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Comparison of the pharmaceutical properties of sustained-release gel beads prepared by alginate having different molecular size with commercial sustained-release tablet

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Spherical alginate gel beads containing pindolol were prepared using three types of sodium alginate with different molecular size. The rate of gelation of sodium alginate in calcium chloride solution was in the range of 1.0 to 1.3 h^{-1} among the used three alginates, but the amount of water squeezed from the alginate gel beads during gelation increased from 5 to 40% with increasing molecular size of the alginate. The beads prepared were similar in diameter (1.2 mm after drying), weight (0.9 mg/bead), calcium content (27–29 μg/bead) and pindolol content (40–45%). Pindolol was rapidly released from all the alginate gel beads at pH 1.2 owing to the high solubility of pindolol, in spite of non-swelling of beads. On the other hand, pindolol release from alginate gel beads at pH 6.8 was dependent on the swelling of the beads and was significantly depressed compared to drug powder. Interestingly, the release rate of pindolol and the swelling rate of beads were markedly slow for gel beads prepared by low molecular size alginate. However, when the alginate gel beads were administered orally to beagle dogs, the serum levels of pindolol showed sustained-release profiles, depending on the molecular size of the alginate. The *in vivo* absorption of pindolol from alginate gel beads did not reflect their *in vitro* release profiles, because of a physical strength of beads in the intestinal tract. Furthermore, the *in vivo* and *in vitro* release of pindolol from alginate gel beads were compared with a commercial sustained-release tablet, Carvisken® showed a rapid release of 50% of content in pH 1.2 fluid and residual 50% of pindolol were easily dissolved at pH 6.8. Although the release characteristics of pindolol from Carvisken® and the alginate gel beads were completely different, the serum levels of pindolol in human volunteers were comparable.

1. Introduction

Swellable hydrogels prepared with hydrophilic polymers have been used for the controlled-release of drugs [1]. The process to control the solute release from the hydrogel is considered to depend upon the association to polymer, diffusion of the solute through the swollen polymer, erosion of the swollen polymer, and so on [2].

Alginate has been applied to the material to achieve a controlled-release of drug due to its hydrogel forming properties [3–5]. Alginate is a natural anionic polysaccharide obtained from marine brown algae, which consists of D-mannuronate (M) and L-guluronate (G) residues, arranged in homopolymeric blocks of each residue (MM and GG), and in heteropolymeric blocks (MG) [6]. The gelation of alginate is caused by forming an egg-box junction to associate the divalent metal ions with the GG block of the alginate polymer chain [7, 8]. Two other blocks, MM and MG take a much lesser part in gelation with divalent metal ions [9].

We previously reported that prednisolone dispersed in a matrix of a spherical gel of alginate showed sustained-release behavior which mainly depended on the swelling of alginate gel beads in both *in vitro* and *in vivo* [10]. The swelling of alginate gel was affected by the GG block content in alginate. Similar release characteristics owing to the swelling property of alginate gel beads were also found for indomethacin [11] and nifedipine [12]. However, the effect of the molecular size of alginate on the *in vitro* and *in vivo* release of drugs has not been clarified. In addition, the release behavior of a basic drug from alginate gel beads has not been proven. The basic compounds may affect swelling of the alginate gel due to the microenviromental pH change generated by dissolving the ionic compound and through the electrostatic interaction of alginate with basic drugs such as propranolol [13]. In the present study, the influence of the molecular size of alginate on the *in vitro* and *in vivo* release of a basic drug from alginate gel beads was studied using pindolol (pKa: 9.3, solubility in water at 25 °C: 2.3×10^{-4} M) as a model drug. Furthermore, the *in vivo* sustained-release characteristics of alginate gel beads were examined in human volunteers and compared with a commercial sustained-release formulation of pindolol, Carvisken®.

2. Investigations, results and discussion

2.1. Preparation of alginate gel beads containing pindolol

It is well known that the GG block of alginate polymer chain is predominantly responsible for divalent metal ions [7–9]. The intermolecular and intramolecular chelations between a divalent cation and alginate result in a gelation. Therefore, the molecular size of sodium alginates might affect gelation and the properties of the fully-cured alginate gel. In this study, three sodium alginates having different molecular sizes with nearly the same content of MM, GG and MG blocks were used (Table 1).

Alginate gel beads were prepared by dropping alginate solution into 0.1 M calcium chloride solution. When the droplets of alginate solution contact with calcium ions, the gelation of alginate started immediately by diffusion of calcium ions into an alginate droplet. Along with the progress of gelation, water within the alginate droplet is squeezed out into the bulk solution. Therefore, the rate of

Table 1: Composition and viscosity of sodium alginates used

Sodium alginate	Composition (%)			MM/GG Ratio	Viscosity* (cp)
	MM	GG	MG		
A	20.6	34.2	45.2	0.60	2.9
B	25.2	38.1	36.7	0.66	13.7
C	27.0	41.7	31.3	0.65	41.8

* 1% Sodium alginate solution at 25 °C

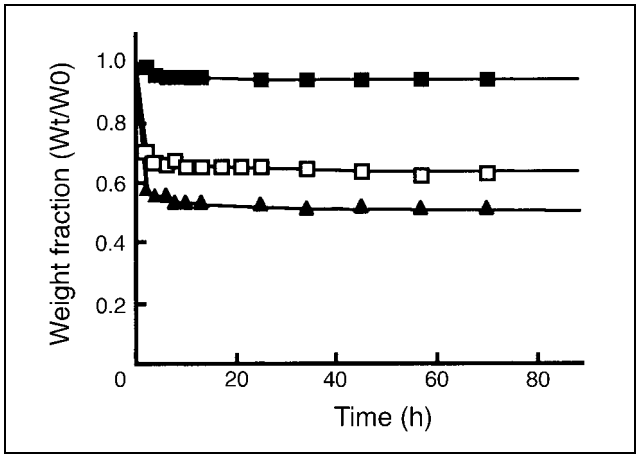

Fig. 1: Weight fraction change of alginate gel beads in 0.1 M $CaCl_2$ at 25 °C. Key: (■) bead A; (□) bead B; (▲) bead C

gelation can be represented by the weight changes of the beads [12]. Fig. 1 shows the weight change of the alginate gel beads. The weight of all beads rapidly decreased within 3 h after dropping alginate solution into 0.1 M calcium chloride; thereafter it decreased gradually. The initial rate of gelation of sodium alginate was 1.02, 1.02 and 1.28 h^{-1} for alginate A, B and C, respectively. However, the squeezed water remarkably increased with increasing molecular size of the alginate. In contrast to only 5% of exclusion of water from alginate A, 40 and 50% of water were squeezed from alginate B and C gel beads, respectively. The weight and radius of fully cured gel beads were larger in the order of alginate A > B > C, as shown in Table 2. The contents of calcium cation which retained in the beads after washing out the adsorbed calcium ions around the alginate gel were nearly the same in all alginates (42–47 μg/bead), that is, a calcium ion associated with 2.2–2.4 of uronate residues. When calcium ion constructs of an egg box structure with GG block, two gluronate residues are required for the interaction with a calcium ion. This clearly suggests that the MM and MG blocks in addition to the GG block also interact with calcium ions when the gelation of alginate was completely performed. The data from squeezed water and calcium content indicated that alginates with large molecular weight (high viscosity) might complicate the intermolecular and intramolecular cross-linkage. Furthermore, the trapped amount of drug in alginate gel was nearly equal in all beads.

2.2. In vitro release studies

The release of pindolol from gel beads prepared by sodium alginates A, B and C was studied at pH 1.2 and 6.8 to predict the release of pindolol in the gastrointestinal tract. All alginate gel beads showed a complete and immediate release of pindolol within 60 min at pH 1.2, although pindolol powder was completely dissolved within 5 min (Fig. 2). At pH 6.8, the release of pindolol from the beads was extensively depressed compared to pindolol

Table 2: Characteristics of alginate gel beads

Beads	Diameter (mm)	Weight (μg/bead)	Ca Content (μg/bead)	Pindolol content (%)
A	1.21	0.91	29.1	44.8
B	1.20	0.88	30.3	39.9
C	1.17	0.91	27.3	40.7

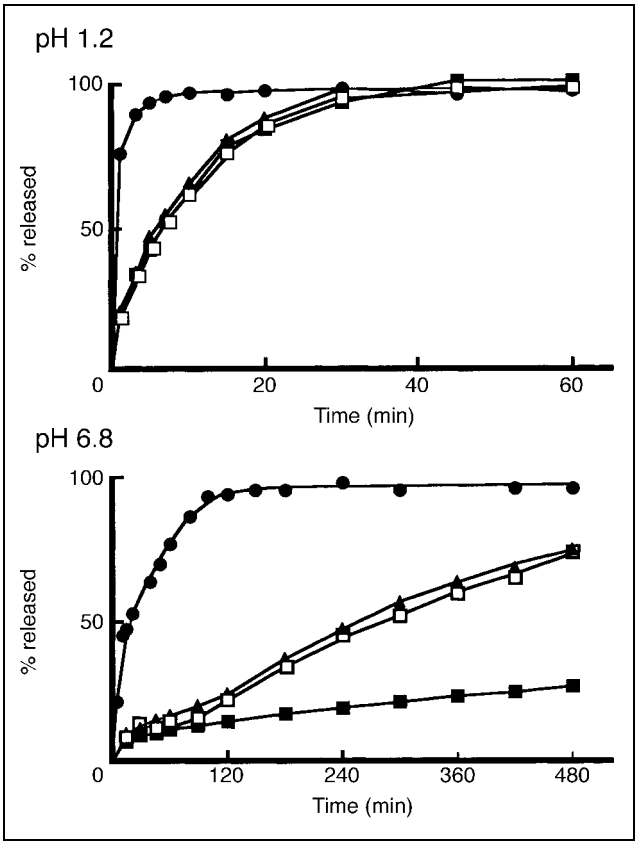

Fig. 2: Release of pindolol from alginate gel beads at pH 1.2 and pH 6.8, 37 °C. Key: (●) pindolol powder; (■) bead A; (□) bead B; (▲) bead C. The data represent the mean ± S.E. (n = 3)

powder. Interestingly, the slowest release rate of pindolol was observed for the beads prepared by the alginate with the smallest molecular size. The beads prepared by alginate B and C showed similar drug release profiles.

Previously, we demonstrated that prednisolone was released from alginate gel beads according to the diffusion of drug from an insoluble matrix of calcium alginate under acidic conditions where swelling of the bead scarcely occurred. The diffusion of prednisolone in non-swelled gel beads at pH 1.2 was depressed by the content of GG block, suggesting that the release of prednisolone (non-ionic form at pH 1.2), was dependent on the matrix structure of the alginate gel beads. On the other hand, under neutral conditions where the swelling of bead occurred, the release of prednisolone depended on the bead swelling, which decreased with increasing GG block content of the alginate [10]. Therefore, we studied the effect of swelling of the alginate gel beads on pindolol release. No bead swelled in pH 1.2 medium, but the gel beads prepared by alginate B and C swelled at pH 6.8. Fig. 3 shows the swelling behavior of the beads at pH 6.8.

The release of pindolol from beads in acidic medium was similar to that of prednisolone according to the drug diffusion from the insoluble polymer matrix of calcium alginate. In addition, pindolol was rapidly released from all the beads, because the solubility of pindolol with a pKa value of 9.3 is quite high at pH 1.2. These data indicated that the drug release from beads under acidic conditions may be explained by the diffusion of drug into the insoluble alginate gel matrix and that the diffusion properties of basic drugs were related to drug solubility rather than the structure of the alginate gel matrix. On the other hand, at pH 6.8 where swelling of the bead occurred, the release of both pindolol and prednisolone was enhanced with an in-

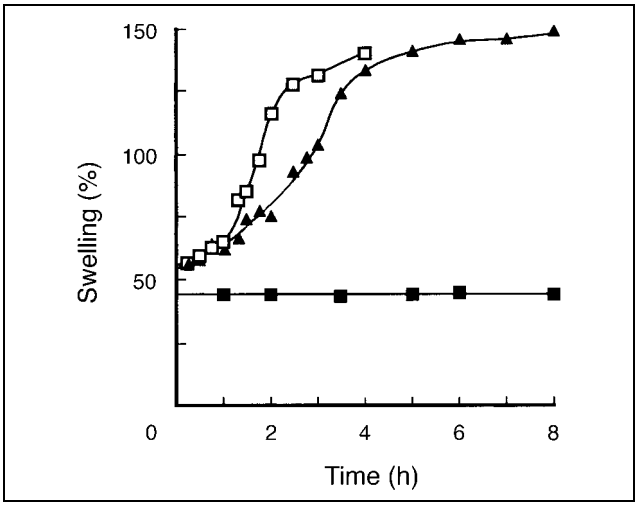

Fig. 3: Swelling of alginate gel beads in pH 6.8 medium at 37 °C. Key: (■) bead A; (□) bead B; (▲) bead C. The data represent the mean ±S.E. (n = 3)

crease in swellability of the bead. In general, the alginate gel was swellable in lower content of GG block, because GG block is strongly bound to calcium ion. However, the GG block content was nearly same among the alginates A, B and C. Although the reason for the poor swellability of bead A is unclear, the following two explanations may be considered: alginates of smaller molecular size can strongly bind to calcium ions and the dehydration of more than 90% of water from the beads during drying affects the gel structure. In addition, no interaction was observed spectrophotometrically between the alginate and pindolol at both pH values (data not shown). These results suggest that the drug release from bead under the neutral condition depends on the swelling of beads rather than the solubility and molecular interaction of alginate with pindolol.

2.3. In vivo absorption studies

The *in vivo* absorption study was performed to prove alginate gel beads as controlled release formulation. The serum level profiles of pindolol and its pharmacokinetic parameters after oral administration of pindolol powder and its alginate gel beads to beagle dogs are shown in Fig. 4 and Table 3. The alginate gel beads were administered once by double dose of pindolol powder, and pindolol powder was administered twice at 6 h interval. When the beads were administered, retarding of the absorption rate and the elimination rate of pindolol was observed compared to the administration of pindolol powder. The MRTs and the VRTs obtained from all beads were significantly larger compared with those from the administration of powder. The AUCs obtained from the administration of beads were not significantly different from the total AUC obtained from the twice administration of pindolol powder. The time-profiles of the serum level of pindolol after oral administration of beads were explained by *in vitro* release behaviors at pH 6.8 rather than those at pH 1.2. However, C_{max} and AUC after administration of beads decreased with increasing molecular size, which was different from the *in vitro* release in pH 6.8. Bead A with the smallest molecular size showed the fastest *in vivo* absorption rate, in spite of the slowest release rate *in vitro*. In the case of bead A, the physical strength of beads might be weak against the physical forced in the gastrointestinal tract, owing to the lowest molecular size. Therefore, *in vivo* absorption of drug in alginate gel beads might depend on the swelling of beads and their physical strength. Furthermore, the variances of serum level and AUC were larger after administration of beads A and C in comparison with bead B. From these results, bead B seems to be the most appropriate for a sustained-release formulation of pindolol.

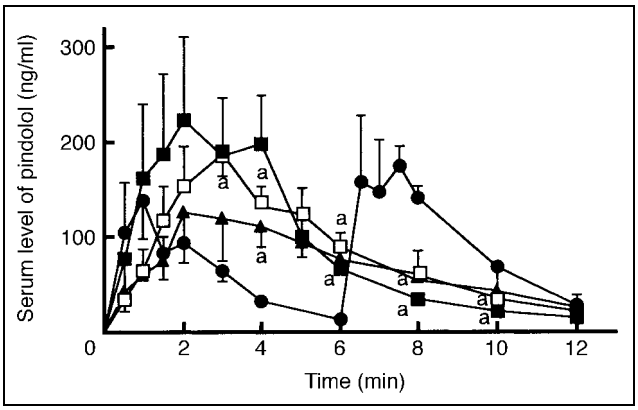

Fig. 4: Serum levels of pindolol after oral administration of alginate gel beads to beagle dogs. Key : (●) pindolol powder; (■) bead A; (□) bead B; (▲) bead C. The data represent the mean ±S.E. (n = 4). (a) $p < 0.05$ vs. pindolol powder; (b) $p < 0.05$ vs. beads B, C and D. Pindolol powder was twice administered 10 mg/kg at 6 h interval. The alginate gel beads were administered at a dose equivalent to 20 mg/kg pindolol

Table 3: Pharmacokinetic parameters for pindolol after oral administration of pindolol powder and its alginate gel beads to 4 beagle dogs

Dosage form	AUC_{0-12} (h · ng/ml)	MRT (h)	VRT (h^2)
Powder	922.2 ± 72.1[a]	2.27 ± 0.20[b]	1.80 ± 0.11[b]
Beads A	1108.5 ± 254.6[c]	4.24 ± 0.49[c]	6.53 ± 1.10[c]
Beads B	989.3 ± 51.9	4.65 ± 0.14[c]	6.89 ± 0.98[c, e]
Beads C	931.7 ± 187.2	4.79 ± 0.66[c]	7.85 ± 1.43[c, e]

[a] Total of twice administrations; [b] Average of twice administrations; [c] $p < 0.05$ vs. pindolol powder; [d] $p < 0.05$ vs. bead C; [e] $p < 0.05$ vs. bead B

2.4. Comparison of the absorption of pindolol in alginate gel beads and a commercial sustained-release tablet

The usefulness of alginate gel beads as sustained-release vehicles drug was evaluated by *in vitro* release and *in vivo* absorption studies of pindolol in human volunteers. The results were compared with those for a commercial sustained-release tablet, Carvisken®. The alginate gel bead B was selected because it was the most appropriate sustained-release formulation in the *in vivo* absorption study in beagle dogs. Fig. 5 shows the *in vitro* release profiles of pindolol from alginate gel bead B and Carvisken®. In contrast to the alginate gel beads, only 50% of pindolol was released from Carvisken® in pH 1.2 medium, although the initial release rate of pindolol was the same for both formulations. Pindolol was immediately released from Carvisken® at pH 6.8 like pindolol powder. These data were explained on the basis that Carvisken® consisted of two phases a rapidly releasing outer phase and an enteric dissolving core.

Although the *in vitro* release characteristics were quite different between alginate gel beads and Carvisken® both formulations showed an appropriate sustained absorption profile after oral administration to human volunteers (Fig. 6). Pindolol powder was administered at half the dose of the sustained releasing formulations. When pindo-

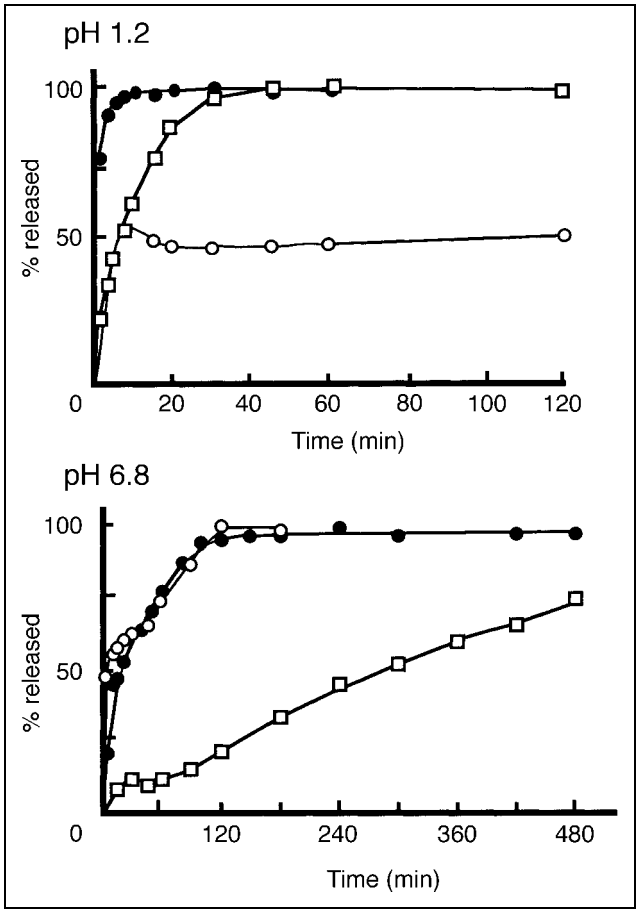

Fig. 5: Release of pindolol from alginate gel beads B and the commercial sustained-release tablet, Carvisken®. Key: (●) pindolol powder; (□) bead B; (○) Carvisken®. The data represent the mean ±S.E. (n = 3)

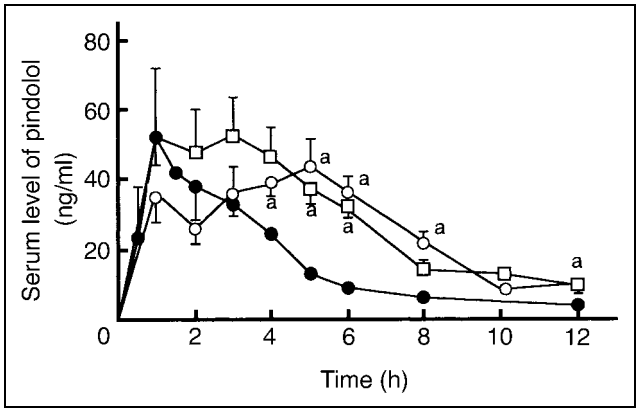

Fig. 6: Serum levels of pindolol after oral administration of powder, alginate gel bead B and Carvisken® to human volunteers. Key: (●) pindolol powder; (□) bead B; (○) Carvisken®. The data represent the mean ±S.E. (n = 4). (a) $p < 0.05$ vs. pindolol powder

lol powder was administered, the serum level of pindolol reached a maximum at 1 h after administration, then diminished gradually. On the other hand, after the administration of the sustained-release formulations, the serum levels of pindolol reached a level comparative to the C_{max} value after administration of pindolol powder and maintained within the effective therapeutic level (10–50 ng/ml) for a period of up to 10 h. As shown in Table 4, the MRTs of two sustained-release formulations were significantly different from pindolol powder, whereas the significant difference was not observed in AUC obtained from each formulation. These results suggest that bead B has

Table 4: Pharmacokinetic parameters for pindolol after oral administration of pindolol powder, alginate gel bead C and Carvisken®

Dosage form	AUC_{0-12} (h · ng/ml)	MRT (h)	VRT (h^2)
Powder	377.5 ± 48.5[a]	3.82 ± 0.32	7.44 ± 0.54
Beads C	336.3 ± 43,3	4.66 ± 0.42[b]	8.16 ± 0.47
Carvisken®	305.7 ± 25.7	5.07 ± 0.30[b]	7.49 ± 0.37

[a] Corrected for the dose by multiplying by 2; [b] $p < 0.05$ vs. pindolol powder

excellent sustained-release characteristics for pindolol which is comparable to that of Carvisken®.

In conclusion, the release of the ionic drug from alginate gel beads depends on its solubility under the gel unswellable condition, and on the swelling property of alginate gel bead under the gel swellable condition. The alginate gel beads behaved as sustained-release vehicles for a basic drug as known for neutral and acid drugs [10, 11, 12]. The alginate gel bead formulations of pindolol demonstrated satisfactory sustained-release characteristics *in vivo* comparable to a commercial sustained-release tablet.

3. Experimental

3.1. Materials

Sodium alginates were donated by Kimitsu Chemical Industries Co., Ltd. (Tokyo, Japan). The physicochemical properties of sodium alginates used in this study are listed in Table 1. Pindolol was purchased from Sigma Chemical Company (St.Louis, MO). The commercial sustained-release tablet containing 20 mg of pindolol, Carvisken®, was purchased from Sankyo Co., Ltd. (Tokyo, Japan). All other reagents and solvents were of analytical grade, and deionized-distilled water was used throughout the study.

3.2. Determination of the gelling rate of alginate gel beads

The gelling rate of sodium alginate was determined from the weight change of alginate gel beads as described by Yotsuyanagi et al.[14]. After dropping of 4% (w/w) sodium alginate solution into 0.1 M calcium chloride, the 10 pieces of beads were taken out periodically and weighed after removing of the surface moisture on a filter paper.

3.3. Preparation of alginate gel beads

Four g of pindolol powder (<100 mesh) were homogeneously suspended in 100 ml of 4% (w/w) sodium alginate. The suspension was dropped using a nozzle (0.65 mm i.d.) into gently agitated 0.1 M calcium chloride solution. The beads were allowed to stand in the solution for 72 h to be fully cured. The separated and washed beads were dried in air for 48 h, followed by-drying *in vacuo* at room temperature for 24 h. The alginate gel beads were washed well by water to take off calcium around the beads. Then the calcium content was measured by means of a polarized Zeeman atomic absorption spectrophotometer (Z-8000 type, Hitachi Ltd., Tokyo, Japan) after ashing with nitric acid.

3.4. In vitro release studies

The dissolution of pindolol powder (<100 mesh) and the release of pindolol from alginate gel beads (equivalent to 20 mg drug) as well as Carvisken® tablets (content of pindolol: 20 mg) were examined according to the paddle method of the Japanese Pharmacopeia XIII dissolution test (100 rpm, 37 °C) using 1000 ml of 1st fluid (pH 1.2) and 2nd fluid (pH 6.8) for disintegration test. A 5 ml aliquot was withdrawn periodically from a glass tube with a glass filter in the middle of the flask and immediately filtered through a 0.45 μm membrane filter (Tokyo Roshi kaisha, Ltd., Tokyo, Japan). The same volume of fresh medium was added to the test medium. The concentration of pindolol in the filtrate was determined spectrophotometrically at 264 nm.

3.5. In vivo absorption studies in beagle dogs

Male beagle dogs (3–5 years, 10–13 kg, non-sterilization) were fed a liquid diet (Besvion; Snow Brand Milk Product Co., Ltd., Tokyo, Japan) for 2 days before being fasted for 24 h. Pindolol powder (2.5 mg/kg, <100 mesh) and alginate gel beads containing pindolol (equivalent to 5 mg/kg of pindolol), filled in gelatin capsules (size No. 0) were administered orally together with 20 ml of water. At appropriate intervals, 6 ml blood sample were withdrawn from the cephalic vein and centrifuged for 30 min at 1,200 × g to obtain serum. During the experimental period, no

food was allowed, but water was available *ad libitum*. The drug samples were administered according to a randomized cross-over design with a 2 weeks washout period.

The analytical procedures for pindolol in serum were as follows. Two ml of serum was vortexed with 1 ml of 1 M sodium carbonate and 6 ml of ether for 10 min. After the mixture was centrifuged at 1,500 × g for 5 min, the ethyl ether phase was taken in a tube containing 2 ml of 0.6 M phosphate buffer (pH 7.5). The mixture was mixed for 10 min, then centrifuged again at 1,500 × g for 5 min. After the ethyl ether phase was discarded, 0.4 ml of 5 N sodium hydroxide and 6 ml of ethyl ether were added to the aqueous phase, then the mixture was vortexed for 10 min and centrifuged at 1,500 × g for 5 min. At each step, the ethyl ether was separated from the aqueous phase by decantation after freezing at −30 °C. The ethyl ether phase was evaporated along with 20 µl of the methanolic solution of acetaminophen (10 µg/ml) as an internal standard and the residue was redissolved in 100 µl of mobile phase of HPLC, 50 µl of which was injected for the determination of pindolol. The HPLC conditions for the determination of pindolol were as follows: pump and UV detector, L-6000 type and 655A type (Hitachi Ltd., Tokyo, Japan); column, LiChrospher RP-Select B (7 µm, 250 × 4 mm i.d., Cica Merck Co., Inc., Tokyo, Japan); mobile phase, 0.02 M KH_2PO_4: methanol (73 : 27); flow rate, 0.7 ml/min; detection, 264 nm. The limitation of determination for pindolol in serum was 2 ng/ml and the coefficients of variation at 5 ng/ml for within day and day-to-day were 3.2 and 4.3%, respectively.

3.6. In vivo absorption study in human volunteers

Four healthy volunteers (23 year, 55–78 kg, non-smokers) fasted for 10 h before the drug was administered in a randomized cross-over manner. The volunteers took pindolol powder (10 mg, <100 mesh), an alginate gel bead C containing pindolol (equivalent to 20 mg of pindolol) and a Carvisken® tablet together with 150 ml of water. The volunteers took a light meal (120 g croissant bread, 300 ml of orange juice and 100 g of apple) at 6.5 h after administration of drug. Blood samples were taken at appropriate intervals and were centrifuged at 1,200 × g for 30 min to obtain serum. The pindolol concentration in serum was determined by HPLC as described above (3.3.).

3.7. Pharmacokinetic analysis

The area under the serum concentration-time curve (AUC) was calculated by the trapezoidal method. Mean residence time (MRT) and variance of residence time (VRT) were calculated using statistical moment analysis [15].

3.8. Statistical analysis

All data are represented as mean ±S.E. The results were evaluated for statistically significant differences by the Dancan's multiple comparison test after a one-way analysis of variance (ANOVA). The probability value was set at 5%.

This research paper was presented during the 2nd Conference on Retrometabolism based Drug Design and Targeting, May 11–14, 1999, Amelia Island, Florida, USA

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