The Use of Pharmacoscintigraphy to Elucidate Food Effects Observed with a Novel Protease Inhibitor (Saquinavir)

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Purpose. To evaluate mechanistically the effect of food on the absorption and gastrointestinal transit of the protease inhibitor saquinavir.
Methods. Pharmacoscintigraphic investigation in eight healthy volunteers.
Results. Gastric emptying occurred rapidly in the fasted state with some capsules leaving the stomach prior to disintegration. Unmeasurable plasma concentrations were observed in several subjects when dosed under fasted conditions. Following post-prandial administration the radioactive marker became re-distributed within the stomach contents and consequently slower gastric emptying resulted. Plasma concentrations under fed conditions were measurable up to 12 hrs after administration in seven of the eight subjects. Six of the eight plasma profiles showed secondary peaks at c. 4 hours post-dose; two of which coincided with the gastrocolonic response following ingestion of lunch.
Conclusions. Bioavailability of saquinavir is significantly improved in the presence of food. Emptying of intact capsules in the fasted state may further reduce bioavailability. In the fed state, capsules disintegrate rapidly and gastric emptying is prolonged which may improve exposure of the drug to target absorption sites. Saquinavir may be absorbed from the colon. Second peaks in the absorption profile can only be attributed to gastrocolonic response following ingestion of a meal in some cases. Increased absorption is more likely to be due to an increase in dissolved drug being available for absorption due to general increased motility and secretion stimulated by ingestion of a meal.

KEY WORDS: saquinavir; protease inhibitor; gastrointestinal tract; food effects; pharmacoscintigraphy.

INTRODUCTION

Saquinavir is a potent and highly selective viral protease inhibitor of Human Immunodeficiency Virus (HIV-1), the causative agent of Acquired Immune Deficiency Syndrome (AIDS). The HIV protease enzyme is an essential component of the replicative cycle of HIV. It is involved in the processing of polyproteins to yield the structural proteins of the viral core (1). Inhibition of this enzyme results in the formation of immature, non-infective virus (2) and prevents maturation of the virus in both acute and chronic infections of HIV-1. For this reason, protease inhibitors possess an advantage over nucleoside analogues which only inhibit replication of the virus in previously uninfected cells but have no effect on virus production by cells in which integration has already occurred ie chronically infected cells (3). Combinations of saquinavir with nucleoside analogues which act at different steps of the viral replicative cycle have shown a greater than additive antiviral activity (4,5).

Saquinavir has been shown to be well tolerated at doses up to 600 mg three times a day, with no association seen between increased dose and toxicity. Saquinavir pharmacokinetics are characterised by a high plasma clearance, a large volume distribution and low bioavailability which shows a large inter and intra subject variation. Studies with ^{14}C labelled- saquinavir have shown that approximately 30% of a 600 mg dose is absorbed and only 4% of this dose reaches the systemic circulation. Administration of the drug in the fed state has been shown to substantially improve bioavailability. Secondary peaks (several hours after dosing) in the plasma concentration/time profile frequently occur shortly after the ingestion of food and it has been postulated that the gastrocolonic response is responsible for this observed increase in absorption following ingestion of food. A pharmacoscintigraphic study combining traditional pharmacokinetic techniques with the non-invasive imaging technique of gamma scintigraphy was conducted to help elucidate the food effects observed with saquinavir; this allowed simultaneous assessment of gastrointestinal (GI) transit and drug absorption (6). The pronounced improvement in bioavailability following fed administration was examined and the effect of a food stimulus (lunch) on absorption was assessed.

MATERIALS AND METHODS

Dosage Form Manufacture

Hard gelatin capsules containing saquinavir (200 mg) were manufactured by Roche Products Ltd, UK. Isotopically enriched samarium oxide (0.67 mg/capsule) was blended with the drug granulate to form a homogenous mix prior to encapsulation. Subsequent irradiation in a neutron source (6 minutes at 10^{12} $n/cm^2/s^{-1}$) converted the non-radioactive tracer (^{152}Sm) into a gamma emitting radioisotope (^{153}Sm) (6). Prior to the clinical phase of the project, trial irradiations were undertaken which demonstrated that neither the drug nor the preparations were affected by exposure to the neutron flux.

Study Design

An open, balanced, randomised two-way crossover study was conducted in eight healthy volunteers (four male, four female; age 21–37 years), who all provided written informed consent. The study was approved by the Quorn Research Review Committee and permission to administer radioactivity to human subjects was obtained from the Department of Health, London.

Procedures

The volunteers arrived fasted at the clinical unit at approximately 7.00 am having fasted overnight. Small anterior and posterior anatomical markers containing 0.1 MBq of ^{99m}Tc were taped to the skin where the mid-clavicular line met the right costal margin. Capsules were administered orally with 150 ml

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water. Each subject received three radiolabelled capsules (a total of 600 mg saquinavir and 1 MBq ^{153}Sm) at approximately 8.00 am.

Anterior and posterior scintigraphic images, each of 50 sec duration, were recorded at approximately 10 min intervals until 12 hrs post-dose using a gamma camera (General Electric Maxicamera) with a 40 cm field of view and fitted with a low energy parallel-hole collimator.

Study Day Meals

On one of the two study periods volunteers remained fasted prior to dosing whilst on the other occasion the capsules were administered following consumption of a heavy breakfast (circa 1300 Kcal; one bowl of cornflakes with 100 ml of whole milk, followed by two rashers of lean bacon, two fried eggs, two slices of toast with butter and 150 ml decaffeinated tea or coffee). Breakfast was consumed within a 30 minute period and administration of the capsules took place within five minutes of completing the meal. All doses were given with 150 ml water. To maintain adequate hydration each subject drank 200 ml of water at two hrs post-dose and received a standard lunch and dinner at four hrs and 10 hrs post-dose, respectively.

Blood Sampling and Drug Analysis

Blood samples (7 ml) were collected either by indwelling cannula irrigated with heparin, or by direct venepuncture at the following time intervals: 0 (pre-dose) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 10.0 and 12.0 hrs post-dose. The samples were collected into heparinised syringes and gently mixed several times. Blood was centrifuged at 1500 g for 10 min and the plasma transferred to pre-labelled polyethylene tubes prior to freezing at -20°C. Plasma saquinavir concentrations were determined by RIA assay by Roche Products Ltd, UK.

Data Analysis

Data were analysed to provide information on the GI transit of the saquinavir formulation. Transit of the formulation was characterised by calculation of the time for 50% of the activity to leave the stomach (Gastric emptying time - $T_{50\%}$) and the time for 50% of the activity to arrive at the caecum (Colon arrival time - $T_{50\%}$). The overlying of the different regions of activity, within the coiled anatomy of the small intestine, prevents an accurate quantification of the formulations in this region. Small intestinal transit was therefore calculated by subtracting the $T_{50\%}$ for gastric emptying from the corresponding value for colon arrival (Small intestinal transit time - $T_{50\%}$). Data were corrected for background activity and radioactive decay. Location of the marker prior to lunch (four hrs post-dose) and the occurrence of gastrocolonic response was recorded.

RESULTS

Fasted Administration

Gastric Emptying

The mean $T_{50\%}$ gastric emptying of the formulation following fasted administration was 0.3 ± 0.3 hrs (range 0.1 to 0.8 hrs) (Table 1). In five of the eight subjects the gelatin capsules disintegrated in the stomach and the radiolabelled marker became distributed throughout the gastric contents prior to emptying. Gastric emptying of fluid has been shown to be rapid and empties from the stomach in either an exponential fashion or as a linear function of the square root of the liquid volume remaining in the stomach (7). In this case, gastric emptying was slightly slower and this slower emptying rate may be attributed to the granular formulation which probably behaves in a manner somewhere between that of a solution and a particulate (8).

Table 1. Gastrointestinal Transit of the Saquinavir Formulation Following Fasted Administration ($T_{50\%}$ hours)[a]

Subject Number	GE	SIT	CA
1	0.3	4.0	4.3
2	0.8	3.6	4.4
3	0.6	5.7	6.3
4	0.1	1.8	1.9
5	0.1	2.5	2.6
6	0.4	2.8	3.2
7	0.1	4.4	4.5
8	0.3	4.2	4.5
Mean	0.3	3.6	4.0
SD	0.3	1.2	1.4
Median	0.3	3.8	4.3

[a] GE = gastric emptying; SIT = small intestinal transit; CA = colon arrival.

In three of the eight subjects the capsules left the stomach (0.1 hrs post-dose) prior to disintegration. Gastric emptying in these cases was probably determined by the physiological mechanism known as the migrating myoelectric complex which occurs over a two hour cycle. The third phase of this cycle is responsible for emptying large non-disintegrating material from the stomach and for this reason has been termed the housekeeper wave (9). In these three subjects phase III activity was probably propagated soon after dosing and gastric emptying of the capsules occurred rapidly thereby preventing prior disintegration of the capsules.

Small Intestinal Transit and Colon Arrival

Mean small intestinal transit time was 3.6 ± 1.2 hrs following fasted administration which is in line with previous small intestine transit data reported in the literature (10); small intestinal transit in subject 3 was 5.7 hrs which was longer than average. Colon arrival occurred on average at 4.0 ± 1.4 hrs post-dose following fasted administration.

Location of the Marker at Four Hours Post-dose

Following fasted administration the radiolabel was contained entirely within the small intestine (in four subjects) or entirely within the colon (in the remaining four subjects) at 4 hours, post-dose (Table 2).

The ingestion of a meal is known to stimulate colonic activity which results in mass movement of material from the terminal ileum into the colon and is termed the gastrocolonic

Table 2. Pharmacokinetic Parameters, Location of Radiolabel and Onset of GCR Following Fasted Administration of Saquinavir[a]

Subject Number	AUC (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	Location 4 hrs (post-dose)	Effect of eating
1	—	—	—	SI	GCR (immediate)
2	—	—	—	Colon	
3	26.87	6.73	1.5	SI	
4	16.81	11.87	1.5	Colon	
5	51.65	18.9	1.0	Colon	
6	13.55	8.58	2.0	Colon	
7	—	—	—	SI	GCR (within 0.5 hrs of feeding)
8	—	—	—	SI	GCR (within 0.5 hrs of feeding)
Mean	27.22	11.52			
Median	21.84	10.22			

[a] GCR = gastrocolonic response; SI = small intestine; — = not calculable.

response (GCR) (11). Incidence of GCR is also recorded in Table 2. A GCR was observed in three subjects following fasted administration. In one subject this response commenced immediately following food ingestion whilst the effect was observed within half an hour of beginning lunch in the other two cases.

Absorption

As expected, plasma concentrations of saquinavir were very low or below the assay sensitivity (mean $AUC_{0\text{-}12}$ 27.22 ng h/mL in those subjects with measurable concentrations) (Table 2) when dosed under fasted conditions. No increase in absorption was observed in any volunteer following the ingestion of lunch.

Fed Administration

Gastric Emptying

When administered following a heavy breakfast the radiolabel was released rapidly from the gelatin capsules and dispersed within the gastric chyme to a variable extent before emptying. Gastric emptying ($T_{50\%}$) occurred between 0.3 and 3.2 hrs (mean 1.8 hrs ± 0.9) post-dose under these conditions (Table 3); the delay in gastric emptying being attributed to a lag phase as the label became distributed throughout the stomach contents. It has been suggested that the rate of gastric emptying is such that the number of calories delivered to the duodenum remains constant (12).

Small Intestinal Transit and Colon Arrival

Mean small intestinal transit time (2.7 ± 1.2 hrs) following fed administration was again in line with previous small intestine transit data reported in the literature (10). One case of prolonged transit (subject 2; 5 hrs) and one case of accelerated transit (subject 5; 0.8 hrs) were observed; cases of both rapid and prolonged transit have been reported previously.

Colon arrival occurred on average at 4.4 ± 0.7 hrs post-dose following fed administration. Since small intestinal transit occurred at a similar rate in both the fed and fasted states the short delay in colon arrival following feeding was attributed to delayed gastric emptying.

Table 3. Gastrointestinal Transit of the Saquinavir Formulation Following Fed Administration ($T_{50\%}$ hours)[a]

Subject Number	GE	SIT	CA
1	2.5	2.0	4.5
2	0.3	5.0	5.3
3	1.8	3.1	4.9
4	0.9	3.2	4.1
5	3.2	0.8	4.0
6	1.3	2.5	3.8
7	1.8	1.7	3.5
8	2.2	2.9	5.1
Mean	1.8	2.7	4.4
SD	0.9	1.2	0.7
Median	1.8	2.7	4.3

[a] GE = gastric emptying; SIT = small intestinal transit; CA = colon arrival.

Location of the Marker at Four Hours Post-dose

Following fed administration the pattern of radiolabel location at 4 hrs post-dose was more complex than with fasted administration (Table 4). In three subjects (subjects 2, 3 and 4) the marker was observed to be entirely within the small bowel whilst all the marker had passed into the colon in subject 6. In subjects 1 and 5, a proportion of the tracer was still present in the stomach whilst the remainder of the marker was distributed in the small intestine and had also commenced entry into the colon. In subjects 7 and 8 all the tracer had left the stomach although entry into the colon was incomplete.

A GCR was observed in two subjects following fed administration. In one case the GCR commenced immediately following food ingestion whilst the effect was observed within half an hour of the subject beginning their lunch in the other case.

Absorption

As expected, mean $AUC_{0\text{-}12}$ when the capsules were administered after a standardised meal were seven times higher (mean $AUC_{0\text{-}12}$197.5 ng h/mL (n = 8)) (Table 4) than with fasted dosing (mean $AUC_{0\text{-}12}$27.22 ng h/mL (n = 4)). Plasma

Table 4. Pharmacokinetic Parameters, Location of Radiolabel and Onset of GCR Following Fed Administration of Saquinavir[a]

Subject Number	AUC (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	Location 4 hrs (post-dose)	Effect of eating
1	137.8	61.73	5.0	c 10% stomach; 60% SI; 30% colon	
2	65.35	19.53	5.0	SI	
3	88.90	22.49	4.5	SI	GCR (within 0.5 hrs of feeding)
4	251.8	71.25	4.5	SI	GCR (immediate)
5	303.6	83.85	6.0	c 25% stomach; 35% SI; 40% colon	
6	122.6	38.14	5.0	Colon	
7	215.4	44.82	3.0	c 40% SI; 60%colon	
8	394.4	151.7	5.0	c 80% SI; 20% colon	
Mean	197.5	61.69	4.75		
Median	176.6	53.28	5.0		

[a] GCR = gastrocolonic response; SI = small intestine.

concentrations were measurable up to 12 hrs in seven of the eight subjects (Figure 1).

Following post-prandial administration, six subjects showed the 'typical' second absorption peak; in each case the peak was observed following lunch at 4 hrs post-dose. In subjects 3 and 4 the increase in absorption of saquinavir was accompanied by a GCR whilst peaks observed for subjects 2, 5, 6, and 7 were not. Second peaks were not observed in subjects 1 and 8.

Adverse Event Summary

Only two subjects experienced adverse events between the time of the first dosing and the post-study medical. Both volunteers reported headaches of moderate severity post-dose on one of the two study days; these were assessed as unlikely to be related to the test medication. Laboratory parameters for all volunteers were normal at post-study screening.

DISCUSSION

Despite low oral bioavailability saquinavir has exhibited substantial and durable *in vivo* antiviral activity both as a single agent and in combination with the nucleoside analogues zidovudine or zidovudine plus zalcitabine (13). Whilst resistance or reduced sensitivity has been reported *in vivo* to other protease inhibitors currently in development leading to loss of therapeutic effect, this problem has not been reported with saquinavir to date.

The low bioavailability of saquinavir observed in this study following fasted administration was consistent with previous data. However, examination of the scintigraphic images revealed that in some cases capsules were emptying intact from the stomach. Such rapid emptying may further affect subsequent absorption especially if the principal sites of absorption reside in the proximal small intestine. *In vivo* dispersion of capsule contents has been shown to be important in drug absorption (14).

As expected the increase in absorption of saquinavir when given after food, together with the characteristic peaks in the absorption profiles following lunch, were observed in this investigation. Although hypothesised that appearance of these peaks coincided with the onset of a GCR previous pharmacokinetic studies could not show whether improved absorption was actually a consequence of bolus movements of intestinal contents into the colon at lunch time. The scintigraphic investigation has shown that correlation with a GCR only occurred in two subjects. In these cases, movement of the marker into the large bowel did suggest some absorption of saquinavir from the colon. However, the cause of the increase in absorption could not be attributed to a GCR in the remaining four cases.

The successful functioning of oral medication depends primarily on how the gastrointestinal tract processes drugs and drug delivery systems. Physiological parameters such as regional pH, motility (and hence residence time), brush border and colonic microflora enzymatic activity all play an important role in the performance of orally administered dosage forms (15).

On average, 10L of fluid enter the gut each day. Approximately 2.5 L of this volume is ingested and 7.5 L is comprised of digestive secretions. The volume of fluid present at any time is controlled by the balance between the rate of absorption and secretion of the small intestinal and colonic epithelia. Following ingestion of food, absorption takes precedence over secretion although this balance is dependent upon a variety of factors including nutrient content in the intestinal lumen and the actions of endocrine substances on the enteric and autonomic nervous systems (16). Following ingestion of a meal, the motility of the small intestine is adapted to optimise exposure of the meal to the intestinal epithelium and the digestive secretions. Postprandial motor activity of the small intestine consists of repetitive and irregular contractions which propagate for very short distances. These contractions aid the digestive process by breaking up the solids and mixing complex food molecules with digestive juices. Saquinavir is a very insoluble drug (solubility in water at 21°C = 0.22 g/100 ml) and it is likely that the second peak in the absorption profiles observed following an additional food stimulus can be attributed, in part, to an increase in dissolved drug being available for absorption which is induced by the increase in motility and secretions generated by lunch (17).

However, following fasted administration of the drug, eating at lunch time will be accompanied by the same increase in motility. Although the drug will have passed to the more distal portions of the gut four hours post-dose second peaks, which were not observed, may have been expected. On fed dosing, absorption is accompanied by secretion of bile salts from the gall bladder which are subsequently reabsorbed in the small bowel (18). Absorption of unconjugated bile acids is

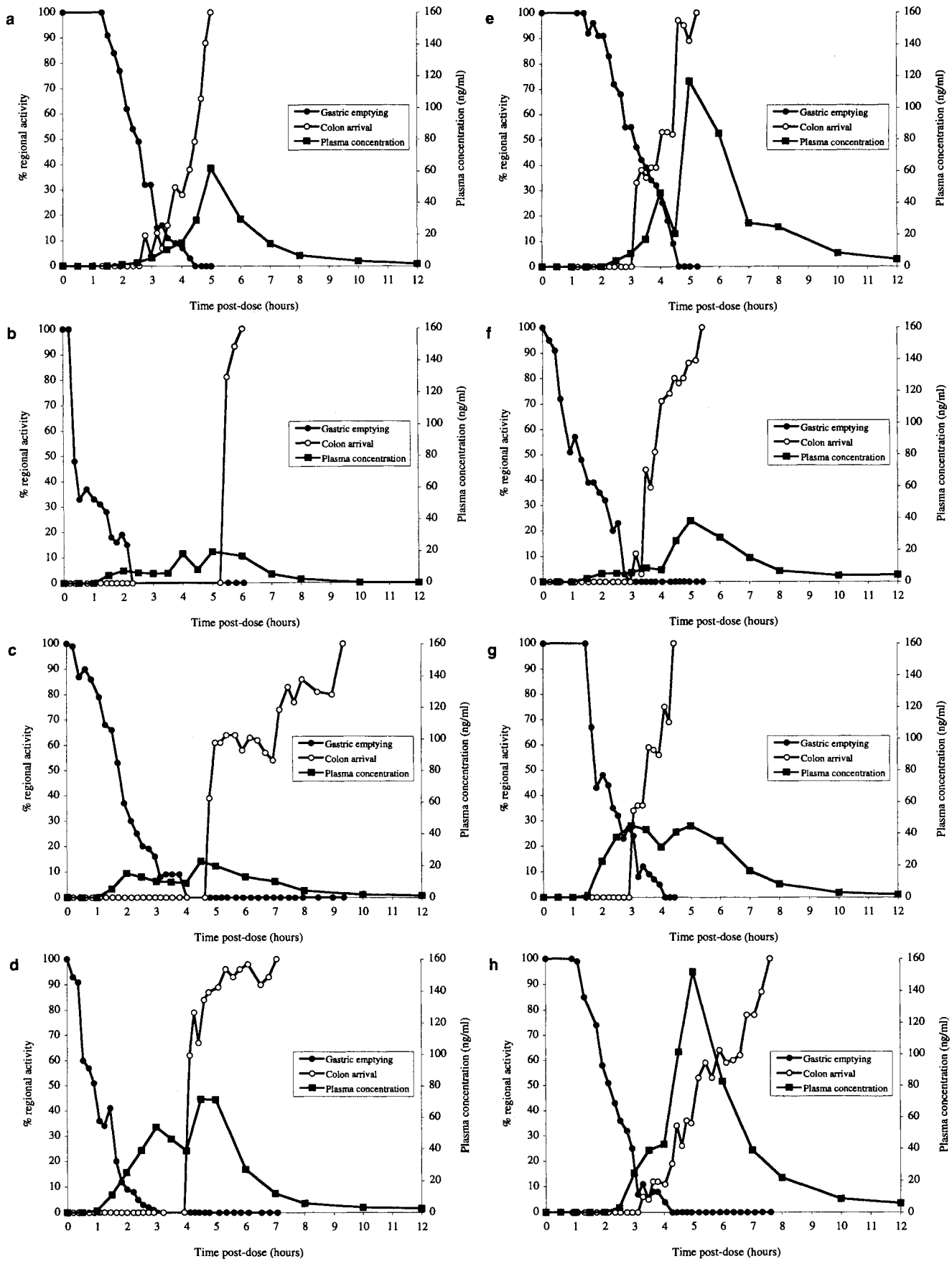

Fig. 1. Pharmacoscintigraphic profile of an immediate release saquinavir preparation following post-prandial administration. (a) Subject 1; (b) Subject 2; (c) Subject 3; (d) Subject 4; (e) Subject 5; (f) Subject 6; (g) Subject 7; and (h) Subject 8.

known to be passive throughout the small intestine and colon whilst active transport is confined to the terminal ileum (19). It has been suggested that some drugs may be reabsorbed along with them, via this active transport system which may contribute to the secondary peak observed with saquinavir. In addition, recent reports have suggested that P-glycoprotein, the cell membrane pump located in the intestinal epithelia, may prevent saquinavir from being systemically available by pumping it back into the lumen of the gut (20). It is, therefore, interesting to speculate if the increased plasma levels of saquinavir at later time points reflect reduction of P-glycoprotein action from the distal small intestine or, alternatively, are indicative of a novel interaction of food with pump activity.

A new soft gelatin formulation of saquinavir with enhanced bioavailability is now in clinical development with the aim of maximising the therapeutic potential of saquinavir (21). Further understanding of the *in vivo* behaviour of this preparation will be important if additional formulation advances are to be made.

CONCLUSIONS

The combination of scintigraphic techniques with a pharmacokinetic investigation has significantly improved our understanding of the pronounced effect that food has on saquinavir absorption. Rapid gastric emptying of intact capsules from the stomach observed scintigraphically may affect subsequent absorption of the drug if the principal sites of absorption reside in the proximal small intestine. Following post-prandial administration re-distribution of the marker within the stomach contents resulting in slower gastric emptying may be responsible for prolonged exposure to saquinavir. Plasma concentrations under these conditions were measurable up to 12 hrs after administration in seven of the eight subjects. Six of the eight plasma profiles showed second peaks after lunch and analysis of the images showed that only two cases could be attributed to a gastrocolonic response following ingestion of food. It is most likely that the increase in motility and secretion following the food stimulus at lunch time increases the amount of dissolved drug available for absorption.

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