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Are chitosan formulations mucoadhesive in the human small intestine? An evaluation based on gamma scintigraphy

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Abstract

Rapid passage through the proximal intestine can result in the low bioavailability of a drug substance with site-specific absorption characteristics in the upper gastrointestinal tract. To overcome this, there is increasing interest in developing gastro-retentive formulations and/or formulations that linger in the proximal parts of the small intestine, e.g. by using mucoadhesive polymers as excipients in formulations. In our recent study, we used neutron activation-based gamma scintigraphy to evaluate the gastro-retentive properties of formulations containing chitosan (Mw 150 kDa) in man. At the same time, we had an opportunity to monitor the transit of the formulations (40 or 95% of chitosan) in the small intestine. Gamma scintigraphic investigations revealed that although the chitosan studied had exhibited marked mucoadhesive capacities in vitro, retention of the chitosan formulations in the upper gastrointestinal tract was not sufficiently reproducible and the duration of retention was relatively short. In 3 volunteers out of 10, the formulation adhered to the gastric mucosa (retention times varied from 1.25 to 2.5 h) and in two volunteers to the upper small intestine (approximate retention time 45 min). In one case, the formulation adhered to the oesophagus. The system failed to increase the bioavailability of furosemide, a drug site-specifically absorbed in the upper gastrointestinal tract. As far as the kind of formulation studied is concerned, preparation of a system that is site-specific to the stomach and/or the upper small intestine seems difficult if the proposed mechanism of action is mucoadhesion. The results suggest that other mechanisms of action should also be studied.

Keywords: Mucoadhesion; Small intestine; Chitosan; Gamma scintigraphy

1. Introduction

Numerous drugs are known to act as substrates for intestinal transporters (Leslie et al., 2005; Steffansen et al., 2004). Membrane transporters may play an important role in determining the bioavailability of the drug substance, absorptive transporters increasing the amount of drug absorbed and efflux transporters such as the P-glycoprotein (P-gp) reducing bioavailability. Intestinal membrane transporters may also be responsible for the site-specific absorption characteristics of the drug substance. This can be explained by the fact that the distribution of many of these transporters varies in different regions of the intestine; for example the expression of P-gp gradually increases down the intestine (Mouly and Paine, 2003). For drugs that are substrates for P-gp, lower activity in the upper intestine could contribute to better absorption in the proximal intestine than in the lower gastrointestinal tract. In general, the better absorption properties of the proximal intestine also include better paracellular permeability and/or dense absorptive transporters.

Despite the superior absorption properties of the proximal intestine, the extent of absorption may be limited because passage through this region is rapid. This inevitably results in the low bioavailability of a drug substance with site-specific absorption characteristics in the upper gastrointestinal tract, particularly if the drug is administered as a slow-release formulation. For example, low bioavailabilities have been reported with slow-release formulations containing the standard drug levodopa for

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the treatment of Parkinson's disease (Klausner et al., 2003; Deleu et al., 2002) and the glucose-lowering agent metformin (Gusler et al., 2001; Marathe et al., 1999; Vidon et al., 1988). To overcome the problem of low bioavailability there is increasing interest in developing gastro-retentive formulations and/or formulations that linger in the proximal parts of the small intestine. Formulations are expected to release the drug site-specifically before or within the "absorption window" of the drug and thus increase its bioavailability.

In developing site-specific formulations interest has focused particularly on mucoadhesive polymers. Gastrointestinal mucoadhesive patch systems (Eiamtrakarn et al., 2002; Shen and Mitragotri, 2002) and microparticles comprising mucoadhesive polymers (Miyazaki et al., 2003; Tao et al., 2003) have been studied. Many polymers, such as the cationic chitosans, have excellent mucoadhesive properties in vitro (Lehr et al., 1992). The mucoadhesive chitosans have been demonstrated to increase the residence times of formulations in the stomach in mice (Remuñan-López et al., 2000) and gerbils (Hejazi and Amiji, 2004) or in the rat intestine (Shimoda et al., 2001). The results of our bioavailability studies on human volunteers are, however, somewhat contradictory to those of animal studies (Säkkinen et al., 2003). In the bioavailability studies, we used furosemide as a model drug in slow-release chitosan granules. Furosemide has a narrow absorption window in the upper gastrointestinal tract (Beermann, 1982). The mechanism for its site-specific absorption behaviour could partially contribute to furosemide being a substrate for intestinal membrane transporters (Flanagan et al., 2001). Because furosemide is site-specifically absorbed from the upper gastrointestinal tract, its bioavailability from a slow-release, mucoadhesive formulation was expected to be as good as, or even better than, from a conventional immediate-release formulation. In most cases, however, the administration of furosemide in chitosan granules resulted in lower bioavailability of the drug, indicating that the formulations studied could not be used as mucoadhesive formulations in the upper gastrointestinal tract in humans.

Studies on the behaviour of chitosan formulations in humans are few, and more studies are therefore needed to demonstrate what happens to chitosan formulations in the human gastrointestinal tract. Several methods currently exist to study the fate of formulations in the human gastrointestinal tract, such as gamma scintigraphy and radiological studies (Newman et al., 2003; Wilding et al., 2001). The greatest advantage of gamma scintigraphy over radiological studies is that it allows visualization over time of the entire course of transit of a formulation through the digestive tract, with reasonably low exposure of subjects to radiation. In a recent study, we used neutron activation-based gamma scintigraphy to visualize the gastro-retentive properties of chitosan formulations in the human stomach. The results are discussed in detail in our latest paper (Säkkinen et al., 2004a). In the same study, we had an opportunity to simultaneously visualize the fate of the formulations in the upper small intestine. In this paper, the findings relating to small intestinal transit are discussed for the first time. To our knowledge, this is the first time that the results of gamma scintigraphic studies on the fate of chitosan granules in the human small intestine have been reported.

2. Materials and methods

2.1. Formulation

The granules contained 95 or 40% of microcrystalline chitosan base (MCCh, mean molecular weight 150 kDa, approximate extent of deacetylation 75%) (Novasso, Finland), and lactose as filler (Pharmatose DCL 21, DMV International, Netherlands). Lactose granules which contained polyvinylpyrrolidone (PVP K25, Fluka Chemie, Switzerland) as binder were used as reference formulation. The radiolabel in the granules was 4 mg of natural-abundance samarium oxide (Sm_2O_3) (purity 99.9%) (Aldrich, USA). The granules were dispensed in size-0 gelatine capsules (Ph.Eur.) by volume. The composition and preparation of the granules has been described in detail elsewhere (Säkkinen et al., 2004a).

Forty-eight hours before administration, the ^{152}Sm in the granules was activated in a thermal neutron flux to the gamma-emitting nuclide ^{153}Sm, using a 250 kW TRIGA Mark II nuclear research reactor (General Atomics, USA) at the Technical Research Centre of Finland (VTT). The neutron flux was 1.2×10^{12} cm^{-2} s^{-1} and the irradiation time 5.5 min. The neutron activation process has been described in detail elsewhere (Säkkinen et al., 2004a).

Table 1
Locations of the formulations in the gastrointestinal tract at different times in 15 volunteers

No.	0.5 h	1 h	2 h	3 h	4 h
MCCh 95%					
1[a]	Stomach	Stomach	Stomach	SI	SI
2	Upper SI	Upper SI	SI	SI	SI
3[b]	Upper SI	Upper SI	SI	SI	Colon
4	Stomach	Upper SI	SI	SI	Colon
5[c]	Oesophagus	Oesophagus	Stomach	SI	n.a.
MCCh 40%					
6[d]	Stomach	Stomach	Upper SI	SI	SI
7	Stomach	Upper SI	SI	SI	n.a.
8[e]	Upper SI	Upper SI	SI	SI	SI
9	Upper SI	Upper SI	SI	SI	SI
10[f]	Stomach	Stomach	Upper SI	Upper SI	Upper SI
Lactose					
11	Upper SI	Upper SI	SI	SI	n.a.
12	Upper SI	Upper SI	SI	SI	Colon
13	Upper SI	Upper SI	SI	SI	n.a.
14	Upper SI	Upper SI	SI	SI	n.a.
15	Stomach	Stomach	Upper SI	Upper SI	n.a.

The superscript letters a–f indicate that the formulation has become attached to the mucosa. The approximate adhesion time was determined as the time for which over 50% of the granules remained at the same place in the gamma images; n.a.: not assigned.

[a] Stomach, 2 h.
[b] Upper small intestine (SI), 0.75 h.
[c] Oesophagus, 1.75 h.
[d] Stomach, 1.25 h.
[e] Upper small intestine (SI), 0.75 h.
[f] Stomach, 2.5 h.

2.2. Gamma scintigraphic study

Three groups of five healthy male volunteers participated in the gamma scintigraphic studies. A capsule containing the granules was administered with 180 ml of water, with the subject in a sitting position, at 8 a.m. or 12 p.m., after the volunteer had fasted overnight for at least 12 h. The volunteers were not allowed to eat or drink during the imaging period. One minute after administration gamma images, each of 1-min duration, were recorded continuously for 30 min, after which six images, each of 1-min duration, were recorded every 15 min for the next 3–4 h. During imaging each subject was in a supine position beneath the gamma camera. At all other times they were able to move freely. Gamma counts were detected using a dual-head gamma camera

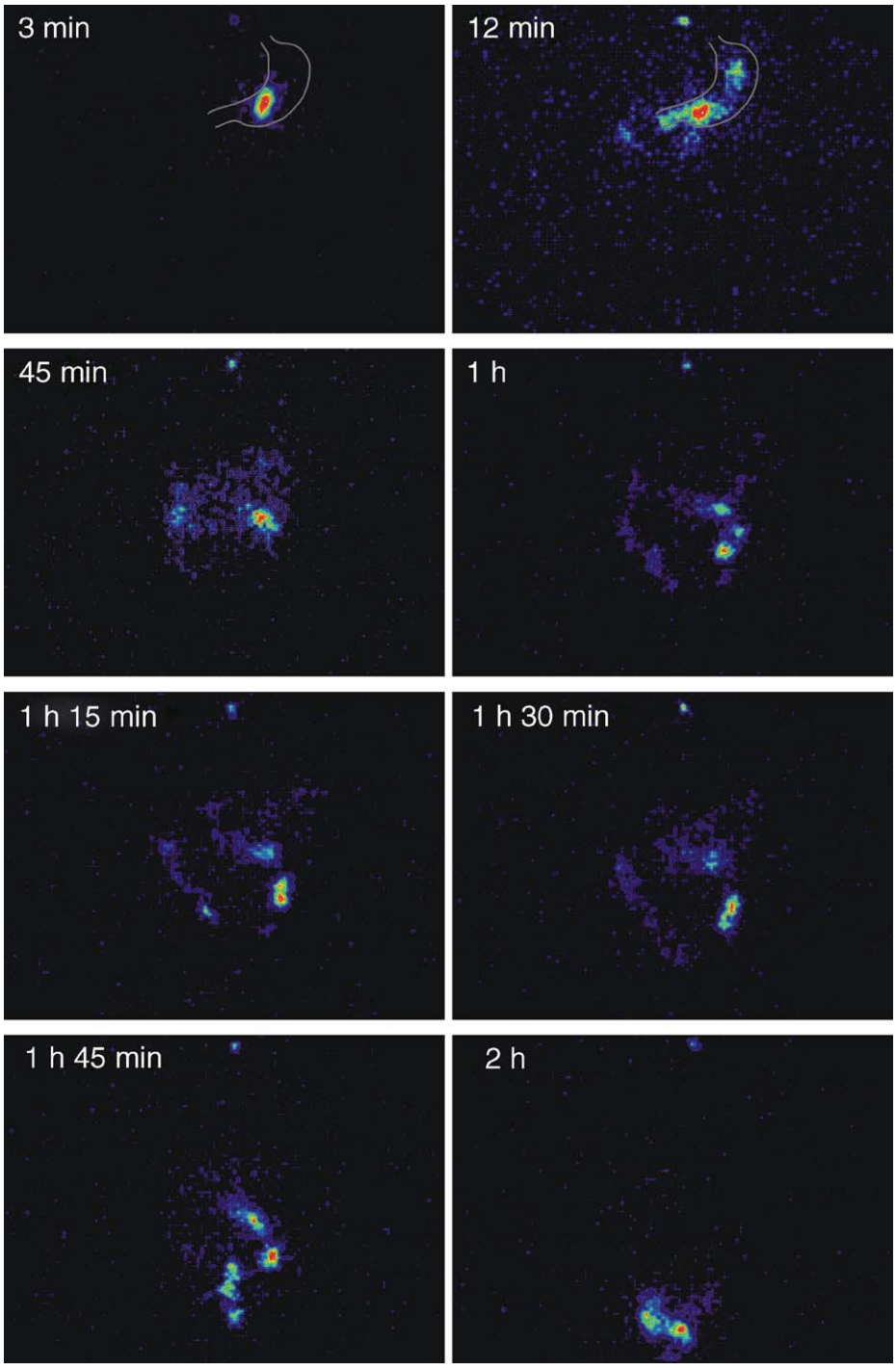

Fig. 1. Gamma scintigraphic images showing the fate of a chitosan formulation (40% MCCh, Mw 150 kDa) in the human gastrointestinal tract (Subject 8). After 3 min, the gelatine capsule containing the granules has not disintegrated. After 12 min, the capsule has disintegrated and the granules are passing through the pylorus into the small intestine. After 1 h, the granules have become attached to the intestinal mucosa in the lower jejunum. After 1 h 45 min, most of the granules have become detached, and after 2 h, are continuing their journey through the intestine.

(ADAC Forte, ADAC Laboratories, USA) equipped with LEGP collimators. The study protocol and approval given to it by various authorities have been described in detail elsewhere (Säkkinen et al., 2004a).

3. Results and discussion

The locations of the formulations in the human digestive tract at different times after their administration are given in Table 1. The chitosan formulations (MCCh 95 or 40%) were evaluated to determine if they acted as mucoadhesive systems. Stagnation of the formulation was expected to take place in the stomach or in the upper small intestine, where the pH is acidic or slightly acidic. In such an environment, the chitosan base would be expected to be ionized, and adhesion could have occurred between the positively charged chitosan and the negatively charged mucus. The reference lactose formulation was expected not to exhibit any adhesive characteristics.

Passage of the chitosan formulation in the digestive tract was prolonged in 6 cases out of 10 (Table 1). Stagnation of the formulation containing 40% chitosan took place in two cases in the stomach (Subjects 6 and 10) and in one case in the upper small intestine (Subject 8). The findings relating to the other formulation (95% MCCh) were fairly similar with the exception that in one case the formulation adhered to the oesophagus before reaching the stomach (Subject 5) (results discussed in a separate case report, Säkkinen et al., 2004b). Stagnation in the upper small intestine took place for approximately 45 min for both formulations (Table 1). This was shorter than the duration of stagnation in the stomach, which varied from 1.25 to 2.5 h. In none of the cases did the adhesion take place after the upper small intestine. The reference formulation exhibited no adhesive properties and the activity in the intestinal region moved constantly forward.

The stagnation of the 40% chitosan formulation in the upper small intestine in Subject 8 is shown in Fig. 1. The number of gamma counts detected in the different regions of the digestive tract in the same subject are presented in Fig. 2. The granules were released from the gelatine capsule within approximately 4 min and became dispersed in the stomach. Gastric emptying began within 10 min, after which it was fairly rapid. Emptying of the granules through the pylorus was seen to take place in frequent boluses, which thereafter passed very rapidly through the duodenum. This is illustrated well in Fig. 2, in which there are several observation points on the declining portion of the gastric emptying curve and very low levels of activity in the duodenal curve. At the same time, the counts from the proximal jejunum started to increase. No evidence was found of the formulation adhering to the mucosa in the stomach or the duodenum. However, 1 h after administration of the formulation the counts from the jejunum reached a plateau level of approximately 400 counts/min for 0.75 h. From the gamma images (Fig. 1), it can be concluded that the formulation had adhered to the intestinal wall, because the activity remained in the same region of the intestine. Similar findings were made for Subject 3, who had received the other formulation (95% MCCh). In the remaining 4 volunteers out of 10, the activity in the intestinal region moved constantly forward, showing that no adhesion had taken place. In these volunteers, the findings were similar to those with the reference formulation, which was expected not to exhibit any adhesive characteristics (Fig. 3).

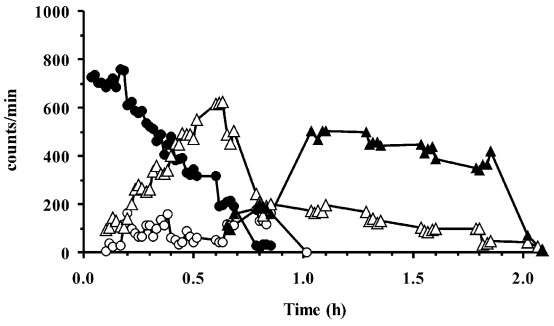

Fig. 2. Gamma counts measured over time from the regions of interest: ●, stomach; ◯, duodenum; △, proximal jejunum; ▲, lower jejunum. Gamma scintigraphic images from the same volunteer are shown in Fig. 1.

The first prerequisite for a site-specific system to the stomach and/or the upper small intestine is that the behaviour of the system should be reproducible. As far as the kind of formulation studied here is concerned, the system seems insufficiently reproducible. Although the results of studies in vitro or in vivo in animals (e.g. rats and dogs) relating to mucoadhesion of polymers have been promising, little tendency to adhesion has so far been found in studies in man (Harris et al., 1990a,b; Davis et al., 1993). Our study also showed that the chitosan grade used exhibited fairly marked mucoadhesive capacity in vitro (Säkkinen et al., 2003), but no similar effect was evident from the in vivo studies. Various physiological and formulation-related factors could explain why the systems did not work as expected (Säkkinen et al., 2004a). Briefly, exposure of chitosan to mucins dissolved in gastrointestinal fluids before it has a chance to interact with the mucus gel layer, and continuous erosion of the mucus gel layer on which adhesion could take place might hinder adhesion to the gastric or intestinal mucosa. Dorkoosh et al. (2004) evaluated a different approach to preparing formulations site-specific to the upper small intestine by using superporous hydrogel composite polymers, which swell rapidly by absorbing gut fluids, and managed to prepare a more reliable formulation that attached itself to the intestinal wall by mechanical fixation. In a gamma scintigraphic study in 5 human volunteers, the attachment of the system to the upper small intestine was evident in all cases (in our study the passage of chitosan formulations in the upper gastrointestinal tract was prolonged in 6 cases out of 10). However, the system employed by Dorkoosh et al. did not yield any longer retention times than the system based on mucoadhesion in our study, and the duration of the retention was relatively short in both studies. The retention times of the superporous hydrogels in the intestine varied from approximately 45 min to 1 h, which is comparable to the adhesion times of our system. The duration of retention was longer if the chitosan formulation adhered to the stomach.

Another prerequisite for a site-specific system is that it should have a sufficiently long retention time at the site of absorption in relation to the drug release rate. Too short a retention

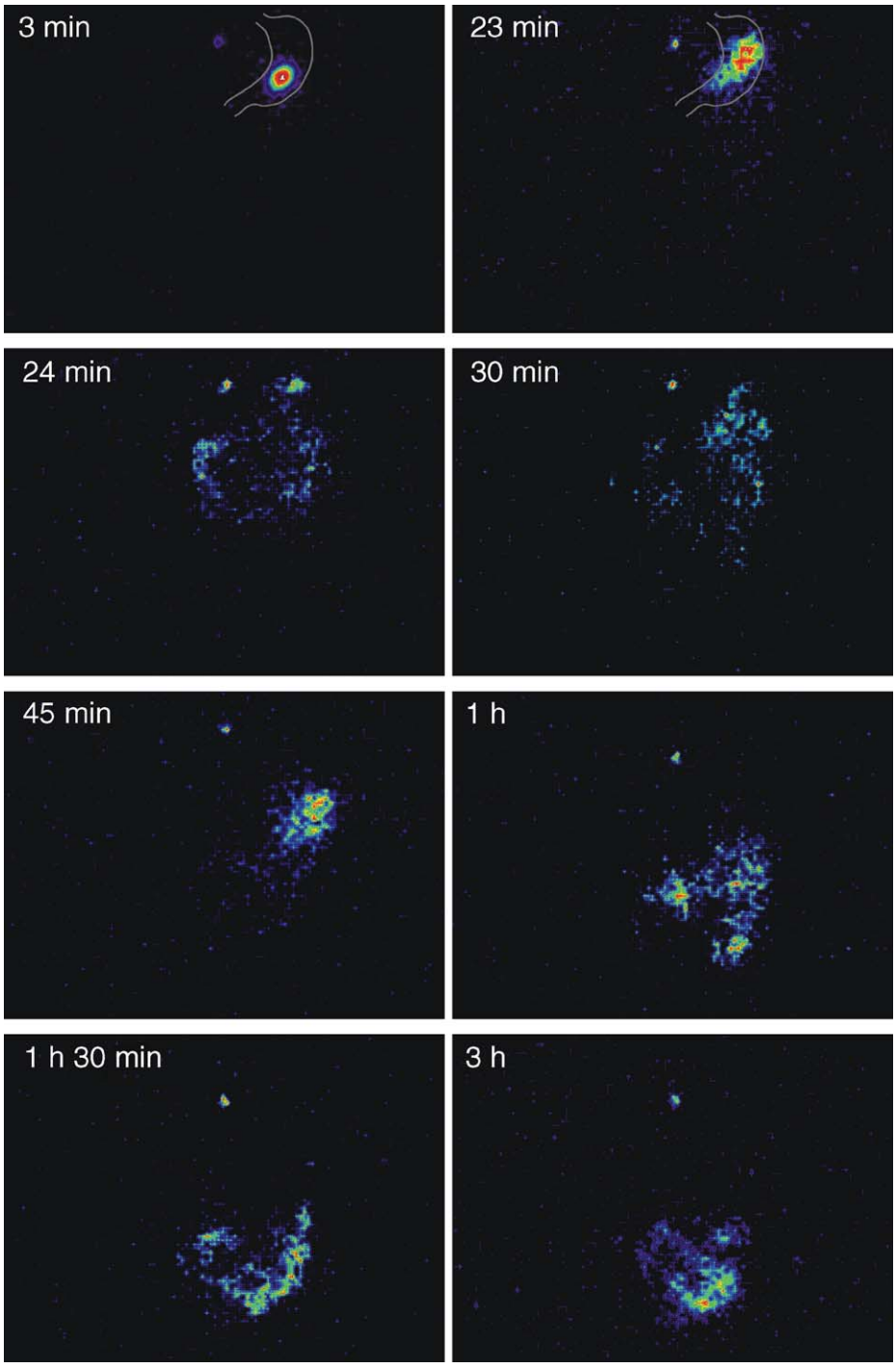

Fig. 3. Gamma scintigraphic images showing the fate of the reference formulation (lactose granules) in the human gastrointestinal tract (Subject 12). The activity in the intestinal region moved constantly forward, indicating that no adhesion had taken place.

time lowers the bioavailability of the drug. In the case of superporous hydrogels, the system released the drug quite rapidly in vivo and the retention was long enough to increase the amount of octreotide absorbed in bioavailability studies in pigs (Dorkoosh et al., 2002, 2004). The average values for F varied from 8.7 ± 2.4 to $12.7 \pm 3.6\%$ for the superporous hydrogels, whereas peroral administration resulted in an average bioavailability of $1.0 \pm 0.6\%$ (reference intravenous administration). In our recent bioavailability study on human volunteers, the results were less promising (Säkkinen et al., 2003). Our study evaluated slow-release furosemide formulations similar to those used in the gamma scintigraphic study reported here (MCCh 95 or 40%). The AUC for furosemide was comparable to that of a conventional, immediate-release tablet after administration of the drug in a formulation containing 40% chitosan (the respective $AUC_{0-\infty}$ values were $3800 \pm 941\ \mu g\,l^{-1}\,h$ and $3450 \pm 1180\ \mu g\,l^{-1}\,h$), but not after administration in a formulation releasing the drug more slowly and containing a higher

amount (95%) of chitosan ($AUC_{0-\infty}$ $2700 \pm 1070\,\mu g\,l^{-1}\,h$). Comparing the results of the bioavailability study with those of the present gamma scintigraphic study shows that only in the first case had the retention time in the stomach or in the upper small intestine been long enough in relation to the drug release rate. Only this formulation (MCCh 40%) allowed furosemide absorption to take place primarily in the upper gastrointestinal tract, in the "absorption window". In the latter case, the retention time in the upper intestine was too short, resulting in limited absorption time and a decrease in the bioavailability of furosemide from the formulation. It is clear from the results that the site-specific systems developed here are also drug-specific.

4. Conclusions

In view of the fact that little or no information exists on the in vivo behaviour of mucoadhesive chitosan formulations in the upper intestine, especially in human beings, the findings of our study are extremely interesting. The results emphasize the importance of in vivo studies. Gamma scintigraphic investigations revealed that although the chitosan studied had exhibited marked mucoadhesive capacities in vitro, the retention at the site of adhesion in the human gastrointestinal tract was relatively short and not sufficiently reproducible. Certain other studies have also suggested that systems based on mucoadhesion may not behave as intended in vivo, although many of the polymers used exhibit good mucoadhesive properties in vitro. The results of the present study suggest that other mechanisms of action should be studied in developing site-specific systems. Bioavailability studies are extremely important if slow-release formulations are being developed for drug substances with a narrow "absorption window". The amounts of drug absorbed need to be studied to demonstrate that the duration of retention of the formulation is sufficiently long. Chitosan failed to increase the bioavailability of furosemide, obviously because the retention time of the chitosan formulation in the stomach or in the upper intestine was too short in relation to the release rate of the drug.

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