Amino acid prodrugs for oral delivery: challenges and opportunities

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Prodrugs are biologically inactive drug molecules that undergo an enzymatic and/or chemical transformation *in vivo* to release the active parent drug. Prodrugs may be developed serendipitously or through rationale drug design with an objective to improve a drug's pharmaceutical properties (solubility, permeability, stability, taste masking and so on), pharmacokinetic properties (absorption, distribution, metabolism and excretion) or site-specific delivery [1–3]. The prodrug approach has been very successful in improving developability of the molecules, with almost 15 of the top 100 best selling small-molecule drugs in 2009 being classified as prodrugs. One interesting example of these blockbuster prodrugs is lisdexamfetamine dimesylate, the oral prodrug of the psychostimulant dextroamphetamine containing the amino acid lysine as a promoiety. This amino acid prodrug was designed to have less abuse potential than other amphetamines due to the slower release of the active parent drug if inhaled or injected. Also valacyclovir belongs to this group of the best-selling drugs. The prodrug approach is commonly used to improve oral bioavailability of drugs whose absorption is limited due to low aqueous solubility and/or permeability. As such, oral prodrugs should provide improved solubility/permeability, have high chemical stability, and rapidly and quantitatively convert to parent drug to maximize drug exposure and minimize unwanted metabolism. This could be achieved by careful selection of the promoiety and *in vitro* and *in vivo* evaluation of the prodrug until a desired profile is achieved.

Amino acids as promoieties

A wide array of promoieties has been used to improve oral bioavailability of drugs [1–4]. The selection of promoiety depends on the purpose of the prodrug (e.g., improve solubility or permeability), type of functional groups available on the parent drug, chemical and enzymatic hydrolysis of prodrug to parent drug, safety of the promoiety and ease of manufacturing. In this regard, the use of amino acids as promoieties has several advantages:

- Fewer safety concerns. Amino acids are building blocks for proteins and are generally regarded as safe [5]. However, there may be specific concerns around certain natural amino acids, for example tyrosine and phenylalanine, and synthetic amino acids, which should be taken into account, especially for compounds with high dose and chronic usage;
- Wide range of functional groups for attachment to parent drug. Most of the amino acid prodrugs are either esters or amides, in which an α-amine or carboxylic group of amino acid is attached to the parent drug. Attachment of amino acids to the parent via a carbonate or carbamate and use of amino acid side chains, which offer a wealth of functional groups (e.g., amine, carboxylic acid, alcohol, thiol) as a prodrug handle, presents tremendous opportunities in prodrug design;
- Large structural diversity. Medicinal chemists have 20 common natural amino acids, a number of less common natural amino acids (e.g., taurine, β-alanine, α-amino butyric acid, γ-aminobutryic acid and ornithine), an extensive array of synthetic amino acids and di-/tri-peptides at their disposal. Amino acids as promoieties perhaps offer the most structural diversity (e.g., aliphatic, aromatic, acidic, basic, neutral, chain length and stereochemistry) and expanse of physicochemical properties [6]. These

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structurally diverse promoieties along with numerous functional links could be effectively used to design prodrugs with desired physicochemical and activation properties such as:

- Commercial availability;
- Well-established prodrug chemistry [3,7];
- Can potentially target carrier-mediated transporters for transport across cell membranes;
- Availability of commercially successful amino acid prodrugs;
- Used to improve 'drugability' of marketed drugs or difficult compounds.

Why use amino acid prodrugs for oral delivery: solubility & permeability enhancement

A large number of drugs and majority of new chemical entities have poor aqueous solubility (Biopharmaceutics Classification System class II or IV) [8], which could limit a drug's oral absorption. The very common prodrug approach has been to increase water solubility by introducing an ionizable/polar promoiety to the parent drug. Conjugation of an amino acid to the parent imparts a charge, either cation or anion, to the parent molecule, which can result in increased solubility at a physiological pH compared with the parent. In addition, the dissolution rate of the prodrug could be further increased by developing a salt form of the prodrug. Several examples of drugs where amino acids have been used as promoieties to improve solubility can be found in the literature, including brivanib, dapsone, camptothecine, CAM-4451, CEP-5214 and quercetin [9], and many have also been advanced to clinical trials. An illustrative example of amino acid prodrugs is *N,N*-dimethyl glycine ester of CEP-5214, which was prepared to provide an increase in aqueous solubility and improve oral bioavailability. CEP-5214 is a potent VEGF receptor tyrosine kinase inhibitor and it suffers from a low bioavailability, which can be attributed to its very low aqueous solubility (10 µg/ml). *N,N*-dimethyl glycine ester, CEP-7055, as the HCL salt demonstrated higher aqueous solubility (40 mg/ml) and, consequently, increased plasma concentration of the parent drug CEP-5214, with bioavailability of 15–20% [10]. The prodrug has been advanced to clinical trials.

Oral absorption of drugs that belong to Biopharmaceutics Classification System class III or IV is limited owing to poor permeability. For such drugs, addition of nonpolar groups could increase permeability. Although there is limited experience in use of amino acids to increase passive permeability, considering the wide-structural diversity in amino acids, this should be achievable. To this end, Beauchamp *et al.* synthesized and tested 18 amino acid esters of acyclovir as potential prodrugs [11]. The antiviral drug, acyclovir, itself was approved as an oral medication in 1985, but it suffered from limited and variable oral bioavailability due to its high polarity. When orally administered to rats, ten of the amino acid prodrugs produced greater amounts of acyclovir in rat urine than the parent compound itself. The lead prodrug, L-valine ester of acylcovir, increased the oral bioavailability of the parent drug, acyclovir, by approximately threefold; 63% as compared to 19% with prodrug and parent, respectively. In further studies in humans, L-valyl ester (later valacyclovir) had three- to five-times higher systemic availability compared with acyclovir [12], which was later attributed to the ability of valacyclovir to be a substrate for intestinal influx transporter for peptides (PEPT1) [13]. Although, the original goal of the valacyclovir study was most likely to simply improve the passive permeability of acyclovir by amino acid prodrugs, this research resulted in, by serendipity, an actively transported prodrug. Valacyclovir was soon followed by another valine ester prodrug, valganciclovir, which increased the oral bioavailability of the parent drug, ganciclovir, by sixfold; 60% as compared to 10% with prodrug and parent, respectively. [14]. The pioneering success with valacyclovir and valganciclovir has led to the development of several L-valine prodrugs of other poorly absorbed drugs, including levovirin valinate, valopicitabine and valtorcitabine [1]. All these amino acid prodrugs are designed to utilize PEPT1-mediated transport in their oral absorption and are undergoing clinical trials. Therefore, the L-valine monoester concept has proved to be an important landmark in the development of PEPT1-targeted oral prodrugs.

The amino acid prodrug strategy targeting PEPT1 has also been effective in improving oral absorption of polar parent drugs using amino acids other than valine as promoieties. Midodrine is a glycine prodrug of desglymidodrine, a selective α1-receptor agonist used in the treatment of orthostatic hypotension. Midodrine has an oral bioavailability of 93%, which is significantly more than the corresponding value for desglymidodrine (50%). Moreover,

an L-alanylamide prodrug (LY544344 or talaglumetad) has been shown to increase systemic parent drug (LY354740) exposure by approximately 13-fold compared with equivalent LY354740 doses in humans.

Activation of amino acid prodrugs

Prodrug activation can occur chemically or enzymatically. Amino acid prodrugs for oral delivery should have high chemical stability in the gastrointestinal environment, but rapidly and quantitatively convert to parent drug prior to the systemic circulation. Chemical stability and bioactivation of amino acid prodrugs depends on the functional link between the amino acid and parent (e.g., ester or amide), site of linkage, structure of parent and promoiety (steric and electronic effects) and stereochemistry [4,15]. Esters and amides are hydrolyzed by the same general mechanism and by the same hydrolases [4]. However, as a general rule, the amide bond is more resistant than the ester bond to chemical and enzymatic hydrolysis. During the development of prodrugs, it is important to understand the structure–activation relationship between prodrugs and hydrolytic enzymes. The mechanisms involved in the chemical hydrolysis of amino acid prodrugs are well understood; however, the field of enzymatic activation has been largely neglected, and very rarely attempts are made to identify if a specific or a group of hydrolases are involved in prodrug activation.

Challenges associated with the development of amino acid prodrugs for oral delivery

Development of amino acid prodrugs can pose some challenges that should be considered during the design stage. The ability of a prodrug approach to mitigate a particular development barrier with the parent is still a trial-and-error and iterative process, which involves synthesis of prodrugs followed by *in vitro* and *in vivo* evaluation. The number of prodrugs synthesized could be reduced by utilizing semi-quantitative and quantitative models. The *in silico* and *in vitro* solubility screens are more predictive than the permeability screens, especially if the prodrug is designed to target a specific transporter. One of the major challenges in prodrug development is to assess rate and extent of prodrug activation *in vivo*, the large diversity of hydrolases and interspecies variability makes extrapolation to humans difficult. The other major challenge is the toxic potential of prodrug and promoiety as compared with the parent drug. This is especially important when prodrug and promoiety exhibit different toxicities as compared with the parent. Amino acids introduce a cation (α-amino pKa: 6.8–7.9) or anion (α-carboxyl pKa: 3.5–4.3) to the parent drug, which could lead to pH-dependent absorption, and potential drug–drug interactions with gastric pH modifiers. Most of the amino acid promoieties introduce an additional chiral center in the molecule that can pose synthetic, purification and analytical challenges. Furthermore, the amino acid prodrugs can bring additional stability challenges; for example, racemization and/or intramolecular cyclization. Amino acid prodrugs increase the molecular weight of the parent, for example hydrolchloride salt of valine ester will increase formula weight by 135 Da and one needs to be mindful of the increase in dose due to the 'nonactive' contribution of the prodrug relative to the bioavailability enhancement with prodrug.

> "*...amino acid prodrugs have a proven track of improving oral delivery of the drugs that have poor solubility and permeability, and more recently providing controlled drug release.*"

Future perspective

The research on amino acid prodrugs has been and will continue to be an integral part of prodrug design and development. This is evident by consistent publications on this topic – prodrug keyword search in PubMed, covering the past decade (2001–2010), resulted in an average 504 articles per year, of which approximately 16% (83 articles per year) were related to amino acid prodrugs. In the future, we expect to see continual research in use of amino acid prodrugs to improve solubility for oral and parentral delivery, improve permeability via passive or active transport mechanisms, controlled drug release, site-specific delivery by targeting specific transporters or enzymes overexpressed in tissues and tumors, and mechanistic understanding of absorption and activation by hydrolases. In conclusion, amino acid prodrugs have a proven track of improving oral delivery of the drugs that have poor solubility and permeability, and more recently providing controlled drug release. The amino acid prodrugs have a proven commercial and regulatory track record, which is beneficial in bringing such drugs to the patient and this track record should be enhanced to the benefit of the patient by future developments in prodrug technology.

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