In Vitro and in Vivo Correlation for Controlled-Release Formulation of d-Chlorpheniramine Maleate

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Four commercial controlled-release tablets of *d*-chlorpheniramine maleate, which showed various drug release properties, were administered to beagle dogs, and the correlation between *in vitro* drug release and *in vivo* absorption was studied. The mean *in vivo* absorption amount–time profile for each product showed good accordance with the *in vitro* drug release profile until 2–3 h after administration. However, absorption of the drug in dogs terminated at about 3 h. This short absorption time may be due to a short intestinal residence time for these dosage forms in the dog. In the present study, the deconvolution method was proved to be useful for *in vitro/in vivo* comparison, which clarified the *in vivo* absorption of controlled-release dosage forms having various release profiles.

Keywords — controlled-release tablets; *in-vitro–in-vivo* correlation; *d*-chlorpheniramine maleate; beagle dog; absorption; deconvolution; convolution; dissolution profile

Introduction

Recently, many controlled-release (CR) dosage forms have been developed and introduced by a number of manufacturers, and methodology for evaluation of performances of these CR products has been discussed.^{1–6} Several mechanisms have been developed for CR dosage forms, with various characteristics of drug release for maintaining the optimum therapeutic drug level. Because of variability of dosage characteristics, development of an *in vitro/in vivo* correlation procedure has been difficult, and for the CR dosage forms, ideal drug-release properties and common indices of bioavailability have not yet been established.

Up to now, there have been many attempts to evaluate drug performance, including studies of *in vitro/in vivo* correlation using single-point dissolution rate (*e.g.* % dissolved in 30 min) and indices of bioavailability such as the area under the plasma level–time curve (*AUC*),^{7–9} and also parameters of moment analysis such as mean residence time (MRT) and mean dissolution time (MDT).^{4,10} However, these variables are insufficient for analysis of CR dosage forms displaying various drug-release characteristics, because they cannot represent the drug-release

properties as a whole. A correlation study of CR dosage forms should be based on sufficient information to characterize CR dosage performance, such as the whole time course of drug release *in vitro* and the whole plasma level–time profile *in vivo*.

The present study examined the correlation between *in vitro* and *in vivo* drug release of CR products using the method of numerical deconvolution. For this study, commercially available sustained-release chlorpheniramine maleate preparations were used because various types of CR dosage forms have been developed for this drug, and numerical deconvolution was proved to be a useful tool for analysis of *in vivo* drug performance.

Materials and Methods

Materials — One commercial immediate-release tablet (product A: Polaramine, lot #G010J, Essex Japan Co., Ltd., Japan) containing 2 mg of *d*-chlorpheniramine maleate, and four commercially available CR tablets (product B: Alerton L, lot #WXAK, Sato Pharm. Co., Ltd., Japan; (product C: Lekrica long-acting tablets, lot #Y537, Yoshitomi Pharm. Ind., Ltd., Japan; (product D: Malemirane-R, lot

#23B7B, Rorer Japan Inc., Japan; (product E: Polaramine Repetabs, lot #3 9F025, Essex Japan Co., Ltd., Japan (technical tie-up with Schering Co., Ltd., U.S.A.) each containing 6 mg of *d*-chlorpheniramine maleate were used in the study.

In Vitro Drug Release — The dissolution test was carried out using the JP XI paddle and rotating basket method with 500 ml of medium. The dissolution media were JP XI 1st fluid for disintegration test (pH 1.2), 0.1 M sodium acetate buffer (pH 4.0) and JP XI 2nd fluid for disintegration test (pH 6.8). A study of the effects of changing the pH of the medium from 1.2 to 6.8 was carried out using JP XI 1st fluid for disintegration test for 1 h followed by a medium of pH 6.8 (pH was changed by adding sodium phosphate, tribasic) for 7 h. The effects of rotation speed on dissolution were studied at 10, 30, 100 and 200 rpm using the JP XI 2nd fluid for the disintegration test. The effect of surfactant on drug release was studied using polysorbate 80 at pH 6.8. The amount of drug dissolved was determined by measuring the absorbance at 262 nm. All the tests were repeated in triplicate.

In Vivo Oral Study — Six healthy male beagle dogs, weighing 8–10 kg, received one immediate-release and four different sustained-release products in a crossover fashion according to a randomized block design with a 7-d washout period between dosing. The dogs were fasted for 16 h prior to and for 8 h after receiving the products. A 12-mg dose of *d*-chlorpheniramine maleate, as six immediate-release tablets or two sustained-release tablets, was administered to each dog, and venous blood samples (3-ml volume) were taken at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 24 and 48 h after dosing. The plasma samples were kept frozen at -20°C until assayed.

Also, the same dogs were intravenously administered a 3-mg dose of *d*-chlorpheniramine maleate dissolved in 2 ml of injectable solution containing 18 mg of sodium chloride, 7 d after the last oral administration. Blood samples were taken at 2.5, 5, 10, 20, 30, and 45 min, and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 24 and 48 h after dosing.

Concentrations of *d*-chlorpheniramine maleate in plasma were determined using the high per-

formance liquid chromatographic (HPLC) method.¹¹⁾

Pharmacokinetic and Statistical Analysis — The plasma concentration–time curves obtained after i.v. administration were fitted to a compartment model using the MULTI¹²⁾ program, in which Akaike's information criterion was used for model selection. The area under the plasma concentration–time curve from zero to infinity (AUC_{inf}) for the i.v. study was calculated using the model parameter values. The MRT and variance of residence time (VRT) were calculated using statistical moment theory.¹³⁾ In the oral study, pharmacokinetic parameters were determined using a model-independent method.¹⁴⁾ The highest observed plasma concentration (C_{max}) and time to reach the peak concentration (T_{max}) were obtained from the plasma concentration–time plot. The AUC for the oral study was determined by the trapezoidal rule. The data were assessed statistically using analysis of variance (ANOVA), and Tukey's test was used to determine significant difference ($p < 0.05$) of means among each of the products, and Dunnett's test was used to determine significant difference ($p < 0.05$) between A and another product.

Calculation of in Vivo Absorption — The amount of absorption following oral administration was calculated by the point-area deconvolution method modified by Iga *et al.*¹⁵⁾ applied to unequal-interval sampling times.

Simulation of Absorption Amount — The simulated amount of the drug absorbed was calculated by convolution using dissolution data (pH 6.8, 100 rpm) and the absorption rate constants (K_a) calculated from the data obtained for an immediate-release product. The elementary part of the computer program is displayed in Fig. 1.

Results

In Vitro Drug Release

The drug release from one immediate-release and four different sustained-release products are shown in Fig. 2. Product A showed immediate and complete drug release within the first 5 min under all conditions. On the other hand, the four

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1000 ' ===== SUBROUTINE GETCI =====
1010 *GETCI      :'absorption rate
1020 READ N
1030 '-----
1040 DIM T(N)          :'time of sampling
1050 DIM D_RATE(N)     :'dissolution rate
1060 DIM GI(N)         :'amount of dissolved drug existing in GI tract
1070 DIM CI(N)         :'amount of drug absorbed into body
1080 '-----
1090 FOR I=1 TO N:READ T(I) :NEXT I          :'read dissolution time
1100 FOR I=1 TO N:READ X(I) :NEXT I          :'read dissolution amount
1110 READ KA                                :'read absorption rate constant
1120 '-----
1130 C0=0:F0=0:T(0)=0:X(0)=0
1140 FOR I=1 TO N
1150   T=T(I)-T(I-1)                        :'time interval between ti-1 and ti
1160   A0=X(I)-X(I-1)                        :'amount of drug released between ti-1 and ti
1170   D_RATE(I)=A0/T                        :'rate of drug release between ti-1 and ti
1180   GI(I)=0:T0=0
1190 '----- convolution ---
1200   FOR J=I-1 TO 0 STEP -1
1210     T1=T(I)-T(J)
1220     WT=(EXP(-KA*T0)-EXP(-KA*T1))/KA      :' area of weight function
1230     GI(I)=GI(I)+D_RATE(J+1)*WT          :' numerical convolution
1240     T0=T1
1250   NEXT J
1260 '-----
1270   CI(I)=CI(I-1)+A0-GI(I)                :'cumulative absorbed amount at ti
1280 NEXT I
1290 RETURN
1300 ' =====
1310 '
1320 '
1490 '----- dissolution data -----
1500 DATA 10                                :' number of sampling
1510 DATA 0.5, 1, 2, 3, 4, 5, 6, 7, 12, 24  :' sampling time (h)
1520 DATA 0.31, 0.42, 0.54, 0.77, 1.04, 3.15, 4.12, 5.54, 6.92, 7.19
                                           :' released amount (mg)
1530 '----- absorption rate constant -----
1540 'DATA 1.19                                :' Ka (/h)

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Fig. 1. BASIC Program for Estimation of Absorption Amount by Convolution

sustained-release products displayed two different types of release: products C and E were the repeat-action type, which showed a long pause of absorption between the first- and second-half

releases, and products B and D were the continuous-release type, which showed maintained release for 4—5 h until most of the drug was dissolved. The CR products of both types

Fig. 2. Mean Release of Chlorpheniramine Maleate from an Immediate-Release Product (A) and Sustained-Release Products (B—E) by the JP XI Paddle Method Using 500 ml of Medium at a Rotation Speed of 100 rpm
Results are expressed as mean $\pm$ S. E. ($n=3$). Product: $\bullet$, A; $\circ$, B; $\blacktriangle$, C; $\triangle$, D; $\times$, E.

Fig. 3. Effect of Change of Rotation Speed on Dissolution Profile of Chlorpheniramine Maleate from Four Sustained-Release Products at pH 6.8
Rotating speed: —, 100 rpm; ---, 30 rpm; ···, 10 rpm.

showed immediate release in the initial stage, when about half of the drug was released. There were small differences among the profiles of drug release in the medium at pH 1.2, 4.0 (data not shown) and 6.8 for all of the products. Product E showed incomplete drug release, only about half being released under all conditions except when the pH was changed successively from pH 1.2 to 6.8. This change of pH did not seriously affect any of the other products. This result suggested that product E would have low bioavailability in patients with low gastric acidity.¹⁶⁻¹⁸⁾ Slight effects of rotation speed on drug release were observed for products C and D (Fig. 3). The drug-release profiles of all products at 200 rpm (data not shown) were very close to those at 100 rpm. There were no significant differences in release behavior among the drugs between the paddle method and the rotating basket method (data not shown), and therefore it was concluded that no mechanical damage caused by friction of the basket occurred for any of the products. Addition of 0.1% polysorbate 80 to the dissolution medium did not significantly affect drug release (data not shown).

In Vivo Drug Elimination

A three-compartment open model was found to adequately describe the i.v. pharmacokinetics of chlorpheniramine in the dogs. The mean estimates are presented in Table I. The mean

TABLE I. Mean Pharmacokinetic Parameter of Chlorpheniramine in Six Dogs after Bolus Intravenous Administration of 3 mg Chlorpheniramine Maleate

Parameter ^{a)}	Mean	S.D.
A_1 (ng/ml)	174.3	188.9
A_2 (ng/ml)	39.3	12.0
A_3 (ng/ml)	22.2	9.8
λ_1 (h ⁻¹)	27.190	14.798
λ_2 (h ⁻¹)	2.236	1.419
λ_3 (h ⁻¹)	0.288	0.080
AUC_{inf} (ng·h/ml)	103.28	14.90
CL_{tot} (l/h)	29.61	4.66
MRT (h)	2.78	0.55

a) The plasma level-time data after i.v. administration was fitted into three-exponential equation: $C_p = \sum_{i=1}^3 A_i e^{-\lambda_i t}$

plasma drug levels in six dogs after oral administration of one immediate-release and four sustained-release products are shown in Fig. 4, and the model-independent parameters and estimated mean absolute bioavailability calculated from the i.v. data are shown in Table II.

In Vivo Drug Absorption

The mean cumulative absorbed amounts of chlorpheniramine maleate after oral administration of each product are shown in Fig. 5. The absorption rate constants (K_a) of chlorpheniramine maleate, calculated using the unabsorbed fraction-time profile of product A, were very

Fig. 4. Mean Plasma Levels of Chlorpheniramine Maleate after Oral Administration of an Immediate-Release (A) and Sustained-Release Products (B—E) to Dogs Using a 12-mg Dose

Results are expressed as mean $\pm$ S. E. for 6 dogs. Product: ●, A; ○, B; ▲, C; △, D; ×, E.

TABLE II. Mean Pharmacokinetic Parameter of Chlorpheniramine after Oral Administration of Immediate-Release Product (A) and Four Different Sustained-Release Products (B—E) to Dogs ($n=6$) with 12 mg Dose

Parameter	Product					ANOVA	Tukey's test
	A	B	C	D	E		
$C_{\max}$ (ng/ml)	40.14 ^{a)} (27.16)	29.75 (20.18)	20.36 (10.79)	20.33 (5.82)	14.27 (7.88)	$p<0.05$	<u>A>B>C>D>E^{c)}</u>
$T_{\max}$ (h)	0.88 (0.34)	0.79 (0.37)	0.71 (0.10)	1.17 (0.92)	0.92 (0.34)	N.S. ^{b)}	
AUC (h·ng/ml)	146.43 (64.55)	114.22 (59.04)	76.59 (30.64)	103.08 (53.19)	58.98 (45.04)	$p<0.05$	<u>A>B>D>C>E</u>
MRT (h)	5.42 (1.52)	6.05 (2.37)	5.62 (2.94)	4.70 (2.27)	6.23 (2.78)	N.S.	
VRT (h ²)	33.47 (27.37)	37.31 (25.61)	30.15 (38.29)	21.30 (25.11)	42.38 (26.23)	N.S.	
$F^d)$ (%)	28.77 (12.35)	21.99 (10.70)	15.79 (5.51)	20.58 (8.68)	11.88 (10.45)		

a) The figures in the parentheses show S.D. b) N.S.: not significant at $p<0.05$. c) The products underlined by a common line did not differ significantly at $p<0.05$. d) Absolute bioavailability.

close to those obtained in the solution study (Table III). It appeared that the drug release from product A occurred rapidly *in vivo*. However, in spite of this immediate drug release, the bioavailabilities shown by product A were considerably low and variable, the mean absolute bioavailability being 28.8% and the standard

Fig. 5. Mean Cumulative Amounts of Absorbed Drug after Oral Administration of an Immediate-Release Product (A) and Sustained-Release Products (B—E) of Chlorpheniramine Maleate

Results are expressed as mean $\pm$ S. E. for 6 dogs. Product: ●, A; ○, B; ▲, C; △, D; ×, E.

deviation 12.3%. This low and variable bioavailability was caused mainly by a presystemic first-pass effect on chlorpheniramine, which has been observed by others.^{19–22} In spite of the large individual variation, Chiou and his co-workers¹⁹ applied the Loo-Riegelman and Chiou methods to an oral absorption study of chlorpheniramine in rabbit assuming no change in the disposition function between i.v. and oral study, and it resulted in a good estimation for the amount of absorption. Accordingly, it is feasible to apply deconvolution for estimating amount of chlorpheniramine absorption, since the intra-individual variation of drug disposition was rather small. In the present study, to reduce the degree of inter-animal variation in the first-pass

TABLE III. Comparison of Parameters for Drug Absorption of Chlorpheniramine after Oral Administration of Immediate-Release Tablet and Aqueous Solution

Parameter	Dosage forms	
	Immediate-release	Solution ^{c)}
K_a (h ⁻¹)	1.9 $\pm$ 0.9 ^{a)}	1.7 $\pm$ 0.4
T_{lag} (h)	0.2 $\pm$ 0.1	0.08 — 0.25 ^{b)}
T_{max} (h)	0.88 $\pm$ 0.34	0.58 $\pm$ 0.16

a) Mean $\pm$ S.D. b) Range of individual values. c) Reference 22.

effect, the cumulative amount of each product was normalized for the amount of product A at 8 h in each dog. With a few exceptions, the normalized cumulative amount–time curves (Fig. 5) reached a plateau at 2–4 h after dosing. These results suggested insufficient drug absorption in dogs from CR dosage forms, because the amount of the drug released after 4 h was not absorbed in dogs. Statistically significant differences in the bioavailabilities of each product were found between the AUC and C_{max} for A and E (Table II) by using Tukey's multiple comparison test. The difference of AUC between A and C was also statistically detected ($p < 0.05$) by another comparison test of Dunnett's method. However, no difference was found in T_{max} and MRT among the products. T_{max} mostly depend on type of drug release but on drug bioavailability. Also, MRT is insufficient parameter to evaluate the bioavailability when the absorption time of drug is short and disposition half life of the drug is long.

Discussion

In Vitro/in Vivo Relationship

The *in vivo* drug absorption profiles in initial stage (1–4 h) were compared with the *in vitro* drug release profiles as shown in Fig. 6. There are two types of *in vitro* curve in Fig. 6: one is intact data of drug release and the other is a simulated absorption profile. The simulated curve was made by convolution using intact release data as input function and drug absorption data of product A as weight function. The initial parts of the intact drug release curves were not so close to the *in vivo* absorption, whereas the simulated absorption curves were very close to the *in vivo* data.

In the *in vitro*/*in vivo* correlation studies by Maturu *et al.*²⁾ and Aiache *et al.*,³⁾ the *in vitro* dissolution–time profiles were directly compared with the *in vivo* absorption amount, and large discrepancies were found in the initial parts between the *in vitro* and *in vivo* data. To get a correlation coefficient between *in vitro* and *in vivo*, the correlation method of Maturu²⁾ and Aiache³⁾ was applied. When the intact dissolution rate was used as *in vitro* data, the correlation coefficients (r) for product A, B, C, D, and E were 0.509, 0.883, 0.618, 0.944, and 0.973. On the other hand, most of these coefficients in-

Fig. 6. Comparison of Mean Cumulative Amount (●) of Drug Absorbed *in Vivo*, Simulated Absorbed Amount (—) Calculated by Convolution, and Mean Drug Released Amount (·····) *in Vitro* (pH 6.8, 100 rpm)

crease to 0.998, 0.940, 0.844, 0.958 and 0.966 when the simulated absorbed amount was used as *in vitro* data. In general, it was said that the drug absorption rate is negligible in a CR formulation study because the drug absorption from CR products is dissolution rate-limited. However, several types of commercial CR products contain an immediate release part in initial stage. Therefore, for an *in vitro/in vivo* comparison, *in vitro* dissolution data should be converted to an absorption profile, especially if the absorption is not dissolution rate-limited but absorption rate-limited.

As described above, it was suggested that the release or absorption of chlorpheniramine terminated by about 3 h in dogs (Fig. 5), whereas the release of chlorpheniramine from most of the CR products maintained for more than 4 h *in vitro* (Fig. 2). In addition to the deconvolution results, this fact was supported by another type of *in vitro/in vivo* comparison using *AUC* and fraction released *in vitro* by 2–3 h. The best *in vivo/in vitro* correlation ($r = 0.966$) demonstrated between the mean *AUC* ($n = 6$) and fraction released in 2 h (Fig. 7). This result is in accord with the insufficient absorption from several CR products in dogs.^{23–25} On the other hand, the absorption of chlorpheniramine from CR product maintained for more than 8 h in men.²¹ The short absorption time in dogs un-

der the fasting condition may be caused by a short reaching time to the colon of dosage forms.²⁶ As previously suggested by Brockmeier *et al.*,⁶ the decrease of drug absorption would be due to a change in consistency of the chyme and gastrointestinal motility along the gastrointestinal tract.

Usage of Deconvolution and Convolution

The drug absorption process can be estimated by several methods using plasma level–time data. However, in the present study, the CR dosage forms used had various release profiles, so the model dependent methods (*i.e.* curve fittings method using a certain absorption model) were not suitable. Deconvolution, containing Wagnet-Nelson and Loo-Reigelman method,¹⁴ is very useful in such a case because it does not need any limited model for drug absorption. By the same reason, if a drug release data is hard to fit a certain mathematical model, convolution is an only tool for simulating the absorption profiles.

In drug absorption study, especially in *in vitro/in vivo* correlation studies, pharmacokinetic parameters such as *AUC*, $C_{\max}$, $T_{\max}$ or MRT give only small parts of information, whereas the absorbed amount–time plots give numerous information for us, such as absorption lag, termination and resuming of drug absorption. In the present study, unfortunately, drug release in later stage (4–8 h) could not be assessed *in vivo*. However, the drug absorption rate in initial stage (0–3 h) could be evaluated reasonably. Convolution is very useful in such a case, and is also useful for simulation of drug plasma levels.²⁷ In conclusion, deconvolution and convolution are very important tools for evaluation and development of CR dosage forms, and these methods can add much information to the data of common pharmacokinetic parameters.

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Fig. 7. Relation between *in Vivo* Mean *AUC* and *in Vitro* Percentage Release in 120 min (pH 6.8, 100 rpm) for Five Products (A–E)

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