Bioequivalence Testing for Locally Acting Gastrointestinal Products: What Role for Gamma Scintigraphy?

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Bioequivalence testing for locally acting gastrointestinal products is a challenging issue for both the pharmaceutical industry and the global regulatory authorities. It is widely accepted that for medicinal products not intended to be delivered into the systemic circulation, pharmacokinetic bioavailability cannot be used. However, it is becoming increasingly accepted that local availability may be assessed, where appropriate, by approaches that qualitatively reflect the presence of the active substance at the site of action. These methods must be specifically chosen for that combination of active substance and route of drug delivery. This paper argues for the use of gamma scintigraphy as a validated measure of local availability and bioequivalence for topically acting products administered to the gastrointestinal tract by the oral and rectal route.

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Ulcerative colitis is an inflammatory disease of the colonic mucosa with unknown etiology, which in almost all cases affects the rectum and often extends to more proximal regions of the colon. Crohn's disease is a transmural inflammatory disease, which can affect the small bowel only (29%), the colon only (30%), or both the small bowel and colon simultaneously (33%). In 8% of individuals, the disease is located in the upper intestine or perianal area.[1] Collectively, these conditions are often referred to under the broader name of inflammatory bowel disease (IBD).

The use of oral anti-inflammatory agents is one of the main treatment approaches for IBD, and the principle therapeutic moiety is mesalazine.[2] While the drug is rapidly and completely absorbed from the upper intestine, when administered as an immediate-release tablet, it is poorly absorbed from the colon.[3] The precise mechanism of action of mesalazine is not known, partly because of the failure to understand the etiopathogenesis of IBD.[4] However, it is generally agreed that its main effect is exerted topically at the inflammatory lesions.[5] As a consequence, high intraluminal drug concentrations are required at the site of inflammation, and therefore the mesalazine products on the market are either prolonged-release (e.g., Pentasa®) or targeted-release (e.g., Asacol®, Claversal®) formulations.

This review article will concentrate on mesalazine, given its extensive use in the treatment of IBD and because a wide variety of formulation types exist, including enteric-coated products, prolonged-release pellets, enemas, and suppositories. However, comparable arguments can be readily established for other locally acting gastrointestinal (GI) products (e.g., steroids). The issue of bioequivalence testing for these locally acting oral products is a complex issue for the regulatory authorities.

The textbook definition of bioequivalence for two oral products, designed for systemic drug delivery, is that they have identical rate and extent of absorption. These assessments are predominantly based on the area under the curve (AUC) of the plasma profile (concentration plotted vs. time) and maximum plasma concentration (C_{max}). The acceptance of bioequivalence of two products requires that the 90% confidence interval for the ratio of test to reference product lies within a predetermined bioequivalence interval; for AUC, the generally accepted interval is 0.8 to 1.25 for

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log-transformed data. A wider interval may be appropriate for C_{max} because of its inherently greater variability. Once bioequivalence of the two products is established, then it is assumed that they will be therapeutically equivalent.

The European regulatory authorities in the form of the Committee of Proprietary Medical Products (CPMP) has stated that for "locally acting products [pharmacokinetic] bioequivalence generally is not a suitable way to show therapeutic equivalence, since plasma levels are not relevant for local efficacy, although they may play a role with regard to safety."[6] Therefore, while measurement of drug plasma levels could prove useful from a safety perspective, it provides little or no information on the in vivo fate of the therapeutic moiety at its target site. An alternative assessment method is therefore required, and the CPMP guidance states that "human pharmacodynamic studies, local availability studies or where appropriate even animal or in vitro studies can be considered, provided that all studies used are adequately validated."[6]

In the draft CPMP guidance on bioavailability and bioequivalence, published in December 1998, it was clarified that "for medicinal products not intended to be delivered into the general circulation, the common systemic bioavailability approach cannot be applied."[7]

However, the guidance explicitly states that "the (local) availability may be assessed, where necessary, by measurements qualitatively reflecting the presence of the active substance at the site of action using methods specifically chosen for that combination of active substance and localisation."

The objective of this review article is to discuss the use of gamma scintigraphy as a validated measure of local availability and bioequivalence for topically acting products administered to the GI tract by the oral and rectal route.

GAMMA SCINTIGRAPHY AND ITS APPLICATION TO BIOAVAILABILITY ASSESSMENT

Gamma scintigraphy was originally developed as a nuclear medicine technique, using gamma ray–emitting radionuclides that localize in the specific organs of the body and are visualized by a gamma camera coupled to a sophisticated data-processing system, providing information on the structure and function of various body systems. However, drug formulations can also be radiolabeled, and therefore gamma scintigraphy has been widely applied to assess the delivery of drugs given by a variety of routes.[8]

With the advent of novel oral drug delivery systems, the requirement to understand exactly what the formulation is doing within the GI tract has increased dramatically. The interaction between drug, dosage form, and gut physiology plays a crucial role in determining the potential of any product, and scintigraphy allows this dynamic process to be visualized in a noninvasive manner.

To follow the fate of a pharmaceutical dosage form, it is not usually possible to radiolabel the drug molecule. This is because, in general, drug molecules are compounds of carbon, hydrogen, oxygen, and nitrogen, and none of these elements has isotopes suitable for gamma camera studies. Instead of labeling the drug molecule, a radiolabel is usually incorporated into the dosage form and is used to define the in vivo performance of the system. Radiolabeling can be achieved either by the direct incorporation of a radiolabeled compound into the preparation or by neutron activation of a dosage form that contains a nonradioactive tracer. The latter method avoids the need to handle radioactive materials during lengthy or complex formulation procedures and permits dosage form manufacture to be conducted under normal production conditions. The quantity of material needed to be incorporated into a formulation to render it suitable for use in a gamma scintigraphic study is very small and does not compromise the performance characteristics of the delivery system.

Gamma scintigraphy has been described as an "elegant technique for phase I investigation of the locality of in vivo release"[9] and has "become the method of choice for investigating the fate of pharmaceutical dosage [forms] in the body."[10] The ability to visualize the drug delivery process in a noninvasive manner acts to fill a significant void in current understanding. Alternative methods of assessing drug delivery, such as pharmacokinetic evaluation for locally acting oral products, could be described essentially as "blunt" measures, lacking a clear outcome.[11] While other approaches for assessing local drug bioavailability have been proposed (e.g., intestinal perfusion[12]), these have the disadvantage of being highly invasive, thereby potentially altering the absorption/secretion balance in the distal bowel. Gamma scintigraphy is, however, a more incisive noninvasive approach, allowing much greater information to be imparted on the in vivo performance of locally acting products without changing intestinal characteristics.

Gamma scintigraphy is widely accepted by the scientific community as a validated measure of product behavior in the intestine. For products containing drug designed for local therapy, where systemic levels are a

poor predictor of product performance, gamma scintigraphy can provide a surrogate measure of local bioavailability. Examples will be provided of the validation of the scintigraphic approach using mesalazine as an example for a range of pharmaceutical products intended for topical therapy in the bowel.

VALIDATION OF GAMMA SCINTIGRAPHY FOR BIOEQUIVALENCE TESTING OF LOCALLY ACTING GASTROINTESTINAL PRODUCTS

Example A: Enteric-Coated Mesalazine Products

Delayed-release enteric-coated preparations have been developed to prevent release of mesalazine until the formulation has reached the terminal ileum and colon. These include several commercially available mesalazine delayed-release systems (e.g., Asacol® and Claversal®).[13] Both preparations use copolymers of methacrylic acid and methyl methacrylate, available commercially as the Eudragit® range of polymers, to deliver drug to the colon.

Claversal® (available in some countries as Mesasal®) has been commercially available in Germany for many years.[14] The tablets contain 250 mg of mesalazine and are coated with the enteric-coating polymer, methacrylic acid copolymer, type A (Eudragit L). This pH-sensitive polymer is resistant to gastric conditions but soluble above pH 6.0 in the intestine. A relatively thick polymer coating is applied to the tablets to delay drug release until the product reaches the terminal ileum and proximal colon.

Studies have been undertaken to investigate the GI transit of the enteric-coated tablets in patients with colitic disease to determine the site of mesalazine release by gamma scintigraphy.[15]

Blood samples were also taken during the studies to enable any of the drug absorbed to be related to the scintigraphic information and to examine the extent of mesalazine absorption. Thirteen patients with quiescent IBD—7 with Crohn's disease and 6 with ulcerative colitis—were studied. The ^{111}In radiolabel was incorporated into the mesalazine granules before compression into tablets and subsequent coating with the polymer. Frequent scintigraphic images and blood samples were acquired for each subject.

Tablet location and the point of tablet dispersion were readily determined (Figure 1). The median time for gastric emptying of the tablets was 2.9 hours, ranging from 0.8 hours to more than 11 hours (Table I). None of the tablets disintegrated in the stomach. On average, the tablets disintegrated 3.2 hours after leaving the stomach, resulting in drug dispersion in the distal small intestine or proximal colon, except in 1 of the surgically treated patients with a right hemicolectomy, in whom dispersed preparation was first detected in the transverse colon.

Table I Transit and Disintegration Times of the Claversal® Tablets in Patients with Inflammatory Bowel Disease (Example A)

	Gastric Emptying (h)	Colonic Arrival (h)	Disintegration Time (h after leaving stomach)
Median	2.9	6.8	3.2

There was a close correlation between the detection of tablet disintegration and the onset of drug absorption ($r = 0.988$, $p < 0.001$) (Figure 2). The patients' individual plasma mesalazine and principal metabolite Ac-5-ASA concentrations correlated with the scintigraphic data throughout the 24 hours. The plasma concentrations of Patient 7, for example, are shown in Figure 1, with the corresponding scintigraphic images. The observation of tablet disintegration at 5.0 hours after dosing corresponds well with the first detection of the drug and its metabolite in plasma. Plasma mesalazine concentrations then increased to a maximum of 0.54 μg/ml at 6.0 hours. The scintigraphic images show that the dispersed radiotracer then moved mainly from the small intestine into the cecum and ascending colon by 7.0 hours, and this coincided with a rapid fall in plasma mesalazine levels. Drug absorption was relatively low in all patients once the preparation had entered the colon.

In conclusion, gamma scintigraphy has been demonstrated to be a very effective and validated approach for visualizing the location of in vivo drug release from enteric-coated mesalazine products.

Example B: Oral Modified-Release (MR) Mesalazine Products

While enteric-coated targeted-release preparations have been shown to release mesalazine in the terminal ileum/cecum, the product known as Pentasa® has been designed as a preparation consisting of a large number of MR ethylcellulose-coated mesalazine microgranules

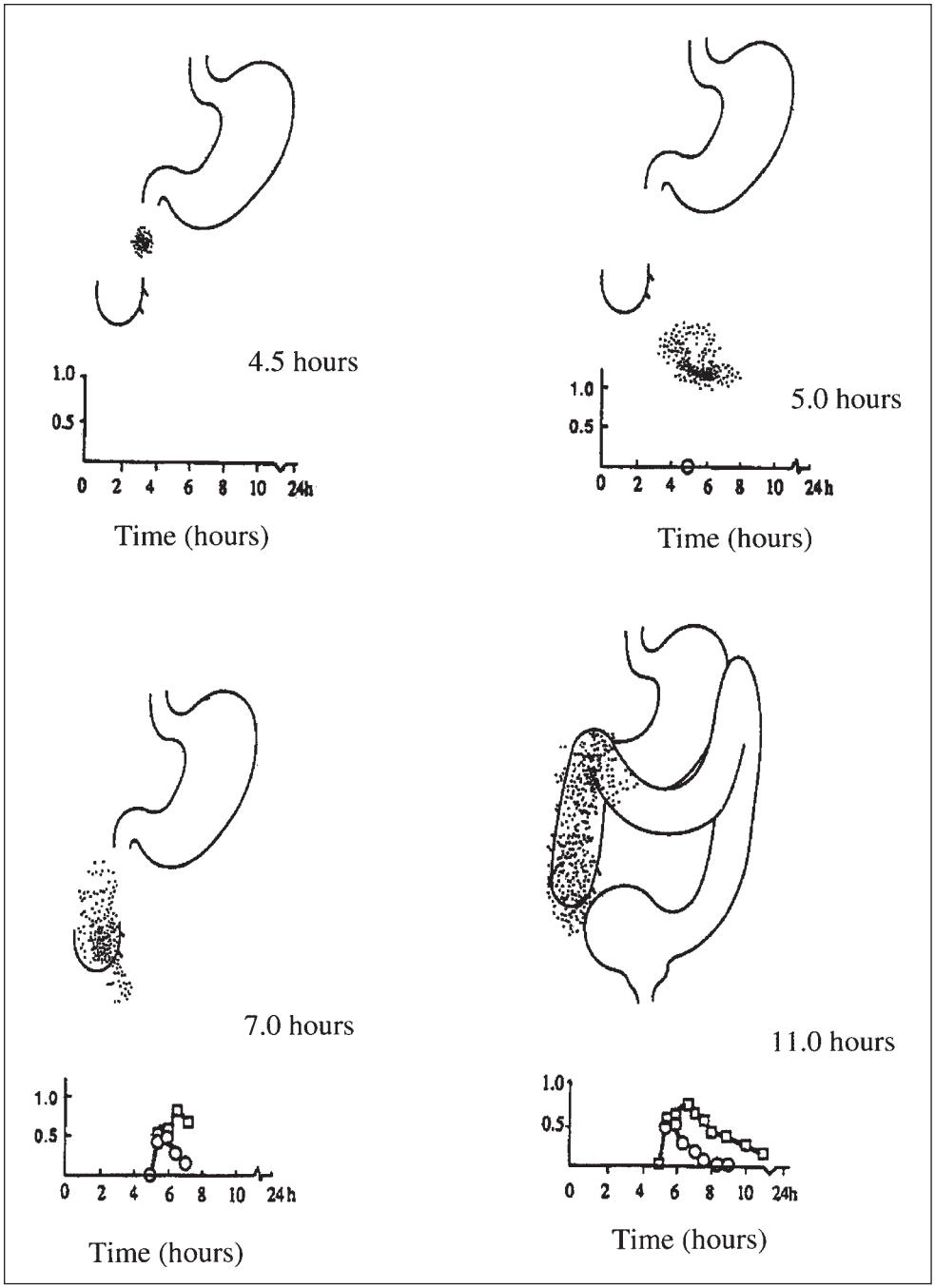

Figure 1. Gastrointestinal transit of the Claversal® tablet in a patient with ulcerative colitis (Patient 7), showing tablet dispersion in the terminal ileum and plasma mesalazine levels (○) and Ac-5-ASA levels (□) (concentrations in μg/ml) (Example A).

intended for delivery of the drug throughout the GI tract.[16]

A combined scintigraphic and pharmacokinetic (pharmacoscintigraphic) study in healthy volunteers has evaluated the location of mesalazine release from Pentasa® tablets in the GI tract under fasted and fed conditions.[17] Disintegration of the tablet preparation occurred in the stomach within 20 minutes, and Ac-5-ASA was detectable in the plasma less than 60 minutes after ingestion. The microgranules were in the colon by 8 hours after dosing, and in all subjects, the preparation became dispersed fully in the large bowel.

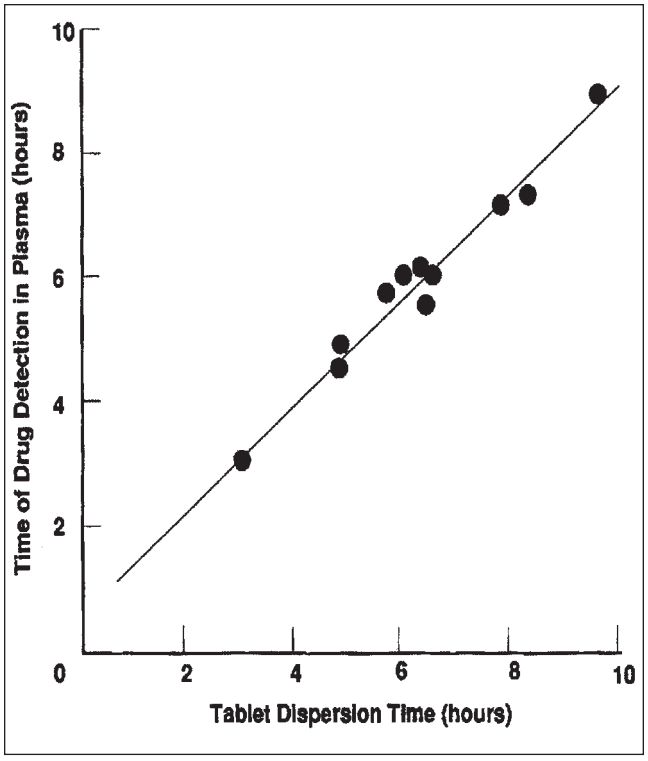

Figure 2. Relationship between tablet dispersion and drug absorption (r = 0.988, p < 0.001) (Example A).

The pharmacoscintigraphic study confirmed that mesalazine release from Pentasa® occurred throughout the GI tract.

A further scintigraphic study was undertaken on the Pentasa® 250 mg tablets to evaluate mesalazine release sites in patients with Crohn's disease. This revealed very similar findings to the investigation in healthy volunteers.[18] Disintegration of the tablets was found to occur in the stomach, and the temporal association between location of the microgranules and mesalazine plasma levels was comparable in the patient population to those reported previously in normal subjects.

However, higher dosages of mesalazine are now being used to treat IBD since clinical trials have demonstrated greater efficacy at higher dosages in both ulcerative colitis (2-4 g/day) and Crohn's disease (2-4 g/day).[19,20]

The 250 mg Pentasa® preparation has been available since 1986 and the 500 mg tablet since 1990 in many European countries. As a consequence of the increase in the recommended daily dose of mesalazine (up to 4 g), a sachet formulation, designed to release drug in the same manner as the Pentasa® tablets, has been developed to improve patient compliance.[21] The sachet formulation consists of an aluminum foil wrapping in which the microparticles are loosely contained. The contents of the sachet are emptied into a glass of water prior to dosing.

A scintigraphic study in healthy volunteers was undertaken to evaluate the disposition, dispersion, and movement of the Pentasa® microgranules in the GI tract following dosing either as tablets (2 × 500 mg) or 1 g sachet (unit-dose) with a view to demonstrating bioequivalence for the new sachet product versus the marketed tablet preparation.[22] All formulations were radiolabeled by the use of neutron activation and involved ^{153}Sm labeled preparation.

Pentasa® preparations were radiolabeled via neutron activation. Dissolution testing at pH 7.5 showed comparable in vitro release properties for the tablet and sachet preparations; $t_{1/2}$ for the tablets and sachet was 100 and 110 minutes, respectively. Eight healthy volunteers provided written informed consent to participate in the two-way randomized, crossover, single-dose study. Subjects were randomized to (1) two Pentasa® 500 mg tablets, each labeled with 0.5 MBq of ^{153}Sm, or (2) one Pentasa® 1 g sachet of microgranules labeled with 1 MBq of ^{153}Sm. Scintigraphic images were acquired at frequent intervals for up to 12 hours using a gamma camera.

The individual transit profiles have been characterized by the half-life ($t_{1/2}$), and the relevant parame-

Table II Gastrointestinal Transit ($t_{1/2}$) (min) for Pentasa® Microgranules Dosed in Either Tablet (2 × 500 mg) or Sachet (1 g) Form (Example B)

	Gastric Emptying		Small Intestinal Transit		Colon Arrival	
	Tablets	Sachet	Tablets	Sachet	Tablets	Sachet
Mean	17	22	213	185	230	206
Standard deviation	5	10	45	83	49	86
Median	19	20	230	195	247	227
Number	8	8	8	8	8	8

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