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Neutron activation based gamma scintigraphic evaluation of enteric-coated capsules for local treatment in colon

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Abstract

The fate of two colon-specific formulations developed in our previous study was investigated using a gamma scintigraphic imaging method. The formulations contained paracetamol and samarium oxide (Sm_2O_3) and either microcrystalline cellulose (MCC) or hypromellose (HPMC K4M) as diluent and were coated with Eudragit® S polymer. The gamma scintigraphic evaluation proved that the products remained intact in the stomach and the upper gastrointestinal tract. The gastric residence time was less that 1 h. Three to four hours after administration the formulations had reached the ileo-caecal junction, i.e. the small intestine transit time was approximately 3 h. The capsules disintegrated in the ileo-caecal junction or in the ascending colon. The capsules containing MCC released the marker momentarily, the capsules containing HPMC K4M gradually spreading it to the whole colon. The gamma images also verified that the HPMC gel disintegrates completely in 12–14 h. While comparing the results to those previously obtained from the bioavailability studies it could be concluded that it is possible to develop colon specific drug products that begin releasing the drug in the ileo-caecal junction or at the beginning of the ascending colon and spread the drug dose to a larger surface area by using enteric coats and hydrophilic polymers.

Keywords: Colon-specific delivery; Modified release; Gamma scintigraphy; Enteric polymers; Hydrophilic polymers

1. Introduction

During the last years in the field of biopharmaceutical research, it has become evident that in vitro studies alone are inadequate when developing modified-release drug formulations. Therefore, in vivo behaviour of these dosage forms needs to be investigated at an early stage. In relation to the development of site-specific formulations, also pharmacokinetic studies in most cases are not adequate to determine actual fate of formulations in gastrointestinal (GI) tract. In order to get final proof concerning the fate of different formulations in humans, the dosage forms should also be studied with an appropriate imaging method. One of the most appropriate means of studying the fates of site-specific formulations in GI tract nowadays is gamma scintigraphy (Newman et al., 2003; Marvola et al., 2004).

In our previous study, we developed enteric-coated capsules for local treatment in colon that could be utilized, e.g. as a carrier for acetaldehyde binding compounds in prevention of alcohol induced colon carcinomas as well as a carrier for β-lactamase in prevention of MRSA infections during drug treatment with β-lactam antibiotics (Väkeväinen et al., 2000; Harmoinen et al., 2004; Mentula et al., 2004; Marvola et al., 2007). We were able to indirectly prove that hypromellose (HPMC) capsules coated with enteric polymer and containing a hydrophilic polymer as a diluent might be suitable for local colon-specific drug treatment. Enteric-coated products are usually designed to remain intact in the stomach and to release the drug dose in the upper parts of small intestine (Agyilirah and Banker, 1991). Enteric coatings can also be utilized in development of colon-specific dosage forms by choosing a polymer that starts dissolving in higher

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pH and/or applying a thicker coating (Hardy et al., 1987). The most commonly used enteric polymers are anionic methacrylates (Eudragit®) of which Eudragit® S dissolves above pH 7. For site-specific treatment in colon, it is also important to decrease the rate of drug release so that the active compound spreads to whole colon content. With capsule formulations, this can be achieved with a right kind of hydrophilic diluent, e.g. HPMC (Ojantakanen et al., 1993; Honkanen et al., 2001).

The formulations in our previous study were subjected to both in vitro dissolution studies as well as to in vivo bioavailability studies. Those studies proved that it was possible to delay the beginning of drug release form the capsules up to 4–5 h by choosing the right kind of enteric coating and hydrophilic diluent. Because it is known that the mean gastric residence time for single solid dosage forms in fasting state is 0.25–2 h and the transit time through the small intestine is approximately 3 h (Wilson and Washington, 2000), it was possible to indirectly conclude from the results that the formulations began releasing paracetamol at the ileo-caecal junction or in the colon. Knowing that the transit times through the colon vary from 7 to 24 h, it could also be concluded that the capsules containing different grades of HPMC as diluent continued to release paracetamol in the colon spreading it probably to transverse and descending colon as well compared to capsules containing microcrystalline cellulose (MCC) as diluent which released paracetamol immediately after the enteric polymer had dissolved. Since these bioavailability tests did not, however, directly prove that the formulations behaved as desired, it became evident that the most promising formulation must also be subjected to neutron activation based gamma scintigraphic evaluation.

The aim of this study was to obtain visual data about the fate of orally administered colon-specific formulations enteric-coated with Eudragit® S and containing either MCC or HPMC K4M as diluent, by gamma imaging the formulations and to prove that these formulations developed in our previous study, do behave as predicted on the grounds of dissolution and bioavailability studies, i.e. that the capsules begin releasing paracetamol at the earliest in the ileo-caecal junction and at the latest in the lower part of ascending colon and that the capsules containing HPMC K4M do actually release the drug compound at a reduced rate to whole colon content.

2. Materials and methods

2.1. Formulations for scintigraphic evaluation

Based on previous bioavailability studies, two formulations were selected for neutron activation based gamma scintigraphic investigation on healthy volunteers. The formulations studied were based on hard HPMC capsule size 0 (Shionogi Qualicaps SA, Spain). They contained 96 mg of paracetamol (Ph. Eur.) and 4 mg of natural-abundance samarium oxide (Sm_2O_3) (Aldrich, USA) and either microcrystalline cellulose (MCC, Avicel PH 102, FMC BioPolymer, USA) or hydroxypropyl methylcellulose (HPMC, Methocel K4M, FMC BioPolymer, USA), q.s. to fill the capsule body. To obtain site-specific start of drug release in the vicinity of ileo-caecal junction the capsules were coated with methacrylate polymer Eudragit® S (Röhm GmbH, Germany).

9.6 g of paracetamol and 400 mg of Sm_2O_3 was weighed out into a measuring cylinder and MCC or HPMC K4M was added to obtain sufficient material for a batch of 100 capsules (68 ml). The powders were then mixed manually and the capsule bodies were filled using a Feton apparatus (Feton International, Belgium). The uniformity of mass was studied from 20 capsules (Ph. Eur.).

A 10% (w/w) solution in ethanol (Oy Primalco Ab, Finland) and water (9:1) of the Eudragit® S-polymer was prepared. Polyethylene glycol (PEG 6000, Sigma–Aldrich Chemie, Switzerland) (1%) was used as a plasticizer and 2.5% of talcum (Ph. Eur.) was added to prevent the capsules from sticking during drying. The capsules were coated dipping them into the Eudragit® solution after which they were allowed to dry on a mesh in a warm airflow. An increase of 13% in the total weight of the capsules was gained.

2.2. Dissolution studies

Study formulations were subjected to a dissolution test both before and after the irradiation to ensure integrity of products during irradiation. They were kept in 5 ml pH 1.2 hydrochloric acid (HCl) buffer (USP 24) for the first 2 h after which they were subjected to dissolution testing using the basket method (USP 24) with rotation rate of 100 min^{-1}. For the first hour 500 ml pH 6.8 phosphate buffer (USP 24) medium at 37 ± 0.5 °C was used and for the remainder of the dissolution test pH 7.4 phosphate buffer (USP 24). Paracetamol concentrations in samples were measured spectrophotometrically at 245 nm. The results gained at pH 7.4 were utilised also for the evaluation of the uniformity of content of each formulation.

2.3. Gamma scintigraphic studies

2.3.1. Neutron activation

The Sm_2O_3 was activated to $^{153}Sm_2O_3$ using the FiR 1 250 kW TRIGA nuclear reactor (General Atomics, USA) at the VTT Technical Research Centre of Finland and a neutron flux of 1.1×10^{12} n cm^{-2} s^{-1}. Six items were irradiated at a time. The irradiation time was 4 min to allow the target radioactivity of 1 MBq to be reached 48 h after irradiation.

2.3.2. Safety requirements

Gamma spectra and radioactivity were measured to determine the safety of the formulations for use in human studies. Safety requirements were in accordance with the guidelines established by STUK (Finnish Radiation and Nuclear Safety Authority). The as-low-as-reasonably achievable (ALARA) principle was observed and exposure to radiation was minimized in all situations. Gamma spectra were measured 24 h after irradiation using a high-purity germanium semiconductor (model 7229P, Canberra, Belgium) to confirm radioactive purity. One capsule from each batch irradiated was studied. The safety requirements were that any net gamma peak area not originating from ^{153}Sm should not exceed 0.3% or the ^{153}Sm main peak at 103 keV and that the total for net peaks areas not originating from ^{153}Sm should not exceed 1% or the ^{153}Sm main peak.

The radioactivity was measured 48 h after irradiation, i.e. immediately before administration to volunteers. A CRC-35R radioisotope calibrator (Capintec, USA) was used for the measurement. Six capsules from each batch were studied. The safety requirement was that ^{153}Sm activity should not exceed 0.7 MBq for a single formulation. Total radioactivity of 1.4 MBq for the two capsules corresponds with an effective absorbed dose of 1 mSv for each study subject.

2.3.3. *In vivo gamma scintigraphy*

Six healthy male volunteers (23–35 years of age) participated in the study. Their weight varied from 63 to 101 kg and BMI from 21 to 26 kg m^{-2}. Before the study, each volunteer was examined physically and subjected to routine haematological testing and urine analysis. Each volunteer was informed about the possible risks and adverse effects of taking the study formulations. Written informed consent to participate in the study was obtained. The investigation was carried out in accordance with International Conference of Harmonization (ICH), Good Clinical Practice Guidelines and the Declaration of Helsinki (World Medical Assembly, 1964) and subsequent amendments. The study protocol had been approved by the Finnish national Agency for Medicines and the Ethics Committee of Helsinki University Hospital (HUS). The studies were carried out at HUCH Division of Nuclear Medicine, which has a radiation safety licence issued by STUK.

Scintigraphic studies were carried out as a single-dose crossover study 48 h after neutron activation to allow decay of unwanted radioisotopes, primarily ^{24}Na. The lower tip of the sternum and the iliac crests of each study subject were marked with a felt-tip pen, and markers containing ^{57}Co were attached to these locations with adhesive tape. The markers were removed after the first set of images in every time point so that they would not disturb detecting the weaker irradiation sent by the formulations. Irradiated capsule containing either MCC or HPMC as an excipient was administered to each volunteer in sitting position at approximately 8 a.m. after an overnight fasting for at least 12 h. Study subjects had been advised to abstain from alcohol and foods or fluids containing caffeine or xantines for 48 h prior to administration of the study formulations. The volunteers were allowed to eat and drink 4 h after the administration of the formulation. Following the administration, anterior and posterior images each of 1 min duration were recorded at intervals of 1 h for 14 h in supine position. Between imaging times the subjects were allowed to move freely. Washout period between the two formulations was 2 weeks. Scintigrams were recorded at 103 keV (window width ± 10%) using a dual-head gamma camera (ADAC Forte, ADAC Laboratories, USA) equipped with low-energy general-purpose (LEGP) collimator.

3. Results and discussion

3.1. *Dissolution study*

In order to determine if irradiation caused any changes to study formulations, irradiated capsules were subjected to dissolution testing before the actual gamma scintigraphic evaluation. It is well known that irradiation can accelerate drug release from formulations containing polymeric excipients (Watts et al., 1993; Säkkinen et al., 2004). The dissolution profiles of paracetamol from the capsules are presented in Fig. 1. With these formulations, no significant difference between non-irradiated and irradiated formulations was perceived. This was most probably due to short irradiation time used.

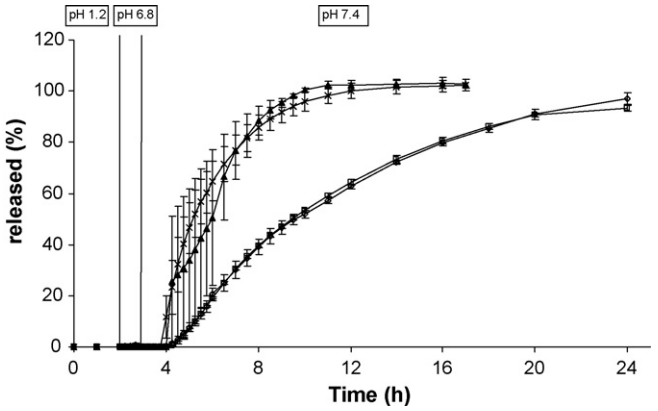

Fig. 1. Dissolution profiles of non-irradiated and irradiated enteric-coated capsules containing MCC or HPMC K4M as diluent. (▲) MCC, non-irradiated; (×) MCC, irradiated; (□) HPMC, non-irradiated; (◊) HPMC, irradiated.

3.2. *Gamma imaging*

Figs. 2 and 3 show the fate of Eudragit® S coated capsules containing MCC or HPMC K4M as diluent in one volunteer. The gastric residence time was less that 1 h. Three to four hours after administration both formulations had reached the ileo-caecal junction, i.e. the transit time through the small intestine was approximately 3 h. As seen in Figs. 2 and 3 both formulations remained intact in the stomach and the upper parts of the gastrointestinal tract and they disintegrated and began releasing radioactive Sm_2O_3 4 h after administration either in the ileo-caecal junction or in the lower parts of ascending colon. The capsules containing MCC released the marker momentarily to the ascending colon, whereas the capsules containing HPMC released the marker gradually spreading it to the whole colon. Eight hours after administration radioactivity detected from capsules containing MCC was still mostly in the ascending colon and 14 h after administration it had only reached the hepatic flexure. With capsules containing HPMC K4M, gel formation was detected. Five hours after administration three separate gel particles were visible. The gel particles distributed to the colon content so that 8 h after administration first gel particle was in the transverse colon, second one in the hepatic flexure and third one still in the upper parts of ascending colon. Ten hours after administration, all the gel particles were in the transverse colon and still clearly detectible. In the 12 and 14 h images, it can be seen that the gel has disintegrated and the radiation detected is spread evenly to the transverse colon and some radiation can also be detected in the upper parts of both ascending and descending colon.

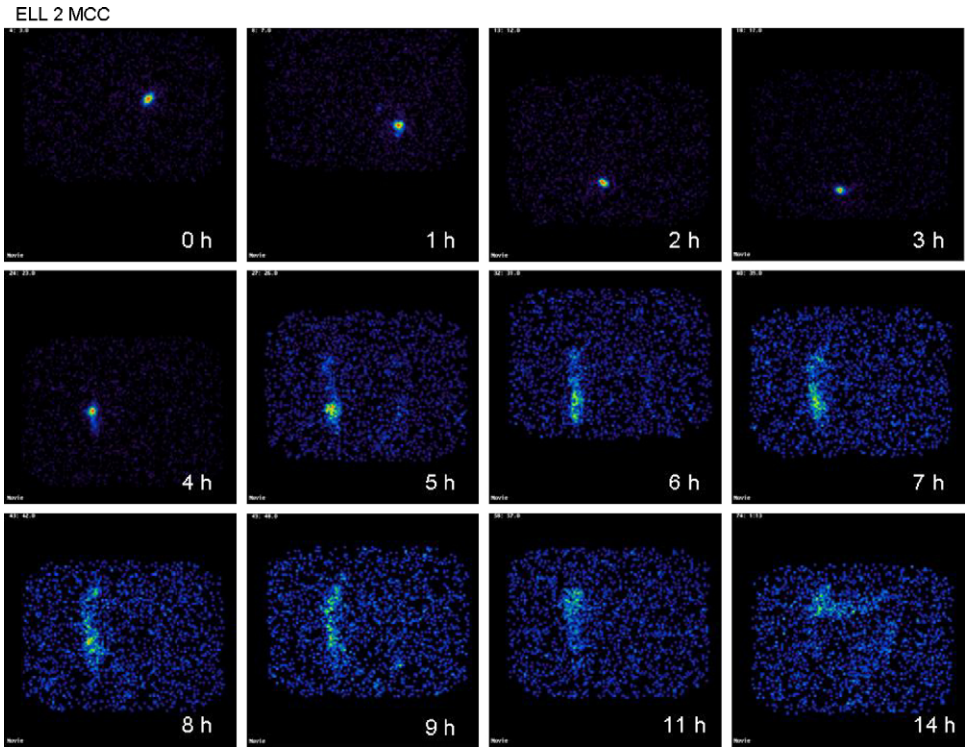

Fig. 2. Scintigrams on a volunteer after administration of one Eudragit S coated capsule containing MCC as diluent.

The study formulations behaved in similar manner in every study subject. All formulations had left the stomach after approximately 1 h and also reached the ileo-caecal junction in 4–5 h (Tables 1 and 2). They also began releasing Sm_2O_3 at the earliest in the ileo-caecal junction and at the latest in the lower parts of ascending colon. Gel formation was detected in all of the capsules containing HPMC K4M as diluent indicating that HPMC does form gel also in vivo in the colon, thus decreasing the release rate of drug compounds.

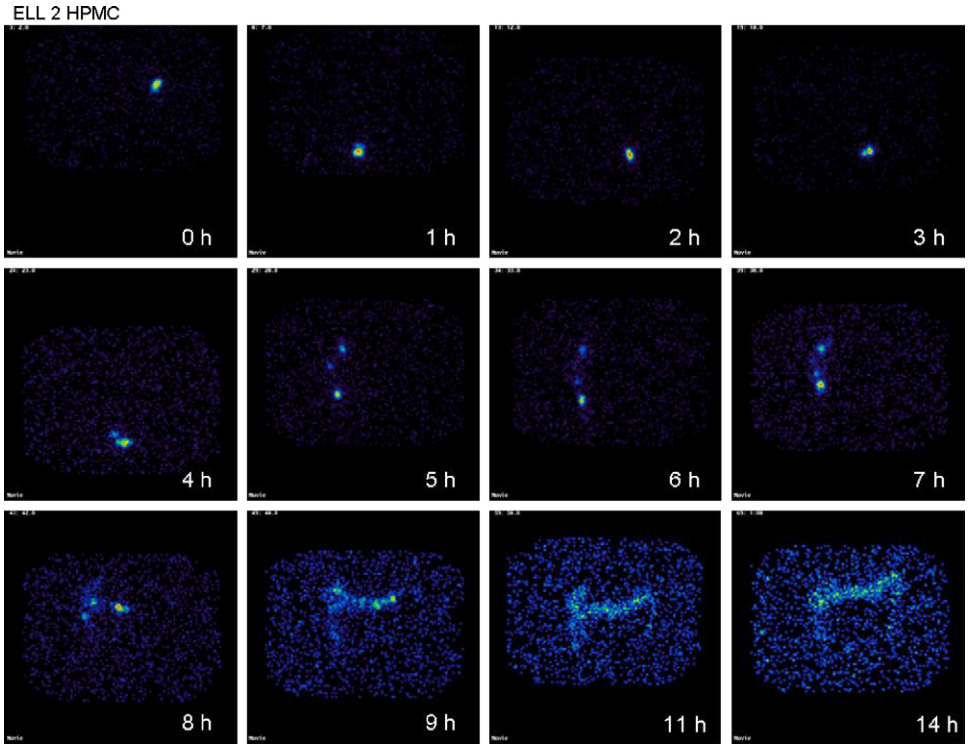

Fig. 3. Scintigrams on a volunteer after administration of one Eudragit S coated capsule containing HPMC K4M as diluent.

Table 1
The time point of each of the six study subject at which the capsule containing MCC as diluent reached a specific part of the GI-tract and the beginning of paracetamol and samarium oxide release from the capsule

Study subject	Stomach emptying	Ileo-caecal junction	Ascending colon	Transverse colon	Descending colon	Initial disintegration
1	0.5	2	3	9	12	3
2	1	3	4	12	13	4
3	1.5	3	4	10	14	4
4	1	3	4	7	13	4
5	0.5	2	3	7	14	3
6	1.5	4	5	9	14	5
Average	1.0	2.8	3.8	9.0	13.3	3.8
S.D.	0.45	0.75	0.75	1.90	0.82	0.75

Site and fate of the formulation in GI-tarct/time after administration (h).

Table 2
The time point of each of the six study subject at which the capsule containing HPMC as diluent reached a specific part of the GI-tract, the beginning of paracetamol and samarium oxide release from the capsule and disintegration of the HPMC gel

Study subject	Stomach emptying	Ileo-caecal junction	Ascending colon	Transverse colon	Descending colon	Initial disintegation	Disintegration of HPMC gel (number of gel pieces)
1	1	4	5	8	9	6	10 (3)
2	0.5	3	4	7	10	4	10 (2)
3	1	4	5	10	13	5	13 (3)
4	0.5	3	4	6	12	5	13 (3)
5	0.5	3	4	8	14	6	14 (2)
6	0.5	2	3	8	14	3	13 (3)
Average	0.7	3.2	4.2	7.8	12.0	4.8	12.2
S.D.	0.26	0.75	0.75	1.33	2.10	1.17	1.72

Site and fate of the formulation in GI-tarct/time after administration (h).

4. Conclusions

While comparing the results obtained from the gamma scinticraphic study to those previously obtained from the bioavailability studies, it was possible to draw a conclusion that the formulations coated with Eudragit® S solution did in fact transport the drug substance to the ileo-caecal junction before releasing it. The results also proved that HPMC does form gel in the colon, thus decreasing the release rate and also spreading the drug compound to whole colon content. This of course is important since if the drug compound is released momentarily in the ascending colon, as was the case with capsules containing MCC as diluent, it is very likely to be absorbed immediately and therefore it will not be spread to other parts of colon and does not reach the aim of local treatment in the whole colon. The gamma images also verified that the HPMC gel disintegrates completely in the colon in 12–14 h proving that the decrease in the amount of paracetamol absorbed from the capsules containing HPMC detected in our previous study was not due to incomplete release of paracetamol from the hydrophilic gel but resulted from lower absorption of paracetamol in the lower parts on the GI tract. Thus, it seems that it is possible to make a capsule formulation suitable for local sustained-release treatment in colon utilising suitable enteric coating materials and hydrophilic polymers as diluents to decrease the drug release rate.

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