Design of Ester Prodrugs to Enhance Oral Absorption of Poorly Permeable Compounds: Challenges to the Discovery Scientist

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Abstract: Many drugs are administered at sites that are remote from their site of action. The most common route of drug delivery is the oral route. The optimal physicochemical properties to allow high transcellular absorption following oral administration are well established and include a limit on molecular size, hydrogen bonding potential and adequate lipophilicity.

For many drug targets, synthetic strategies can be devised to balance the physicochemical properties required for high transcellular absorption and the SAR for the drug target. However, there are drug targets where the SAR requires properties at odds with good membrane permeability. These include a requirement for significant polarity and groups that exhibit high hydrogen bonding potential such as carboxylic acids and alcohols. In such cases, prodrug strategies have been employed.

The rationale behind the prodrug strategy is to introduce lipophilicity and mask hydrogen bonding groups of an active compound by the addition of another moiety, most commonly an ester. An ideal ester prodrug should exhibit the following properties:

1) Weak (or no) activity against any pharmacological target,
2) Chemical stability across a pH range,
3) High aqueous solubility,
4) Good transcellular absorption,
5) Resistance to hydrolysis during the absorption phase,
6) Rapid and quantitative breakdown to yield high circulating concentrations of the active component post absorption.

This paper will review the literature around marketed prodrugs and determine the most appropriate prodrug characteristics. In addition, it will examine potential Discovery approaches to optimising prodrug delivery and recommend a strategy for prosecuting an oral prodrug approach.

INTRODUCTION

For patient convenience, most drugs are administered by the oral route. However, there are significant hurdles confronting the delivery of a drug from the oral route, which often means that all of the administered compound does not reach its intended site of action. The extent to which the compound can overcome the hurdles to oral drug delivery and reach the systemic circulation is quantified by the term oral bioavailability. Optimising the oral bioavailability of a candidate molecule is a key objective of an oral drug discovery program. Clearly, compounds exhibiting low oral bioavailability are likely to require high doses to achieve the desired effects, since systemic exposure to the active compound will be limited. In addition, low oral bioavailability agents are prone to exhibit greater variation in exposure than higher bioavailability agents. This is particularly an issue in the area of drug-drug interactions where concomitantly administered drugs can lead to unacceptable systemic exposure to a drug.

The factors limiting the oral bioavailability of drug molecules are well established. They fall into 2 broad categories: absorption from the g.i. tract and first-pass extraction by the gut wall and liver. Incomplete oral absorption can be a major cause of poor oral bioavailability and much research has focused on the physicochemical properties required to ensure high oral absorption.

The major physicochemical determinants of extensive passive transcellular oral absorption have been extensively studied. The Lipinski 'rule of 5' mnemonic [1] suggests that in order to exhibit good oral absorption in humans, a drug should possess a molecular weight lower than 500, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors and a clog P less than 5. In addition, it is well known that in order to be transcellularly absorbed, a drug must possess a degree of lipophilicity (in general log $D_{(7.4)}$ greater than 0). These physicochemical constraints are related to the requirement of the drug to pass through the lipophilic environment of the gut wall cell membrane.

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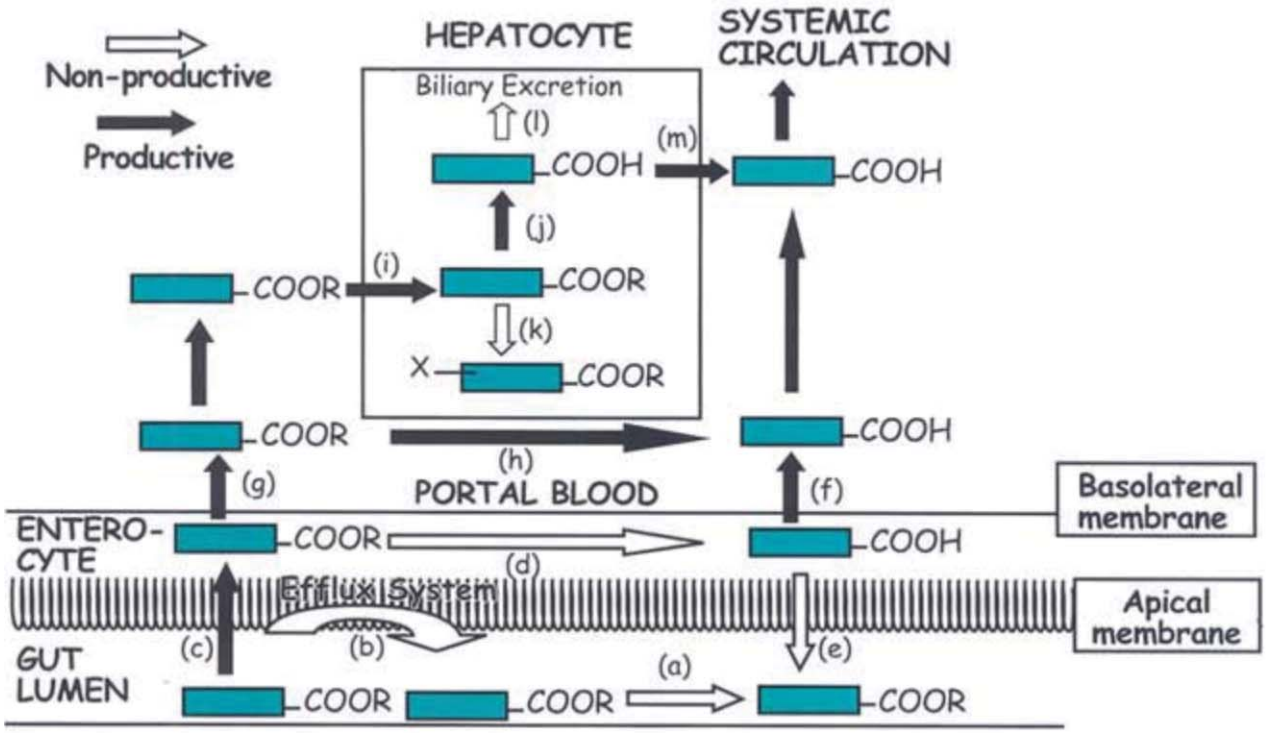
However, there are pharmacological targets (eg GpIIb/IIa antagonists, ACE inhibitors, viral reverse transcriptase inhibitors etc.) for which some chemical series require physicochemical properties that are at odds with good transcellular permeation. Thus, drug intervention at these targets can require compounds that are highly polar, have high molecular weight and/or high hydrogen bonding functionality. In order to make such compounds amenable to oral delivery, it is necessary to temporarily address these physicochemical constraints in a manner that can be readily reversed post absorption. This is the rationale behind the prodrug approach.

The major aim of a prodrug approach is to mask polar or ionisable groups within a molecule. This increases the overall lipophilicity of the molecule and promotes membrane permeability and oral absorption. However, increases in lipophilicity produced by a prodrug approach do not inevitably lead to major improvements in oral bioavailability. This is due to the multiplicity of the barriers facing oral delivery. These are outlined in Fig. (**1**).

In order to be absorbed, the prodrug must be in solution in the contents of the gut. Thus, it must exhibit sufficient aqueous solubility to dissolve the entire dose. Once in solution, the prodrug needs to avoid extensive chemical and enzymic degradation, and must pass through the membrane of the gut wall cells (enterocyte). It is well known that efflux transporters are present in the enterocyte membrane [2], which are capable of intercepting drugs during membrane passage and placing them back into the gut lumen. Thus, successful prodrugs may need to avoid affinity for these transport proteins. Enterocytes are metabolically competent cells that express a wide range of drug metabolising enzymes including esterases [3], cytochrome P450 isoforms [3-5] and UDP-glucuronyl transferases [3,6]. Prodrug ester hydrolysis in the enterocyte can be productive, if the active principle



(a) Chemical instability or degradation by gut lumen esterases,
(b) Efflux of ester from the enterocyte membrane by a transporter mechanism (such as P-glycoprotein),
(c) Permeation of ester through the apical membrane of the enterocyte,
(d) Breakdown of the ester to the acid by enterocyte esterases,
(e) Return of acid to gut lumen (possibly by an efflux system),
(f) Crossing of the basolateral membrane of the enterocyte by the acid,
(g) Crossing of the basolateral membrane of the enterocyte by the ester,
(h) Hydrolysis of the ester by esterases in the portal vein blood,
(i) Hepatic extraction of the ester,
(j) Hydrolysis of the ester by hepatocytes esterases,
(k) Non-esterase metabolism of the ester (such as CYP-mediated metabolism),
(l) Biliary excretion of the acid,
(m) Return of the liberated acid to the systemic circulation (probably transporter mediated).

Fig. (1). Barriers confronting the oral delivery of a prodrug.

can pass through the basolateral membrane of the enterocyte and into the blood. However, if the active principle is a substrate for apical membrane transporters, it is most likely to be returned to the gut lumen. Any cytochrome P450 or conjugation metabolism on passage through the enterocyte will also be observed as incomplete oral bioavailability of the active principle.

Post absorption, the ideal prodrug should rapidly and quantitatively be converted to the active principle. Our analysis suggests that this is rarely the case, due to the multiplicity of enzyme systems available to metabolise drugs on first-pass through the liver. Esterase enzymes present in the blood are capable of hydrolysing ester prodrugs. However, these esterase enzymes have a strict structure activity relationship and do not metabolise all esters. Therefore some ester prodrugs do require hepatic ester hydrolysis and this poses several issues for quantitative release of active principle. Following hepatic ester hydrolysis, the active principle will be released in the hepatocyte. Since the aim of a prodrug strategy is to improve membrane permeation, there is potential for the active principle to be trapped within the hepatocyte and require active efflux from the cell. Should the active principle be a preferential substrate for canalicular transporters, it will be extensively excreted into the bile. In order to be returned to the blood, it may require efflux by sinusoidal transporters. In addition, the hepatocyte also expresses high levels of Phase I and II metabolising enzymes that may metabolise the prodrug or the active principle in a non-productive manner. Thus, any metabolism or excretion of either the prodrug or the active principle, which does not lead to the transfer of the active principle to the blood, will be non-productive and lead to a lowering of potential oral bioavailability.

Overall, the barriers confronting the oral delivery of prodrugs are considerable. In addition, to improving membrane permeability of a polar active principle, a prodrug should avoid transporter mediated efflux and be designed to yield the active principle into the systemic circulation. Incomplete membrane permeation, efflux, non-esterase metabolism or biliary elimination will lead to a reduction in the potential oral bioavailability of the active principle. Thus, in order to be successful, a prodrug approach must consider the balance of all these issues.

This paper examines many literature examples illustrating the issues facing the prodrug approach. It will attempt to review the oral bioavailability of marketed prodrugs and outline examples of prodrug approaches that have failed. Finally, an attempt will be made to outline a strategy for the Discovery scientist to successfully prosecute a prodrug approach.

REVIEW OF MARKETED ESTER PRODRUGS

A number of examples of marketed ester prodrugs are listed in Tables **1** to **6**. The prodrugs show a wide diversity in chemical structure, molecular weight and lipophilicity. However, relatively few different classes of ester groups have been employed as the prodrug moiety. In general, the highest oral bioavailability values that can be achieved clinically using ester prodrugs are 40-60%, indicating that complete oral bioavailability in a prodrug programme is very unlikely.

ALKYL ESTERS

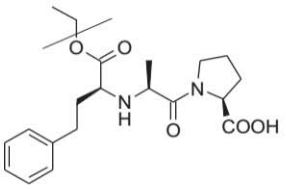
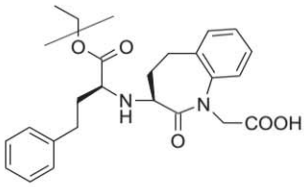
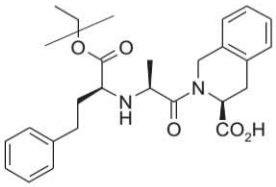
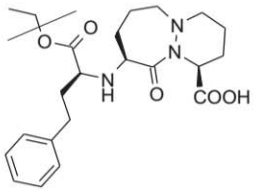
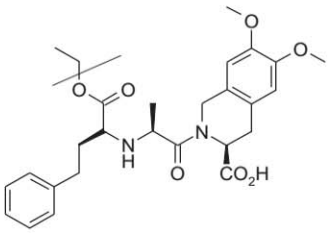
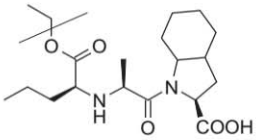
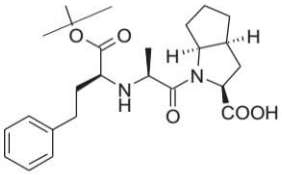
The most common prodrug moiety in marketed drugs is the esterification of an acid group with a simple alkyl alcohol. Major examples are illustrated in Table **1**. One of the most successful classes of alkyl ester prodrugs is the diacidic angiotensin converting enzyme (ACE) inhibitors, where one of the acidic functions is masked as an ethyl ester. The oral bioavailability values for the diacidic active principles (eg benazeprilat, quinaprilat etc) are not widely reported but are likely to be low. For instance, the oral absorption of enalaprilat is only 3% [7] and the bioavailability of cilazaprilat is 19% [8]. In contrast, the oral bioavailability values of most of the monoacid prodrug agents (enalapril, benazepril, quinapril etc.) are significantly higher than 20%. However, even within this class the oral bioavailability values exhibit a significant range, from 17.5 % for moexipril to 57% for cilazapril.

There are a number of reasons why the monoacid ester pro-drugs have higher bioavailability than their respective di-acid active principle and why there is a large variability in bioavailability between different ACE inhibitors. Firstly, changing one of the acid groups to an ethyl ester increases lipophilicity with a resultant increase in transcellular absorption. However, a number of the monoester ACE inhibitors exhibit greater oral absorption than would be expected purely from their lipophilicity values. For example, enalapril is relatively hydrophilic but is well absorbed in humans. It appears that as well as increasing lipophilicity, the monocarboxylic acid ester ACE inhibitors are substrates for intestinal uptake transporters [9-15]. In contrast although a number of di-acid ACE inhibitors can interact with the intestinal uptake transporters the second negative charge of these compounds makes their transport by these proteins unfavourable [12,16], and this in part explains their lower bioavailabilty.

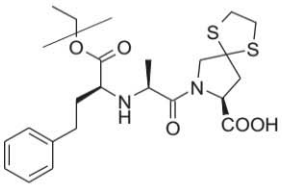
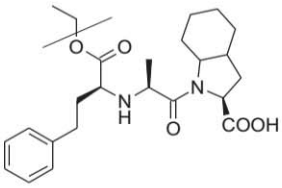
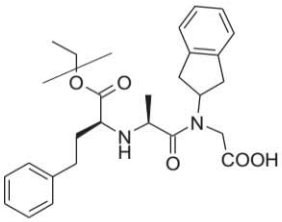
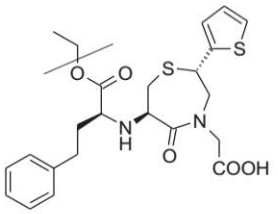
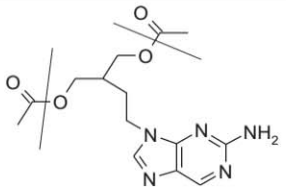
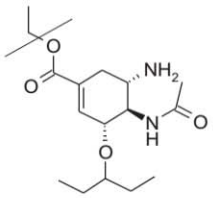
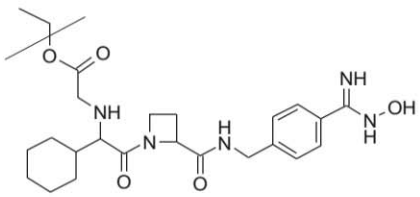
The ACE inhibitor ethyl esters tend to be stable to hydrolysis in human blood and require the action of hepatic (and possibly intestinal and/or kidney) esterases to liberate the active principle [17-20]. Thus, for enalapril the prodrug is 53-74% absorbed following oral dosing in man and the bioavailability of enalaprilat is 36-44%. This is consistent with the release of diacid from ester after intravenous administration, which is calculated to be 60%. The relatively slow hydrolysis of the ethyl ester prodrugs illustrates the potential benefit offered by blood labile prodrugs, which would give even higher concentrations of enalaprilat per mg dose of prodrug. Thus, the oral bioavailability values for individual ACE inhibitors will be dependant on several factors such as intrinsic lipophilicity of the monoester, affinity for the absorptive transporter and the rate of release of the active principles by cellular esterases.

Ximelagatran is a more recent example of an ethyl ester prodrug. It contains two modifications to the direct acting thrombin compound melagatran. In addition to the ethyl ester group, the amidino group of melagatran is hydroxylated in the prodrug. Thus, two metabolic reactions are needed to liberate the active principle [21]. The modifications incorporated into ximelagatran increase the octanol: water partition by 170-fold compared to melagatran. This increased lipophilicity is reflected by an 80-fold greater flux through a

Table 1. Human Absorption and Bioavailability Data for Selected Alkyl and Aryl Ester Prodrugs

Prodrug [references]	Structure	Absorption of prodrug (%)	Oral bioavailability of active principle when given as prodrug (%)
Enalapril [7,19]		53-74	36-44
Benazepril [81]		≥ 37	≥ 17
Quinapril [104]		60	46-52
Cilazapril [8]		≥57	57
Moexipril [137, 22]		23	≈17.5 estimate from urine moexiprilat excretion after iv and oral moexipril doses
Perindopril [92,105]		ND	19-35 higher values seen in elderly patients
Ramipril [82]		48-56	28-34

(Table 1) contd….

Prodrug [references]	Structure	Absorption of prodrug (%)	Oral bioavailability of active principle when given as prodrug (%)
Spirapril [106]		50-70	42
Trandolapril [93]		≥ 40-60	40-60
Delapril [91,107,108]		35-55	ND
Temocapril [109]		>40	ND
Famciclovir [110]		≥77	54-77
Oseltamivir [111]		>80	79
Ximelagatran [21,22]		ND	18-24

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