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Possibility of a patch system as a new oral delivery system

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Abstract

A new oral patch system has been designed to increase the residence time of model drugs within the gastrointestinal tract. The system consisted of three layers (1) water-insoluble backing layer (2) drug-carrying adhesive layer composed of a model drug, fluorescein (FL) or fluorescein isothiocyanate-dextran (FD), and gel-forming polymer and (3) pH-sensitive enteric polymer. These three layers system was prepared as 3.0 mm diameter patches. As references, tablet containing FL or FD was prepared. In vitro dissolution studies showed that the mean dissolution time (MDT) of model drugs from patch preparation was 0.739 ± 0.021 h for FL and 0.407 ± 0.021 h for FD, which were longer than from tablet, 0.327 ± 0.008 h for FL and 0.270 ± 0.019 h for FD. The two test preparations were orally administered to beagle dogs in a crossover manner at a FL dose of 30 mg/dog and the measured plasma FL concentrations were used for pharmacokinetic analysis. With FL patch preparation, area under the plasma drug concentration vs. time curve (AUC) was 2.12 ± 0.24 μg h/ml and mean residence time (MRT) was 4.60 ± 0.18 h, which were greater than those of tablet, AUC was 1.52 ± 0.16 μg h/ml and MRT was 3.18 ± 0.09 h, respectively. Oral patch preparation also increased both AUC and MRT of FD, a model macromolecular drug, which was formulated into both patches and tablets and administered to dogs (30 mg/dog). The AUC and MRT of FD from the patch preparation were 1.11 ± 0.13 μg h/ml and 5.58 ± 0.55 h and from tablets were 0.53 ± 0.08 μg h/ml and 4.09 ± 0.29 h, respectively. These results suggest that oral patch preparation has a potential as a new oral delivery system to obtain a long residence time of drug in the gastrointestinal tract.

Keywords: Patch preparation; Oral delivery; Absorption; Mean residence time; Fluorescein; Fluorescein isothiocyanate-dextran; Rats

1. Introduction

In the past few decades, scientists have tried to deliver protein drugs orally by various dosage forms such as azo-polymer coated gelatin capsule (Saffran et al., 1986), microemulsion (Patel et al., 1991; Morishita et al., 1998), liposomes (Takeuchi et al., 1996, 2001; Claudio et al., 2000), microspheres (Damge et al., 1990; Paolo et al., 2000) and

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the shuttle system (Farid et al., 2001). We have been also studying a patch system for the oral delivery of protein drugs. The patches consisted of three layers (1) the backing layer made of a water-insoluble polymer, (2) the drug-carrying layer, and (3) the enteric polymer membrane. The potential of the oral patch preparation as a new oral protein delivery system was demonstrated with granulocyte-colony-stimulating factor (G-CSF) in our previous study (Eaimtrakarn et al., 2001a), where three layers film system was used. By means of film patch, a pharmacological availability of 23% was obtained for G-CSF as compared to the i.v. injection of the same dose of G-CSF in dogs. This was the highest value among our other delivery systems including colon delivery system and enteric effervescent system (Takada et al., 1989; Takada and Ushirogawa, 1991; Takada et al., 1994; Ushirogawa et al., 1992; Takaya et al., 1995). The adhesion efficiency of the film patches expressed as the retention and the transit of the patches in the small intestine was also studied in rats (Eaimtrakarn et al., 2001b). The results showed that film patches adhered to the rat small intestinal wall. The adhesion site was found to depend on the dissolution pH of the enteric polymer layer of the film patch and a retention time of more than 2.0 h at the small intestinal adhesion site was observed.

In the above studies, film patches were prepared and used for in vivo evaluation experiments. As suggested from the G-CSF study, the loading drug dose was low, i.e. the oral G-CSF does was 125 μg/dog, because of the limited drug loading space of the film patches. However, other macromolecular drugs such as heparin need much clinical dose than G-CSF. Therefore, to solve this problem, three layers patch system having larger drug loading space has been designed. Namely, among the three layers, the space of the drug-carrying layer composed of drug and gel-forming polymer has been increased and the patch system was prepared as follows: At first, the drug-carrying layer made of both gel-forming polymer and drug was spread onto the surface of water-insoluble backing layer. The pH-sensitive enteric surface layer was next covered on the drug-containing layer. The three-layered preparation was sealed and cut into 3-mm diameter patches by a heat-sealing punching method. Finally, the patches were filled into gelatin capsules.

To perform an early-stage evaluation of this system, model drugs like fluorescein (FL) and fluorescein isothiocyanate (FITC)-dextran (FD) were used and patch preparations containing FL or FD were prepared. To evaluate the gastrointestinal transit characteristics of this new oral preparation in the dog, tablets containing the same amount of model drugs were used as reference preparations.

2. Materials and methods

2.1. Materials

Hydroxypropyl methylcellulose phthalate (HP-55) was obtained from Shin-etsu Chemical Industry Co., Ltd (Tokyo, Japan). Carboxyvinyl polymer (Carbopol® 974P) was obtained from Chugai Boyeki Co., Ltd (Tokyo, Japan). Ethylcellulose (ETHOCEL$_{PREMIUM}$, 100 cps) (EC) was obtained from Nissin Kasei, Co., Ltd (Osaka, Japan). Polyoxyethylated, 60 mol, hydrogenated castor oil derivative (HCO-60®) was supplied by Nikko Chemicals Co., Ltd (Tokyo, Japan). Citric acid (CA), sodium hydrogen carbonate, disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Microcrystalline cellulose (Avicel®PH 101) was obtained from Asahi Kasei Co., Ltd (Osaka, Japan). FL, sesame oil and saccharose were purchased from Kanto Chemicals Co., Ltd (Tokyo, Japan). FITC labeled dextran (FD, MW. 4,400) was obtained from Sigma Chemical (St. Louis, USA). All other chemicals used were reagent grade and used as received without further purification. Hard gelatin capsules, size #00, were obtained from Yoshida Co., Ltd (Himeji, Japan). Male beagle dogs and standard solid meal of commercial food (Labo D stock®) were obtained from Nippon Nousan Co., Ltd (Yokohama, Japan). The film spreading apparatus (Bakerapplicator®) and the heat-sealing punching equipment were developed in our depart-

ment in technical collaboration with Imoto Seisakusho Co., Ltd (Kyoto, Japan).

2.2. Methods

2.2.1. Preparations

Three layer patches were prepared as follows: The backing layer made of EC was prepared by a solvent evaporation technique. EC solution (20%w/v) was made by dissolving EC in a mixture of methylene chloride and methanol (4:1). Bakerapplicator® was used for spreading EC solution on a Teflon plate; 30×30 cm^2. EC film was obtained after solvent evaporation at room temperature for 5 min. The thickness of the EC film obtained was 23.6 ± 1.5 μm. The enteric polymer membrane was also prepared by the same method as described above using HP-55 solution (12%w/v). The thickness of the enteric film was 16.9 ± 1.1 μm. The drug-carrying layer was prepared by mixing 15 mg of Carbopol, 30 mg of FL, 160 mg of CA and 100 mg of HCO-60. In the case of FD patches, 30 mg of FD, 7.5 mg of Carbopol, 125 mg of CA and 75 mg of HCO-60 were mixed as mentioned above. After mixing well, the drug-carrying layer was weighed. Then, it was uniformly spread on the surface of the backing layer (5×5 cm^2). The pH-sensitive surface layer was covered on the drug-carrying layer. The three-layered preparation was sealed and cut into 3-mm diameter patches by a heat-sealing punching equipment. The patches (120 patches) were filled into gelatin capsule of size #00 along with 400 mg of effervescent powder, the mixture of CA and sodium hydrogen carbonate (5.5:4.5), and 0.5 ml of sesame oil. As reference, tablet was prepared by mixing 30 mg of FL or FD with 350 mg of microcrystalline cellulose (Avicel®PH101) and directly compressed into a tablet size 0.5 inches diameter at a compression force of 0.4 ton using a hand press machine (Shimadzu model SSP-10A, Japan).

2.2.2. In vitro dissolution test

The in vitro dissolution tests on FL or FD containing patch and tablet preparations were performed using a JP XIII paddle apparatus (50 rpm) with 900 ml of pH 7.4 phosphate buffer, maintained at 37 °C, as the dissolution medium. Samples of 5.0 ml were collected at 5, 10, 15, 20, 30, 60, 120 and 180 min and were replaced with 5.0 ml of fresh medium. The time to 50% dissolution of model drug into the dissolution medium, $T_{50\%}$, was determined from percent drug dissolved vs. time profiles. All the experiments were carried out in triplicate. Samples were measured spectrofluorometrically at an excitation wavelength of 468 nm and an emission one of 512 nm after suitable dilution using a Shimadzu RF-1500 spectrofluorophotometer (Kyoto, Japan).

2.2.3. Pharmacokinetic studies of FL and FD from test preparations after oral administration to dogs

Adult beagle dogs weighing 10.3–12.7 kg were fasted overnight for at least 12 h with free access to water. Test preparations were administered at 10:00 A.M. with 20 ml of warm water in a cross-over manner with 1 week washout period. At 30 min before drug administration, a control blood sample (1.0 ml) was taken from the jugular vein. At 8 h after administration, a solid meal of commercial food (450 g) was given. Water was given ad libitum and no additional food was given during the study. All the experiments were carried out at the same time of the day to exclude the influence of circadian rhythm. After oral administration of the test preparations, 1.0 ml of blood samples were obtained from the jugular vein at 0, 1, 2, 3, 4, 5, 6, 8 and 10 h. The plasma fraction used for FL or FD assay was obtained by centrifuging the blood samples at 12 000 rpm for 10 min.

2.2.4. Analytical methods

2.2.4.1. Plasma FL concentration. The FL concentration in the plasma was determined by a spectrofluorometry. To 200 μl of dog plasma sample, 0.5 ml of methanol was added. After mixing well, the resulting mixture was centrifuged at 12 000 rpm for 10 min. One milliliter of 0.1 N NaOH solution and 2 ml of distilled water were added to the supernatant and the fluorescence intensity was measured using a Shimadzu RF-1500 spectrofluorometer at an excitation wavelength of 468 nm and an emission one of 512 nm.

2.2.4.2. Plasma FD concentration. The concentration of FD in the plasma was determined according to the method of Sakai et al. (1999) by an HPLC system (Shimadzu LC-10AS liquid chromatograph), using a fluorescence detector (Shimadzu RF-10AXL) at an excitation wavelength of 491 nm and an emission one of 520 nm. The analytical column was Chemcosorb 5-ODS-H. The mobile phase consisted of 5 mM phosphate buffer, pH 7.4, and acetonitrile (88:12) and the flow rate was 1 ml/min. Methanol (1.0 ml) was added to 200 μl of plasma, mixed well, and centrifuged. The supernatant was transferred to a new tube and evaporated to dryness. The residue was dissolved with 200 μl of mobile phase and 100 μl of the reconstituted sample was injected into HPLC system.

2.2.5. Pharmacokinetic analysis

The mean dissolution time (MDT) of FL or FD from the test preparations was calculated by a moment analysis method (Tanigawara et al., 1982). Pharmacokinetic parameters were determined from the plasma FL or FD concentration vs. time data. The maximum drug concentration (C_{max}) and the time to reach maximum concentration (T_{max}) were obtained as measured values. The area under the plasma drug concentration vs. time curve (AUC) and the area under the first-moment vs. time curve (AUMC) after oral administration were calculated using the linear trapezoidal rule up to the last measured drug concentration. The mean residence time (MRT) after oral administration was calculated by AUMC/AUC.

2.2.6. Statistics

All values are expressed as their mean ± SE. Differences in pharmacokinetic parameter values of FL or FD between two preparations were statistically evaluated by one side student's *t*-test. Statistical differences were assumed to be significant when $P < 0.05$.

3. Results and discussion

The results of the dissolution studies of FL from the patch and tablet preparations containing FL, 30 mg are shown in Fig. 1. The dissolution rate of FL from patch preparation was slower than that from tablet. The time to 50% dissolution, $T_{50\%}$, was 27.0 ± 4.5 min for patch preparation and 12.8 ± 0.5 min for tablet. Approximately 80% of FL in the patch preparation was dissolved into the dissolution medium by 0.5 h and thereafter complete dissolution was obtained within 1 h. The mean dissolution time, MDT, of FL from the test preparations was 0.739 ± 0.021 h for patch preparation and 0.327 ± 0.008 h for tablets. These results indicate a little bit slow dissolution of FL from patch preparation. Fig. 2 shows the result of the dissolution study on FD preparations. From the dissolution pattern, the difference between the two curves was very small. The MDT of FD was 0.407 ± 0.021 h for patch preparation and 0.270 ±

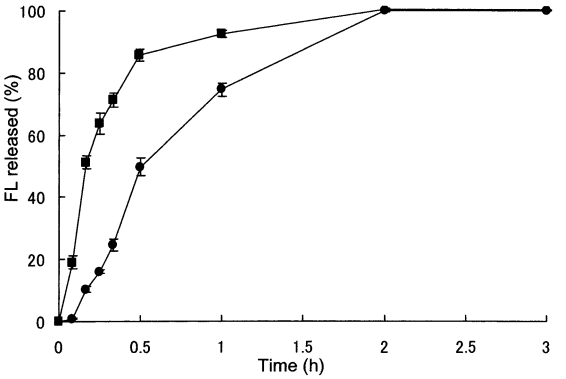

Fig. 1. Dissolution profiles of FL, from (●) patch and (■) tablet preparations. Each point shows the mean ± SE.

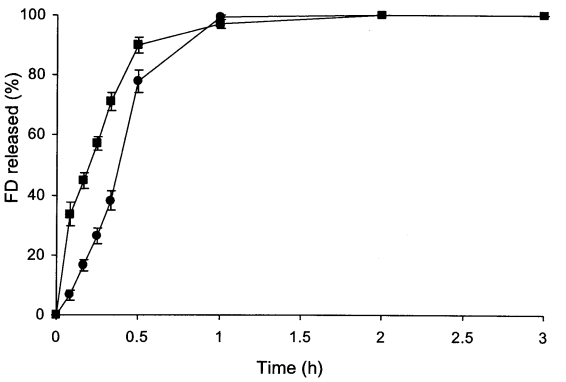

Fig. 2. Dissolution profiles of FD from (●) patch and (■) tablet preparations. Each point shows the mean ± SE.

0.019 h for tablet. As compared to the FL patch preparation, faster dissolution of FD was obtained from the patch preparation.

After establishing the in vitro dissolution characteristics of the test preparations, pharmacokinetic studies were carried out in beagle dogs. The two test preparations were orally administered to four beagle dogs at the FL dose of 30 mg/dog. The plasma FL concentrations vs. time profiles are shown in Fig. 3. The calculated pharmacokinetic parameter values are shown in Table 1. In the case of FL tablets, mean C_{max} was 0.42 ± 0.05 μg/ml, T_{max} was 2.25 ± 0.23 h, AUC was 1.52 ± 0.16 μg h/ml and MRT was 3.18 ± 0.09 h. However, with FL patch preparation, C_{max} was 0.39 ± 0.07 μg/ml, T_{max} was 2.86 ± 0.61 h, AUC was 2.12 ± 0.24 μg h/ml and MRT was 4.60 ± 0.18 h. Although there was no significant difference in the C_{max} and T_{max} between patch and tablet preparations, AUC was significantly increased, about 1.4 times, with the patch preparation as compared to the tablet. As the patch system was composed of three layers and the surface layer was made of enteric polymer film, HP-55, absorption lag-time of approximately 1 h was detected. HP-55, a pH-sensitive polymer, was used as the enteric polymer membrane to control the dissolution of the patches at the small intestinal absorption site. As the dissolution threshold pH of HP-55 is 5.5, the surface layer of the patches was thought to be dissolved just after they were transferred into the small intestine, because the luminal pH of the upper small intestine was reported to be higher than 6.0 (Helliwell 1993). Therefore, the dissolution of FL from the patches was prevented in the stomach. After the patches entered into the small intestine, the surface enteric layer, HP-55, was dissolved and consequently FL was released there. Therefore, an absorption lag-time of 1.0 h was obtained with patch preparation.

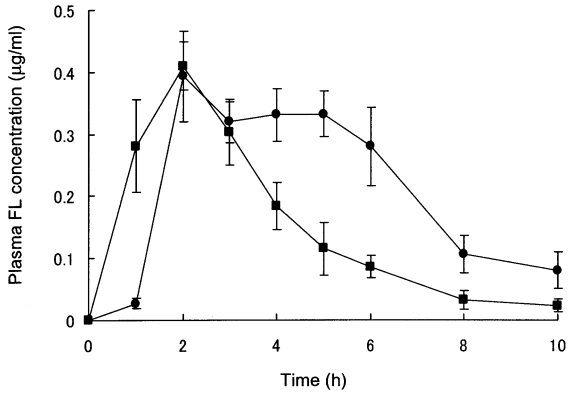

Fig. 3. Mean plasma FL concentration vs. time profiles obtained after oral administration of (■) tablet and (●) patch preparations to four beagle dogs at a FL dose of 30 mg. Each point shows the mean ± SE.

Table 1
Pharmacokinetic parameters of FL after oral administration of tablet and patch to dogs

	Tablet	Patch
C_{max} (μg/ml)	0.42 ± 0.05	0.39 ± 0.07
T_{max} (h)	2.25 ± 0.23	2.86 ± 0.61
AUC (μg h/ml)	1.52 ± 0.16	2.12 ± 0.24*
MRT (h)	3.18 ± 0.09	4.60 ± 0.18*

C_{max}, the maximum plasma concentration of FL; T_{max}, the time when FL reached to its maximum value; AUC, the area under the FL concentration vs. time curve; MRT, mean residence time.

* Shows significant difference against tablet ($P < 0.05$).

Heinamaki et al. (1988) reported that Eudragit L-Eudragit E tablets (enteric tablets) were found in the distal regions of the dog stomach within 1.5 h under fasted condition. Eudragit L is also used as an enteric coating material and its dissolution threshold pH is 6.0. On the other hand, the gastric emptying time (GET) of solid preparation in dogs was reported by Hayashi et al. (1999). According to their results, approximately 90% of the granules were emptied within 1 h after administration to dogs even in the fed state. By taking these results into the consideration, we may state that the patches did not disintegrate in the dog stomach and FL was released from the patches after the patches were transferred into the small intestine. Thereafter, FL was absorbed into the systemic circulation and was detected. As three layers patch system was used in this study, the drug-carrying layer appeared after the surface layer of patches was dissolved and adhesion of the drug-carrying layer to the small intestine was thought to occur, because adhesive polymer, Carbopol, was formulated into the drug-containing layer (Machida et al., 1979). Mucoadhesive polymers showed their

ability to retain drugs in close contact with mucosal membrane for long periods of time (Long et al., 1985; Lee et al., 2000; Lehr et al., 1990, 1992). In this study, the increase in AUC of FL was ascribed to the increased residence time of patches at the absorption site in the small intestine as the MRT of FL from patch preparation was about 1.5 times increased than tablet. Also, the MRTs corrected with the dissolution process, MRT–MDT, were 3.86 h for patch preparation and 2.85 h for tablet. The adhesive polymer used, Carbopol, was thought to form gel structure in the small intestine. Consequently, adhesion to the intestinal wall was thought to prolong the residence time of FL by patches.

FD, a model macromolecular compound having a molecular weight of 4 kDa was used for evaluating the oral patch preparation. Fig. 4 shows the plasma FD concentration vs. time profiles obtained after oral administration of patch and tablet preparations containing 30 mg of FD to dogs. The pharmacokinetic parameter values are shown in Table 2. The patch preparation and tablet had C_{max} values of 0.20 ± 0.07 and 0.16 ± 0.05 μg/ml and T_{max} values of 4.75 ± 0.48 and 2.50 ± 0.29 h, respectively. AUC and MRT values of the patch preparation were 1.11 ± 0.13 μg h/ml and 5.58 ± 0.55 h, respectively, which were significantly higher than that of tablet (AUC $= 0.53 \pm 0.08$ μg h/ml, MRT $= 4.09 \pm 0.28$ h). The values of

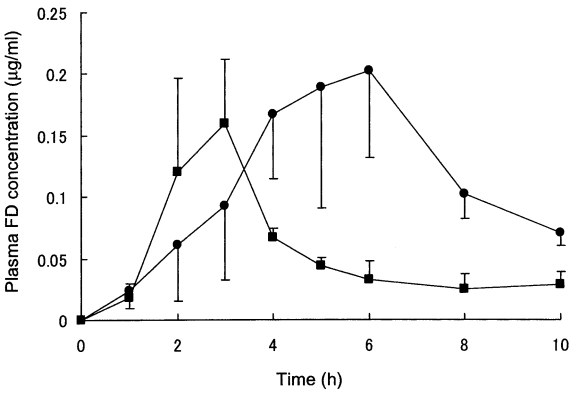

Fig. 4. Mean plasma FD concentration vs. time profiles obtained after oral administration of (■) tablet and (●) patch preparations to four beagle dogs at a FD dose of 30 mg. Each point shows the mean ± SE.

Table 2
Pharmacokinetic parameters of FD after oral administration of tablet and patch to dogs

	Tablet	Patch
C_{max} (μg/ml)	0.16 ± 0.05	0.20 ± 0.07
T_{max} (h)	2.50 ± 0.29	4.75 ± 0.48
AUC (μg h/ml)	0.53 ± 0.08	1.11 ± 0.13*
MRT (h)	4.09 ± 0.28	5.58 ± 0.55*

C_{max}, the maximum plasma concentration of FD; T_{max}, the time when FD reached to its maximum value; AUC, the area under the FD concentration vs. time curve; MRT, mean residence time.

* Shows significant difference against tablet ($P < 0.05$).

MRT–MDT were 5.17 h for patch preparation and 3.82 h for tablet. These results suggest that higher relative bioavailability of FD was obtained with patch preparation by increasing the residence time of the patches in the small intestine. Therefore, we may state that three layer patch system can be applied to a macromolecular drug to increase the retention and transit in the gastrointestinal tract. Based on the obtained results of these basic studies, we are now developing an equipment to produce large amount of patches for the oral delivery of protein and peptide drugs.

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