Colonic Absorption and Bioavailability of the Pentapeptide Metkephamid in the Rat

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The concept of delivering systemically active peptide drugs to the colon in order to improve their oral absorption requires reasonable peptide permeability of the large intestinal wall and stability against the activity of the colonic microflora. In addition, the role of hepatic extraction needs to be addressed. In this study the absorption of the pentapeptide metkephamid following single pass perfusion of rat ascending colon was investigated by monitoring its disappearance from the large intestine and simultaneous appearance in the portal vein, the hepatic vein and the aorta. In addition its stability against colonic microflora was tested in vitro using pig caecal contents. Metkephamid was absorbed from the large intestine and appeared in the blood circulation; peptide concentrations in the portal vein increased over-proportionally with increasing perfusate concentrations (0.1 - 4.6 mmol/L) from 0.19 μg/mL ± 0.12 (SD, n = 7) to 31.6 μg/mL + 20.65 (SD, n = 4), respectively, and thus suggesting a saturable transport or metabolism. Concentrations in the hepatic vein were significantly lower than in the portal vein, hepatic extraction ratios were 0.35 ± 0.14, 0.61 ± 0.18 and 0.62 ± 0.28 (SD, n = 4) for 0.1, 0.5 and 1.0 mM metkephamid perfusate concentrations, respectively. In the anaerobic colon metabolism model the degradation half-life of the peptide was 14.9 hours, thus, indicating relative stability in the bacterial environment of the colon. The results of the present study encourage further investigations on colonic delivery of peptide drugs.

KEY WORDS: intestinal absorption; colonic delivery; peptides; intestinal microflora.

INTRODUCTION

Metabolic degradation of enzymatically labile peptides in the intestinal tract is currently considered one of the most important barriers that prevent entry of peptides into the systemic circulation. Among the approaches to circumvent this biochemical barrier, modifications of the peptide structure (1–2), coadministration of peptidase inhibitors (3) and restriction of the peptide release to an intestinal site devoid of secreted digestive enzymes (4) has been suggested. From a metabolic activity point of view, there are indications that the colonic mucosa is fairly low in the activity of endogenous digestive enzymes (5,6) that affect peptide stability in the small intestine. This has led to an increasing development of colonic delivery systems for peptide drugs. However, several, mainly biological aspects in the field of colonic peptide delivery remain unclear.

(i) The bioavailability of colically delivered peptides, being a function of their membrane permeability, metabolic degradation and residence time in the absorbing segment is insufficiently characterized although absorption of a variety of therapeutics from the large intestine has been demonstrated, including beta-blockers, ACE-inhibitors, NSAID’s, Xanthin derivatives (7).

(ii) Although there is conclusive evidence that extensive digestion of polysaccharides occurs in the colon, little is known about the digestion of proteins and peptides by colonic bacteria. Only a few species of colonic bacteria, e.g. Bacteroides ruminicola, can utilize proteins as a sole source of carbon and energy, and these species are found only in very low numbers in the colon (8). For this reason it is often assumed, that digestion of protein in the colon is much less extensive than the digestion of polysaccharides. On the other hand, all bacteria produce proteases which are involved in the turnover of cell protein and these proteases could well contribute to the breakdown of therapeutic peptides and proteins in the colon. A recent investigation by Macfarlane et al (9) points out the possibility that considerable bacterial protein fermentation in humans could potentially account for 17% of the short-chain fatty acids found in the caecum and 38% found in the sigmoid/rectum.

(iii) The scarce information available today on the role of hepatic extraction of orally administered peptide and protein drugs indicates that a contribution of the liver to the presystemic elimination by metabolism and/or biliary excretion may be quite variable depending on the structure of the compound investigated (10).

The aim of the present study is to address some of the above mentioned questions utilizing the pentapeptide metkephamid as a model compound. More specifically the investigations were designed to clarify whether

- the peptide is absorbed from the ascending colon and appears intact in the portal vein,
- metkephamid is extracted by a hepatic first-pass effect and appears in the systemic circulation following colonic administration,
- there is significant metabolic peptide degradation in the presence of viable hindgut microflora.

MATERIALS AND METHODS

Materials

Metkephamid (Tyr-D-Ala-Gly-Phe-N-Me-Met-NH2 CH2COOH, mol. wt. 660.8) was kindly provided by Ely Lilly (Indianapolis, IN). O-Phthalaldehyde, 2-mercapto-ethanol, urethane (ethylcarbamate) were obtained from SIGMA Chemical (St. Louis, MO). Propionic acid, phosphoric acid, potassium chloride, calcium chloride, magnesium chloride, sodium bicarbonate, monobasic sodium phosphate, sodium acetate were obtained from Fluka (Buchs, Switzerland), methanol from Romil Chemicals, Loughborough, England. Phenol red solution was purchased from E.
Merck (Zürich, Switzerland). All buffer and mobile phase components were analytical grade and used as received.

Assay Method

Metkephamid was assayed by HPLC on a Merck-Hitachi system (Dietikon, Switzerland) equipped with a L-6200A gradient pump, a L-4250 UV-VIS detector, a F-1050 fluorescence spectrophotometer, an AS-2000 autosampler and a D-2500 chromato-integrator. The methods for quantification of the peptide from perfusate and blood have been published previously (5).

Sodium and potassium in the intestinal perfusion solutions were quantitatively determined by flame photometry (Instrumentation Laboratory Flame Photometer 243, Instrumentation Laboratory Inc., Lexington MA, USA). Chloride was quantified argentometrically using a chloride analyzer (model 925, Corning Ltd., Halstead, GB). The volume marker phenol red was analyzed at 546 nm using a photometer (model 4010, Boehringer Mannheim, Mannheim, FRG) following dilution of a 0.15 mL sample with 0.5 mL 1M sodium hydroxide solution.

Perfusion Solutions

Perfusion solutions for colonic perfusion experiments consisted of a buffer prepared according to Argenzio et. al [11]. It was composed of 20 mM potassium chloride, 2.5 mM calcium chloride, 2.5 mM magnesium chloride, 20 mM monobasic sodium carbonate, 30 mM monobasic sodium phosphate, 60 mM sodium acetate, 10 mM propionic acid and 56.4 mM phenol red and metkephamid (0.1 mM–4.6 mM). The pH of the buffer was adjusted to 5.5 with phosphoric acid. 15 minutes prior to perfusion, the buffer was oxygenated with O2. Perfusion solutions were isosmotic.

Perfusion Studies

Perfusion studies were performed using the single-pass perfusion technique. Male rats, strain SIVZ-50 (Institut für Zuchthygiene, Veterinärmédizinische Fakultät der Universität Zürich), weighing 280–320 g, were fasted 15–20 h prior to the experiment. Anaesthesia was induced by an i.m. injection of 1.5 g/kg body weight of urethane. The rats were put on a heating pad to maintain body temperature. The peritoneum was opened by a midline incision. Segments of the ascending colon, about 4–6 cm in length, starting approximately 1 cm caudal of the ileocaecal valve, were cannulated with a silicon tube (OD: 4mm, ID: 2mm) following a midline longitudinal incision. Blood supply to the perfused segments was maintained during the study. The segments were thoroughly cleaned of faecal matter by passing an appropriate volume of plain perfusate buffer through the segment. Perfusion of the colon was from cranial to caudal. The inlet cannula was attached to a 50 mL syringe (Becton-Dickinson, Basel, Switzerland) which was placed on a perfusion pump (Perfusor® VI, Braun Melsungen AG, Neuhausen, Switzerland). Perfusate solution was delivered continuously at a rate of 0.2 mL/min for 90–120 minutes through the segment. The inlet tubing was thermostated at 39°C by a water bath so that the perfusate entered the intestinal segment at body temperature. After the surgery the intestinal segment was placed back into the abdominal cavity which was then closed with wound clips to prevent water loss from the animals’ body. Care was taken to avoid any kinks. Initially the eluate from the colonic segments was collected in 15 minute intervals to determine the time necessary to reach a steady state flow in terms of peptide, water and electrolyte absorption. In later studies, the eluates from t = 0 to t = 30 min were discarded and from t = 30 min to t = 90 min were combined and assayed and compared to peptide and electrolyte concentration in perfusion buffers prior to absorption. After 90 min of perfusion, 1.0 mL blood samples were taken from the portal vein and the aorta abdominalis of the animal for all perfusate concentrations of metkephamid, whereas samples from the hepatic vein were available only at 0.1, 0.5 and 1.0 mM perfusate concentrations. Samples were immediately stored on ice and processed as described previously (5). Thereafter the colonic segment perfused was removed from the animal, its length was measured and the segment was dried at 50°C for 24 hours to determine the tissue dry weight.

Peptide Stability in the Presence of Hindgut Anaerobic Microflora

Metkephamid stability in the presence of anaerobic and metabolically functional microflora was evaluated at 1 mM and 0.1 mM peptide concentrations in the incubation fluid using the Cositec (Colon Simulation Technique) apparatus described by Breves et al. (12, 13). Briefly, hindgut contents were obtained from caecally fistulated pigs and gauze filtered caecal fluid was used to start the system. Nylon bags containing freeze-dried particles from the caecum were introduced into the incubation vessels as a pre-digested substrate to be fermented. In each experiment equilibration for 8 days was followed by 6–8 sampling days, with volatile fatty acids (VFA) and gas production, microbial protein synthesis and digestibilities of organic matter and fibrous carbohydrates being measured.

Data Analysis

Water absorption (mL/100 mg dry weight · h) was calculated from the difference in concentrations of the volume marker phenol red in the perfusion solutions before and after single-pass perfusion, and was then calculated as:

\[
\text{Water absorption } W = \frac{(1 - F) \times PR \times 100 \times SI}{DW}
\]

where F is the ratio of the absorbance at 546 nm of the perfusate solution before and after perfusion of the isolated gut segment, PR is the perfusion rate (mL/min), SI is the sampling interval (min) and DW is the dry tissue weight of the perfused segment (mg). The solute absorption (µmol/100 mg dry weight · h) from the analysis of perfusate samples was corrected for net water absorption/secretion and was calculated according to

\[
\text{Solute absorption } S = \frac{(C_0 - (C_m x F)) \times PR \times 100 \times SI}{DW}
\]

where C_m and C_0 represent the outlet and inlet concentrations (µmol/L) of the solute respectively. Positive values for