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Anti-hypertensive Effect of Oral Controlled-release Microspheres Containing an ACE Inhibitor (Delapril Hydrochloride) in Rats

YOHKO AKIYAMA, MINORU YOSHIOKA*, HIDETOSHI HORIBE*, YOSHIYUKI INADA†, SHINICHIRO HIRAI*, NOBUYUKI KITAMORI‡ AND HAJIME TOGUCHI

*DDS Research Laboratories, * Pharmaceutical Development Laboratories and † Pharmaceutical Research Laboratories I, Takeda Chemical Industries Ltd, 17-85 Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, and ‡ Research Center, Grelan Pharmaceutical Co. Ltd, 3-4-3 Sakae-machi, Hamura City, Tokyo 205, Japan*

Abstract—An oral controlled-release drug delivery system based on microspheres of polyglycerol esters of fatty acids (PGEFs), was applied to an anti-hypertensive, delapril hydrochloride. The in-vitro release profile was controlled by selecting a PGEF with an appropriate hydrophilic–lipophilic balance value for the matrix. The microspheres from which 80% of the drug was released in 6 h were orally administered to rats. The plasma concentration of the active metabolite was sustained after administration of the microspheres in comparison with administration of a solution. The in-vivo release profile was in good agreement with the in-vitro release profile. When the microspheres were administered, the pharmacological effect of delapril hydrochloride on the angiotensin I-induced pressor response was also sustained showing consistency with the plasma concentration–time curve.

Several controlled-release, long-acting preparations of drugs for treatment of hypertension such as nifedipine (Chung et al 1987), propranolol (Nace & Wood 1987) and captopril (Seta et al 1988a) have been developed because the avoidance of initially high plasma concentrations of the drugs decreases the risks of undesired side-effects. Furthermore, the extension of dosage intervals could lead to an improved compliance and to improved therapeutic effects when steady plasma concentrations are maintained, particularly in long-term therapy. Seta et al (1988b) reported that an oral sustained-release dosage form of captopril using an oily semisolid matrix maintained a steady plasma concentration and the inhibition of pressor response to angiotensin I for extended periods in comparison with the conventional tablet in dogs. This effect might have resulted from the lack of an absorption window for captopril and the slower transit of the oily semisolid matrix in the gastrointestinal tract. Generally, however, the in-vivo evaluation of oral controlled-release dosage forms using dogs as animal models is difficult for drugs with an absorption window because the transit time in the small intestine in dogs is shorter than in man (Dressman 1986). The pharmacological evaluation of oral controlled-release dosage forms using rats has not been reported because of the smaller dimensions of their gastrointestinal tract, although the transit time in the gastrointestinal tract is similar to that in man (Mori et al 1989).

We have studied oral controlled-release microspheres using polyglycerol esters of fatty acids (PGEF) from which the in-vitro drug-release rate could be regulated by selecting a PGEF with an appropriate hydrophilic–lipophilic balance (HLB) for the matrix. A multiple-unit dosage form spreads uniformly in the gastrointestinal tract and causes little local irritation as compared with a single-unit dosage form (Bechgaard & Nielsen 1978; Davis et al 1984). Delapril hydrochloride, *N*-(*N*-((*S*)-1-ethoxycarbonyl-3-phenylpropyl)-L-alanyl)-*N*-(indan-2-yl) glycine hydrochloride, is an agent that blocks the renin–angiotensin system and may be used in the treatment of hypertension.

The purpose of this study was to apply oral controlled-release microspheres using PGEF to delapril hydrochloride and evaluate the in-vivo effect of the microspheres using rats. The plasma concentrations and the pharmacological effects after administration of either a solution or microspheres to rats were examined.

Correspondence: Y. Akiyama, DDS Research Laboratories, Pharmaceutical Research Division, Takeda Chemical Industries Ltd, 17-85 Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.

Materials and Methods

Materials

Delapril hydrochloride was supplied by the Chemistry Research Laboratories of Takeda Chemical Ind., Ltd (Inada et al 1986; Oka et al 1988). Tetraglycerol pentastearate, tetraglycerol tristearate and tetraglycerol monostearate were obtained commercially (Sakamoto Yakuhin Koygo Co. Ltd). The HLB value of each ester was calculated from Griffin's equation as 2·6, 4·7 and 8·4, respectively. Angiotensin I and angiotensin II were obtained commercially (Protein Research Foundation, Japan). All other chemicals were of reagent grade.

Microsphere preparation

Delapril hydrochloride was dispersed in melted tetraglycerol pentastearate and tetraglycerol monostearate in combination or tetraglycerol tristearate singly at 85°C, and microspheres were prepared by spraying and chilling the melted mixture using a 15-cm aluminium disc (spray-chilling method) described in our previous paper (Akiyama et al 1993). Microspheres in the size range 250–350 μm obtained by sieving, were used for release studies and animal experiments. The formulations are shown in Table 1.

Table 1. Formulation of delapril microspheres.

	Formula		
	I	II	III
Delapril hydrochloride	2·0	2·0	2·0
Tetraglycerol pentastearate	7·0	6·4	—
Tetraglycerol monostearate	1·0	1·6	—
Tetraglycerol tristearate	—	—	8·0
HLB value	3·3	3·7	4·7

Solubility determination

Solubility measurements of delapril hydrochloride were determined in 0·5 M HCl-sodium acetate and 0·2 M phosphate buffers at 25°C. The drug concentrations in the equilibrated solutions were determined spectrophotometrically.

In-vitro dissolution test

In-vitro release was measured using a USP XXII paddle apparatus (100 rev min^{-1}) at 37°C in 900 mL first fluid (pH 1·2) and second fluid (pH 6·8) specified in the Japanese Pharmacopoeia XII; polysorbate 20 (0·1%) was included to improve wettability. The amount of drug dissolved was determined spectrophotometrically.

Administration of solutions and microspheres to rats

Male Sprague-Dawley rats, 300–400 g, were allowed free access to tap water and food. The apparatus for administration of microspheres consists of a syringe, gastric sonde and polyethylene tube (1·77 mm i.d. and 2·80 mm o.d., 40 mm in length, PE260, Nippon Becton Dickinson Co., Ltd) as shown, in Fig. 1. Microspheres were placed in the polyethylene tube, the end of which was covered with a thin hydroxypropyl cellulose film and administered to the rat stomach using the tube attached to a gastric sonde and 0·5 mL water. For the solution experiments, 0·5 mL solution containing delapril hydrochloride was administered using a gastric sonde. Blood samples were withdrawn periodically from the tail vein.

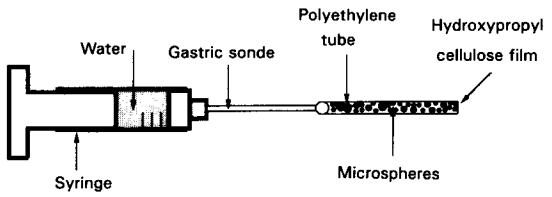

FIG. 1. Apparatus for administration of microspheres to rats.

Determination of plasma concentration of the active metabolite of delapril

The plasma concentrations of *N*-(*N*-((*S*)-1-carbonyl-3-phenylpropyl)-L-alanyl)-*N*-(indan-2-yl) glycine (M1), one of the active metabolites of delapril were determined by the HPLC method reported by Itoh et al (1985). The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were obtained from individual plasma–concentration curves. The area under the plasma–concentration curve ($AUC_{0-7\,h}$) was calculated by the trapezoidal method and the mean residence time (MRT) was calculated by a model-independent statistical moment analysis (Yamaoka et al 1978).

Inhibition of pressor response to angiotensin I

Rats were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.) and polyethylene cannulae were placed in the abdominal aorta via the femoral artery (PE-10) to measure blood pressure and the vena cava via the femoral vein (PE-50) for angiotensin I or angiotensin II injection. Both catheters filled with saline containing heparin were passed subcutaneously to an exit on the dorsal part of the neck. The rats were then placed in plastic cages and allowed to move freely; tap water and solid food were freely available and measurements of pressor responses after administration of delapril hydrochloride were started the next morning. The aortic cannula was connected to a pressure transducer (San-ei 45277, Japan), and the mean blood pressure was recorded on a polygraph (San-ei 360, Japan). The pressor responses to angiotensin I (300 ng kg^{-1}) and angiotensin II (100 ng kg^{-1}), both of which were injected into the venous cannula, were measured twice, and the average response to each drug was used as the control for all calculations. After the blood pressure had returned to pretreatment levels, solutions or microspheres containing drug were administered orally. Thereafter, angiotensin I or angiotensin II was injected repeatedly at given times and the inhibition of the pressor response to angiotensin I was determined. The angiotensin II challenge was used to correct values of the inhibition of the pressor response to angiotensin I due to changes in vascular responsiveness during the course of the experiment, thus values of percent inhibition were calculated as:

$$100\left(1-\frac{I_t}{I_c}\times\frac{II_c}{II_t}\right)$$

where I_c and II_c are the pressor responses (mmHg) to angiotensin I and angiotensin II in the control period, respectively, and I_t and II_t are the pressor responses at time (t) after the solutions or microspheres had been administered.

Results and Discussion

The structures of delapril and its metabolite M1 are shown in Fig. 2. Delapril is an amphoteric compound with pK_a values of 3·26 and 5·65. The solubility did not differ greatly from pH 3–6 but was higher outside this pH range (Fig. 3). The

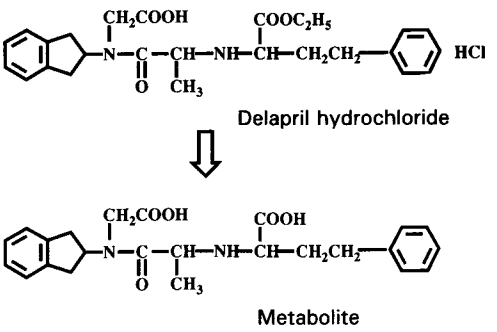

FIG. 2. Chemical structures of delapril and its metabolite.

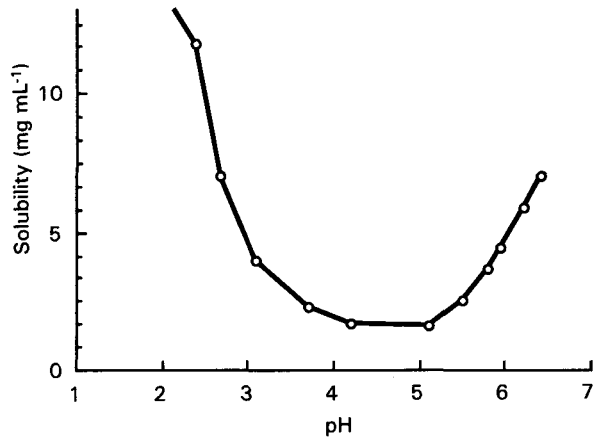

FIG. 3. Solubility–pH profile of delapril.

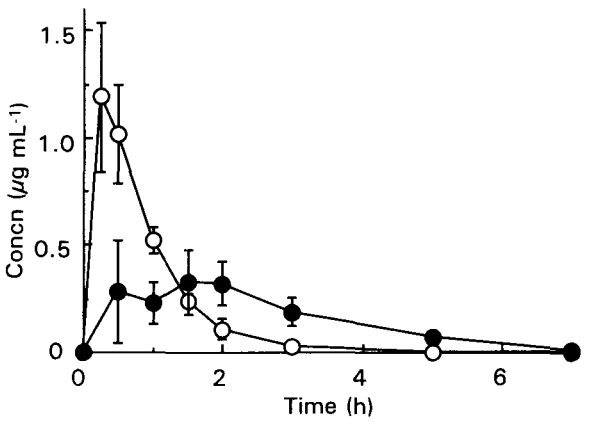

FIG. 5. Plasma concentration of delapril metabolite in non-fasted rats receiving delapril hydrochloride (10 mg kg^{-1}). ○ Solution, ● microspheres.

Table 2. Effect of HLB on delapril release in pH 6·8 fluid.

	Formula		
Time (h)	I	II	III
0·00	0·0	0·0	0·0
0·33	24·4	26·5	35·0
0·37	30·5	33·6	59·6
1·00	39·2	48·4	86·1
2·00	53·7	74·2	96·6
3·00	66·5	86·8	98·3
4·00	74·7	90·5	99·4
5·00	78·5	91·8	100·0
6·00	80·1	92·8	100·0

Data are expressed as percentage of dissolved delapril.

in-vitro release rate of delapril from microspheres was affected by the size of the microspheres and the drug content in the microspheres. In addition, delapril was released more rapidly from the microspheres with the higher HLB values (Table 2). Thus, the desired release rate can be obtained by selecting a PGEF with an appropriate HLB value as described in our previous paper (Akiyama et al 1993). The pH of the dissolution medium (pH 1·2 or 6·8) did not affect the release rate (Fig. 4).

Delapril is a prodrug and has little pharmacological activity until it is hydrolysed to its active metabolites (Inada et al 1986). Since M1, the diacid form (Fig. 1), is the most active metabolite, the plasma concentrations of this metabolite were determined.

The plasma concentration profile of the metabolites after a single administration is shown in Fig. 5.

The plasma concentration of metabolite I peaked around 1–2 h after administration of the microspheres, while it peaked 15 min after administration of the solution. Fig. 5 shows that the plasma concentration was sustained after the administration of microspheres, and the relevant pharmacokinetic parameter, C_{max}, t_{max}, $AUC_{0\text{-}7\,h}$ and MRT are

Table 3. Pharmacokinetic parameters of delapril metabolite, M1, after administration of delapril hydrochloride solutions (10 mg kg^{-1}) and microspheres to non-fasted rats (n = 3).

	Solution	Microspheres
C_{max} (μg mL^{-1})	1·20 ± 0·34	0·46 ± 0·15
t_{max} (h)	0·33 ± 0·08	1·33 ± 0·44
$AUC_{0\text{-}7\,h}$ (μg h mL^{-1})	1·19 ± 0·18	1·08 ± 0·39
MRT (h)	0·85 ± 0·08	4·44 ± 1·26

Mean ± s.e.

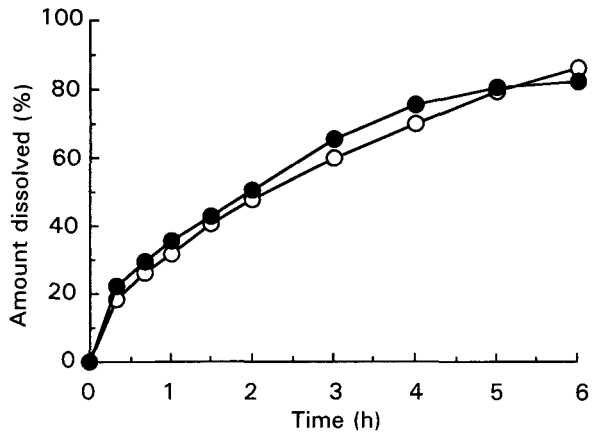

FIG. 4. Release profiles of delapril. ○ pH 1·2, ● pH 6·8.

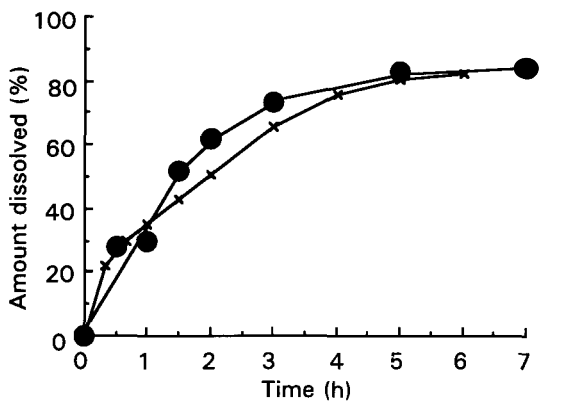

FIG. 6. Comparison of in-vitro (×) and in-vivo (●) release profiles.

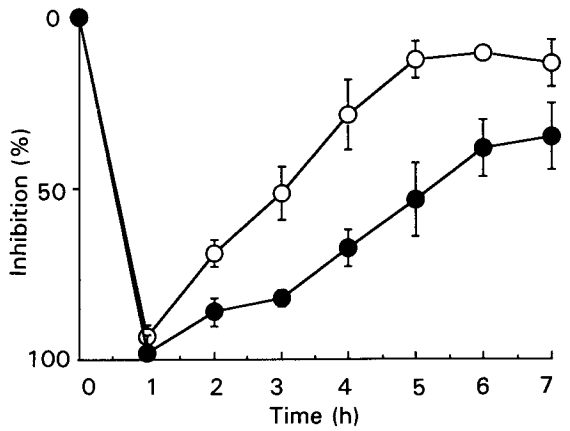

FIG. 7. Inhibitory effects of delapril on angiotensin I-induced pressor response in rats. ○ Solution, ● microspheres.

shown in Table 3. $AUC_{0\text{-}7\ h}$ after administration of the microspheres was almost the same as that after administration of the solution. MRT was prolonged after administration of the drug in the form of microspheres compared with that following the administration of the solution.

The in-vivo release profile of delapril from the microspheres was estimated using a deconvolution method (Iga et al 1986). In this report, the deconvolution method was used where the plasma concentration of the drug after an impulse input was expressed as a multiexponential function. The plasma profile after administration of the oral solution of delapril hydrochloride was described by a one-compartment model (biexponential function):

$$C_p = 2{\cdot}3(e^{-1{\cdot}5t} - e^{-7{\cdot}0t}) \quad (1)$$

The in-vivo release profile evaluated from the deconvolution method using equation 1 agreed well with the in-vitro release profile in the pH 6·8 medium (Fig. 6). This suggested that delapril was released from the microspheres both in-vivo and in-vitro at a similar rate, assuming that the drug is hydrolysed to the acid as soon as it is absorbed.

The inhibition of the pressor response to angiotensin I was measured after administration of aqueous solutions or microspheres (Fig. 7). When the aqueous solution (10 mg kg^{-1}) was administered, the maximum inhibition of pressor response to angiotensin I was already induced by 1 h and decreased rapidly; the inhibition after administration of the microspheres containing the same dose of delapril lasted longer than that after administration of the solution. The duration of the inhibitory effects after the microsphere administration is considered to be a result of the prolongation of the plasma concentrations of the metabolite.

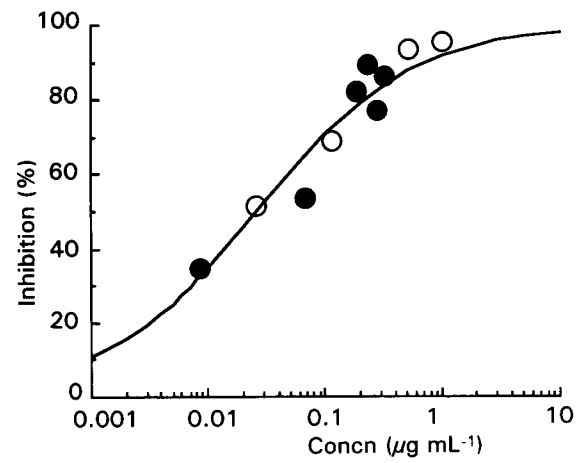

FIG. 8. Relationship between pharmacological effects and plasma concentrations. ○ Solution, ● microspheres.

As the inhibition of pressor response to angiotensin I is an index of anti-hypertensive activity, the controlled-release microspheres are expected to have potent and long-lasting anti-hypertensive activities compared with the aqueous solution.

The inhibition of pressor response to angiotensin I was plotted as a function of the plasma concentration after administration of the drug preparations (Fig. 8).

Several pharmacodynamic models referring to the relationship between effects (pharmacological response) and drug concentrations at the site of action (represented by the plasma concentration in most cases) have been presented (Holford & Sheiner 1981, 1982).

A mathematical description of a biological property of a drug action which has a maximum effect is obtained with the Hill function:

$$E = E_{max}(C^n/(EC50^n + C^n)) \quad (2)$$

where E is intensity of the effect, C is concentration, E_{max} is the maximum effect attributable to the drug, EC50 is the concentration producing 50% of the maximum effect and n is a parameter affecting the shape of the curve. Endoh et al (1985, 1989) reported that the relationship between the mean arterial blood pressure and the amount of angiotensin II in the rat after intravenous administration of the ACE inhibitor captopril could be clarified by Hill's equation (eqn 2). Hill's equation was applied to the inhibition of pressor response to angiotensin caused by the ACE inhibitor delapril, since this relationship postulates no effect when drug is absent and a maximum effect when a certain drug concentration is attained. This could be described by a sigmoid E_{max} model with a value of 0·7 for n, that is:

$$E = 100(C^{0\cdot7}/(EC50^{0\cdot7} + C^{0\cdot7})) \quad (3)$$

The curve expressed by equation 3 is depicted in Fig. 8 as a solid line. The pharmacological response could be predicted from the concentration–response relationship, and our experiments using rats showed that the controlled-release microspheres were more effective than the solution containing the same dose.

Gastrointestinal transit can be divided into gastric emptying and transit through the intestine; gastric emptying after a meal differs from that in the fasting condition because there is a cyclic pattern of motility (Code & Marlett 1975; Hinder & Kelly 1977; Rubinstein et al 1988).

Gastric emptying time in man is approximately 2 h for both tablets and granules under the fasting conditions, and 4 h for tablets and 2 h for granules in the fed condition; small intestinal transit time is about 3 h independent of the dosage form or fasting conditions (Davis et al 1986; Khosla et al 1989). In dogs, the transit time in the small intestine is reported to be less than half that in man (Dressman 1986), although dogs have been extensively used for evaluation of

dosage forms (Kaniwa et al 1988; Mizuta et al 1990). Monkeys, guinea-pigs or rabbits are not so useful (Takahashi et al 1985; Kokue et al 1988), because of difficult handling or prolonged gastric emptying under fasting conditions.

In rats, the gastric empyting time for granules is 1–2 h and is prolonged when food is present in the stomach (Mori et al 1989). The mean transit time of granules through the small intestine is about 3 h irrespective of the presence of food. As the transit profile through the gastrointestinal tract in rats is similar to that in man, rats are useful as experimental animals to estimate the absorption of controlled-release granules in man.

One of the reasons why rats have not been used as an animal model is that administration of an oral dosage form designed for human use is impossible because of the small dimensions of the gastrointestinal tract of rats. However, as microspheres described here have the form of a powder, rats could be used effectively for estimating the absorption of the drug in such experimental formulations.

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