Intravenous Administration: Injections were prepared by dissolution of an antibiotic (210 mg) in 0.3 ml of 1 N HCl and 7 ml of isotonic NaCl solution and administered as an intravenous infusion for 3 min through the left ear vein. Blood samples were withdrawn at suitable intervals from the right marginal ear vein for 24 h after administration. The samples were immediately centrifuged to obtain plasma (8000 rpm, 4 min), and the plasma was placed in a fully wrapped small test tube and stored in a refrigerator until antibiotic assay.

Oral Administration: Multiple and single oral administration of powder and microcapsules of antibiotics were done with 30 ml water and 22 g/kg of SD-3 which had been prepared by adding 70 parts of water to 30

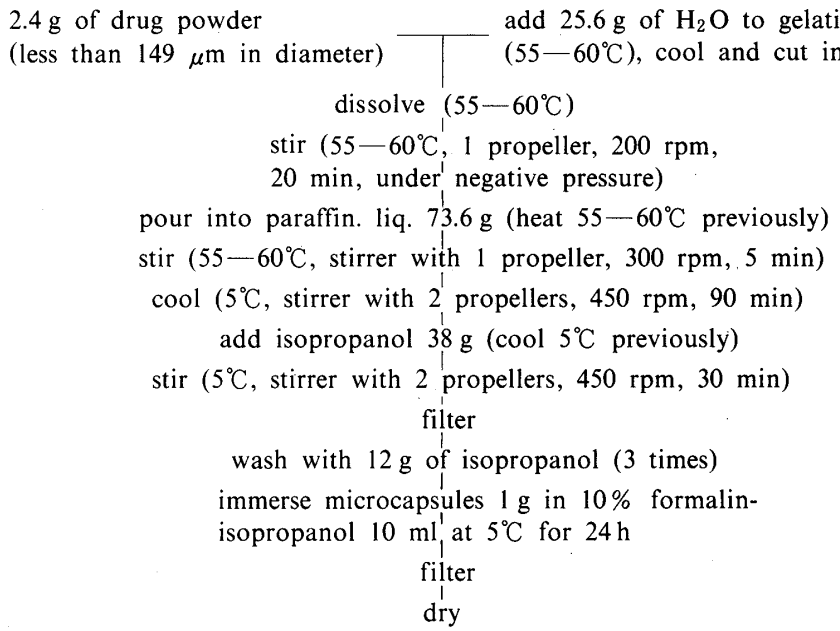


Chart 1. Preparation of Gelatin Microcapsules

parts of the special solid diet (SD-1). The multiple oral administration of powder at 0 and 6 or 12 h was done as follows: 30 mg/kg for AP and AO, and 20 mg/kg for CE at each time. The single oral administration of microcapsules at 0 h was done as follows: 60 mg/kg for AP and AO, and 40 mg/kg for CE.

Analytical Methods for Antibiotics in Plasma——AP:[8] To 0.5 ml of plasma, 2 ml of 10% trichloroacetic acid aqueous solution was added for deproteinization. The mixture was centrifuged (3000 rpm) for 10 min, then the clear supernatant (2 ml) was used for analytical measurement. A Shimadzu spectrofluorometer, Model RF-500, was employed for analysis of the sample, which was prepared as follows. The above clear supernatant (2 ml) was mixed with 0.5 ml of citrate buffer containing 7% HCHO (pH 2.7), and warmed on a water bath at 90°C for 120 min. The mixture was allowed to cool, then 1 ml of 2 N NaOH was added. As the excitation and emission wavelengths for determination of AP, 346 nm and 422 nm were selected. This method was also applied for the determination of AP content in microcapsules, and the dissolution test for AP powder and microcapsules.

AO:[9] To 1 ml of the plasma, 1 ml of 10% trichloroacetic acid aqueous solution was added, and the mixture was centrifuged at 3000 rpm for 10 min. The clear supernatant (1 ml) was collected. One ml of 0.05 N NaOH was added, and the mixture was heated on a water bath at 90°C for 90 min. The sample was cooled, 2 ml of 2-methoxyethanol was added, and the mixture was measured by a fluorometric method (excitation wavelength of 345 nm and emission wavelength of 425 nm).

CE:[10] The procedure was similar to that for AP and AO. The mixture, which was composed of 1 ml of plasma sample and 0.5 ml of 2 N NaOH, was permitted to stand for 10 min. Then 0.5 ml of 2 N HCl and 3 ml of citrate-NaOH buffer (pH 5) containing 1% HCHO were added and the mixture was heated at 90°C for 30 min. The mixture was cooled to room temperature. The fluorometric method was employed for analysis (345 nm excitation wavelength and 420 nm emission wavelength).

Results and Discussion

Dissolution Pattern of Antibiotics

Three kinds of microcapsules containing 20% (w/w) antibiotics were prepared using the simple microencapsulation method (Chart 1). The antibiotic content in these microcapsules was determined, and the experimental values agreed well with the theoretical percentages (20% (w/w)). For AO and CE, the dissolution pattern from powder coincided with that from the microcapsules. However, it was concluded that the dissolution rates from microcapsules of AP decreased only in the neutral medium. The representative dissolution pattern for AP is shown in Fig. 1.

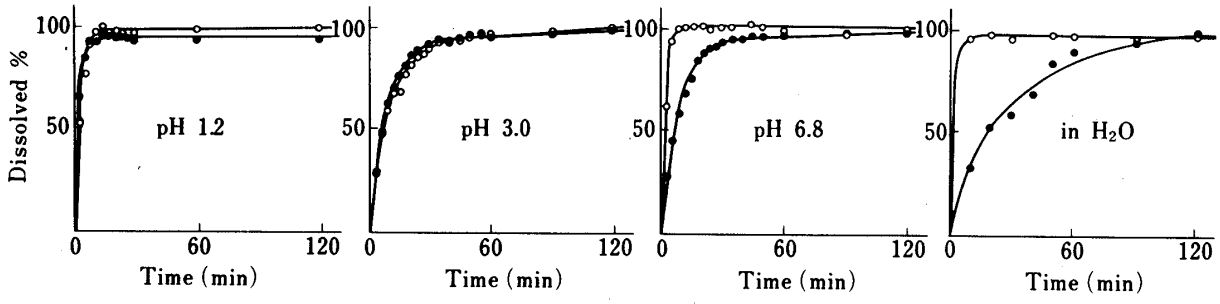


Fig. 1. Dissolution Curves for Ampicillin from Powder (—○—) and Microcapsules (—●—) at 37°C and 50 rpm using J.P.X Dissolution Apparatus (Paddle Method)

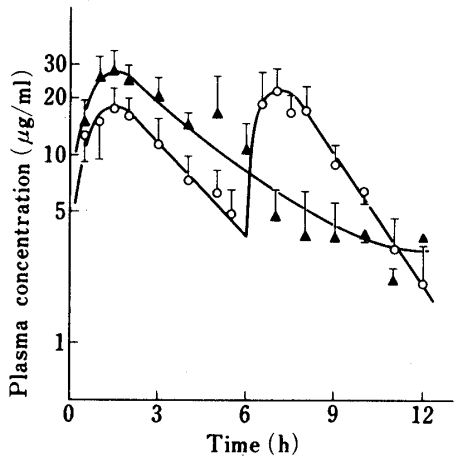


Fig. 2. Plasma Concentration of Amoxicillin following a Single Dose of Microcapsules (—▲—, 60 mg/kg; n=3) containing 20% (w/w) Amoxicillin and Multiple Doses of Powder (—○—, 30 mg/kg; n=3) of Amoxicillin

Each point represents a mean and the vertical line indicates the standard deviation.

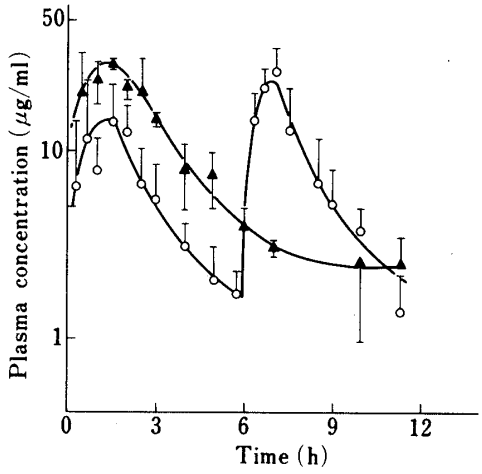


Fig. 3. Plasma Concentration of Cefalexin following a Single Dose of Microcapsules (—▲—, 40 mg/kg, n=3) containing 20% (w/w) Cefalexin and Multiple Doses of Powder (—○—, 20 mg/kg; n=3) of Cefalexin

Each point represents a mean and the vertical line indicates the standard deviation.

Administration Studies of Amoxicillin (AO) and Cefalexin (CE)

AO is a semi-synthetic penicillin. The rate and extent of gastro-intestinal absorption are greater than those of AP. CE is a cephalosporin which is absorbed orally. However, CE has a very short biological half-life ($t_{0.5}$, 54 min) requiring that the drug be administered every 6 h. Administration of two doses of the powder at 0 and 6 h (20 mg/kg) was compared with a single dose of microcapsules at 0 h (40 mg/kg) using gastric-emptying-controlled rabbits. The results are presented in Figs. 2 and 3. No significant difference was observed between the two preparations, powder and microcapsules. The plasma level and the $AUC^{0\to24h}$ after administration of microcapsules were twice those after administration of the powder and the times of peak plasma levels for the two preparations were almost identical as shown in Figs. 2 and 3. These results indicate that the microcapsules do not provide a useful sustained release of the drug; although the absorption rate constant was reduced to half by gelatin-microencapsulation of AO and CE, such a degree of slowing down was not enough to produce a significant prolongation of drug effect.

Administration Studies of Ampicillin (AP)

Plasma levels were higher after a single oral dose of microcapsules of AP than after two

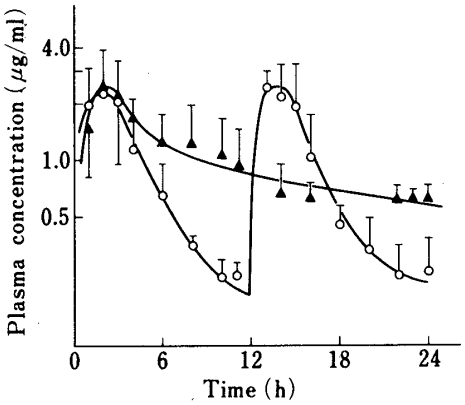


Fig. 4. Plasma Concentration of Ampicillin following a Single Dose of Microcapsules (—△—, 60 mg/kg; $n=3$) containing 20% (w/w) Ampicillin and Multiple Doses of Powder (—○—, 30 mg/kg; $n=3$) of Ampicillin

Each point represents a mean and the vertical line indicates the standard deviation.

oral doses of powder, and comparison of $AUC^{0\to24h}$ values showed an increase of 1.1 times. The results are shown in Fig. 4.

The solubility and biological half-life values of the oral antibiotics are summarized in Table I. It is clear that all oral antibiotics listed in Table I have comparatively low solubilities and short biological half-lives. The apparent absorption rate constants of all pharmaceuticals used in this series of studies were calculated according to the Wagner–Nelson method[11] for pharmaceuticals which can be represented by a one-compartment open model and by the Loo–Riegelman method[12] for pharmaceuticals which can be represented by a two-compartment open model. The results are summarized in Table II.

However, this calculation was based on the assumption that the overall elimination rate constants calculated after the intravenous administration trial remained constant during the experimental period after oral administration. A sustained release effect for both sulfa-

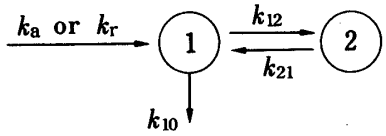
TABLE I. Solubility and Biological Half-lives in Man of the Oral Antibiotics used in This Study

Drug	Solubility in H_2O (g/l) at 37℃	Half-life ($t_{0.5}$, h)
Ampicillin (AP)	25.8	0.8
Amoxicillin (AO)	15.2	1.2
Cefalexin (CE)	25.7	0.9

TABLE II. Mean Rate Constants[a] (h^{-1}) of Drugs used in the Previous and Present Studies

Drug	Absorption		Distribution		Elimination
	k_a Powder	k_r Microcapsule[b]	k_{12}	k_{21}	k_{10}
Sulfamethizole	0.42	0.12	—	—	1.4
Ampicillin	0.38	0.13	—	—	2.5
Amoxicillin	0.75	0.39	1.2	1.2	3.8
Cefalexin	0.87	0.57	0.83	0.64	2.8
Sulfanilamide	1.2	0.53	—	—	0.29
Sulfisomidine	0.35	0.33	0.56	0.31	0.16

a) These rate constants were calculated by the Wagner-Nelson or the Loo-Riegelman method.



b) Microcapsules containing 20% (w/w) drugs were used.

methizole (SM) and ampicillin (AP) was evident, as described in the previous[1] and the present reports. The plasma profiles of these two pharmaceuticals could be described by a pharmacokinetic one-compartment open model. It is clear that the elimination rate constant (k_{10}) exceeds the apparent absorption rate constant. A so-called flip-flop phenomenon could be observed in the blood concentration *vs.* time curves. When the flip-flop phenomenon is observed in the blood concentration curve of a drug, indicating excessively slow absorption and excessively rapid elimination after oral administration, that drug may be a good candidate for a microcapsule formulation having sustained-release properties. The difficulty in producing sustained-release microcapsules and the effects of the pharmacokinetic properties of drugs will be discussed in the following paper.

References and Notes

1) *a*) This paper forms part VIII of a series entitled "Evaluation of Microcapsules"; *b*) The preceding paper, part VII: M. Komatsu, K. Tagawa, M. Kawata and S. Goto, *Chem. Pharm. Bull.*, **31**, 262 (1983).
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