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The delayed dissolution of paracetamol products in the canine fed stomach can be predicted in vitro but it does not affect the onset of plasma levels

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Abstract

Although it is generally believed that paracetamol can be used as a marker of gastric emptying, there have been reports in the literature that show delayed dissolution of immediate release paracetamol tablets using standard in vitro setups and food-simulating media, delayed disintegration of paracetamol products in the fed stomach, and no correlation of paracetamol absorption with gastric emptying in the fed state. In this study, we confirmed that dissolution of Panodil® and Apotel® tablets is delayed in food-simulating media regardless of the in vitro hydrodynamics and on a formulation dependent manner. Further, we assessed the usefulness of in vitro dissolution data in the prediction of delayed disintegration time in the fed stomach and we examined the importance of delayed gastric disintegration on the onset of plasma levels using the canine model. In vitro dissolution data in cow's milk reflected the delayed disintegration of Panodil® tablets in the fed stomach. In vitro dissolution of Apotel® tablets in milk was delayed less than of Panodil® and the effect of dosing conditions on the in vivo disintegration was not apparent. However, for the products tested in this study, there was no correlation between intragastric disintegration and onset of plasma levels probably because gastric emptying in also delayed in the fed state.

Keywords: Paracetamol; Acetaminophen; Disintegration; Dissolution; Absorption; Dogs

1. Introduction

Paracetamol is a highly soluble compound that despite its low permeability characteristics (Lindenberg et al., 2004) has been repeatedly found to show gastric emptying controlled absorption kinetics in humans

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(Willems et al., 2001) and has been suggested to be used as marker of gastric emptying (Peh and Yuen, 1996). However, some studies have indicated that paracetamol absorption in the fed state may not be controlled by gastric emptying (Petring et al., 1986; Petring and Flachs, 1990). Other studies have shown that disintegration and/or dissolution in the fed stomach can be substantially delayed (Kelly et al., 2003), which can sometimes lead to a delay in the onset of absorption (Walter-Sack et al., 1989). In agreement with these findings, in vitro studies have shown that dissolution of paracetamol tablets in media simulating the initial gastric composition in the fed state can be surprisingly slow (Macheras et al., 1986; Galia et al., 1998).

The objectives of this study were three-fold. The first was to confirm that delayed dissolution of paracetamol in food-simulating media is related primarily to the formulation and is observed regardless of the in vitro hydrodynamics. The second was to confirm that in vitro dissolution data collected in milk adequately reflect intragastric disintegration times in fed dogs. The third was to assess the importance of delayed intragastric dissolution on the onset of plasma levels using the canine model and two tablet formulations with different disintegration characteristics in the stomach.

2. Materials and methods

2.1. Dosage forms

Two IR tablets, Panodil® (film-coated tablet, 500 mg/tablet, Lot # 00F23 (L) 000282 A, SmithKline Beecham A/S, Denmark) and Apotel® (uncoated tablets, 500 mg/tablet, Lot # 00300603, Unipharma Pharmaceutical Laboratories S.A., Greece) and a suspension, ben-u-ron® (200 mg per 5 ml, Lot # 305052, Novartis Consumer Health GmbH, Germany) were studied.

2.2. In vitro dissolution tests

In vitro dissolution data were collected at 37 °C with the USP II (rotating paddle) apparatus and with the USP IV (flow-through) apparatus (US Pharmacopeia, 2003) in simulated gastric fluid, SGF (Galia et al., 1998), whole milk (3.5% fat), fasted state simulating intestinal fluid (FaSSIF) and fed state simulating intestinal fluid (FeSSIF) (Galia et al., 1998). To reduce expenses, in this study, soybean phosphatidylcholine and crude taurocholic acid were used for the preparation of FaSSIF (Vertzoni et al., 2004). Experiments were run in triplicate.

In one set of experiments, a Distek® rotating paddle dissolution tester (model 2100B, North Brunswick, NJ, USA) with the paddle rotating at 100 rpm was used [earlier studies have shown that, compared to 100 rpm, 50 rpm result to minor changes on the dissolution profile and sometimes lead to unwanted coning effects (Galia et al., 1998)]. Four milliliter samples (with volume replacement) were obtained 5, 10, 15, 20, 30 and 60 min after the beginning of the experiments. Samples in SGF, FaSSIF or FeSSIF were immediately filtered through regenerated cellulose filters (0.45 μm, Titan®, Scientific Resources Inc., USA) and after addition of the internal standard (2-acetamidophenol) they were injected into the HPLC. Samples in milk were immediately filtered through nylon membrane filters (5.0 μm, Titan®, Scientific Resources Inc., USA). The internal standard and 10% perchloric acid (1:1, v/v) were then added and mixed with the filtrates. After centrifugation at 8 °C the supernatant was filtered through regenerated cellulose filters (0.45 μm, Titan®, Scientific Resources Inc., USA) before injection into the HPLC. In another set of experiments an Erweka® flow-through dissolution tester (model DFZ60, Erweka GmbH, Heusenstamm, Germany) equipped with USP cells for testing tablets (Ø22.6 mm) or with USP cells for testing powders and granulates was used. Cells were connected to an Erweka® piston pump (model HKP60). Dissolution in SGF was studied at 12 ml/min and it was too fast to be followed by FaSSIF. Experiments in milk followed by FeSSIF were run at 6 ml/min. Flow rates were decided to achieve a balance between the fluid volumes into which the tablet releases its contents intralumenally and the physiological gastric and small intestinal residence times (Davenport, 1982; Nicolaides et al., 2000). Tablet dissolution was initiated after positioning a 5 mm-size glass bead in the tip of the cell and 1.7 g of 1 mm-size glass beads above the 5 mm glass bead, with a glass fiber filter (MNGF1: 0.7 μm pore size, 22 mm diameter, Macherey-Nagel, Germany) placed on the top of the cell. Dissolution of the suspension was tested without the presence of 1 mm-size glass beads, although a glass fiber filter [MNGF5 with 0.4 μm

pore size or MN 85/70 BF with 0.6 μm pore size, Macherey-Nagel, Germany] was also placed on the top of the cell in this case. At the beginning of each experiment, the tablet was mounted onto a holder whereas an aliquot of the suspension was poured into the cell. Upon exiting the flow cell the fluid was collected in a volumetric cylinder. Cylinders were replaced at 10 min intervals. Samples were treated as described above for the rotating paddle apparatus prior to injection into the HPLC.

The paracetamol content of each sample was determined with an HPLC-UV method described previously (Vertzoni et al., 2003a).

2.3. Canine studies

Two sets of in vivo experiments were performed in dogs fasted for 16 h from food but not water before each administration. The study was approved by the Animal Ethics Committee of Gothenburg (Ethics Approval Number, 1082001).

In the first set of experiments, in vivo disintegration data of Panodil® and Apotel® were collected in 4 and 3 male Labradors (25–30 kg), respectively, on a crossover basis. A tablet was placed in a net and inserted through a gastric fistula approximately 5 cm into the fundus of the stomach. Water (200 ml) or milk (3% fat, 200 ml) was administered to the dogs by an orogastric tube immediately before inserting the tablet in the stomach. Tablet disintegration was observed by removing the net from the stomach at different times (at 10, 15, 20 or 30 min after the administration of water and at 30, 40, 45 or 60 min after administration of milk), drying the net and its contents, and weighing its contents. The initial weight of the tablet minus the weight of the remaining of the tablet in the net equals the "% disintegration". Each experiment results to a single % disintegration value. At a given formulation/dosing conditions, an experiment was performed to the same dog 1–3 times.

In the second set of canine experiments, four non-fistulated male Labradors (25–35 kg each) were used on a crossover basis. On different occasions each dog was administered one Panodil® tablet or one Apotel® tablet with either 200 ml water or with 200 ml milk (3% fat) via an orogastric tube. Blood samples were drawn from a suitable foreleg vein. After centrifugation, the serum samples were stored at −20 °C until assayed. Two hours after dosing the dog was allowed to drink water ad libitum. Four hours after drug administration the dog was offered a standard meal (approx. 600 g pellets, ID from Kruuse or GLP/CAD from Leo Pharma AB). The washout period between phases was at least 1 week for each dog. Paracetamol assay in canine plasma was performed according to a published method (Ameer et al., 1981).

2.4. Data treatment

Since the use of a single model was not possible to apply to all data sets and dissolution in most cases was too fast for an index to be used, differences between in vitro data sets collected in media other than milk, were evaluated using the times for 50% dissolution, $T_{50\%}$, that were estimated from individual data sets with linear interpolation.

In vitro profiles in fed state simulating media were compared with the difference factor, $f_{1,\text{area}}$, using a bootstrap procedure and the estimated median (90% confidence intervals) of $f_{1,\text{area}}$ (Vertzoni et al., 2003b).

Area under a plasma concentration versus time data set at 1 h post-dosing ($AUC_{0-1\,h}$) and at 8 h post-dosing ($AUC_{0-8\,h}$) was estimated with the loglinear trapezoidal rule. Comparisons of $AUC_{0-1\,h}$ values and $AUC_{0-8\,h}$ values were performed with two-way repeated measures analysis of variance (Factor A: dogs; Factor B: dosing conditions, Sigmastat for windows, Version 2.03, SPSS Inc.) and significant differences were assessed at the 0.05 level.

3. Results

3.1. In vitro dissolution data

Fig. 1 shows that, with the exception of dissolution of tablets in milk, dissolution of all three products tested in this study is rapid and complete in vitro, regardless of composition of the medium and/or hydrodynamics; individual $T_{50\%}$ values ranged from 2.8 to 7.5 min (when dissolution was studied with the rotating paddle apparatus) and from 4.4 to 14.8 min (when dissolution was studied with the flow-through apparatus). Dissolution of paracetamol from tablets (but not from suspension) is significantly slower in milk, regardless of hydrodynamics; $T_{50\%}$ values for the dissolution of Panodil®

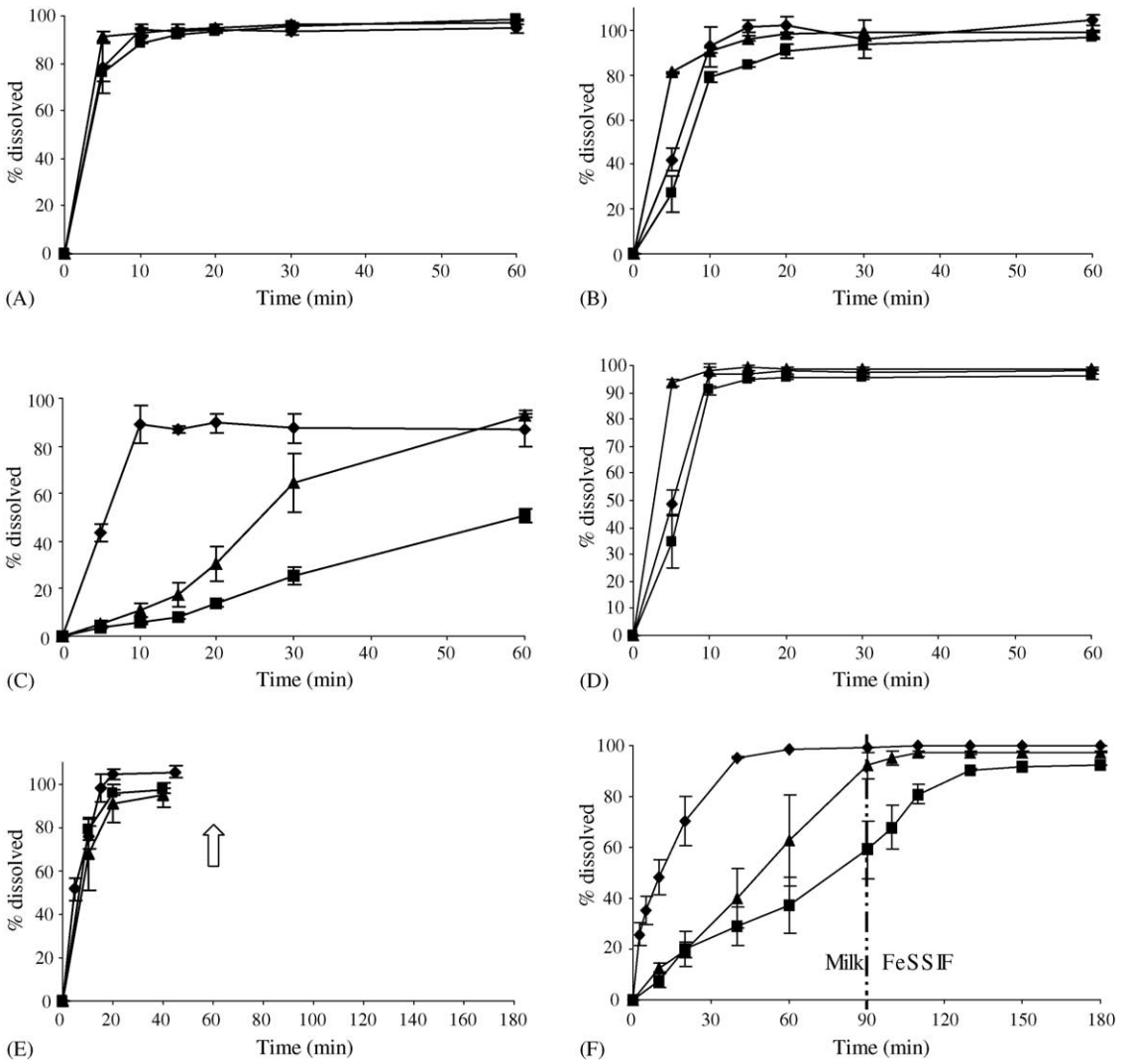

Fig. 1. Mean cumulative percent dissolution data ± S.D. for paracetamol from Panodil® (■), Apotel® (▲), and ben-u-ron® suspension (♦) obtained under the following conditions: (A) USP apparatus II/SGF; (B) USP apparatus II/FaSSIF; (C) USP apparatus II/milk 3.5%; (D) USP apparatus II/FeSSIF; (E) USP apparatus IV/SGF—the arrow indicates the time that FaSSIF would have followed if dissolution was not complete; (F) USP apparatus IV/milk (0–90 min, 6 ml/min) followed by FeSSIF (90–180 min, 6 ml/min).

tablets in milk were 59.1 ± 3.1 and 77.7 ± 15.0 min using the paddle and the flow-through apparatus, respectively, whereas the corresponding numbers for Apotel® were 26.1 ± 3.4 and 50.7 ± 13.4 min, respectively. These numbers suggest that, regardless of the hydrodynamics, in milk Panodil® dissolves significantly slower than Apotel®; regardless of what profile is designated as reference, estimated median $f_{1,area}$ values were 0.56–1.29 whereas the 90% confidence intervals ranged from 0.40 to 1.45. Based on the lowest limit of confidence intervals, dissolution profiles of the two products in milk differ by at least 40%.

3.2. In vivo disintegration data

With the used experimental procedure, the time for complete in vivo disintegration could not be observed. When nothing was present in the net no data could be

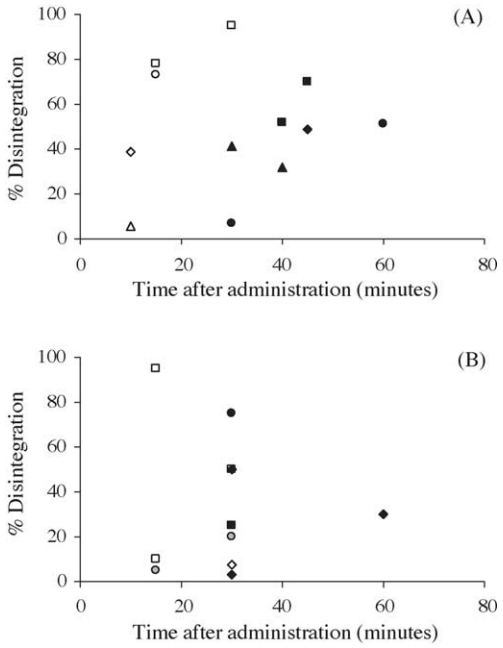

Fig. 2. Percentage disintegration data of Panodil® tablets (A) and Apotel® tablets (B) in the stomach of Labradors. Each symbol corresponds to a single experiment. A symbol with a given shape (regardless of filling and for both graphs A and B) corresponds to a specific dog. Open or lightly filled symbols refer to the fasting conditions (after administration of 200 ml water) and closed symbols refer to fed conditions (after administration of 200 ml milk).

collected because 100% disintegration could have occurred much earlier than the time the net was taken out of the stomach. Empty net was observed in many cases up to 30 min after the administration of either product with water. There was no case in which we observed 100% disintegration 60 min after the administration of either product with milk.

In agreement with in vitro dissolution data, in vivo disintegration data presented in Fig. 2 suggest that intragastric disintegration is slower in the fed than in the fasting state for Panodil®. For Apotel® fasted versus fed state effects are minimal. A confounding factor in the observation of disintegration from this product is that it tended to soften rather than disintegrate. The different food effects on the in vivo disintegration of the two products are in agreement with the substantially faster, compared to Panodil®, in vitro dissolution profile of Apotel® in milk.

3.3. Plasma data

Mean plasma profiles are shown in Fig. 3. $AUC_{0–8\,h}$ values did not vary significantly with the formulation and/or dosing conditions. $AUC_{0–1\,h}$ values were significantly bigger in the fasting than in the fed state ($p = 0.030$); mean $AUC_{0–1\,h}$ values of the pooled data were 4.46 mg ml^{-1} h in the fasting state and 2.11 mg ml^{-1} h in the fed state. However, the interaction of product with dosing conditions for the $AUC_{0–1\,h}$

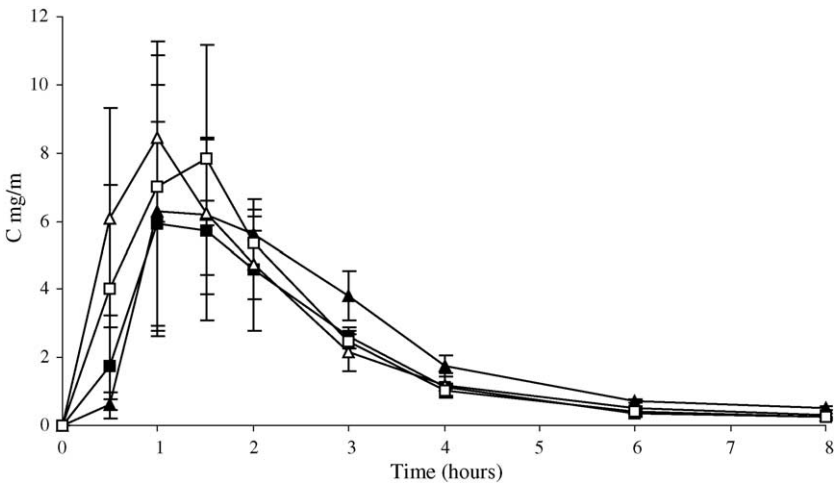

Fig. 3. Mean ± S.D. paracetamol levels in plasma after single administration of one Panodil® tablet (squares) and one Apotel® tablet (triangles) to four fasted dogs with 200 ml of water (open symbols) and with 200 ml of milk (3% fat) (closed symbols).

values was not significant ($p = 0.144$). These data suggest that the extent of absorption is not affected by the formulation and/or the dosing conditions and that the overall rate of absorption is slower in the fed state but is not formulation dependent.

4. Discussion

The longer disintegration times of Panodil® tablets in the fed canine stomach (Fig. 2) were correctly predicted from the delayed in vitro dissolution data of this product in milk (Fig. 1), regardless of the hydrodynamics. For Apotel®, in vitro simulation of fed gastric conditions with milk as the dissolution medium led to lower dissolution rates than were observed in SGF. However, the difference was much less than with the Panodil® tablets. Administering Apotel® on a milk fed stomach led to, at best, only a modest trend to lower disintegrating times.

It was recently reported (Abrahamsson et al., 2004) that food could significantly delay tablet disintegration in stomach by a formation of a film around the tablet, a phenomenon that is dependent both on the tablet's excipients and composition of the administered food. In line with that data, Panodil® (unlike Apotel®) is film-coated and contains PVP, the disintegrant activity of which is substantially affected by the presence of food components (Abrahamsson et al., 2004). Further, the presence of proteins in the meal seems to be critical with respect to the effect on disintegration times (Abrahamsson et al., 2004).

Despite the substantially delayed disintegration/dissolution of Panodil® (only ∼40% had dissolved by 60 min in USP II and ∼60% dissolved by 90 min in USP IV apparatus), the absorption rates of Apotel® and Panodil® in the fed state were delayed to similar extents, suggesting that gastric emptying controls the absorption process in the fed state as well as in the fasted state.

Two issues should be considered before interpreting the importance of these findings for human studies. The first relates to the relevance of milk as a food simulator, given the difference in energy content between milk and the types of meal frequently administered in bioavailability/bioequivalence BA/BE studies (Klein et al., 2004). Based on data collected with metoprolol products in dogs (Abrahamsson et al., 2004), disintegration times in stomach after administration of a nutritional liquid with energy content similar to milk were similar to those observed after administration of a typical meal administered in BA/BE studies (Abrahamsson et al., 2004). The second issue relates to species differences on gastric emptying rates. Fasted dogs empty granules (∼1 mm) and liquids slightly faster or similarly to fasted humans (Dressman, 1986; Aoyagi et al., 1992). Nutrient fluids are emptied slower than non-nutrient fluids and the decrease in the rate of gastric emptying is roughly similar in dogs and humans (Dressman, 1986). Non-digestible solids with sizes of about 11 mm (i.e. non-disintegrated tablets) will remain in the fed canine stomach longer than in humans whereas for smaller sizes (e.g. granules) species difference is less pronounced (Aoyagi et al., 1992). Therefore, the lack of correlation between delay in intragastric disintegration of Panodil® and Apotel® with the onset of absorption can be also the case for humans. This hypothesis is supported by data showing that the substantially delayed disintegration of Panadol® tablets (a product that is almost identical to Panodil® tablets) in the human stomach 30 min after the administration of a high-fat meal did not significantly affect early exposure to paracetamol (Kelly et al., 2003).

5. Conclusions

Compared to in vitro dissolution data in a medium simulating the fasted gastric conditions, dissolution in milk is significantly delayed on a formulation dependent manner, regardless of the hydrodynamics. In vitro dissolution data in milk predict the formulation for which disintegration in the fed stomach is delayed at most. However, no correlation could be observed between gastric disintegration and onset of plasma levels in dogs, probably because gastric emptying is also delayed in the fed state.

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