Biopharmaceutical Evaluation of Sustained-Release Ethylcellulose Microcapsules Containing Amoxicillin Using Beagle Dogs[1)]

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A beta-lactam antibiotic, amoxicillin, was microencapsulated with ethylcellulose using a solvent evaporation process in liquid paraffin containing sorbitan tristearate as a dispersing agent, and the microcapsules obtained showed first-order drug release.

Usage of the previous log–log relationship of cefadroxil between *in vitro* dissolution half-lives (T_{50}) and the experimental release rate constants (k_r') of the drug *in vivo*, and the nomogram for the design of satisfactory sustained-release preparations resulted in the prediction that ethylcellulose microcapsules containing 60% amoxicillin would show the most effective sustained-release pattern. Prepared microcapsules containing various amounts of amoxicillin were administered to beagle dogs and it was found that above prediction was correct. In addition, a more precise log–log correlation concerning amoxicillin was also undertaken, good linearity was observed and the decline was very similar to that of cefadroxil.

Keywords amoxicillin; microcapsule; ethylcellulose; sustained release

Amoxicillin, an oral β-lactam antibiotic, has been used in the treatment of various infectious diseases,[2)] but frequent administration is essential owing to its rapid elimination from the body. Therefore, a sustained-release preparation of amoxicillin may be advantageous since reduced frequency of administration is more convenient for patients and may thereby improve compliance.

In the previous paper,[3)] we reported on two cephem antibiotics microencapsulated with ethylcellulose. A good sustained-release pattern was achieved in the case of ethylcellulose microcapsules containing 70% cefadroxil and the log–log relationship between *in vitro* dissolution half-lives (T_{50}) and the *in vivo* release rate constants (k_r') calculated on the basis of the proposed pharmacokinetic model of the drug was established. A linear correlation was obtained and should prove very useful for studies on the design of sustained-release dosage preparations.

In this study, we investigated whether the log–log relationship obtained for cefadroxil is applicable to the design of sustained-release microcapsules containing amoxicillin.

Experimental

Materials Amoxicillin was kindly provided by Fujisawa Pharmaceutial Co., Osaka, Japan, and sieved through a 270 mesh (58 μm) sieve. Ethylcellulose from Tokyo Kasei Co., Tokyo, Japan, viscosity of 90—110 cps grade, was used. Other reagents were all of special reagent grade.

Preparation of Ethylcellulose Microcapsules A solvent evaporation process in liquid paraffin using sorbitan tristearate as a dispersing agent was employed as described previously.[4)]

Dissolution Experiments The procedures and apparatus were the same as described in the previous paper.[4)]

Measurement of Plasma Levels of Amoxicillin[5)] A mixture of 1 ml plasma and 1 ml of 10% trichloroacetic acid was shaken for 3 min. After centrifugation at 3000 rpm for 15 min, 1 ml of the upper layer was taken. To this solution, 1 ml of 0.05 N NaOH was added, then the mixture was boiled on a water bath for 90 min, and cooled. After addition of 2 ml of 2-methoxyethanol, the fluorescence was measured (excitation wavelength, 345 nm; emission wavelength; 425 nm) using a Shimadzu RF-500 spectrofluorophotometer.

Animals Male beagle dogs weighing 13.5—15.0 kg were fasted overnight and used for the experiments. They were allowed free access to water, and no food was given until the last blood sample had been taken.

Single Intravenous Administration Amoxicillin powder (125 mg) was dissolved in 0.3 ml of 1 N NaOH and 5 ml of isotonic NaCl solution and administered to four beagle dogs intravenously *via* the cephalic vein. Blood samples were withdrawn from the cephalic vein with a heparinized syringe up to 8 h after the administration, and the collected blood was centrifuged at 3000 rpm for 15 min to obtain plasma. The plasma amoxicillin level was measured according to the above fluorometric method.

Oral Administration A crossover design was used for five treatments with four beagle dogs. All dogs were fasted overnight and the preparations were administered. A solution containing 125 mg of amoxicillin in 10 ml of isotonic NaCl solution was orally administered with 20 ml of purified water. Special hard gelatin capsules (Kasho Co., 1/80 OZ, J Type) were filled with amoxicillin powder or microcapsules containing amoxicillin. Double oral administration of the powder (125 mg × 2) at 0 and 6 h and single oral administration of the microcapsules corresponding to 250 mg of amoxicillin at 0 h were made with 30 ml of water. Blood samples were withdrawn from the cephalic vein of the front leg up to 12 h after the administration.

Calculation of Pharmacokinetic Parameters Fittings were done in the calculations of the pharmacokinetic parameters of injections, oral administrations of drug solutions and solid-state preparations by using the nonlinear least-squares regression program MULTI.[6)]

Results and Discussion

Preparation and Dissolution of Ethylcellulose Microcapsules Containing Amoxicillin As described in the previous paper,[5)] microencapsulation of amoxicillin using a solvent evaporation process in water containing sodium lauryl benzenesulfonate as a dispersing agent was unsuccessful because of a great loss of drug during the preparation process as a result of amoxicillin dissolving in water. In the present investigation, a solvent evaporation process in liquid paraffin containing sorbitan tristearate as a dispersing agent was employed and less than 1% drug loss was occurred in the preparation of ethylcellulose microcap-

TABLE I. Components, Stirring Rates with the Propeller for Preparation of Ethylcellulose Microcapsules Using the Solvent Evaporation Process in Liquid Paraffin, Yield and Contents of Amoxicillin in Products

		Treatment No.			
		1	2	3	4
Ethyl cellulose (g)		3.0	2.4	1.8	1.2
Drug (g)		3.0	3.6	4.2	4.8
Stirring rates (rpm)		200	220	220	220
Yield (%)		83	86	85	85
Drug contents (%)	Found	49.5	60.7	70.2	79.6
	Calcd	50.0	60.0	70.0	80.0

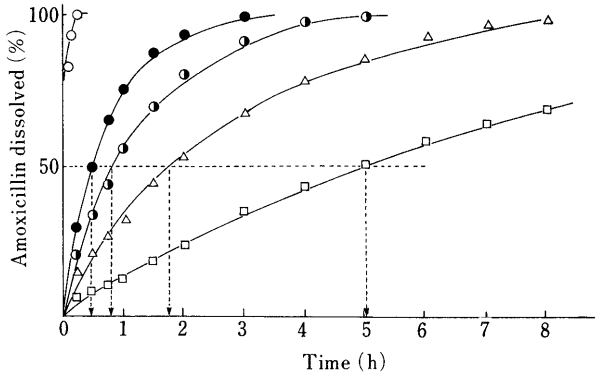

Fig. 1. Dissolution Patterns of Amoxicillin from Powder (○) and Microcapsules Containing 50% (□), 60% (△), 70% (◑) and 80% (●) Amoxicillin Prepared Using the Solvent Evaporation Process in Liquid Paraffin, at 37 °C, 50 rpm and pH 6.8

The dotted lines and arrows represent T_{50} (h).

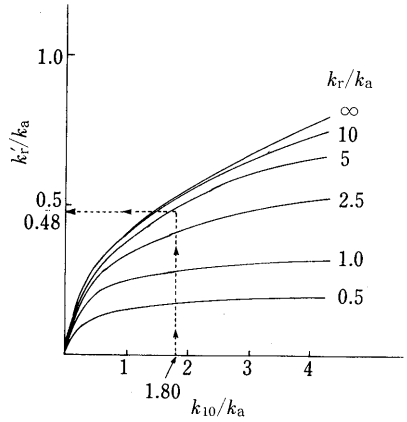

Fig. 2. Interrelationship between k_r'/k_a and k_{10}/k_a in the Case where D^m/D is 2

See the text or previous paper[3] for explanation of symbols.

TABLE II. Pharmacokinetic Parameters for Amoxicillin Following i.v. Injection, Oral Administration of Solution and Powder Based on the Previous Pharmacokinetic Model[3]

Parameters	i.v. injection	Oral administration	
		Solution	Powder
C (μg/ml)	60.1	61.2	62.3
	(4.2)	(4.2)	(3.9)
C' (μg/ml)	—	60.2	61.9
		(3.2)	(2.4)
C'' (μg/ml)	—	—	59.3
			(4.4)
α (h^{-1})	2.91	2.45	2.46
	(0.31)	(0.13)	(0.18)
β (h^{-1})	0.424	0.396	0.383
	(0.040)	(0.022)	(0.041)
k_{12} (h^{-1})	1.08	0.814	0.809
	(0.14)	(0.123)	(0.112)
k_{21} (h^{-1})	0.901	0.765	0.738
	(0.154)	(0.071)	(0.075)
k_{10} (h^{-1})	1.34	1.27	1.28
	(0.12)	(0.05)	(0.05)
k_a (h^{-1})	—	0.713	0.710
		(0.054)	(0.082)
k_{gi}' (h^{-1})	—	0.075	0.073
		(0.007)	(0.012)
k_r or k_r' (h^{-1})	—	—	3.06
			(0.36)
k_{gi} (h^{-1})	—	—	0.836
			(0.103)
SS[a]	0.042	0.104	0.310
	(0.012)	(0.031)	(0.034)

The parameters were estimated using the pharmacokinetic analysis program MULTI for microcomputers.[6] The figures in parentheses represent standard deviations. In the case of injection, oral administration of solution and powder, one-, two-, three-line fittings were performed, respectively. *a*) The residual sum of squares (SS) was calculated from $SS=\sum W_i\cdot(C_i(\text{obs.})-C_i(\text{cal.}))^2$ where W_i is the weight of the points. $(C_i)^{-2}$ was adopted as the weight where C_i is the value of the i-th point.

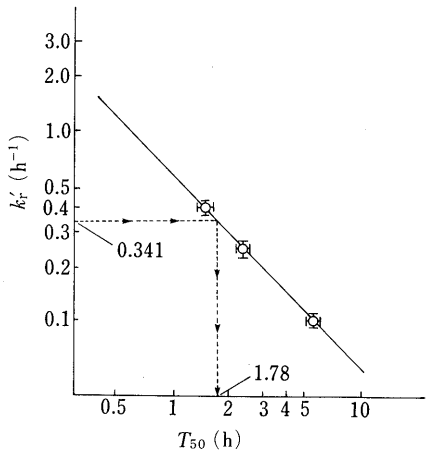

Fig. 3. log–log Relationship of Cefadroxil between *in Vitro* Dissolution Half-Lives (T_{50}) at pH 6.8 and the Experimental Release Rate Constants *in Vivo*

The value of 0.39 is satisfactory for sustained release of cefadroxil (see the previous paper[3]) and 0.341 is the calculated value for sustained release of amoxicillin on the basis of the nomogram as shown in Fig. 2.

sules containing various specified percentages of amoxicillin. The experimental conditions and results of preparations of ethylcellulose microcapsules containing amoxicillin are summarized in Table I.

Figure 1 shows the dissolution patterns at pH 6.8 and 37 °C from ethylcellulose microcapsules containing 50, 60, 70 and 80% amoxicillin. The release rates of amoxicillin increased as amoxicillin content (%) in the ethylcellulose microcapsules increased and it was found that in all cases first-order release patterns were realized ($r>0.998$).

Design of Satisfactory Sustained-Release Microcapsules Containing Amoxicillin In the previous paper,[3] we introduced a simple nomogram for the design of ideal sustained-release preparations even though it is only applicable to preparations with first-order release.

Intravenous injection of amoxicillin solution and oral administrations of amoxicillin solution and powder in beagle dogs were performed and the pharmacokinetic parameters were calculated according to the previous paper[3] using the multi-line fittings program MULTI. Values of $0.710\,h^{-1}$ and $1.28\,h^{-1}$ were calculated for the absorption (k_a) and elimination (k_{10}) rate constants of amoxicillin powder, respectively (Table II).

Judging from our previous nomogram,[3] the desirable k_r'/k_a, *i.e.*, the control ratio of the release rate constants of the sustained-release preparations to the absorption rate constants, becomes 0.48, as shown in Fig. 2. Therefore, the desirable k_r' value could be calculated to be $0.341\,h^{-1}$ (*i.e.*, $0.48\times0.71=0.341$).

The log–log relationship between *in vitro* dissolution half-lives (T_{50}) and *in vivo* release rate constants (k_r') of cefadroxil from the ethylcellulose microcapsules was established in the previous paper.[3] A good linearity was obtained as shown in Fig. 3.

Considering a similarity in aqueous solubility[7] and pharmacokinetic properties[5] of cefadroxil and amoxicillin, the above log–log relationship may also be applicable to amoxicillin. The ethylcellulose microcapsules containing 60% amoxicillin (1.75 h *in vitro* dissolution half-life at 37 °C and pH 6.8 was obtained) seem to show the most favorable sustained-release pattern among the various microcapsules prepared, because the theoretical half-life value of 1.78 h corresponds to the release rate constant of 0.341 h^{-1} (Fig. 3).

Plasma Concentration Curves of Amoxicillin Following Double Oral Administrations of Powder and Single Oral Administration of Ethylcellulose Microcapsules The plasma amoxicillin level after oral dosing of the standard regular-release preparation (powder) at an antibiotic dose of 125 mg/body at 0 and 6 h was compared with those of sustained-release preparations (ethylcellulose microcapsules) administered in a single dose of 250 mg/body at 0 h. The results are shown in Fig. 4.

Even though area under the blood concentration–time curve (AUC) obtained after the administrations of microcapsules containing 70 or 80% amoxicillin was almost equivalent to that obtained by double oral administrations of amoxicillin powder, a good sustained-release pattern was not obtained in each case, since the maximum plasma concentration (C'_{max}) observed after the administration of microcapsules was not controlled compared with that after the first administration of amoxicillin powder. In the case of ethylcellulose microcapsules containing 60% amoxicillin, C'_{max} was comparable to that after the first administration of amoxicillin powder. If C_{max} is the desirable maximum plasma concentration of drug in the body following a single dosing of the regular-release preparations based on many years' experience, the same maximum concentration may be required for the sustained-release preparations, *i.e.*, $C_{max}=C'_{max}$. This experimental fact is thus very favorable as one of the desirable sustained-release properties.[8] On the other hand, the administration of ethylcellulose microcapsules containing 50% amoxicil-

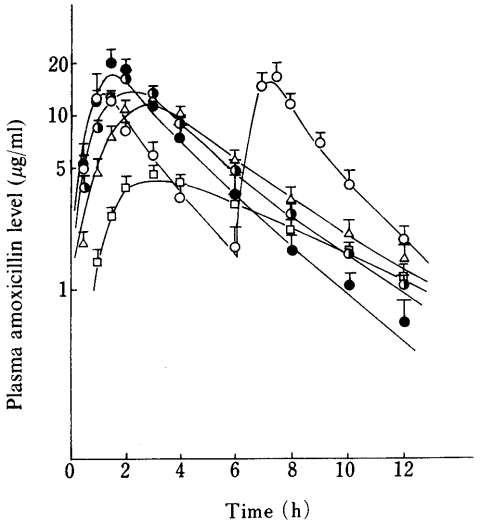

Fig. 4. Plasma Amoxicillin Level after Single Oral Administration of Ethyl Cellulose Microcapsules Containing 50% (□, 250 mg/body; $n=4$), 60% (△, 250 mg/body; $n=4$), 70% (◑, 250 mg/body; $n=4$) and 80% (●, 250 mg/body; $n=4$) and Double Doses of Powder (○, 125 mg/body × 2) $n=4$) of Amoxicillin in Beagle Dogs

Each point represents a mean and the vertical line indicates the standard deviation. The plasma concentration–time curves are computer-fitted lines.

TABLE III. Pharmacokinetic Parameters for Amoxicillin Following Oral Administration of Solution and Solid-State Preparations Based on the Previous Pharmacokinetic Model[3]

Parameters	Powder	Microcapsules			
		AMPC-80[a]	AMPC-70[a]	AMPC-60[a]	AMPC-50[a]
C (μg/ml)	62.3	60.7	63.5	63.1	64.2
	(3.9)	(3.6)	(4.3)	(5.2)	(1.8)
C' (μg/ml)	61.9	61.7	62.1	61.5	60.4
	(2.4)	(2.4)	(5.2)	(2.8)	(3.8)
C'' (μg/ml)	59.3	116.6	114.4	119.3	124.2
	(4.4)	(4.8)	(6.9)	(8.2)	(5.4)
α (h^{-1})	2.46	2.36	2.41	2.25	2.55
	(0.18)	(0.12)	(0.24)	(0.17)	(0.12)
β (h^{-1})	0.383	0.420	0.397	0.374	0.416
	(0.041)	(0.021)	(0.019)	(0.011)	(0.022)
k_{12} (h^{-1})	0.809	0.737	0.808	0.738	0.864
	(0.112)	(0.124)	(0.196)	(0.011)	(0.062)
k_{21} (h^{-1})	0.738	0.816	0.765	0.694	0.826
	(0.075)	(0.062)	(0.067)	(0.044)	(0.042)
k_{10} (h^{-1})	1.28	1.22	1.25	1.22	1.28
	(0.05)	(0.07)	(0.07)	(0.09)	(0.03)
k_a (h^{-1})	0.710	0.737	0.682	0.704	0.677
	(0.082)	(0.061)	(0.031)	(0.015)	(0.042)
k'_{gi} (h^{-1})	0.073	0.087	0.065	0.082	0.064
	(0.012)	(0.013)	(0.019)	(0.008)	(0.012)
k_r or k'_r (h^{-1})	3.06	1.36[b]	0.844	0.377[b]	0.144[b]
	(0.36)	(0.07)	(0.171)	(0.021)	(0.014)
k_{gi} (h^{-1})	0.836	0.123	0.057	0.052	0.171
	(0.103)	(0.054)	(0.007)	(0.024)	(0.059)
SS[c]	0.310	0.316	0.275	0.244	0.204
	(0.034)	(0.054)	(0.047)	(0.081)	(0.066)

The parameters were estimated using the pharmacokinetic analysis program MULTI for microcomputers.[6] The figures in parentheses represent standard deviations. In all cases, three line fittings were done using data obtained after injection, oral administration of solution and solid-state preparations. *a*) For example, AMPC-70 means the microcapsules containing 70% (w/w) amoxicillin. Significantly different from k_a value of powder at *b*) $p<0.001$. *c*) The residual sum of squares (SS) was calculated from $SS=\sum W_i\cdot(C_i(\text{obs.})-C_i(\text{cal.}))^2$, where W_i is the weight of points. $(C_i)^{-2}$ was adopted as the weight where C_i is the value of the i-th point.

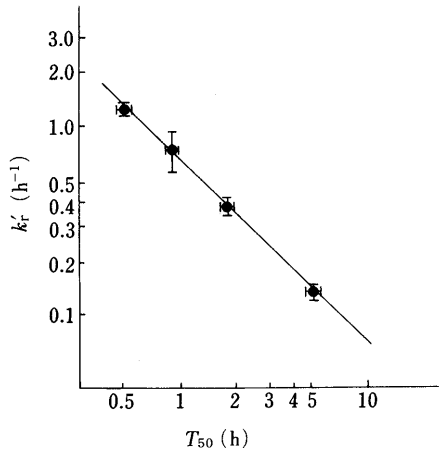

Fig. 5. log–log Relationship of Amoxicillin between *in Vitro* Dissolution Half-Lives (T_{50}) at pH 6.8 and the Experimental Release Rate Constants *in Vivo*

lin caused a considerable decrease in AUC or C_{max}, even though a sustained-release effect was observed to some extent.

Pharmacokinetic Evaluation of Sustained-Release Ethylcellulose Microcapsules Containing Amoxicillin Using Beagle Dogs Pharmacokinetic analysis was performed according to the previous paper.[3] The parameters calculated are listed in Table III. The *in vivo* release rate constants (k_r') from microcapsules correlated well with the *in vitro* dissolution half-lives at 37 °C and pH 6.8. The k_r' value for ethylcellulose microcapsules containing 60% amoxicillin was obtained as $0.377\,h^{-1}$. This value is close to the value of $0.341\,h^{-1}$ that was predicted using our nomogram.[3]

As shown in Fig. 5, the log–log relationship of k_r' calculated on the basis of the previous pharmacokinetic model[3] *vs.* T_{50} determined by *in vitro* dissolution test concerning amoxicillin showed a good linearity and the situation and slope of the correlation line are very similar to those of cefadroxil. Therefore, such a quantitative correlation may be applicable to other β-lactam antibiotics.

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