IN VITRO AND IN VIVO EVALUATION OF A SUSTAINED RELEASE PREPARATION CONTAINING PHENYLPROPANOLAMINE HYDROCHLORIDE

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The relationship between dissolution behaviour and plasma concentration after administration of a sustained-release preparation containing phenylpropanolamine hydrochloride was studied in 11 male volunteers. The testing for dissolution was carried out using the JP X rotating basket procedure, and human plasma samples were assayed for phenylpropanolamine hydrochloride by a specific high-performance liquid chromatography. Three hours after dosing, the mean peak plasma concentration was 104.1 ng/ml and the plasma concentration declined with a half-life of 4.85 h. The time course of plasma and dissolution data were analyzed by a non-linear least squares curve fitting method to compare *in vivo* and *in vitro* release behaviour. Plasma concentration changes of phenylpropanolamine hydrochloride were correlated with *in vitro* release behaviour of the sustained-release preparation.

Keywords — phenylpropanolamine hydrochloride; sustained-release; dissolution test; human plasma; pharmacokinetics

INTRODUCTION

Phenylpropanolamine hydrochloride (PPA-HCl), used as a nasal decongestant, is rapidly and completely absorbed in the intestine, and has been widely used as a long-acting dosage form owing to its biological half-life of 2–3 h.¹⁾ Many studies on the absorption kinetics of several prolonged-release products containing PPA-HCl have been reported.^{2–5)}

Lindberg *et al.*,²⁾ for example, showed that the *in vitro* release rate from a prolonged acting tablet reflected the actual net rate excretion-*versus*-time profile using Wiegand and Taylor's equation.⁶⁾ Tomida *et al.*³⁾ studied *in vitro* dissolution test method for wax-coated beads and compared the results with an *in vivo* data. These previous reports on the comparison of *in vitro* and *in vivo* data were restricted to the urinary excretion profiles of slow-release PPA-HCl.

Barrett *et al.*⁴⁾ and Lönnerholm *et al.*⁵⁾ studied bioavailability of PPA-HCl during a dose interval in steady-state after the administration of a sustained-release preparation. However, no comparison of *in vitro* and *in vivo* data was made. Little work, therefore, has been done on the quantitative relationship between *in vitro*

release and plasma concentration changes after the administration of a sustained-release preparation showing a rapid-release phase followed by a slow-release phase.

In this study, the *in vitro* and *in vivo* release rates of a sustained-release preparation in healthy male volunteers were investigated on the basis of a pharmacokinetic model using a non-linear least squares curve fitting method.

EXPERIMENTAL

Dosage Form — The sustained-release preparation was in the form of uncoated and ethylcellulose-coated beads (590–840 μ m diameter) filled in hard gelatin capsules containing 40 mg of PPA-HCl. This product was prepared in the laboratory.

Twenty five mg of PPA-HCl was usually applied 3 or 4 times daily as the rapid-release dosage form.⁷⁾ Taking this conventional usage into consideration, the authors decided the rapid-release dose and the slow-release rate constant arbitrarily to dissolve 25 mg of PPA-HCl in 1–2 h.

PPA-HCl is often used by allergic rhinitis patients with an anti-histaminic drug and an anti-

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cholinergic drug, as a combination product. The sustained-release preparation used in this study also contained 4 mg of chlorpheniramine maleate and 0.2 mg of belladonna alkaloids.

In Vitro Evaluation — Dissolution testing was carried out according to the JP X rotating basket method. A covered 1000 ml beaker containing with 900 ml of dissolution medium was immersed in a 37 °C constant temperature bath and the solution was stirred at 100 rpm. Speeds of 50 and 150 rpm were also used to examine the effect of rotating speed on the dissolution behaviour.

Since sustained-release preparations pass through the upper gastrointestinal tract, the *in vitro* dissolution test was performed at its pH range. Davis *et al.*⁸⁾ showed the gastric emptying profile of pellets for fasting or light meal-consuming volunteers using a scintigraphic method. Although there was intersubject variation, more than 80% of the pellets emptied from the stomach in 2 h. Therefore, the exposure to the acid medium was limited to 2 h in this study.

The dissolution medium for the JP X first solution was at pH 1.2 and the acetate buffer solutions were pH 4.0 and then pH 5.0 during the initial 2 h. Subsequently, the beaker was removed and replaced with the JP X second solution pH 6.8.

In Vivo Evaluation — Eleven healthy male subjects, 22–41 years old and 57–77 kg (mean 68.0 kg) were studied. They did not receive any drugs, nor any alcoholic or caffeine-containing beverages. The subjects were not allowed food overnight, from 12 h before to 3 h after administration. Each subject received one sustained-release capsule containing 40 mg of PPA-HCl with 150 ml of water. Serial blood samples were collected over a 24 h period and the plasma from the blood frozen immediately and kept frozen until assayed.

Determination of Phenylpropanolamine in Human Plasma — The paired ion chromatographic method described by Misaki was modified.⁹⁾ Chlorprenaline hydrochloride was used as the internal standard. Two ml of plasma samples were mixed with 2 µg of the internal standard, 0.5 ml of 1 N NaOH and 0.35 g of NaCl. The mixture was extracted twice by shaking

for 10 min with 4 ml of dichlormethane and ethylether (30 : 70). The two organic extracts were combined and extracted with 0.5 ml of 0.5% phosphoric acid by shaking for 30 min. Following centrifugation for 10 min at 3000 rpm, 100 µl of the aqueous layer was injected into an high-performance liquid chromatography (HPLC).

Chromatography was performed with a Nucleosil C₁₈ (10 µ) column (25-cm × 4.6-mm i.d.) with a mobile phase of acetonitrile and 0.2% phosphoric acid aqueous solution containing 0.01 M sodium lauryl sulphate (40:60). This mobile phase was used at a flow rate of 2 ml/min and the temperature of the column was maintained at 35 °C. The wavelength of detection was 210 nm. Peak height ratios were converted to concentrations using a calibration curve for a PPA-HCl concentration range of 0.02–5 µg/ml.

Kinetic Analysis — In Vitro: The time course data of percentages dissolved were fitted to Eqs. 1 and 2.

$$y(\%) = 0 \quad \text{for } t \leq \Delta t \quad (1)$$

$$y(\%) = k_r \cdot (t - \Delta t) + b \cdot (1 - e^{-k_i \cdot (t - \Delta t)}) \quad \text{for } \Delta t < t \leq \tau \quad (2)$$

y , percentages dissolved (%);

k_i , first-order release rate constant from a rapid-release dose (h⁻¹);

k_r , zero-order release rate constant from a slow-release dose (%/h);

b , total percent dissolved from a rapid-release dose (%);

τ , time taken for a slow-release dose to dissolve completely (h);

Δt , lag time (h).

The value of b was limited to ≤ 50 in this calculation.

In Vivo: The time course data of the plasma levels were fitted to Eqs. 3, 4 and 5 represented by Chart 1,¹⁰⁾ where k_i and k_r were first-order and zero-order release rate constants, respectively. These pharmacokinetic parameters were obtained by a non-linear least squares method.*

$$C_p(\text{ng/ml}) = 0 \quad \text{for } t \leq \Delta t \quad (3)$$

* "NONLIN '74"; Metzler C.M.; The Upjohn Co.

$$C_p \text{ (ng/ml)} = \frac{10^3 \cdot f \cdot D_{fi}}{V_d} \cdot k_i \cdot k_a \left[\frac{e^{-k_a(t-\Delta t)}}{(k_e - k_a)(k_i - k_a)} + \frac{e^{-k_i(t-\Delta t)}}{(k_a - k_i)(k_e - k_i)} \right. \\ \left. + \frac{e^{-k_e(t-\Delta t)}}{(k_i - k_e)(k_a - k_e)} \right] + \frac{10 \cdot k_r \cdot D_T}{V_d} \left\{ \frac{1}{k_e} [1 - e^{-k_e(t-\Delta t)}] \right. \\ \left. + \frac{1}{k_a - k_e} [e^{-k_a(t-\Delta t)} - e^{-k_e(t-\Delta t)}] \right\} \\ \text{for } \Delta t < t \leq \tau \quad (4)$$

$$C_p \text{ (ng/ml)} = \frac{10^3 \cdot f \cdot D_{fi}}{V_d} \cdot k_i \cdot k_a \left[\frac{e^{-k_a(t-\Delta t)}}{(k_e - k_a)(k_i - k_a)} + \frac{e^{-k_i(t-\Delta t)}}{(k_a - k_i)(k_e - k_i)} \right. \\ \left. + \frac{e^{-k_e(t-\Delta t)}}{(k_i - k_e)(k_a - k_e)} \right] + \frac{10 \cdot k_r \cdot D_T}{V_d} \left\{ \frac{1}{k_e} [1 - e^{-k_e(\tau-\Delta t)}] \right. \\ \left. + \frac{1}{k_a - k_e} [e^{-k_a(\tau-\Delta t)} - e^{-k_e(\tau-\Delta t)}] \right\} \cdot e^{-k_e(t-\tau)} \\ + \frac{10 \cdot k_r \cdot D_T}{V_d \cdot (k_a - k_e)} \cdot [1 - e^{-k_a(\tau-\Delta t)}] [e^{-k_e(t-\tau)} - e^{-k_a(t-\tau)}] \\ \text{for } \tau < t \quad (5)$$

C_p , plasma concentration (ng/ml);
 V_d , distribution volume (l);
 k_a , absorption rate constant (h^{-1});
 k_e , elimination rate constant (h^{-1});
 D_T , a total dose (mg) = 40 mg in this preparation;

D_{fi} , a rapid-release dose (mg) = 20 mg in this preparation;
 f , a fraction of drug absorbed from a rapid-release dose.
 Other abbreviations were used as well as *in vitro* kinetic analysis.

RESULTS AND DISCUSSION

In Vitro Evaluation

The dissolution profiles of PPA-HCl are shown in Figs 1 and 2. The difference in percentages of the dissolved-time data was not significant at pH 1.2, 4.0, and 5.0 for the initial 2 h, and at subsequent pH 6.8 for 6 h. The effect of the rotating speeds was not observed in the range of 50–150 rpm. These test conditions did not affect the drug release from the sustained-release preparation.

In the following discussion, the condition of the dissolution test at a rotating speed of 100 rpm with a medium of pH 1.2 for the initial 2 h, and subsequent pH 6.8, was used as a typical *in vitro* evaluation.

The simulated curve of percentages of the dissolved-time data using Eqs. 1 and 2 for the typical dissolution test condition is shown as a

FIG. 1. Effect of pH for the Initial 2 h on the Dissolution Profiles at a Rotating Speed of 100 rpm. Each points represent the mean $\pm$ SEM ($n = 3$). Δ , pH 1.2; $\blacksquare$, pH 4.0; $\blacktriangledown$, pH 5.0; —, simulated curve for pH 1.2.

FIG. 2

Upper: Effect of the rotating speed on the dissolution profiles at pH 1.2 for the initial 2 h, and at subsequent pH 6.8 for the remaining time.

Each points represent the mean $\pm$ SEM ($n = 3$). $\square$, 50 rpm; $\triangle$, 100 rpm; $\bullet$, 150 rpm; —, simulated curve.

Lower: Dissolution profiles for the rapid-release beads alone and for the slow-release beads alone are shown as a dotted line ($\cdots$) and a broken line ($---$), respectively.

solid line. The rapid-release curve, fitted to a first-order term in Eq. 2, is shown as a dotted line, and the slow-release curve, fitted to a zero-order term in Eq. 2, is shown as a broken line.

A lag time of 4–8 min was observed for these curves, corresponding to the time required for the gelatin shell to dissolve. The lag time of the rapid-release curve was nearly equal to that of the slow-release curve in Fig. 2. Based on the *in vitro* release behaviour, equality of lag time between a rapid-release phase and a slow-release phase was assumed in this study.

In vitro release parameters obtained for the dissolution curves in Fig. 2 are listed in Table I.

CHART 1. Kinetic Model for Sustained-Release

The difference in the release rate constants was not significant, but the first-order release rate for the rapid-release phase in the sustained-release preparation was slightly slower than the first-order release rate for the rapid-release beads alone. This may be caused by the slightly slow disassociation of the capsule. The zero-order release rate constant for the slow-release preparation agreed well with the zero-order release rate constant in the slow-release beads alone.

In Vivo Evaluation

Figure 3 shows the plasma concentration *versus* time data of PPA-HCl. The mean peak plasma concentration of PPA-HCl was 104.1 ± 8.0 ng/ml (mean $\pm$ SEM) at 3 h and the plasma concentration declined at a half-life of 4.85 ± 0.17 h.

The changes in plasma levels with time were described by an open one compartment model (Chart 1),²⁾ and the pharmacokinetic parameters for each individual are listed in Table II.

The simulated curve of plasma concentration-time data with Eqs. 3, 4 and 5 is shown as a solid line in Fig. 3. The relatively good agreement between the observed and the calculated values for plasma levels validates the pharmacokinetic model.

An *in vivo* zero-order release rate constant,

TABLE I. *In Vitro* Release Parameters for Sustained-Release Beads in a Typical Dissolution Test Condition^{a)}

Sample beads	k_r (mg/h)	k_i (h^{-1})	τ (h)	Δt (h)
Rapid + slow	3.63 ± 0.17^b	8.61 ± 0.72	5.04 ± 0.12	0.146 ± 0.028
Rapid	—	10.92 ± 1.82	—	0.064 ± 0.007
Slow	3.50 ± 0.05	—	5.26 ± 0.07	0.100 ± 0.032

a) The dissolution test was carried out at a rotating speed of 100 rpm with a medium at pH 1.2 for the initial 2 h, and then at pH 6.8. b) Data are expressed as the mean $\pm$ SEM ($n = 3$).

TABLE II. Pharmacokinetic Parameters for Phenylpropanolamine Obtained from Plasma Concentration-Time Data

Sub No.	Age	Body weight (kg)	$f \cdot D_{fi} / V_d$ (ng/ml)	k_r / V_d (ng/ml · h)	k_e (h ⁻¹)	k_i (h ⁻¹)	τ (h)	Δt (h)	k_a (h ⁻¹)	k_r (mg/h)	SS ^{a)}
1	22	57	82.42	18.57	0.175	80.00	4.31	0.309	9.82	4.51	86.8
2	24	68	66.22	13.84	0.143	5.19	4.07	0.213	4.99	4.18	189.7
3	27	77	87.69	13.12	0.109	75.14	2.80	0.297	10.00	2.99	375.1
4	25	67	103.52	29.06	0.148	31.44	3.30	0.179	0.969	5.61	131.9
5	34	70	91.27	11.19	0.131	7.29	4.15	0.259	7.10	2.45	189.7
6	34	69	90.95	20.76	0.143	4.43	2.98	0.163	2.81	4.57	272.9
7	36	64	58.98	22.08	0.158	5.85	4.12	0.095	4.92	7.49	23.2
8	41	68	76.89	12.99	0.134	4.72	4.48	0.293	4.99	3.38	55.1
9	41	67	118.85	26.68	0.176	4.03	3.94	0.150	4.22	4.49	27.9
10	23	65	132.24	35.52	0.166	34.93	2.96	0.463	10.00	5.37	414.6
11	26	76	132.26	4.70	0.137	3.69	4.60	0.068	2.04	0.71	470.9
$\bar{x}$	30.3	68.0	94.66	18.96	0.147	23.34	3.79	0.226	5.62	4.16	—
SEM	2.0	1.6	7.11	2.58	0.006	8.36	0.19	0.033	0.93	0.51	—

a) Residual sum of squares (SS) was calculated by $SS = \sum W_i \cdot (C_i(\text{obs.}) - C_i(\text{cal.}))^2$, where W_i was weight of points. A weight of unity was employed.

k_r was calculated from parameters in Table II on the assumption that the fraction of the drug absorbed (f) from the rapid-release dose ($D_{fi} = 20$ mg) was 1.0. This assumption is supported by the result that urinary recoveries of PPA-HCl after oral administration in man were about 80–100% in 24 h.^{2,3,5)}

There was a considerable intersubject variation in first-order release rate constants (k_i) from a rapid-release dose as shown in Table II. This may be attributed to the intersubject variation in the drug release process in the stomach, such as the disassociation of the capsule contents from the gelatin shell.

The values of k_i were not smaller compared with the absorption rate constants (k_a) in almost all subjects, indicating that the rate-limiting step in the absorption of PPA-HCl as a rapid-release phase was not the drug release process.

Comparison of *in vitro* and *in vivo* parameters in Tables I and II gave a relatively small difference in zero-order release rate constants (k_r) from the slow-release dose (D_{fs}) (*in vitro*, 3.63 ± 0.17 mg/h; *in vivo*, 4.16 ± 0.51 mg/h). Consequently, a zero-order release phase contributed to the duration of plasma levels.

In Fig. 3, the “dash-dot” line shows the curve calculated by substituting *in vitro* release rate

FIG. 3. Plasma Concentration-Time Data (●) of Phenylpropanolamine Hydrochloride after a Single Dose in 11 Male Volunteers (Mean $\pm$ SEM)

The figure shows the simulated curve from the plasma concentration-time data (—), the calculated curve with Eqs. 3–5 by substituting *in vitro* release rate constants, 3.63 mg/h and 8.61 h⁻¹ for k_r and k_i in Chart 1 (---) and the predicted individual contributions to the plasma concentration-time profile from D_{fi} (···) and D_{fs} (—·—).

constants (3.63 mg/h, 8.61 h⁻¹) for k_r and k_i in Chart 1. Both the *in vivo* and *in vitro* curves have almost the same profiles throughout the period

of measurement.

It was demonstrated, therefore, that the release behaviour of PPA-HCl in the gastrointestinal lumen was considerably reflected in the *in vitro* dissolution test according to the JP X rotating basket procedure.

In addition, individual contributions from the rapid-release dose and the slow-release dose to the plasma concentration-time profile were separated on the basis of the pharmacokinetic parameters in Table II. Plasma levels can be maintained above the peak value of the dotted line for a relatively long period of time as seen in Fig. 3. This sustained-release action was almost in agreement with the objective for which it was designed.

These profiles (Fig. 3) based on the pharmacokinetic model were helpful in understanding sustained-release action.

In this study, it was demonstrated that the *in vitro* dissolution model of a sustained-release preparation with a rapid-release phase followed by a slow-release phase, can be introduced into *in vivo* pharmacokinetics. A reasonable good correlation was found between the *in vitro* dissolution behaviour and the *in vivo* performance of PPA-HCl. This may be mainly due to biological characteristics of PPA-HCl such as rapid and complete absorption by the oral route. However, the evaluating process in the present study appears to be useful for designing sustained-release formulation of drugs without poor absorption and/or first-pass characteristics.

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