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Leading article

Intestinal absorption of peptide drugs: advances in our understanding and clinical implications

The route of administration of most drugs, including antibiotics, is often decided empirically based on bioavailability data from animal studies. Increasing knowledge, however, of the transport mechanisms participating in solute uptake from the human intestine has improved our understanding of the absorption of peptide drugs (including a number of antibiotics). This article will briefly discuss dipeptide transport in humans and then focus on the intestinal absorption of peptide drugs, in particular ß-lactam antibiotics, and the therapeutic implications of research in this field. ß-lactam compounds are weakly acidic peptides and thus expected to be poorly absorbed from the gastrointestinal tract. Some of the drugs in this group, however, have surprisingly good oral bioavailability because of the utilisation of carrier mediated transport systems for small peptides within the brush border. Peptide compounds, particularly hormones and their analogues, are increasingly used therapeutically, although oral bioavailability is often a problem. Specific pharmacological design to facilitate intestinal absorption is potentially of great benefit. Design of drugs specifically to exploit carrier systems is important as the clinical usefulness of a compound is greatly enhanced if it is well absorbed when given by mouth.

Mammalian peptide transport systems

To reach the bloodstream from the gut lumen, solutes must either first cross the brush border membrane of the enterocyte microvilli, traverse the enterocyte cytoplasm, and exit by the basolateral membrane or enter by the paracellular route. Protein is predominantly absorbed from the mammalian gut as amino acids and oligopeptides, although some larger peptides may also be absorbed intact.[1] Absorption of these molecules entails both passive diffusion through the paracellular pathways, and facilitated by carrier mediated transport. It is the use of these carrier mediated peptide transport systems in the absorption of peptide drugs that has recently provoked interest.

Peptide transport in mammalian gut was first reported by Newey and Smyth more than 30 years ago.[2,3] Peptide transport plays a major part in the absorption of amino acids and is therefore of nutritional significance. After luminal and brush border digestion by proteases, proteins are hydrolysed to a mixture of free amino acids, dipeptides, and a few tripeptides, which are suitable substrates for absorption into the enterocyte.[4] Much of the evidence for separate dipeptide and amino acid transport has been provided by studies in patients with Hartnup disease and cystinuria. In these conditions there is a genetic deletion, which results in an intestinal transport defect for neutral amino acids in Hartnup disease[5] and dibasic amino acids in cystinuria.[6] Despite these transport defects, however, the amino acids in question are absorbed normally or near normally when presented to the mucosa in the form of dipeptides. Furthermore, in most studies on dipeptide transport, uptake of the amino acid components of dipeptides is faster than that of the corresponding free amino acids, showing that different transport systems are involved.[7,8] Absorption of intact oligopeptides larger than 2 or 3 amino acids may also occur, although the data are conflicting. An elegant study by Fricker *et al* used a fluorescent labelled analogue of somatostatin, an octapeptide, to explore the absorptive pathways of the molecule through the rat small intestine.[9] They showed that the molecule is quickly absorbed into rat jejunal enterocytes and that paracellular pathways played little or no part in uptake.

RELATION BETWEEN MUCOSAL HYDROLYSIS AND DIPEPTIDE TRANSPORT

There is a close relation between dipeptide transport and mucosal hydrolysis because both in vivo and in vitro experiments have shown that dipeptide uptake is associated with the appearance of free amino acids in the media bathing the mucosal preparations.[7,8,10] There are several explanations, which are not mutually exclusive; some dipeptides are probably hydrolysed by brush border peptidases and absorbed as amino acids while others are transported unhydrolysed into the cell and hydrolysed by peptidases within the cytoplasmic compartment.[11,12] The cytoplasmic peptidases cleave dipeptides preferentially, and are complementary to the brush border enzymes; small peptides that are poor substrates for brush border enzymes are excellent substrates for the dipeptide carrier and for cytoplasmic peptidases. A comprehensive study in humans with intestinal perfusion and in vitro techniques concluded that in the human intestine, the predominant mechanism for assimilation of glutamine dipeptides is absorption as the intact dipeptide rather than hydrolysis.[13]

CHARACTERISATION OF PEPTIDE TRANSPORT SYSTEMS

In vitro experiments with hamster jejunum have shown that the dipeptide glycylsarcosine can be transported into the enterocyte against both a chemical and electrochemical gradient, and that this active process is sodium dependent,[14] although this may not be the case in humans.[15] Uptake of other peptides has similarly been shown to be energy dependent, and saturable.[16,17] While some simple diffusion of dipeptides does occur, active transport is more important. Available evidence suggests that there is a single transporter for dipeptides,[17–19] although some workers have proposed the existence of more than one transport system.[20,21] Subsequent studies have shown that peptides are cotransported with protons (H^+) and that the protons are then recycled out of the enterocyte by Na^+–H^+ exchange.[22]

Intestinal absorption of ß-lactam antibiotics

The passive diffusion of any molecule through the lipid bilayer of a membrane is inversely proportional to its hydrophilicity and charge. ß-lactam antibiotics are generally weak acids (negative logarithm of the acidic dissociation constant (pKa) values between 2 and 3) with low lipophilicity. pH Partition theory would predict that as ß-lactam antibiotics are completely ionised under physiological conditions they would not easily cross the enterocyte

membrane. It is therefore somewhat surprising to find that the aminopenicillins (amoxicillin and cyclocillin) and aminocephalosporins (ceflacor, cephalexin, and cephadroxil) are almost completely absorbed from the gut.[23] An α-amino group is the key feature, as analogues with similar physicochemical properties, but without a free α-amino group, are not absorbed. Such selectivity suggests an active process. In 1970 Quay showed that cephalexin could be actively transported across rat jejunum in vitro.[24] It has since been shown that cephalexin competitively inhibits the uptake of the tripeptide glycylsarcosarcosine, leading to speculation that cephalexin is absorbed by a dipeptide or tripeptide carrier system. Not all workers have obtained similar results however and while some have shown that the uptake of ß-lactam antibiotics is saturable, others have shown that these compounds are absorbed passively by a non-saturable process. These discrepancies are probably explained by differences in experimental systems, and in the antibiotics studied; those that are rapidly transported have saturable kinetics that are easy to identify whereas those with slow absorption have kinetics that are more difficult to define.

ß-lactam antibiotics utilise intestinal dipeptide carrier systems

The absorption of ß-lactam antibiotics is conventionally determined using whole animals, in situ perfusion, or excised gut sacs. Perfusion studies with rat small intestine have sometimes yielded conflicting results. For example, cephalexin may be transported by both active and passive processes. These anomalies have been explained in terms of an experimentally induced bias as a consequence of an unstirred diffusion boundary layer surrounding the intestinal epithelium. Active and passive transport both show dependence upon the flow rate and intestinal length perfused. Sinko and Amidon[25] overcame these problems by using a modified boundary length method under steady state conditions and showed that jejunal absorption of aminocephalosporins in rat perfusions was mainly the result of an active process, but with in some cases a small passive component. Quay[24] had earlier suggested that cephalexin could be actively transported across rat jejunum in vitro and it was subsequently shown that cephalexin competitively inhibited the uptake of the tripeptide glycylsarcosylsarcosine.[26] Amino-ß-lactam antibiotics can be regarded as peptide mimetics of D-alanyl-D-alanine. Most of the amino-ß-lactam antibiotics, with the exception of ampicillin, inhibit the absorption of each other, suggesting that they share a common carrier. It is now clear that the amino-ß-lactams cyclocillin, amoxycillin, cephradine, and cephalexin are absorbed by a carrier mediated transport process from the rat small intestine and that the carrier is almost certainly a sodium independent proton driven dipeptide symport. The brush border membrane dipeptidases do not participate in the uptake process.[27] A study of the absorption kinetics of amoxycillin in humans provides further evidence of a saturable carrier mediated uptake of this antibiotic.[28] The uptake mechanism for other amino-ß-lactam antibiotics has been more difficult to define in animals because of their slower relative absorption. It has been shown that a free N-terminal α-amino group is a requirement for recognition and transport of dipeptides and peptide like therapeutic agents.[29] Figure 1 shows the transport mechanism of ß-lactam antibiotics across the intestinal epithelium.

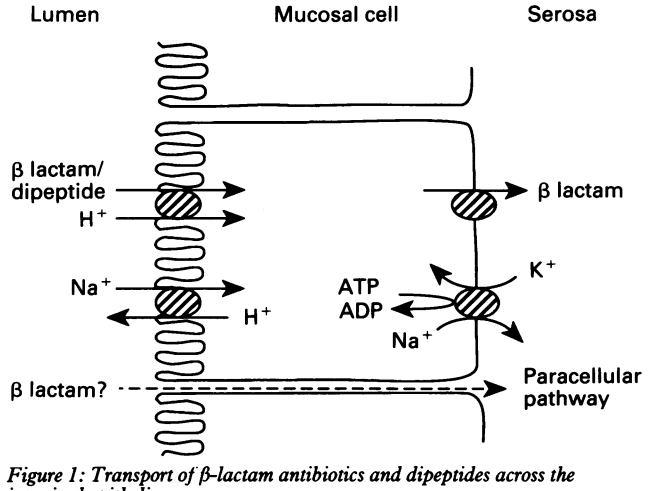


Figure 1: Transport of ß-lactam antibiotics and dipeptides across the intestinal epithelium.

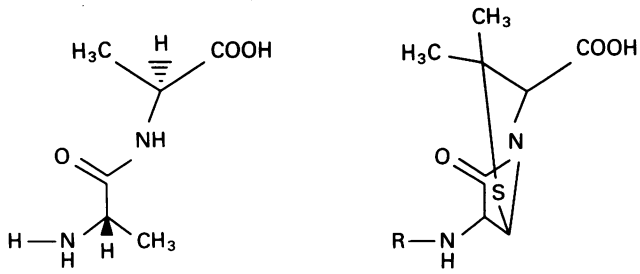
Mechanisms of ß-lactam transport

Studies with brush border membrane vesicles (BBMVs) have distinguished the components of the uptake system for solutes. The uptake of amino-ß-lactams in rat or rabbit BBMVs is driven by a sodium independent proton gradient. The physiological function of this carrier is to translocate small peptides,[30 31] and is distinct from the sodium dependent transport system used by amino acids.[22 32 33] BBMVs prepared from human jejunum have also been shown to possess a proton driven dipeptide porter that can transport amino-ß-lactams.[34] Photoaffinity labelling studies with rabbit brush border membranes showed that amino-ß-lactams and dipeptides both interact with an integral membrane glycoprotein of 127 kD.[35-37] ß-lactams that are not absorbed from the small intestine also bind to the luminal side of this protein but are not transported across the brush border membrane. This carrier also translocates orally absorbed peptide like renin inhibitors[38] and there is convincing evidence that this 127 kD protein is indeed the intestinal peptide transporter (or a component thereof).[39]

Initially only ß-lactams possessing an α-amino group were thought to use the carrier mediated transport systems. The recent aminothiazolyl cephalosporins cefixime and ceftibutene, however, are both well absorbed from the gut although they lack the α-amino group and have a low lipophilicity. Studies by Tsuji[40] show that an inward proton gradient also drives the transport of cefixime[41] and the *cis* isomer of ceftibuten.[42] The *trans*-isomer of ceftibuten is not absorbed from the gut or driven by a proton gradient into BBMVs, suggesting that uptake is stereoselective. The C-3 substituent of orally absorbed cephalosporins is lipophilic and small or absent; bulky side chains at this position militate against passage through the mucosa.

Most, if not all orally absorbed ß-lactam antibiotics share a common transport system in the intestinal brush border, although the precise structural features essential for the recognition and translocation of these antibiotics are not yet known. For penicillins, it is the 6 position α-amino side chain alone that dictates uptake; in the cephalosporins both 3 and 7 positions play a part. The dipeptide carrier system may not be the only uptake mechanism for ß-lactams; certain lipid soluble penicillins that do not have a 6 α-amino substituent cross the brush border membrane by rate limited diffusion through the unstirred water layer above the membrane. Although the available data suggest that a dipeptide carrier that translocates amino-ß-lactams does not have an affinity for these agents, they may use other, as yet uncharacterised, porters. Figure 2 illustrates the structural similarities between dipeptides and ß-lactam antibiotics.

It is assumed that once across the brush border membrane ß-lactams diffuse freely through the enterocyte cytoplasm. The mechanism by which they leave the enterocyte is unknown. An outwardly directed proton symport for gylcylproline, however, has recently been shown in the



L-alanyl-L-alanine Generalised penicillin

Figure 2: Schematic representation of the chemical structure of L-alanyl-L-alanine and generalised penicillin, showing the similarities between the molecules.

basolateral membrane from rabbit small intestine.[43] There is a potential alternative but probably non-selective route across the intestinal epithelium, the so called paracellular pathway through the tight junction between enterocytes (Fig 2). Whether peptides or ß-lactam antibiotics can use this route is unknown and experimentally difficult to confirm.

ROLE OF CALCIUM IONS IN ANTIBIOTIC UPTAKE

Coadministration of nifedipine, a calcium channel blocker, enhances the rate of amoxycillin absorption.[44] This might be explained by the role of calcium in the control of the Na^+/H^+ electrolyte antiporter, which by pumping out sodium in exchange for protons, contributes in part to the maintenance of the proton gradient.[45] A nifedipine induced loss in intraenterocyte calcium could stimulate sodium expulsion by the antiporter resulting in the enhanced inward proton gradient being available to drive antibiotic uptake,

ABSORPTION OF ESTER PRODRUGS

As only a small proportion of the large number of ß-lactams synthesised show significant oral absorption, alternate strategies have been developed to permit oral dosing. Among these, the most successful has been the esterification of the free carboxylate group.[46] The mechanisms by which these ester prodrugs are absorbed and de-esterified to produce high free drug concentrations in the bloodstream is not well understood. Increased lipophilicity is not the sole explanation because subtle modifications of either the C-3 or C-7 substituent can dramatically reduce the oral bioavailability.

In conclusion, current clinical practice demands that new antibacterial agents be available in both oral and injectable formulations. Unfortunately, those ß-lactam antibiotics with the highest antibacterial activity often possess a chemical structure of low lipid solubility and groups that ionise under physiological conditions, features that militate against their ability to diffuse passively across the gastrointestinal tract. Fortuitously, some are recognised by carrier mediated systems for dipeptides present in the small intestine. We are now beginning to understand the comparative contributions of passive and active transport in the absorption of ß-lactam antibiotics, to identify specific carriers, and the forces driving their uptake and transfer into the bloodstream. Studies with animal and human BBMVs, taken in conjunction with conventional data from rodents and primates could greatly facilitate the selection of those candidates most likely to be orally bioavailable in subsequent human trials. In the longer term, an understanding of the molecular basis of the interaction of ß-lactams and peptides with their translocators could permit the rational design and synthesis of new drugs with enhanced oral absorption.

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1 Takaori K, Burton J, Donowitz M. The transport of an intact oligopeptide across adult mammalian jejunum. *Biochem Biophys Res Commun* 1986; **137**: 682–7.
2 Newey H, Smyth DH. The intestinal absorption of some dipeptides. *J Physiol (Lond)* 1959; **148**: 48–56.
3 Newey H, Smyth DH. Intracellular hydrolysis of dipeptides during intestinal absorption. *J Physiol (Lond)* 1960; **152**: 367–80.
4 Silk DBA. Peptide transport. *Clin Sci* 1981; **60**: 607–15.
5 Silk DBA, Dawson AM. Intestinal absorption of carbohydrates and protein in man. In: Crane RK, ed. *International review of physiology. Gastrointestinal physiology III*. Baltimore: University Park Press, 1979; **19**: 151–204.
6 Milne MD. Disorders of intestinal amino acid transport. *J Clin Pathol* 1971; **24** (suppl 5): 41–4.
7 Silk DBA Peptide absorption in man. *Gut* 1974; **15**: 494–501.
8 Matthews DM, Adibi SA. Peptide absorption. *Gastroenterology* 1976; **71**: 151–61.
9 Fricker G, Bruns C, Munzer J, Briner U, Albert RM, Kissel T, *et al.* Intestinal absorption of the octapeptide SMS 201–995 visualized by fluorescence derivatization. *Gastroenterology* 1991; **100**: 1544–52.
10 Adibi SA. Intestinal phase of protein assimilation in man. *Am J Clin Nutr* 1976; **29**: 205–15.
11 Gray GM. Intraluminal and surface membrane digestion of dietary carbohydrate and protein. In: Go VLW, Brooks FP, DiMagno EP, Gardner JD, Lebenthal E, Schede GA, eds. *The exocrine pancreas: biology, pathobiology, and diseases*. New York: Raven Press, 1986: 375.
12 Adibi SA, Kim YS. Peptide absorption and hydrolysis. In: Johnson LR, ed. *Physiology of the gastrointestinal tract*. New York: Raven Press, 1981: 1073.
13 Minami H, Morse EL, Adibi SA. Characteristics and mechanism of glutamine-dipeptide absorption in human intestine. *Gastroenterology* 1992; **103**: 3–11.
14 Addison JM, Burston D, Matthews DM. Evidence for the active transport of the dipeptide glycylsarcosine by hamster jejunum in vitro. *Clin Sci* 1972; **43**: 907–11.
15 Rajendran VM, Ansari SA, Harig JM, Adams MB, Khan AH, Ramaswamy K. Transport of glycyl-L-proline by human intestinal brush border membrane vesicles. *Gastroenterology* 1985; **89**: 1298–304.
16 Matthews DM, Addison JM, Burston D. Evidence for the active transport of the dipeptide carnosine (L-alanyl-L-histidine) by hamster jejunum in vitro. *Clin Sci Mol Med* 1984; **46**: 693–705.
17 Sleisenger MH, Burston D, Dalyrymple SA, Wilkinson S, Matthews DM. Evidence for a single 'common carrier' for uptake of a dipeptide and tripeptide by hamster jejunum in vitro. *Gastroenterology* 1976; **71**: 76–81.
18 Matthews DM, Gandy RH, Taylor E, Burston D. Influx of two dipeptides glycylsarcosine and L-glutamyl-L-glutamic acid into hamster jejunum in vitro. *Clin Sci* 1979; **56**: 15–23.
19 Taylor E, Burston D, Matthews DM. Influx of glycylsarcosine and L-lysyl-L-lysine into hamster jejunum in vitro. *Clin Sci* 1980; **58**: 221–5.
20 Lane AE, Silk DBA, Clark ML. Absorption of two proline-containing peptides by rat small intestine in vivo. *J Physiol (Lond)* 1975; **248**: 143–9.
21 Fairclough PD, Silk DBA, Clark ML, Matthews DM, Marrs TC, Burston D, *et al.* Effect of glycylglycine on absorption from human jejunum of an amino acid mixture simulating casein and a partial enzyme hydrolysate of casein containing small peptides. *Clin Sci Mol Med* 1977; **53**: 27–33.
22 Ganapathy V, Leibach FH. Is intestinal peptide transport energized by a proton gradient? *Am J Physiol* 1985; **249**: G153–60.
23 Kwan K, Rogers JD. Pharmacokinetics of beta-lactam antibiotics. In: Demain AL, Solomon NA, eds. Antibiotics containing the beta-lactam structure. Part II. Berlin: Springer Verlag, 1983: 247–370.
24 Quay JF, Foster S. Cephalexin penetration of the surviving rat intestine. *J Physiol (Lond)* 1970; **269**: 241.
25 Sinko PJ, Amidon GL. Characterization of the oral absorption of beta-lactam antibiotics. I Cephalosporins: determination of intrinsic membrane absorption parameters in the rat intestine in situ. *Pharmacol Res* 1988; **5**: 645–50.
26 Addison JM, Burston D, Dalrymple JA, Matthews DM, Payne JW, Sleisenger MH, *et al.* A common mechanism for the transport of di- and tri-peptides by hamster jeunum in vitro. *Clin Sci Mol Med* 1975; **49**: 313–22.
27 Kramer W, Dechent C, Girbig F, Gutjahr U, Neubauer H. Intestinal uptake of dipeptides and ß-lactam antibiotics. I. The intestinal uptake system for dipeptides and ß-lactam antibiotics is not part of a brush border membrane peptidase. *Biochim Biophys Acta* 1990; **1030**: 41–9.
28 Westphal JF, Deslandes A, Brogard JM, Carbon C. Reappraisal of amoxycillin absorption kinetics. *J Antimicrob Chemother* 1991; **27**: 647–54.
29 Bai P-F, Subramanian P, Mosberg HI, Amidon GL. Structural requirements for the intestinal mucosal-cell peptide transporter; the need for an N-terminal α-amino group. *Pharmacol Res* 1991; **8**: 593–9.
30 Lowther J, Hammond SM, Russell K. H^+-coupled transport of aminocephalosporins by intestinal BBMVs from rabbit. *Biochem Soc Trans* 1989; **17**: 880.
31 Hori R, Okano T, Kato M, Maegawa H, Inui K-I. Intestinal absorption of cephalosporin antibiotics; correlation between intestinal absorption and BBM transport. *J Pharm Pharmacol* 1988; **40**: 646–7.
32 Okano T, Inui K-I. Maegawa H, Takano M, Hori R. H^+-coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal BBM. *J Biol Chem* 1986; **261**: 1410–3.
33 Ganapathy V, Burckhardt G, Leibach FH. Characteristics of glycylsarcosine transport in intestinal rabbit BBMVs. *J Biol Chem* 1985; **258**: 14189–92.
34 Lowther J, Hammond SM, Russell K, Fairclough PD. Uptake of cephalosporins by human BBMVs. *J Antimicrob Chemother* 1990; **25**: 183–4.
35 Kramer W. Identification of identical binding polypeptides for cephalosporins and dipeptides in intestinal BBMVs by photoaffinity labelling. *Biochim Biophys Acta* 1987; **905**: 65–74.
36 Kramer W, Girbig F, Leipe I, Petzoldt E. Direct photoaffinity labelling of bining proteins for beta-lactam antibiotics in rabbit BBM with [^{3}H]benzylpenicillin. *Biochem Pharmacol* 1988; **37**: 2427–35.
37 Kramer W. Intestinal absorption of beta-lactam antibiotics, topological studies of the intestinal uptake system for beta-lactam antibiotics and small peptides. *Arch Pharmacol* 1990; **341** (suppl): R40.
38 Kramer W, Girbig F, Gutjar U, Kleeman H-W, Leipe I, Urbach H, *et al.* Interaction of renin inhibitors with the intestinal uptake system for oligpeptides and beta-lactam antibiotics. *Biochim Biophys Acta* 1989; **1027**: 25–30.
39 Kramer W, Girbig F, Gutjahr U, Kowalewski S, Friedhelm A, Schiebler W. Intestinal absorption of ß-lactam antibiotics and oligopeptides. *Eur J Biochem* 1992; **204**: 923–30.
40 Tsuji A, Terasaki T, Tamai I, Hirooka H. H^+ gradient-dependent and carrier-mediated transport of cefixime, a new cephalosporin antibiotic, across BBMV from rat small intestine. *J Pharmacol Exp Ther* 1987; **241**: 594–601.

41 Inui K-I, Okano T, Maegawa H, Kato M, Takano M, Hori R. H^+-coupled transport of cephalosporins by dipeptide carriers in rabbit BBM; difference of transport characteristics between cefixime and cephradrine. *J Pharmacol Exp Ther* 1988; **247**: 235–41.
42 Yoshikawa T, Muranashi N, Yoshida M, Oguma T, Hirano K, Yamada H. Transport characteristics of ceftibuten, a new oral cephem in rat intestinal BBMV: proton coupled and stereospecific transport of ceftibuten. *Pharma Res* 1989; **6**: 302–7.
43 Dyer J, Beechey RB, Gorvel J-P, Smith RT, Wooton R, Sherazi-Beechey SP. Glycyl-L-proline transport in rabbit enterocyte baso-lateral membrane vesicles. *J Biochem* 1990; **269**: 565–71.
44 Westphal J-F, Trouvin J-H, Deslandes A, Carbon C. Nifedipine enhances amoxilcillin absorption kinetics and bioavailability in humans. *J Pharmacol Exp Ther* 1990; **255**: 312–7.
45 Semrad EB, Chang EB. Calcium mediated cyclic AMP inhibition of Na/H exchange in small intestine. *Am J Physiol* 1987; **352**: C312–5.
46 Williams PEO. Factors affecting the oral absorption of esterified antibiotics. *Biochem Soc Trans* 1985; **13**: 511–3.