Intestinal pH as a Factor in Selection of Animal Models for Bioavailability Testing

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Abstract □ Previous *in situ* studies showed that intestinal drug absorption is strongly influenced by intestinal pH. Three animal species considered for bioavailability testing were studied *in situ* to determine intestinal pH. Results from the rat and dog correlated well with results in humans, while results from the rabbit did not. The rabbit appears to be a poor candidate for attempted animal–human bioavailability correlations.

Keyphrases □ Intestinal pH, rats, dogs, and rabbits—*in situ* determination, factor in selection of animal model for bioavailability testing □ Bioavailability correlations, animal–human—intestinal pH of rats, dogs, and rabbits as a factor in model selection □ Animal models for bioavailability testing—intestinal pH as factor for model selection, *in situ* determination in rats, dogs, and rabbits

New drug entities are routinely tested in animal models. Following initial and toxicological tests, candidate dosage forms are frequently tested in animals to determine the best dosage forms for further clinical testing. Although it is recognized that there are many differences between animals and humans and between animal species, little information is available concerning differences in absorption between these groups. With the recent increase in emphasis on bioavailability testing, the search for better animal models for these tests has intensified.

The Food and Drug Administration (1) recently suggested that animal models be developed that correlate bioavailability in animals with similar results in humans. However, before this plan can be accomplished on a widespread basis, more information must be available on the similarities and dissimilarities of these animal models. Dogs have occasionally been used in these studies, but other animals have also been suggested (2).

Previous studies (3) in animals showed that the absorption of drugs from the intestine is highly dependent on intestinal pH. This study was undertaken to determine intestinal pH in several animal species suggested for bioavailability studies.

EXPERIMENTAL

The *in situ* method of Doluisio *et al.* (4) was used in these experiments. Briefly, in each animal model the intestine was exposed by midline incision. The intestinal section to be studied was prepared by making a small slit in the intestinal wall at each end of the segment, inserting a small polyethylene cannula in each slit, and ligating these cannulas into place to form a cannulated intestinal segment. Care was taken to keep trauma to the intestine to a minimum and to maintain an intact blood supply.

A plastic syringe barrel was then attached to the exposed end of each cannula, and the intestinal solution was placed in the intestinal loop. The intestinal solutions used were isotonic saline in the rat, isotonic phosphate buffer (0.055 *M*) in the rabbit, and isotonic phosphate buffer (0.095 *M*) in the dog. All solutions were checked for isotonicity and adjusted to pH 6.0 prior to instillation. At time intervals of 10–20 min, the solution was gently forced into one syringe barrel by air pressure from the other, and the pH was determined by a micro-pH electrode. The solution was then returned to the intestine.

This procedure ensured that the contents of the intestine were thoroughly mixed during the experiment. Although the solutions used differed in buffer capacity, the intestine has a large intrinsic ability to buffer its contents. The equilibrium pH values reported in Table I were recorded at 50, 60, and 100 min in the dog, rat, and rabbit, respectively.

In the rat studies, the duodenal section consisted of approximately 10 cm of duodenum measured from the pylorus to 4 cm distal to the ligament of Trietz. The bile duct was either left intact (normal bile) or ligated (no bile). The rat upper jejunal section consisted of 45 cm of jejunum beginning 4 cm distal to the ligament of Trietz, and the lower jejunal section consisted of an equal length of jejunum measured proximally beginning 5 cm proximal to the cecum.

In the rabbit studies, the duodenal section consisted of 25 cm of duodenum measured distally beginning 1 cm below the bile duct, and the jejunal section consisted of an equal length measured distally beginning 45 cm below the pylorus. In the dog experiments, the jejunal section consisted of 26 cm of jejunum measured distally beginning 26 cm below the ligament of Trietz.

Although the upper jejunal sections used are not exactly comparable in the three species due to experimental limitations, intestinal pH in the dog upper jejunum was found constant in four different sections studied.

RESULTS AND DISCUSSION

The pH values reported in Table I indicate that there is little difference in pH between the duodenum and upper jejunum and between the presence and absence of bile flow in the rat. In the rabbit, duodenal and jejunal pH values are also quite similar but are considerably higher than those found in either the rat or dog. Human intestinal pH values in normal subjects are reported to be (5): duodenum, 4.7–6.5, upper jejunum, 6.2–6.7, and lower jejunum, 6.2–7.3. Thus, pH values reported in humans correspond well with those found in the rat and dog but not with those found in the rabbit. Based on pH considerations alone, the rabbit does not appear to be a good animal model for attempting human–animal correlations.

The pH values reported here are equilibrium values in the bulk intraluminal fluid and may not necessarily be representative of the pH found in the "unstirred layer," the thin aqueous film located directly next to the absorbing membrane (6). However, previous studies (3) in the *in situ* model showed a good correlation between the rate of drug absorption and intraluminal pH over a wide pH range. Therefore, it is not unreasonable to speculate that drug ab-

Table I—Intestinal pH Values *In Situ*

Intestinal Section	pH Values, Mean ± *SD*		
	Rat	Rabbit	Dog
Duodenum (no bile flow)	6.6 ± 0.2 (6)[a]	7.8 ± 0.1 (15)	—
Duodenum (normal bile flow)	6.5 ± 0.3 (6)	—	—
Upper jejunum	6.4 ± 0.1 (4)[b]	7.5 ± 0.1 (19)	6.2 ± 0.1 (10)
Lower jejunum	7.2 ± 0.1 (5)	—	—

[a] Number in parentheses indicates number of experiments. [b] At 120 min.

sorption may be influenced by the differences in pH described here.

Under any circumstances, the intraluminal pH will have an important effect on the disintegration and dissolution characteristics of dosage forms administered to intact animals. This is particularly true of special dosage forms such as suspensions, coated tablets, and timed-release products where pH is an inherent part of the product design. Just as differences in biliary recycling between species can influence the pharmacokinetics of drugs administered in intact animals, intraspecies differences in intestinal pH can also be expected to influence the site and extent of absorption as well as the intestinal contribution to the volume of distribution (7).

When selecting an animal model for pharmacokinetic or bioavailability studies, intestinal pH should be considered as a potential determinant of the outcome. Of course, some drugs are potentially too toxic (*e.g.*, methotrexate) to be tested for bioavailability in normal human subjects, so for these drugs the development of suitable animal models is especially important.

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COMMUNICATIONS

Effect of an Antacid on Absorption of Digoxin in Dogs

Keyphrases □ Digoxin—effect of antacids on absorption, dogs □ Absorption—digoxin, effect of antacid, dogs □ Antacids—effect on absorption of digoxin, dogs

To the Editor:

Recently, Khalil demonstrated that the *in vitro* dissolution of digoxin tablets[1] was suppressed by a commercial antacid containing aluminum hydroxide (0.31 g/5 ml) and magnesium trisilicate (0.60 g/5 ml). The present study examined the effect of this commercial antacid[2] on the absorption of digoxin in dogs after oral administration.

Following overnight fasting, each of four mongrel dogs was given either two digoxin tablets[3] (1.0 mg) or 20 ml of the commercial antacid plus two tablets at weekly intervals in accordance with a crossover design. The antacid was given by stomach intubation, and 50 ml of water was given to each dog following administration of the dosage formulation.

Blood (4 ml) was withdrawn from the radial vein at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 7, and 24 hr following drug administration (Table I). The plasma samples were analyzed using a commercial digoxin assay kit[4]. A standard curve was obtained for each animal using its blank plasma.

Analysis of variance on each data point indicated that there was no significant difference ($p \leq 0.01$) between concentrations at any time point following the

Table I—Mean Plasma Digoxin Concentrations (Nanograms per Milliliter) following Digoxin or Commercial Antacid plus Digoxin Administrations

	0.5 hr	1.0 hr	1.5 hr	2.0 hr	2.5 hr	3.0 hr	4.0 hr	7.0 hr	24.0 hr
				Antacid plus Digoxin Tablets					
Mean	2.13	6.43	6.52	5.81	5.25	5.45	5.00	2.93	0.99
SD	3.14	2.43	1.75	1.83	1.59	2.57	1.38	0.82	0.10
				Digoxin Tablets					
Mean	1.51	4.23	5.33	6.64	5.50	5.24	4.98	3.52	1.26
SD	2.03	2.59	3.45	1.82	2.07	2.54	1.69	0.76	0.39

[1] Lanoxin tablets BP.

[2] Gelusil, Warner Chilcott Co., Canada.

[3] Lanoxin tablets, 0.5 mg, Burroughs Wellcome Co., Canada.

[4] Bio-R.I.A., Montreal, Quebec, Canada.