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Gastro intestinal tracking and gastric emptying of solid dosage forms in rats using X-ray imagining

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ABSTRACT

The aim of this research was to study the gastrointestinal transit and gastric emptying of non-disintegrating solid dosage forms in rats using X-ray imaging. Commercial gelatin minicapsules were filled with barium sulfate and enterically coated using Eudragit S100. The capsules were administered orally to rats followed by a solution of iodine based contrast agent iopromide. Images were obtained using a standard X-ray camera and digital film processing. Capsules were followed through the GI tract from the stomach to the small intestine, cecum and large intestine and the capsule location could be easily identified. Gastric emptying of different sized capsules was studied. The effect of fasting and time of administration on gastric retention was also studied. It was found that shortened capsules of 3.5 and 4.8 mm length were emptied from the stomach whereas the commercial length 7.18 mm capsules were retained. Surprisingly, 2.5 h post administration more rats retained the capsules in the stomach in the fasted state than in the fed state. We found that X-ray imaging can be used for simple visualization and localization of solid dosage forms in rats in the fed state using shortened commercial minicapsules on rats.

1. Introduction

In the course of our research on azo polymers for colon drug delivery (Saphier and Karton, 2010), we looked for a convenient and non-invasive technique for the visualization and localization of colon targeted single unit solid oral dosage forms in rats. There is a dearth of publications describing *in vivo* experiments of this nature. This is in contrast to the many publications that use rat cecal contents as an *in vitro* model (Bromberg et al., 2005; Ghandehari et al., 1997; Kalala et al., 1996; Ravi et al., 2008; Sakuma et al., 2001; Samyn et al., 1995; Soozandehfar et al., 2000; Tozaki et al., 2001; Wiwattanapatapee et al., 2003). Some researchers have used celiometry to study the advancement and disintegration site of pellets and minitablets or minicapsules (up to 1.6 mm diameter or 3.5 mm length respectively) (Hu et al., 1999; Tozaki et al., 1997, 2001, 2002; Tuleu et al., 1999), in rats.

There are several imaging techniques used in the field of oral drug delivery but only few studies have followed the *in vivo* fate of per os (PO) dosage forms in animals (Albrecht et al., 2006). γ Scintillation is common in human studies (Goto et al., 2004; Tuleu et al., 2007). This technique was also used in rats but only for liquids and small solid particles (Haruta et al., 2001; Jain et al., 2006; Quan et al., 2008). The accurate determination of a dosage form in the gastrointestinal tract by scintigraphic analysis requires an experienced analyst. The anatomical region passed by a dosage form during gastrointestinal transit is assigned relative to defined markers. In addition, the added short-lived gamma-emitting radionuclide markers require dosage form preparation close to the experiment time or neutron activation (Digenis and Sandefer, 1991). Simultaneous *in vivo* visualization and localization of solid dosage form in the rat gastrointestinal tract have been achieved by magnetic resonance imaging (MRI) (Christmann et al., 1997). However, labeling techniques of oral solid dosage forms for MRI applications have not been well established as those of γ scintillation and imagining of the whole GI tract under different conditions is still difficult (Albrecht et al., 2006; Kremser et al., 2008).

Radiology is one of the oldest methods for imaging. It is cheap, simple to perform and easy to analyze and has been used in the past to follow the fate of enterically coated dosage forms in humans (Fell and Digenis, 1984; Meka et al., 2009) but it is infrequently used for humans because of the large radiation exposure during repeated imaging. On the other hand, radiation exposure is not of great concern in laboratory rats and is therefore an attractive alternative technique for animal imagining. The

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advances made in regard to digitization and speed of image development makes it today fast and easy to obtain high resolution multiple images using relatively cheap and readily available equipment, like those existing in any veterinary or dental clinic (Harvey et al., 2001). Therefore, we decided to visualize the fate of an enteric-coated minicapsule through the GI tract of the rat using radiology.

Gastric emptying of liquids, pellets or disintegrating solids in rats has been extensively studied under different conditions, some using different imagining techniques (Haruta et al., 2001; Levin et al., 2005; Perry et al., 1993; Torjman et al., 2005) however there are very few imagining studies on non-disintegrating solid dosage forms (Albrecht et al., 2006; Christmann et al., 1997; Kremser et al., 2008).

In this study, we have investigated the gastrointestinal transit of enterically coated hard gelatin minicapsules in rats by X-ray imagining. We have shown that this technique is still relevant today bringing high quality images, which can be easily interpreted. Using X-ray imaging we have studied the influence of capsule length and feeding conditions on gastric emptying and were able to follow the enteric-coated capsules through the GI tract.

2. Materials and methods

2.1. Contrast agents

To obtain an image of the digestive organs, a liquid contrast agent was used. At first, water (purified) was used to prepare 20% and 50% (w/w) emulsions of Barium Sulfate (Sigma–Aldrich, USA). During our preliminary experiments, we turned to an iodine based solution Ultravist® 300 (Schering AG, Germany) (1 ml contains 0.623 g iopromide, 300 mg I/ml).

2.2. Preparation of enterically coated minicapsules

Hard Gelatin PCcaps minicapsules #9 (Capsugel, Belgium) size 2.64 (cap width) mm × 7.18 mm were used. Capsules were cut to reduce their size either from one side to obtain capsule length of 4.8 ± 0.2 mm or both sides to obtain capsule length of 3.5 ± 0.5 mm using a sharp blade. A stand and funnel provided as a kit with the capsules was used for filling the capsules with barium sulfate.

Capsules were coated by dipping six times in an isopropanol solution with 8% (w/w) Eudragit S100 (Degussa, Germany) containing 5% (w/w) water and 0.6% triethylcytrate as plasticizer. Average coating obtained was calculated to be 4.4 ± 1.1 mg/cm^2.

2.3. Animal model

The animal studies met the requirements of the Institutional Animal Care and use committee and were conducted according with the NIH Guide for care and Use of Laboratory Animals (1996). Male Sprague-Dawley rats (350–400 g) were obtained from Harlan Laboratories (Israel). The animals were kept in plastic cages, two per cage, and maintained in air-conditioned quarters with a target room temperature of 20 ± 2 °C, and an alternating 12:12-h light–dark cycle starting at 6 am. Rats were fed *ad libitum* a standard laboratory rodent diet in pellet form (Altromin, Lage, Germany).

The oral administration of minicapsules to rats was performed using a delivery device provided with the purchased minicapsules. Rats received an enterically coated minicapsule followed by an oral contrast agent (2 ml). Insertion of capsule and liquid were performed under light isoflurane anesthesia. Additional contrast agent (2 ml) was given every 2 h.

Induction of anesthesia was achieved by placing the rats in an induction chamber using a 4% isoflurane (Abbott Laboratories Ltd, England) concentration in 100% oxygen using anesthetic machine (Vetland, USA). Maintenance of anesthesia was performed using 1.5% isoflurane.

All rats were allowed free access to water at all times. Rats were fasted 16 h prior to the administration of capsules. Fasting rats were placed on grid floors to prevent coprophagy.

Group I ($n = 8$) received medium and short length capsules together and long capsules separately. The experiment was performed under fasting conditions.

Group II ($n = 20$) was used in a crossover study designed to study the effect of the feeding regimen on gastric retention of the capsules. Each rat was given medium length capsules 6 times, varying the conditions so the rats would be alternatively fasting or freely fed with at least one weak washout time.

Each rat received an additional capsule (#7), given at the beginning of their night time (6 pm) instead of the regular morning time.

At the end of the experiments the rats were sacrificed by exposure to CO_2.

2.4. Radiological experiments

An X-ray camera model DM100p (Domgmun, Korea) was used. The camera was placed 1 m above the film cassette. Optimal exposure was 0.05 s and 60 kVp. The rats were placed on a kappa board, which is transparent to X-rays, at 20 cm above the film. The dorsal recumbency position was used in the studies. Digital film development was performed using Orex PcCR 812-Xi with Dent-A-View software ver. 1.7.1.01 (Orex, Israel).

Anaesthetized rats were placed on the kappa board and their mouth and nose were fitted into a mask through which they continued to receive isoflurane at 1.5% concentration during imaging. Between consecutive X-ray radiographs, rats were returned to their cages and allowed free access to food and water.

2.5. Statistical analysis

Number of rats in which capsules were emptied or left in the stomach, in fasting or freely fed conditions was analyzed by a χ^2 test.

3. Results

3.1. Simultaneous imagining of the rat's gastrointestinal tract and enteric-coated minicapsule

In preliminary studies, rats were given capsules without additional liquid contrast agent. The capsules were clearly observed but it was difficult to determine their location, as the GI tract was not highlighted (Fig. 1A). Liquid contrast agent (iopromide) given orally to rats by gavage following capsule administration enabled excellent visualization of the capsule on the background of the GI tract allowing for determination of the capsule location (Fig. 1B). Iopromide solution remained for a longer period of time than barium sulfate emulsions in the GI track thus enabling visualization for longer periods post administration. Furthermore, as the solution is homogenous, the images are more reproducible than with barium sulfate emulsions. Additional iopromide solution was given every 2 h.

3.2. Capsule size

In preliminary experiments rats were administered full size (length 7.18 mm) enterically coated commercial minicapsules filled with barium sulfate. Different conditions were attempted but the rats showed no sign of evacuating the capsule from the stomach for the periods of time followed, between 6 and up to 12 h. The

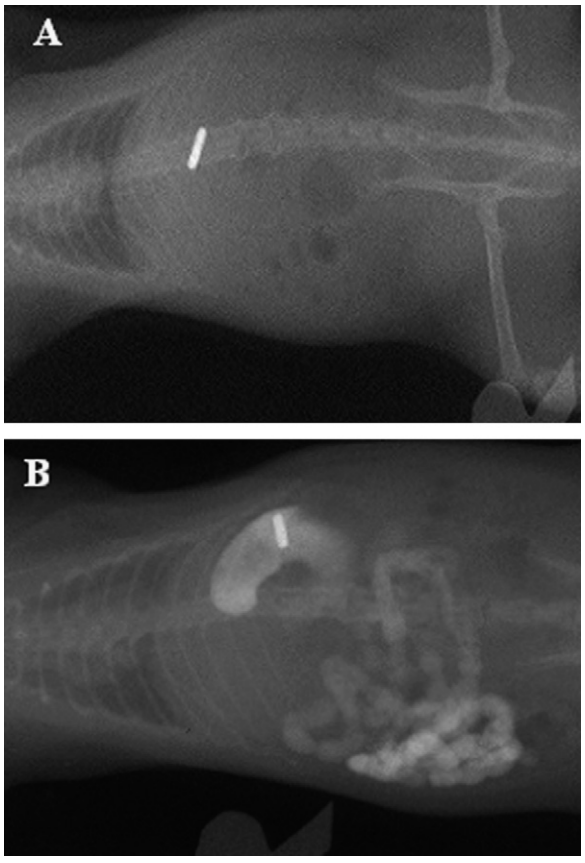

Fig. 1. Visualization of capsule with (B) and without (A) background contrast of the GI tract.

anatomy of the pylorus was examined in relation to the size of the capsule however no evidence was found to indicate any physical hindrance.

The commercial capsules were shortened. Three sizes of capsules were tested: long (Fig. 2A), medium B (Fig. 2B) and short (Fig. 2C).

A group of eight rats was given two capsules each, one small (Fig. 2C) and one medium size (Fig. 2B) capsule. Images were taken at 3 and 5 h post administration. Five out of the eight rats emptied both capsules intact from the stomach to the intestine within the first three hours. No additional emptying was observed at 5 h post administration. In all rats, there was no difference between the small and medium size capsules regarding the passage of the capsule from the stomach. Capsules left in the stomach did not disintegrate for the duration of the experiment (5 h). The same rats were given full size (Fig. 2A) capsules. None of the large capsules left the stomach 5 h after administration. Only medium size capsules were used in the following sections in all experiments.

3.3. In vivo visualization and localization of capsule

A series of X-ray images was used to follow the fate of the enterically coated capsules. The medium size minicapsule containing barium sulfate could be seen in the stomach (Fig. 3A) as a strong white ellipsoid object on the weaker white color of the highlighted stomach. The capsule could be followed as it moved to the intestine (Fig. 3B). At 4 h after administration, a white signal of part of the capsule and a white trail of spots were seen towards the end of the small intestine, close to the cecum (Fig. 3C), indicating the beginning of the capsule's disintegration near this location. The capsule was completely disintegrated in the cecum (Fig. 3D) half an hour after the onset of disintegration.

Table 1
Gastric emptying at 2.5 h under different feeding conditions (medium size capsules were used).

Number of experiments	Conditions	Number of gastric emptying events at 2.5 h
57	Fasting	1
57	Free access to food and water	19
20	Administration of capsules in the evening (8 pm) instead of morning —non-fasting condition	3

3.4. Gastric retention

Although the method for following the enterically coated capsules throughout the GI tract using X-Ray imaging had now been established, gastric retention remained a problem, having high variability between different rats and days of experiment. In order to perform an *in vivo* experiment on a large group of animals, in a reasonable time frame, it is desirable that capsules leave the stomach in 2–3 h after administration. Moreover, in our experience, rats that did not evacuate the capsule from the stomach after 2–3 h, tended to retain the capsule for several more hours. We have serendipitously found that fasting of rats aggravated the problem of gastric retention under the above-described conditions. Therefore, in order to investigate this confusing result further, a crossover study was designed with a group of 20 rats. Almost all (56/57) of the rats under fasted conditions retained the capsule in their stomach for at least 2.5 h. On the other hand, about a third of the capsules (19/57) passed the stomach during the 2.5 h period in non-fasting rats $\chi^2(1) = 17.52$, $p < 0.001$ (Table 1). Evening administration did not seem to improve the ratio of capsules that left the stomach in that time limit.

4. Discussion

The rat GI tract was first imaged by radiology using barium sulfate solution in 1993. According to the authors this was the first time a full imagining of the GI tract of the rat was taken, with a series of images following the advancement of the fluid contrast solution (Perry et al., 1993). Another series of images of the GI tract can be found from 2002 (Popesko et al., 2002). Several publications used X-ray imagining for the GI tract of rats (Chickering et al., 1997; Lee et al., 2002; Takada et al., 2003) but to the best of our knowledge none followed the fate of non-disintegrating solid dosage forms.

In this study we have shown that simultaneous visualization of capsule and background of the GI tract is possible using X-ray contrast agents. The passage of capsules through the gastrointestinal tract was easily detected, even to the untrained eye. Since the organs were highlighted, each picture could be read independently.

Preliminary experiments with smaller concentrations of Barium Sulfate inside the capsule were investigated (results not presented). The capsules were visible at 50% barium sulfate (w/w) and even at 25% for most images. This will enable us in the future to reduce capsule density and perform pharmacokinetic evaluation of drug release from coated capsules simultaneously with localization by imaging.

Gastric retention of non-disintegrating solid dosage forms in animal models has important implications for their use in preclinical trials. This subject has been investigated in several animal models such as dogs, rabbits (Aoyagi et al., 1992) and pigs (Davis et al., 2001; Hossain et al., 1990; Snoeck et al., 2004) as well as in

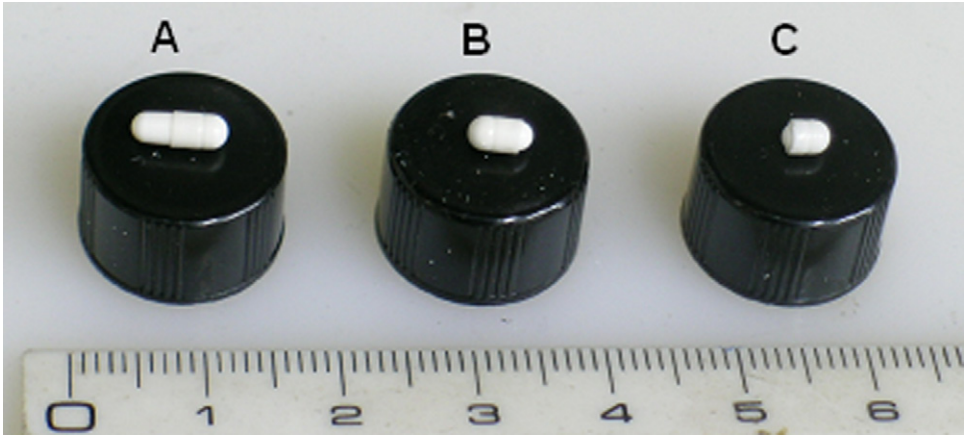

Fig. 2. Commercial (Capsulgel) hard gelatin capsules for rats, hand-cut to smaller size in the laboratory.

humans (Davis et al., 1988; Kenyon et al., 1994; Khosla and Davis, 1990). Gastric retention presents an obstacle in working with single unit dosage forms in small animals like rats and may be one of the reasons for the low number of *in vivo* experiments performed on this animal model. Rats are not considered suitable for the testing of non-disintegrating solid dosage forms by some researchers because of their small size (Davis et al., 2001). In order to overcome the difficulties of PO administration of non-disintegrating solid dosage forms to this size of animals, sometimes invasive techniques for inserting the dosage form directly into the organs of interest have been used (small intestine VS colon) (Mooter et al., 1995; Ogura et al., 2007). Invasive techniques may alter the normal patterns of gastric motility and therefore it is more desirable to find non-invasive methods.

Albrecht et al. (2006) reported that enterically coated gelatin minicapsules of 2.65 mm diameter and 8.4 mm height could empty from the rat stomach (4 rats, Sprague-Dawley, weighing 350–400 g). Without a prokinetic agent this took between 2 and 8 h. In our preliminary experiments similar capsules were used and although we tried several different protocols, no event of gastric emptying of the capsules was observed for at least 6 h. The next morning capsules were either intact in the stomach or disintegrated in the cecum or completely absent. As mentioned in the above reference, observing a large group of animals for prolonged periods using imaging apparatus is impractical for both animals and staff. Instead of using a prokinetic agent, we decided to reduce the size of capsules by reducing their length. Since shorter capsules were commercially unavailable, capsules were shortened by hand using a sharp blade knife. The smaller capsules easily exited the stomach. The effect of size of a non-disintegrating single unit solid dosage form on gastric emptying has been investigated in humans (Khosla and Davis, 1990) as well as large animal models such as pigs (Hossain et al., 1990). In these publications, the gastric emptying time of larger forms were shown to be longer. To the best of our knowledge, the effect of length of an enterically coated capsule on the gastric emptying of rats has not been investigated before.

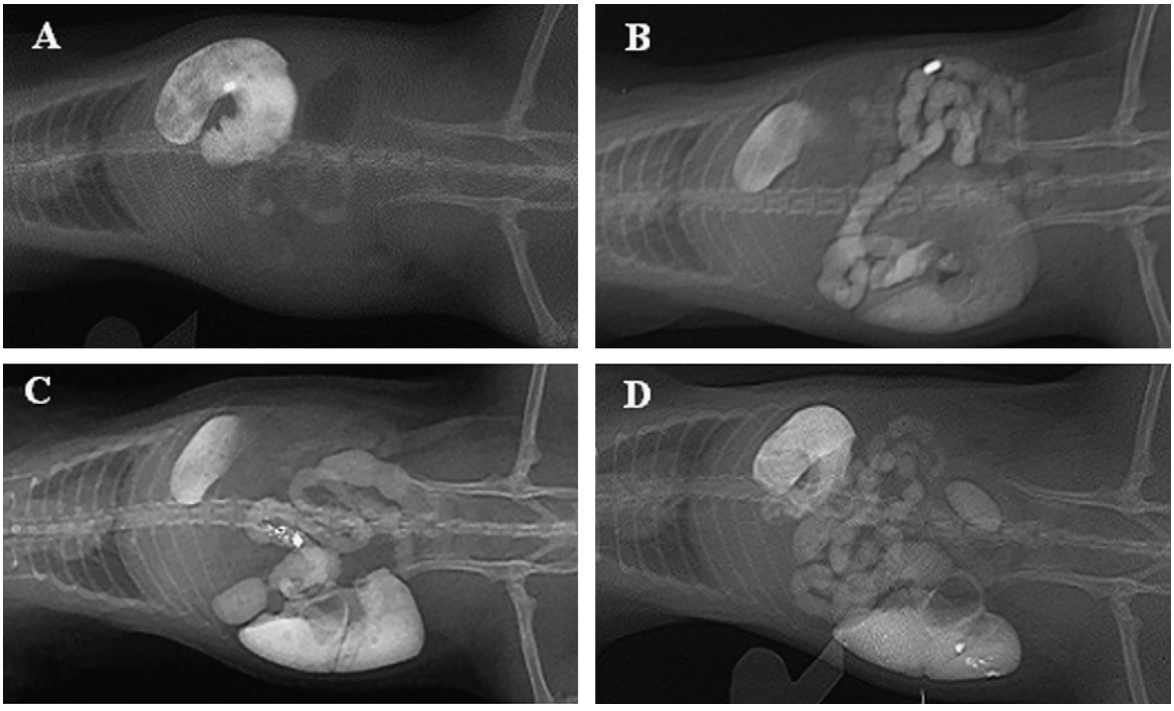

Fig. 3. X-ray images of the gastrointestinal tract of a rat, showing the movement of an enterically coated capsule from the stomach (A) 10 min after administration, to the intestine (B) 2 h 15 min. (C) Opening of the capsule at the ileum close to the entrance to the cecum after 4 h. (D) Barium sulfate dispersed in the cecum 4 h 35 min.

We have found that a reduction of a few millimeters in capsule size enabled performing routine *in vivo* studies in rats.

During our method development, we found that more capsules emptied under our time limit from the fed stomach than from fasted rats. This result was surprising since food is generally considered to delay gastric emptying. In addition, we noticed that there was a large variability in regard to gastric retention in different rats and on different days of experiments. We therefore preformed an experiment with a large number of animals ($n = 20$) and repetitions (alternating between fed state and fasted state three times). The results obtained were statistically significant. It is clear that in rats more capsules leave the stomach in the first 2.5 h when fed than when fasted. Other experiments have also shown that gastric emptying is not always faster in the fasted state for example in stomach-emptying-controlled rabbits enteric-coated granules were expelled faster when the rabbits received food immediately after drug administration compared to fasting (Aoyagi et al., 1992).

Food is known to delay gastric emptying of tablets (Aoyagi et al., 1992; Fadda et al., 2009) and capsules in humans (Kenyon et al., 1994; Martinez et al., 2002). The gastric retention of tablets in fed dogs is even more pronounced, creating a delay of more than 10 h (Aoyagi et al., 1992). This corresponds to the disruption time of the interdigestive migrating motor complex (MMC) after feeding. In humans and dogs, MMC is usually observed every 90–120 min in the interdigestive state. In contrast, in rats, the MMC cycle is short (less than 20 min) and not as regular as that of humans and dogs. Sometimes phase III-like contractions are not clearly observed even after 24-h fasting in rats. This has been suggested to possibly occur because of the free feeding commonly applied in laboratory rats (Ariga et al., 2007), as opposed to fixed or scheduled regimens of meals in humans (three meals a day) or dogs (one meal a day). The phase III contractions are important for the gastric emptying of large solids and their absence even after 24-h fasting could well be the reason for the difficulty of gastric emptying of the capsules in the fasted state. In case of fasting, adding grid floors to prevent coprophagy also adds stress, which may additionally delay gastric emptying (Nakade et al., 2005).

It has been recently shown for humans, that the simple distinction of fasted and fed is an oversimplification, and that other patterns of feeding may lead to different results regarding GI motility (Fadda et al., 2009). Rats are known to be nocturnal animals, eating mainly at night. Therefore, by the beginning of our experiments around 10 pm, they have probably been "voluntarily" fasting for several hours (their day time schedule starts at 6 am). The fact that "fed" rats have been fasting for several hours prior to the experiment may also help explain our results. Administration of capsules at night, after rats have probably not eaten most of the day gave intermediate results. In order to study this point further, a more ordered regimen of feeding may be applied, which could lead to more frequent phase III contractions, and more rapid gastric emptying (Ariga et al., 2007).

5. Conclusion

In this study we have shown that the location and fate of enterically coated capsules in the GI tract of rats can be easily followed using simple X-ray imagining technique. Shortening the smallest commercially available gelatin minicapsules, intended for preclinical trials in rats, alleviated the problem of gastric retention.

Gastric emptying of the capsules was unexpectedly faster from the fed state. This result demands further investigation using a more ordered feeding regimen, similar to that of humans, which may induce stronger phase III contractions. From preliminary data we have been collecting, differences in species and even sources of rats may also affect the rate of gastric empting. These differences are currently under investigation.

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