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International Journal of Pharmaceutics 137 (1996) 225–232

international journal of pharmaceutics

Influence of physical factors in gastrointestinal tract on acetaminophen release from controlled-release tablets in fasted dogs

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Received 14 July 1995; revised 7 December 1995; accepted 12 March 1996

Abstract

Four types of controlled-release (CR) acetaminophen (AAP) tablets, namely A, B, C and D, were prepared to investigate the influence of physical factors in the gastrointestinal (GI) tract on AAP release in fasted dogs. CR-A was designed as a completely resistant formulation to mechanical destructive force but to show an agitation speed-dependent dissolution rate. CR-B, CR-C and CR-D were designed to have different wet strengths but to show similar dissolution rates. The absorption profiles of the four CR forms in dogs showed biphasic patterns, with phase change about 2 h after oral dosing. The first phase of the absorption profile of CR-A and in vivo release directly observed were close to its in vitro profiles at a speed of 25–50 revs./min, indicating that agitation intensity in the dog GI tract may be relatively low. The first phase of the absorption profiles of the CR-B, CR-C and CR-D differed from each other, despite the fact that dissolution rates in vitro were similar. The tablet with low wet strength showed a faster absorption rate, indicating that it would be destructed by GI mechanical forces. Furthermore, absorption during the second phase was extremely low for all CR tablets. We confirmed on necroscopy that the suppression of drug absorption in the second phase was caused by the termination of AAP release from the tablets in the colon. These results will be useful in evaluating the in vivo performance of CR tablets in fasted dogs.

Keywords: Acetaminophen; Controlled-release; Colonic release; Gastrointestinal transit; Mechanical destructive forces; Agitation intensity

1. Introduction

In vitro dissolution tests which can predict in vivo drug release from controlled-release (CR) dosage forms may serve not only as quality control checks, but also as development tools in obtaining desired in vivo release behavior. However, because drug release from CR dosage forms is affected by various gastrointestinal (GI) factors, in vitro drug dissolution from CR dosage forms is not always similar to that in vivo. Among these factors, while the effect of pH on drug release has been extensively investigated, little is known about physical factors in GI motility such as agitation intensity (Katori et al., 1995) and me-

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PII S0378-5173(96)04524-3

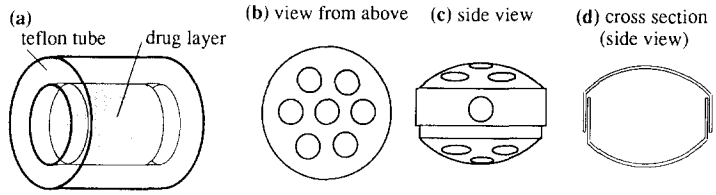

Fig. 1. Projected structure of CR-A (a) and structure of PVC case (b–d).

chanical destructive forces (Aoki et al., 1993). Investigation of the effect of physical factors on various CR dosage forms therefore requires the establishment of in vitro systems which can predict in vivo release behavior. Moreover, the bioavailability of CR dosage forms is also affected by GI transit time (Cressman and Sumner, 1971; Uchida et al., 1986). As CR dosage forms move down the GI tract, absorption and release of drugs in the lower GI play important roles in extending the absorption and enhancing the bioavailability of CR forms. Investigation of drug release behavior in the colon from CR forms is therefore also important.

The beagle dog is widely used as an animal model in pharmacokinetic study of oral dosage forms. Most pharmacokinetic studies are conducted under fasting conditions. In this study, we investigated the influence of agitation intensity and mechanical destructive forces on drug release from CR tablets in fasted dogs using four types of CR tablet with differing in vitro characteristics. We also investigated colonic release from these CR tablets.

2. Materials and methods

2.1. Materials

Acetaminophen (AAP) was purchased from Yoshitomi Pharmaceutical Industries Ltd. (Japan). Hydroxypropylmethylcellulose 2910 (HPMC, viscosity 9 cps and 50 cps) and ethylcellulose (EC, viscosity 10 cps) were obtained from Shin-Etsu Chemical Co., Ltd. (Japan). Other reagents used were of analytical reagent grade.

2.2. Preparation of CR tablets

Four different AAP 100 mg CR tablets were prepared. CR-A: AAP and HPMC-9cps were mixed (0.35:1) in a mortar, and the mixtures were compressed directly into a cylindrical matrix core using 5-mm diameter flat-faced punches. The matrix core was inserted into a teflon tube of 5 mm inner and 7 mm outer diameter and 8 mm length (Fig. 1a). CR-B: AAP and HPMC-50cps were mixed (1:1) in a mortar, and the mixtures were compressed directly using 8-mm diameter round-faced (8 mmR) punches. CR-C: AAP and EC ground with a jet mill were mixed (1:0.5) in a mortar, and the mixtures were compressed directly using 8-mm diameter round-faced (8 mmR) punches. CR-D: CR-C was covered with a polyvinylchloride (PVC) case (Fig. 1b–d) made of sheets of PVC 0.5 mm thick containing 16 holes of 2 mm diameter.

2.3. In vitro dissolution test

The in vitro dissolution test of AAP from CR tablets was determined using Dissolution Apparatus No. 2 (paddle) of JP XI. The test media were 500 ml of 1st fluid (pH 1.2), 2nd fluid (pH 6.8) for JP XI disintegration test or 0.1 M acetate buffer (pH 4.0). The amount of dissolved AAP was determined spectrophotometrically at 280 nm.

2.4. Wet strength

CR tablets were removed from the dissolution medium at 1, 2 and 4 h after the start of the in vitro dissolution test (pH 6.8, 100 revs./min). Crush strength in the wet condition was deter-

mined using a Reo Meter (Fudoh Industries, Japan).

2.5. In vivo absorption study

Six male beagle dogs weighing 9.5 to 15.5 kg were fasted for 20 h before administration. They were allowed free access to water but food was withheld until the last blood sample was taken. AAP 100 mg (solution or CR tablets) was administered orally with 20 ml water. A minimum 1-week washout period was provided between each administration. Blood samples were collected at frequent intervals up to 10 h after dosing. Plasma samples were immediately separated and frozen at −20°C until assay.

2.6. Plasma extraction

An aqueous solution of 60 μg/ml 2-acetaminophenol was prepared for use as an internal standard. Internal standard solution 0.1 ml and 5 ml of ethyl acetate were sequentially added to 0.5 ml of plasma in a test tube. The tube was shaken for 10 min, and centrifuged for 5 min at 2000 revs./min. The upper organic layer was transferred to a clean test tube, then evaporated. The dried residue was redissolved with 0.1 ml of mobile phase for HPLC assay.

2.7. HPLC assay of AAP

AAP in plasma was determined by HPLC with UV detection according to a previously reported procedure (Ameer et al., 1981). Separation on an octadecylsilane column (Nucleosil, 150 mm length × 4.6 mm diameter, 5 μm) was achieved at ambient temperature at a flow rate of 1 ml/min. The mobile phase contained water/acetonitrile/methanol (88:6:6, v/v). UV detection was at 254 nm.

2.8. Deconvolution

AAP absorption after oral dosing of CR tablets was calculated from oral dosing of solution and of CR tablets by the point-area deconvolution method (Iga et al., 1986). Differences in each absorption amount at 10 h after dosing were statistically evaluated by the ANOVA.

2.9. Necroscopy study

Two male beagle dogs weighing 12.0 and 15.5 kg were fasted for 20 h before first administration with free access to water. CR-A and CR-B were individually labeled by marking on the teflon tube and by the addition of coloring agent 0.5% to allow tracing in the GI tract. Both CR-A and CR-B were administered with 20 ml water to both dogs at a predetermined time. Six hours after first dosing, the dogs were sacrificed, and the entire GI tracts was immediately removed, opened and the CR tablets were recovered from their GI tracts. By analyzing the residual amount of AAP in the tablets, the released amount was calculated as the difference between initial and residual contents.

3. Result and discussion

3.1. In vitro characteristics of CR tablets

As gastric pH in the dog shows large individual variation (Lui et al., 1986), physical factors of the GI must be investigated using pH-independent drug release. We used AAP (pK_a 9.5) as a model drug because its solubility in water is almost constant (approximately 15 mg/ml) over a physiological range of pH (1–7). Drug release rate was controlled using the non ionic polymers HPMC and EC. As shown in Fig. 2, pH of the test media did not affect AAP release from either HPMC matrix (CR-B) and EC matrix (CR-C), indicating that GI pH does not affect drug release from CR tablets.

CR-A was designed as agitation speed-dependent formulation using a matrix core composed of AAP and low viscosity HPMC from which AAP release would be affected by the agitation. This core swells approximately 1 mm at each end on contact with dissolution medium. To eliminate the effect of mechanical destructive forces on AAP release, the matrix core was inserted into a teflon tube 3 mm longer than the matrix core to protect the swelling layer. As shown in Fig. 3a, AAP

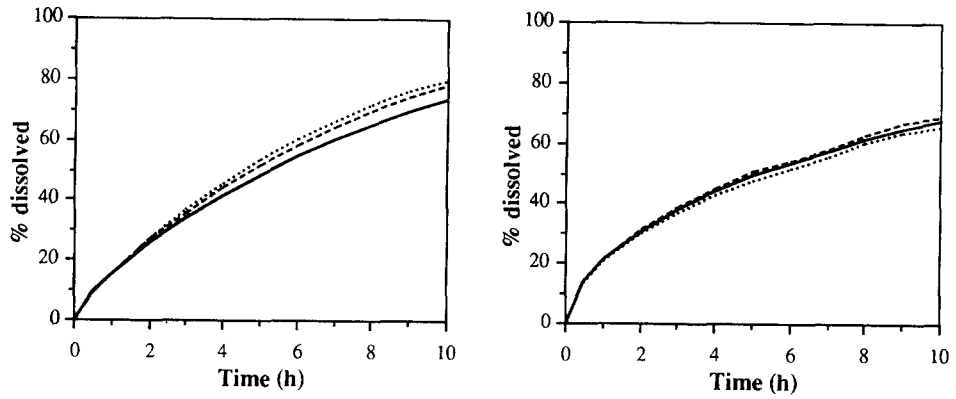

Fig. 2. Effect of pH on in vitro dissolution of AAP from CR-B (a) and CR-C (b) using the paddle method at 25 revs./min. Key: ....., pH 1.2; ---, pH 4.0; ___, pH 6.8.

dissolution rate from CR-A was remarkably affected by the paddle rotation speed. Even when CR-A was subjected to up to 2000 g pressure, it was not destructed (Table 1). Taken together, these results indicated that CR-A was a suitable formulation for the investigation of agitation intensity.

CR-B was a hydrophilic gel-forming matrix tablet composed of AAP and HPMC. Hydration of the polymer results in the formation of an outer layer that controls the release rate of drug (Ford et al., 1991). CR-C was a water insoluble matrix tablet from which drug release is controlled by diffusion. CR-D was designed as a completely resistant type of CR-C to mechanical destructive forces by enclosing CR-C in a PVC case (Fig. 1b–d). The PVC case had 16 holes of 2 mm diameter to permit the entry of liquids. Fig. 4 shows the dissolution profiles of AAP from CR-B, CR-C and CR-D as tested by the paddle method at 25 revs./min. CR-C and CR-D showed almost identical dissolution profiles, showing that the PVC case was not a barrier to AAP release from CR-D. Further, drug dissolution from CR-B was close to those of CR-C and CR-D at up to 10 h. AAP dissolution rate from CR-C and CR-D was not effected by paddle rotating speed over the range 25–100 revs./min (Fig. 3c and d, respectively). In the case of CR-B, paddle rotation speed had a slight effect on drug dissolution rate (Fig. 3b). Table 1 shows changes in the strength of CR tablets in wet conditions. The strength of CR-B and CR-C decreased markedly in a time-dependent manner, reaching 56 g and 257 g after 4 h, respectively, showing CR-B's wet strength decreased more than that of CR-C. With CR-B, a gel layer was formed on the surface of the tablet correlating with water penetration. Wet strength was dependent on the strength of the non-gelated residual core; the gel layer would have been weaker still. In the case of CR-C, this formulation maintained its shape for up to 10 h of in vitro dissolution testing. Wet strength was dependent on the strength of its remaining matrix structure. In contrast, the strength of CR-D was dependent on that of the PVC case, with wet strength remaining at more than 2000 g for up to 4 h. To investigate the influence of mechanical destructive forces on drug release, a series of CR-B, CR-C and CR-D was used. The results showed that these formulations, which provided different wet strengths but similar dissolution rates in vitro, would be suitable formulations for this study.

3.2. *In vivo absorption of CR tablets*

Fig. 5 shows AAP absorption profiles calculated by deconvolution after dosing of CR tablets to fasted dogs. All four tablets showed biphasic characteristics, with phase change about 2 h after dosing. Table 2 shows the GI transit at necroscopy of CR-A and CR-B after oral dosing to

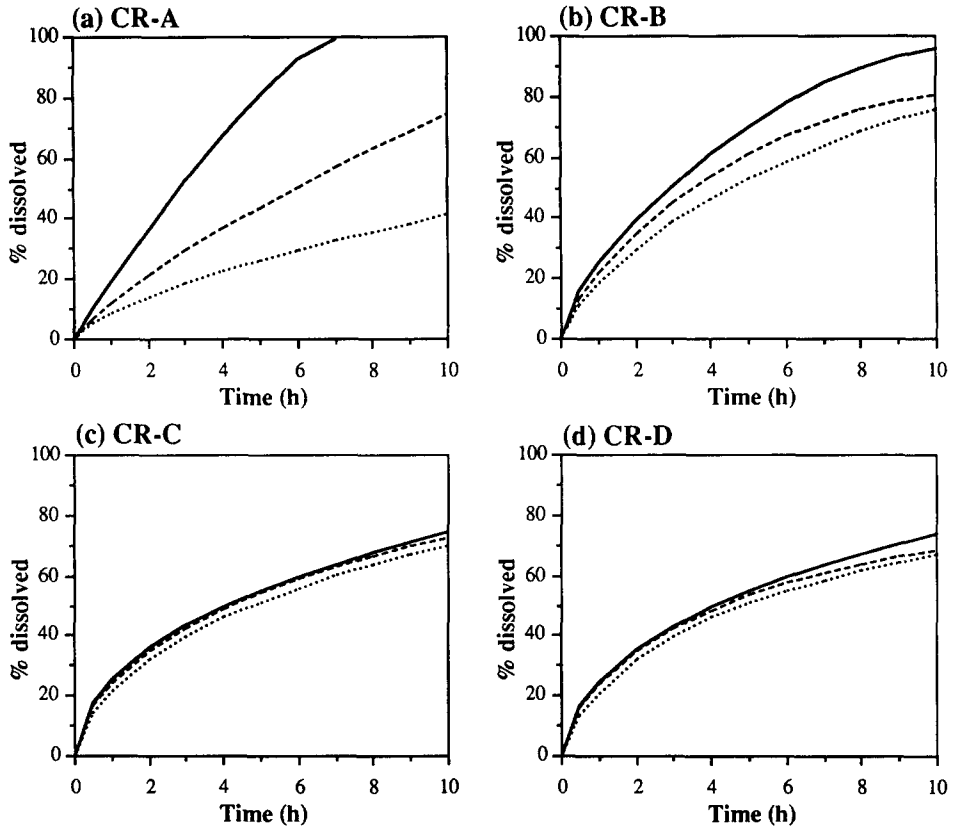

Fig. 3. Effect of rotation speed on in vitro dissolution of AAP from CR-A (a), CR-B (b), CR-C (c) and CR-D (d) using the paddle method in JP XI 2nd fluid (pH 6.8). Key: dot line, 25 revs./min; dash line, 50 revs./min; solid line, 100 revs./min.

fasted dogs. Both tablets reached the colon within 2 or 3 h after oral dosing. This result is in accord with that of a previous study of Sagara et al. (1992), who reported that the colon arrival time of solid forms in fasted dogs is 2.8 h after oral dosing. Thus, the first phase would reflect the drug absorption mainly from the small intestine, and the second phase that from the colon.

Table 1
Wet strength of CR tablets

Wetting time (h)	Wet strength (g)			
	CR-A	CR-B	CR-C	CR-D
0	>2000	>2000	>2000	>2000
1	>2000	613 ± 111	700 ± 50	>2000
2	>2000	250 ± 29	423 ± 31	>2000
4	>2000	56 ± 46	257 ± 5	>2000

3.3. Agitation intensity in the dog GI

AAP absorption from CR-A in fasted dogs continued for only 2 h after dosing (Fig. 5). Mean in vitro AAP dissolution from CR-A at 1 h with a paddle speed of 25, 50, 100 revs./min was 8.5%, 12.0% and 19.2%, respectively. Mean AAP absorption from CR-A at 1 h calculated by deconvolution was 7.2%, which was close to its in vitro dissolution at a speed of 25 revs./min. In necroscopy study, in vivo AAP release from CR-A at 1 h (12.1%) and in the early stage (0–2 h) pattern were close to its in vitro dissolution profile at a paddle speed of 50 revs./min (Fig. 6). Taken together, these results indicate that the design of

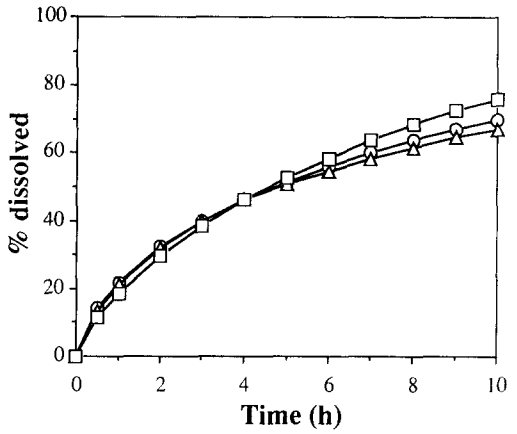

Fig. 4. Comparison of the dissolution of AAP from CR-B (□), CR-C (○) and CR-D (△) using the paddle method at a rotation speed of 25 revs./min in JP XI 2nd fluid (pH 6.8).

in vitro dissolution tests correlating with in vivo conditions must consider that agitation intensity in the paddle method is relatively low at 25–50 revs./min.

3.4. Mechanical destructive force in the dog GI

Despite their almost identical in vitro dissolution profiles at low agitation intensity (Fig. 4), the first phase of AAP absorption of CR-B, CR-C and CR-D markedly differed from each other in vivo (Fig. 5). Absorption rate was fastest for

Table 2
Location of recovery of CR-A and CR-B in the GI after oral dosing to fasted dogs

Time (h)	CR-A		CR-B	
	Dog A	Dog B	Dog A	Dog B
1	Jejunum	Stomach	Jejunum	Stomach
2	Colon	Stomach	Colon	Stomach
3	Colon	Colon	Colon	Colon
4	Colon	Colon	Colon	Colon
5	Colon	Colon	Colon	Colon
6	Colon	Colon	Colon	Colon

CR-B, followed by CR-C and CR-D in this order. In other words, a correlation was seen between lower wet strength and faster initial AAP absorption rate. As CR-D was covered with a PVC case, its release rate should not have been affected by GI destructive force. CR-C, a water insoluble matrix tablet, would be broken down slightly in the dog GI; in fact, in vivo absorption at 10 h after dosing of CR-C was slightly increased to 1.2-fold that of CR-D, but this difference was not significant ($P > 0.05$). In contrast, the hydrophilic gel-forming polymer matrix tablet CR-B, with its low wet strength, should be broken down in part by GI destructive forces and its in vivo release rate remarkably accelerated; results showed that the in vivo absorption at 10 h after dosing of CR-B was significantly ($P < 0.01$)

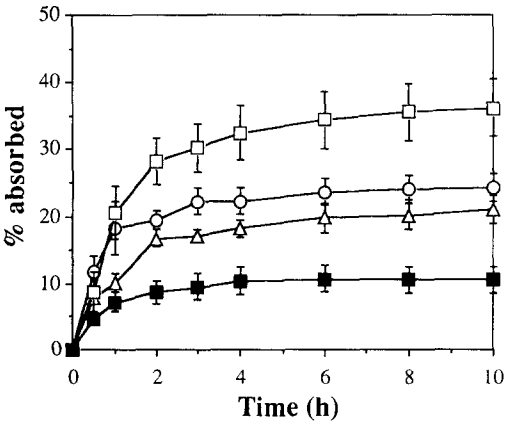

Fig. 5. Absorption profiles after oral dosing of CR tablets to fasted dogs. Each result shows mean ± S.E. in six dogs. Key: ■, CR-A; □, CR-B; ○, CR-C; △, CR-D.

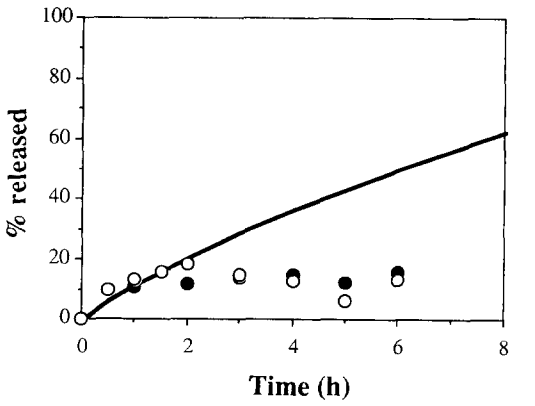

Fig. 6. In vivo release of AAP from CR-A after oral dosing to dogs (●, dog A; ○, dog B) and in vitro dissolution determined by the paddle method at 50 revs./min (solid line).

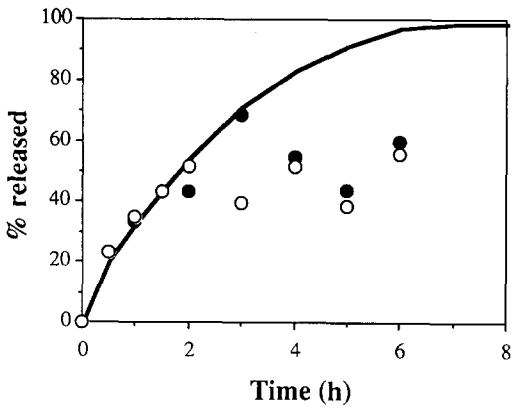

Fig. 7. In vivo release of AAP from CR-B after oral dosing to dogs (●, dog A; ○, dog B) and in vitro dissolution determined by the paddle method at 200 revs./min (solid line).

greater (1.7-fold) than that of CR-D. Ogata et al. (1984) reported that the mechanical destructive force in dogs is greater than that in humans. In humans, CR-B also showed a higher absorption rate than CR-C (Shameem et al., 1995), indicating that, as in dogs, drug release from hydrophilic gel-forming matrix is determined largely by breakdown due to mechanical force.

The physical condition of CR-B in the GI tracts of fasted dogs was observed at necroscopy. In contrast with the in vitro state, CR-B recovered from the GI tracts had only a thin gel layer on its surface. This finding indicates that a large portion of the gel layer was broken down by GI destructive forces. This would promote AAP release because the gel layer controls the drug release rate from hydrophilic matrix (Ford et al., 1991). The in vivo drug release of CR-B up to 2 h after dosing was faster than its in vitro dissolution at a paddle speed of 100 revs./min, and was close to that at 200 revs./min, as shown in Fig. 7. As in vivo drug release from CR-A, a completely resistant formulation to mechanical destructive force, was close to its in vitro dissolution at a speed of 25–50 revs./min, the difference would be due to mechanical destructive forces of the GI. Consequently, the design of in vitro dissolution tests correlating with in vivo conditions must take full account of mechanical destructive forces, and the forces, for example, should be settled that AAP release from CR-B accelerates about 1.7-fold of that at paddle 25 revs./min and that from CR-C about 1.2-fold.

3.5. Colonic release from CR tablets in dogs

Fig. 5 also indicates that drug absorption were terminated in all CR tablets at about 2 h postdosing. This result is in accord with previous findings of insufficient drug absorption from several CR products in dogs (Cressman and Sumner, 1971; Uchida et al., 1986; Katori et al., 1991). The question was whether the tablets had passed beyond the main site of AAP absorption or whether AAP release had actually terminated. The answer was obtained from the following necroscopy studies. Both CR-A and CR-B reached the colon within 2 h or 3 h after dosing (Table 2). AAP release remained almost constant from CR-A (Fig. 6) and CR-B (Fig. 7) after reaching the colon, although a large amount of AAP remained in CR tablets. This in vivo release termination agrees with the AAP absorption termination in the second phase calculated by deconvolution (Fig. 5). Kimura et al. (1994) reported that absorption of AAP within 10 min from the colon was 23%, and the AUC after dosing from the colon was over 80% compared to that from the jejunum and ileum. This absorption rate is much higher than the drug release rate of the CR tablets which we used, indicating that the absorption rate from the colon would not the restricted factor for the CR tablets. Further, our results demonstrated that the AAP absorption from CR tablets was markedly inhibited in the colon. It is widely considered that, when drug absorption is not sustained after dosing of a CR form, the absorption rate of the drug in the lower GI must be very low. However, our study confirmed that the cause of low AAP absorption in the colon is the suppression of drug release from CR tablets. This would be due to the environment in the colon, namely the small volume of GI fluid and viscous colonic contents, which would restrict fluid movement around the tablets and retard thereby drug dissolution. These findings indicate that only colonic permeability of drugs but also the colonic release from CR forms must be considered. From the

results for CR-A, it is difficult to predict colonic release from in vitro dissolution by the paddle method because the colonic release rate was less than in vitro dissolution at a paddle speed of 25 revs./min. Prediction of colonic release may therefore require a new in vitro system which uses a small amount of fluid or a limited tablet surface area.

4. Conclusions

Agitation intensity in the dog GI was relatively low, but CR tablets with low wet strength were broken down by GI destructive forces, accelerating the drug release rate. Wet strength is an effective means to predict erosion in the GI. Further, in fasted dogs, CR tablets reached the colon within 2–3 h after dosing, but little drug was released here. The design of dissolution tests for CR tablets correlating to in vivo situations must consider these physical factors and colonic drug release. Furthermore, these results indicate that the drug absorption for longer periods will be possible if favorable drug release can be achieved in the colon.

Acknowledgements

This study was supported in part by the Japan Human Sciences Foundation.

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