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In order to describe and predict the impact of intestinal metabolism on peptide absorption, intestinal chymotrypsin activity, flow rate, and pH were characterized in fasted, duodenally fistulated dogs as a function of gastrointestinal (GI) motility phase. GI motility was classified as either active or quiescent. Cumulative volume, F(t), and volumetric flow rate, Q(t), curves were constructed and the data were sorted according to motility phase. The mean ± SE active phase pH was 6.4 ± 0.3, whereas the quiescent phase pH was 7.3 ± 0.3. The difference between the mean active and the mean quiescent phase pH values was significant. The active and quiescent phase flow rates (ml/min) were also significantly different, at values of 1.2 ± 0.2 and 0.28 ± 0.07, respectively. The active phase flow rates were consistent among the dogs studied; however, the quiescent phase flow rates were highly variable among the dogs. The variability of the quiescent phase flow rates was expected since phase II of the GI motility cycle is characterized by intermediate, irregular spike activity. The mean active and quiescent phase chymotrypsin activities were 1.87 × 10−5 ± 0.53 × 10−5 and 1.56 × 10−7 ± 0.65 × 10−7 M, respectively. The active phase values were not statistically different among dogs; however, the quiescent phase values were found to be highly variable among dogs. The difference between the active and the quiescent phase chymotrypsin mean levels, however, was not statistically significant. The chymotrypsin levels determined in dogs were found to be approximately 10 times greater than those reported in humans. The significance of fasted-state chymotrypsin levels is discussed with respect to the impact of GI metabolism on peptide and peptide-like drug absorption in dogs. Further, given the intestinal metabolic differences between dogs and humans, the suitability of using the dog model for predicting the oral absorption of peptides in humans is discussed.

KEY WORDS: chymotrypsin; dog; human; intestinal metabolism; oral absorption; pancreatic serine protease; peptide; peptide-like drugs.

INTRODUCTION

The therapeutic use of orally delivered peptides and peptide analogues is advantageous since these compounds are usually potent, safe, and rapidly eliminated from the body. Moreover, the oral route is generally less expensive and more convenient for the patient than other routes of administration. The oral delivery of peptides, however, is compromised by chemical and proteolytic instability as well as by intestinal membrane transport limitations. Small peptides (3 amino acid residues or smaller) are absorbed primarily by a carrier-mediated absorption mechanism, whereas larger peptides (4 to 10 amino acid residues) may be absorbed by paracellular and endocytotic mechanisms (1). Even though the intestinal transport of large peptides (>10 amino acid residues) and proteins may be possible, presystemic metabolism by intestinal proteolytic enzymes presents a significant obstacle for their oral delivery. In fact, the oral bioavailability of larger peptides is usually less than 10% (1).

The preclinical absorption screening of orally delivered, peptidic drug candidates is commonly performed in dogs, however, metabolic and physiological differences between dogs and humans limit the usefulness of the dog model unless these differences are well characterized. While extensive comparisons have been made between dogs and humans with regard to intestinal pH (2–4), the presystemic metabolism of peptides by intestinal proteolytic enzymes has not been well characterized and could differ significantly from humans. In this report, the results of studies characterizing the upper GI pH, volumetric flow rate, and activity of chymotrypsin in mongrel fistulated dogs as a function of fasted GI motility phase are presented. The significance of fasted-state chymotrypsin levels is discussed with respect to the impact of GI metabolism on peptide and peptide-like drug absorption in dogs. Furthermore, the suitability of using the dog model for predicting intestinal peptide absorption in humans, given the intestinal metabolic differences between dogs and humans, is also discussed.

MATERIALS AND METHODS

Chemicals and Reagents

BTEE (benzoyl-L-tyrosine ethyl ester), calcium chloride, and hydrochloric acid were obtained from Sigma Chemical Company (St. Louis, MO). HPLC-grade methanol was obtained from J. T. Baker Chemical Company (Phillipsburg, NJ).

Experimental

Five healthy female, mongrel dogs weighing 18 to 23 kg were implanted with a chronic duodenal Thomas fistula. This procedure has been described by Meyer et al. (5) and Siros et al. (6). The fistula was placed 12–19 cm distal to the pylorus (proximal to mid-duodenum). A recovery period of 2 weeks was allowed after surgery. The dogs were fasted for 12 to 18 hr prior to each experiment. Water was given ad libitum except for 2 to 3 hr prior to the experiment. During the experiments the dogs were fully conscious and retained in slings (Alice King Chatman Medical Arts, Los Angeles, CA). A small volume of water (50 ml) was given during phase I by a natural swallowing technique. Phase I was ascertained by observing the discharge from the cannula as described by Gupta and Robinson (7). Mucus and bile discharges were taken as phase II activity, and 20 min after cessation of any

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discharge from the cannula, the motility was assumed to be phase I. The intestinal discharge was collected from the duodenal fistula at 10 min intervals. If the motility phase was considered active, the sampling time would be shortened to 2 to 5 min. Sampling continued for 210 min. Effluent sample color, enzyme activity, pH at 37°C (Beckman P31 pH meter, Beckman Instruments, Inc., Fullerton, CA), and volume were recorded for each sample. Enzyme determinations were performed as described below. Two to four experiments were completed in each dog.

Chymotrypsin Assay

Chymotrypsin activity was determined using the method of Hummel (8). Briefly, the rate of hydrolysis of the substrate BTEE (benzoyl-L-tyrosine ethyl ester) is determined from the change in absorbance at 256 nm. One unit is equivalent to 1 μmol of substrate hydrolyzed per min at pH 7.8 and 25°C. The concentration of protein is determined by multiplying the absorbance at 280 nm by 0.49 (the molar absorptivity of the protein). Finally, the activity per milligram of protein was calculated from the following equation:

Enzyme activity per mg = 
\[ \frac{\text{rate of change absorbance (per min at 256 nm) \times 3000}}{964 \times \text{amount enzyme used (mg)}} \]

where 964 is the molar absorptivity of N-benzoyl-DL-tyrosine.

Data Analysis: Statistical Methods

The statistical analysis was performed using SYSTAT: The System for Statistics, SYSTAT, Inc., Evanston, IL (version 4). The Kolmogorov–Smirnov–Lilliefors (KSL) test (11) was used to examine the probability distributions for all three parameters (chymotrypsin activity, flow rate, and pH). The KSL tests for normality without assuming a particular mean or standard deviation for the distribution. Moreover, the KSL test standardizes the data in such a way so that it is concerned only with the shape of the distribution and not the absolute scale. This enabled the testing for a log normal distribution by simply taking the log transformation of the data and testing using KSL. Interdog parameter statistics were examined using one-way ANOVA and a Tukey-Kramer analysis. T-tests were used to test the difference between the means of chymotrypsin activity, flow rate, or pH as a function of GI motility phase.

RESULTS AND DISCUSSION

The 50 ml of water given at the beginning of the study was not expected to affect the motility cycle (7). It was observed, however, that during the first 30 min of the study some dogs appeared to empty the volume given immediately. This apparent active phase motility pattern was probably an artifact since other intestinal parameters such as pH and effluent color did not change as would be expected during a true active phase motility pattern. It has been observed that certain dogs can be classified as “quick” gastric emptiers and others as “slow” emptiers. This may explain the active phase artifact. A total volume balance taken at the end of each study revealed that approximately 50 to 400% of the volume given was recovered at the duodenal fistula. Given that water secretion and reabsorption are dynamic processes.