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Stability of peptide drugs in the colon

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ABSTRACT

This study was the first to investigate the colonic stability of 17 peptide molecules (insulin, calcitonin, glucagon, secretin, somatostatin, desmopressin, oxytocin, Arg-vasopressin, octreotide, ciclosporin, leuprolide, nafarelin, buserelin, histrelin, [D-Ser^4]-gonadorelin, deslorelin, and goserelin) in a model of the large intestine using mixed human faecal bacteria. Of these, the larger peptides – insulin, calcitonin, somatostatin, glucagon and secretin – were metabolized rapidly, with complete degradation observed within 5 min. In contrast, a number of the smaller peptides – Arg-vasopressin, desmopressin, oxytocin, gonadorelin, goserelin, buserelin, leuprolide, nafarelin and deslorelin – degraded more slowly, while octreotide, histrelin and ciclosporin were seen to be more stable as compared to the other small peptides under the same conditions. Peptide degradation rate was directly correlated to peptide lipophilicity ($\log P$); those peptides with a higher $\log P$ were more stable in the colonic model ($R^2 = 0.94$). In the absence of human faecal bacteria, all peptides were stable. This study highlights the impact of the colonic environment – in particular, the gut microbiota – on the metabolism of peptide drugs, and identifies potential peptide candidates for drug delivery to the colon.

1. Introduction

Peptide and protein biopharmaceuticals provide an exciting new avenue of therapeutic opportunities for many disease states ranging from rheumatoid arthritis to inflammatory bowel disease. Indeed, these molecules offer enhanced activity, potency and reduced toxicity as compared to existing low molecular weight therapeutics (Park and Kwon, 2011; Smart et al., 2014). However, whereas peroral delivery remains the preferred route of drug administration, this route encompasses multiple caveats insofar as the delivery of biopharmaceuticals are concerned, ranging from fluctuations in pH levels to changes in enzyme activity within the gastrointestinal milieu, collectively contributing to the structural breakdown of peptides. Our group recently investigated the stability of 17 peptide drugs in the proximal regions of the human gastrointestinal (GI) tract (Wang et al., 2015), revealing that in gastric fluid, large peptides were rapidly metabolized, whereas smaller peptides showed better stability under the same conditions. However, in small intestinal fluids, both the small and large peptides degraded rapidly, albeit those peptides featuring a cyclic structure or containing a disulfide-bridge demonstrated greater stability. Contrasted with the upper gut, the large intestine (colon) offers a potentially attractive alternative site for peptide and protein delivery owing to its more benign environment due to lower protease levels, longer transit times and higher responsiveness to permeation enhancers that may be more refractive to the breakdown of protein molecules (Ashford and Fell, 1994; Maroni et al., 2012; Rubinstein, 1995).

Despite these perceived advantages, however, the colon is colonized by at least 3000 different bacterial species (McConnell et al., 2009), with up to 10^{11}–10^{13} bacteria/g gut contents (Sousa et al., 2008); it is these bacteria which may be liable to influence peptide stability and contribute to their premature degradation. Indeed, the gut microbiota secretes a diverse range of metabolic enzymes involved in the metabolism of drugs through a number of reactions ranging from reduction to hydrolysis, decarboxylation, dehydroxylation and deglucoronidation (Langguth et al., 1997; Sousa et al., 2008). These bacteria also secrete enzymes capable of degrading drugs of low molecular weight (Sousa et al., 2014; Yadav et al., 2013), as well as peptides and proteins (Tozaki et al., 1995).

At present, there is limited information available in the literature regarding the stability of peptides in the colon, with published studies reporting data on only one or two drugs in rat colonic contents (Tozaki et al., 1997; Ungell et al., 1992). Moreover, the rat is not always representative of the human situation in the context of colonic drug stability (Hatton et al., 2015). A database containing human colonic stability data on a large number of peptides is

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Table 1
Primary structure of the 17 peptides.

Peptide	Amino acid sequence
Ciclosporin	D-Ala-NMe*-Leu-NMe-Leu-NMe-Val-NMe-Bmt**-Abu***-NMe-Gly-NMe-Leu-Val-NMe-Leu-Ala (cyclic)
Desmopressin	Deamino Cys-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH_2
Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
Arg-vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2
Octreotide	D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-L-threoninol
Nafarelin	Glu-His-Trp-Ser-Tyr-D-2-Nal-Leu-Arg-Pro-Gly-NH_2
Buserelin	Glu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NHEt
Goserelin	Glu-His-Trp-Ser-Tyr-D-Ser (tBu)-Leu-Arg-Pro-AzaGly-NH_2
Histrelin	Glu-His-Trp-Ser-Tyr-D-His(Bzl)-Leu-Arg-Pro-NHEt
Leuprolide	Glu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt
[D-Ser]^4-Gonadorelin	Glu-His-Trp-D-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
Deslorelin	Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-NHEt
Somatostatin	Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH
Secretin	His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Glu-Gly-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2
Calcitonin	H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2
Glucagon	His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met- Asn-Thr
Insulin	Gly-Ile-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn :A-Chain (21 amino acids) Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr : B-Chain (30 amino acids)

* NMe-: N-methyl-.
** Bmt: 4-(2-butenyl)-4-methyl-L-threonine.
*** Abu: 2-Aminobutyric acid.

therefore warranted in order to aid identification of structural peptide features that may obviate susceptibility to *in vivo* metabolic effects. Similarly, such a database could also be used to better inform the selection of colonic delivery candidates, and aid in the development of peptide formulation strategies. Consequently, the aim of this study was to investigate the stability of 17 peptide molecules with a wide range of physicochemical properties within an *in vitro* model closely representative of the human colon. Based on a mixed human faecal inoculum, the model mimics the luminal environment of the large intestine. It has been widely used to evaluate the stability of small molecule drugs in the colon (Basit et al., 2002; Varum et al., 2013; Yadav et al., 2013), and the *in vivo* relevance of this type of colonic model has been shown by Tannergren et al. who established a good correlation between drug degradation and fraction absorbed in the colon (Tannergren et al., 2014).

Based on their physicochemical properties, the peptide drugs were separated into three groups: (a) small peptides containing disulfide bridges (desmopressin, oxytocin, Arg-vasopressin and octreotide) or cyclic structures (ciclosporin), (b) small linear peptides (nafarelin, buserelin, goserelin, histrelin, leuprolide, [D-Ser]^4-gonadorelin and deslorelin) and (c) large peptides (somatostatin, secretin, calcitonin, glucagon and insulin) (Table 1).

2. Materials and methods

2.1. Materials

Oxytocin acetate, buserelin acetate, goserelin acetate, nafarelin acetate, desmopressin acetate, leuprolide acetate, histrelin, [D-Ser]^4-gonadorelin, human recombinant insulin and Arg-vasopressin acetate were obtained from Sigma–Aldrich, UK. Octreotide, deslorelin, somatostatin, glucagon, secretin and salmon calcitonin were obtained from Oxford Expression Technologies, UK. Ciclosporin was obtained from Abcam plc, UK. Sodium chloride and dipotassium hydrogen phosphate were obtained from Fisher Chemical. Magnesium sulphate heptahydrate and calcium chloride hexahydrate were obtained from VWR. Sodium bicarbonate and trifluoroacetic acid (TFA) were sourced from Sigma Aldrich while haemin, L-cysteine HCl, vitamin K and resazurin were obtained from Sigma Life Sciences. Bile salts and tween 80 were from Fluka Analytical and Fluka Chemika respectively. All other chemicals and solvents were of analytical reagent grade and used without further purification.

2.2. Establishment of the model of the colon

An *in vitro* colonic model based on a mixed faecal inoculum was used to mimic the luminal environment of the human large intestine (Basit et al., 2002; Sousa et al., 2014; Yadav et al., 2013). The model was established inside an anaerobic workstation (Electrotek 500TG workstation, Electrotek, West Yorkshire, UK) maintained at 37 °C and a relative air humidity of 70%. Three healthy human volunteers were given previously weighed plastic receptacles into which faecal samples were collected. The volunteers were not on medication and had not taken antibiotics for at least the previous six months. The faecal material was transferred to the anaerobic workstation and diluted with freshly prepared basal medium (Hughes et al., 2008) to obtain 10% w/w slurry by homogenization using an Ultra Turrax (IKA T18 Basic) homogenizer at a speed of 18,000 rpm. The homogenized bacterial media was sieved through an open mesh fabric (SefarNitex™, pore size 350 μm) to remove any unhomogenised fibrous material. The pH and buffer capacity of the human faecal slurry was 6.8 and 28.3 mM/L/pH unit, respectively, which closely match the conditions of the human colon (Diakidou et al., 2009; Sousa et al., 2014).