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Rapid communication

Duodenum-specific drug delivery: In vivo assessment of a pharmaceutically developed enteric-coated capsule for a broad applicability in rat studies

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ABSTRACT

Targeting new oral drug formulations in the intestine has a broad applicability in animal studies. Enteric-coated capsules are gastroresistant and specific drug delivery systems useful for the evaluation of new pharmaceutical formulations during pre-clinical validations in rats. The purpose of this study was to develop and validate in a large-scale, reliable, reproducible capsules, to offer a safe and standardized duodenum-specific delivery system adapted for studies in rats. The reproducibility of the coating method, the coating layer uniformity and thickness, the external capsules integrity and their enteric properties after in vitro dissolution in simulated gastric and intestinal media were already evaluated and validated. This study presents the in vivo tests of the gastroresistance and of the location of the disintegration. Micro-computerized tomography and a pharmacokinetic study of acetaminophen-filled capsules showed that the enteric-capsules were resistant in the stomach with no apparent leak of the capsules, and were disintegrated in the early duodenum 1–1.5 h after oral administration. A positive impact on the bioavailability of acetaminophen in coated capsules was attested. In conclusion, this work, developed with a rigorous pharmaceutical technology, presents a tool adapted for duodenum-specific delivery of new formulations in rats.

The oral route remains the most considered way to administer drugs, which can either act locally in the gastro-intestinal (GI) tract or be absorbed at the level of the intestine. However, in some cases the oral drug administration is problematic: for instance when the drug lacks stability in gastric fluid, when it causes irritations of the GI tract or when it has to be targeted at a specific site. Because there are pH variations all along the GI tract, such problems can be solved by incorporating drugs into solid oral dosage forms coated with pH sensitive enteric polymers (Pinto, 2010). Commonly used polymers are polymethacrylates, e.g. Eudragit® (Corá et al., 2006; Cole et al., 2002), cellulose-based polymers, e.g. hydroxypropylmethylcellulose acetate succinate (Ghosh et al., 2011) or polyvinyl derivatives, covering a pH range dissolution from 5.5 to 7, suitable for delivery from the duodenum to the colon.

Such polymers are used by pharmaceutical industries and on a large scale in animal studies for the delivery of acid labile formulations. Formulations are anti-inflammatory drugs specifically delivered into the colon for the treatment of inflammatory bowel disease (Krishnamachari et al., 2007), chemotherapeutic agents against colorectal cancer (Ji et al., 2009; Zambito et al., 2005), or drugs specifically delivered into the duodenum: vaccines (Uddin et al., 2009; Mercier et al., 2007) or drugs in particles, such as: insulin (Sonaje et al., 2010), immunosuppressants (Dai et al., 2004), enzymes for enzyme replacement therapy for pancreatitis (Naikwade et al., 2009). This non-exhaustive list of applications shows a broad applicability of gastroresistant and specific drug delivery systems for the assessment of new pharmaceutical formulations during a pre-clinical validation in animal models.

Rats are the most widely used and best validated small animal model for basic studies on bioavailability, pharmacokinetics and pharmacodynamics. In this way, publications report gastroresistant capsules filled with freeze-dried compounds and adapted to rats (Saphier et al., 2010; Sonaje et al., 2010; Kremser et al., 2008). Such capsules have several advantages. First, the solid form increases the amount of intact molecules delivered to a specific site of the intestine, and consequently increases the bioavailability. Secondly, the coating process of capsules is independent of the capsule content (Cole et al., 2002). In humans, the formulation of enteric-coated capsules is well defined according to good manufacturing practices for pharmaceutical products. However, in the rat, all previously quoted studies reported capsules formulation in a

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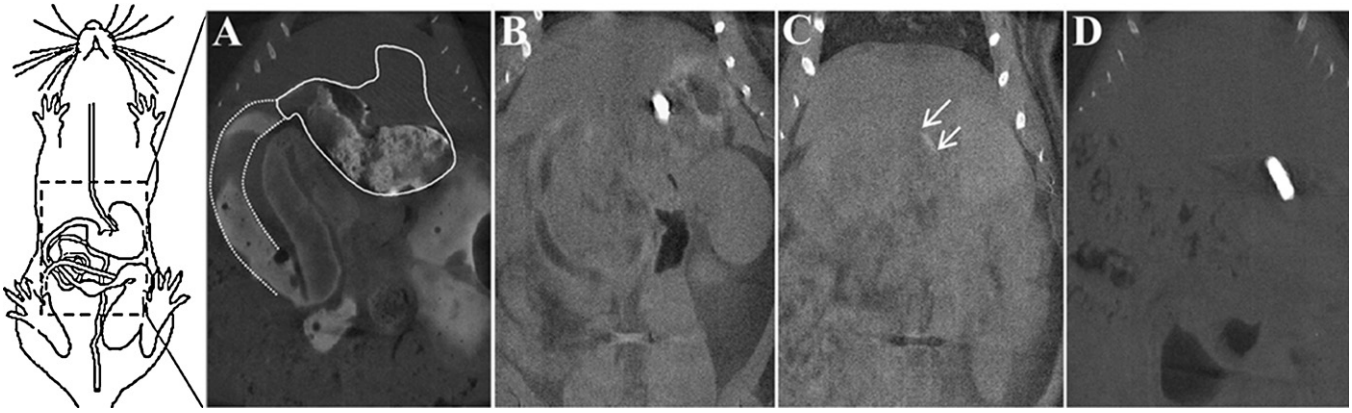

Fig. 1. Micro-computerized tomography (μCT) visualizations of capsules in rats. (A) Contrast of the stomach and the beginning of the intestine with a solution of $BaSO_4$ orally administered. Mid coronal sections showing the kinetic of disintegration of an uncoated capsule within the rat stomach 15 min (B) and 1 h (C) after oral administration. (D) Visualization of a coated capsule still intact in the stomach 5 h after administration.

small-scale which did not respond to these criteria. The innovative aspect of our work was to develop and evaluate a pharmaceutical enteric coating process based on a transposition of an industrial technology to small-sized capsules required for rats. The aim was to obtain in a large-scale, reliable, reproducible capsules with commonly used material and moieties for manufacturing with respect to good manufacturing practices for pharmaceutical products, to offer a safe and standardized tool adapted for pre-clinical trials in rats. This development was led in association with Capsugel (Capsugel Division of Pfizer Inc., Colmar, France) which optimized and developed an enteric-coated capsules model. We then evaluated this model in vivo in rats.

Capsules were PCcaps™ size 9 (Capsugel). 700 g of capsules were coated with a ready-to-use enteric formula based on Eudragit® L 100-55 (Acryl-Eze®, Colorcon, UK) designed to achieve enteric properties, as it dissolves at pH 5.5 in the duodenum. A homogeneous aqueous dispersion of Acryl-Eze® was used for the capsules coating in a fluid bed apparatus equipped with a wurster and a bottom spray nozzle. The coating layer weight was between 6.5 and 7 mg/mm^2. The reproducibility of the coating method, the coating layer uniformity and thickness, the external capsules integrity and their enteric properties after in vitro dissolution in simulated gastric and intestinal media (pH 6.8) were evaluated and validated by Capsugel. Capsugel verified that the capsules coated with Acryl-Eze complied with the European Pharmacopoeia requirements for enteric-coated formulation (less than 10% dissolved in 2 h in simulated gastric medium and not less than 80% dissolved at 45 min in simulated intestinal medium).

The gastroresistance of PCcaps™ was tested in vivo to identify the delay and the location of the disintegration site by using micro-computerized tomography (μCT) and a pharmacokinetic study. Animal studies were performed in accordance with the guidelines of the local authorities and French laws. Male Wistar rats weighing 250 ± 50 g were purchased from Depré (Saint-Doulchard, France), housed under a 12 h light, 12 h dark cycle and fed with a standard laboratory rodent diet (Safe, Augy, France). The oral administration of capsules was performed using a delivery device provided with the capsules.

μCT imaging was performed with PCcaps™ filled with barium chloride (Sigma–Aldrich, 22 mg/capsule) and coated using conditions described above. To enable a good distinction of the stomach and the intestine, two rats were injected intraperitoneally with 1 mL of iodine (Iomeron® 350, BYK, Le Mée sur Seine, France) to contrast the intestine and force-fed with 700 μL of $BaSO_4$ (Micropaque®, Guerbet, Roissy CdG, France) to contrast the stomach (Fig. 1A). Two groups of rats received coated (n = 12) or uncoated capsules (n = 6) of barium chloride. In order to have an early duodenal drug delivery, we tried to limit the gastric retention time. As GI motility can be activated when a meal is ingested, rats were fasted for 14 h with water ad libitum before capsules administration, and were refed thereafter. Acquisitions were done 0.25, 1, 2, 3 and 5 h after force-feeding. Imaging was performed by using an X-ray μCT scanner constructed at the DRS/IPHC, Strasbourg, France. The X-ray tube was operated at 40 kV, 250 μA and produced 768 views over 360°. Visualization and analysis were performed using Anatomist/BrainVISA software (http://brainvisa.info).

Fig. 1B shows that the disintegration of uncoated capsules occurred after 15 min and was complete in less than 1 h, as we can see a clear decrease of the contrast and the size of the mass of barium chloride (Fig. 1C). Enteric capsules were still intact after 5 h in gastric environment (Fig. 1D). The μCT experiments in rats confirmed the gastroresistance property of the coated capsules until at least 5 h. However, we did not observe the gastric emptying of the capsules. This result was not quite expected because the size of the capsules is adapted to rats, as there is no physical hindrance with the anatomy of the pylorus. We believe that the perturbation of the transit was due to the stress of the experiment. Stress, although difficult to quantify, is also known to slow down GI motility and subsequently delay the gastric emptying (Enck et al., 1989).

To investigate by another way the delay of disintegration of Eudragit® L 100-55 coated capsules, a pharmacokinetic study with acetaminophen as drug marker was done. Capsules were filled with 5 mg of acetaminophen (Sigma) and 11 mg of lactose (Meggle). Rats were fasted for 8 h prior to the experiment, and the per os administration of a coated (n = 5) or uncoated (n = 7) acetaminophen capsule before refeeding was done at the beginning of the dark period. As rats are known to be nocturnal animals, they eat mainly during this period and are more active, which facilitates the gastric emptying. Blood samples were withdrawn in the left carotid *via* a polyethylene tube to the following time schedule: 0 (pre-dose), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h post-dose. Concentrations of acetaminophen in the serum were determined by ELISA assay (Immunoanalysis Corp., Pomona, CA).

Mean plasma concentration curves of acetaminophen are shown in Fig. 2A and corresponding pharmacokinetic parameters in Table 1. The acetaminophen uncoated capsule T_{max} occurred rapidly at 18 ± 6 min post-dose and was delayed at 114 ± 12 min in rats which received a coated capsule. At this time, as shown by the μCT experiments, there was no apparent leak of the capsules in the stomach which were still intact. Therefore, they were likely

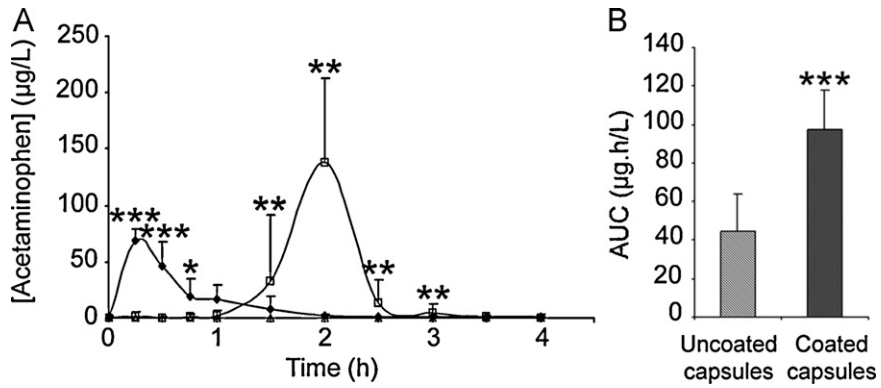

Fig. 2. (A) Mean (± sd) plasma acetaminophen concentrations following per os administration of empty capsules (open triangles, $n = 4$), acetaminophen-filled uncoated (lozenges, $n = 7$) or coated capsules (open squares, $n = 5$). (B) Area under the curves (AUC) up to 4 h (Student's T-test $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

Table 1
Summary of pharmacokinetic parameters for acetaminophen uncoated and coated capsules in rats following oral administration.

Pharmacokinetic parameters	Acetaminophen capsules		Statistical significance
PCcaps	Uncoated	Coated	
Dose (mg)	5	5	
T_{max} (h)	0.3 ± 0.1	1.9 ± 0.2	$p = 0.003$
C_{max} (µg/mL)	65 ± 15	163 ± 25	$p < 0.001$
AUC (µg h/mL)	44 ± 20	97 ± 20	$p = 0.001$

to be disintegrated in the early duodenum. The mean C_{max} value of acetaminophen was 2.5 higher in the coated capsules group than in the uncoated capsules group and the difference between the $AUC_{0\rightarrow 4\,h}$ of uncoated and coated capsules groups was statistically significant (Fig. 2B, $p < 0.001$). The bioavailability of acetaminophen administered in coated capsules was about 220% compared to acetaminophen administered in uncoated capsules, suggesting an impact of enteric capsules on the rate of drug absorption and an effective gastric protection.

In conclusion, this work, developed with a rigorous pharmaceutical technology, presents a tool adapted for duodenum-specific drug delivery of new formulations in rats. The large-scale production is an undeniable advantage for the screening of new compounds in pre-clinical trials.

Furthermore, the methods used in the present work offer a wide range of possibilities for the development and the assessment of capsules coated with various enteric polymers specific to the distal part of the intestine or the colon for example.

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