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A novel concentration dependent amino acid ion pair strategy to mediate drug permeation using indomethacin as a model insoluble drug

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ABSTRACT

Assessment of oral drug bioavailability is an important parameter for new chemical entities (NCEs) in drug development cycle. After evaluating the pharmacological response of these new molecules, the following critical stage is to investigate their *in vitro* permeability. Despite the great success achieved by prodrugs, covalent linking the drug molecule with a hydrophobic moiety might result in a new entity that might be toxic or ineffective. Therefore, an alternative that would improve the drug uptake without affecting the efficacy of the drug molecule would be advantageous. The aim of the current study is to investigate the effect of ion-pairing on the permeability profile of a model drug: indomethacin (IND) to understand the mechanism behind the permeability improvement across Caco-2 monolayers. Arginine and lysine formed ion-pairs with IND at various molar ratios 1:1, 1:2, 1:4 and 1:8 as reflected by the double reciprocal graphs. The partitioning capacities of the IND were evaluated using octanol/water partitioning studies and the apparent permeabilities (P_{app}) were measured across Caco-2 monolayers for the different formulations. Partitioning studies reflected the high hydrophobicity of IND (Log P = 3) which dropped upon increasing the concentrations of arginine/lysine in the ion pairs. Nevertheless, the prepared ion pairs improved IND permeability especially after 60 min of the start of the experiment. Coupling partitioning and permeability results suggest a decrease in the passive transcellular uptake due to the drop in IND portioning capacities and a possible involvement of active carriers. Future work will investigate which transport gene might be involved in the absorption of the ion paired formulations using molecular biology technologies.

1. Introduction

Oral drug bioavailability is an important parameter for new drug candidates under the drug discovery investigation programme. Once the new drug candidate is identified based on its pharmacological response, the following critical stage is to investigate its *in vitro* permeability. Further development is unlikely to take place if the drug is found to have poor permeability or absorption and as a result a great deal of interest is dedicated to study and enhance drug permeability (Egan and Lauri, 2002).

pH partitioning theory for charged drug candidates states that lipid membrane bilayers only allow the absorption of unionised (molecular form) drug candidates while the charged species do not pass through the membrane (Shore et al., 1957). However, investigations over the last couple of decades on oral drug absorption have demonstrated the role of other factors in drug absorption. Daniel et al. (1985) suggested that the acidic microclimate of the drug at the surface of the epithelium has an effect on the passive uptake of uncharged weakly acidic drugs which was known as pH shift. Although passive diffusion is the main mechanism of absorption for majority of drugs, carrier mediated transporters, found in mucosal and serosal membranes can be involved in the transport of acidic drugs. Tsuji et al. (1994) investigated the mechanism of absorption of monocarboxylic acid drugs through various ion channel transporter systems. The results showed that carboxylic acid drugs were primarily carried across the intestinal cells through H^+ gradient dependant transporter systems when compared to Na^+ dependent carrier systems. Although the study by Tsuji et al. (1994) identified the role of a specific carrier system in the transport of acidic drugs, the contribution of active influx to net transport of the drug was not clear suggesting the wider role of alternative/complimentary carrier systems in the absorption of weakly acidic drugs. The next set of studies

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published by Ganapathy et al., in 1995 identified H^+ coupled oligopeptide transporters (PEPT)-1 (SLC15A1) expressed in human small intestine and was shown to mediate the absorption of anionic angiotensin converting enzyme inhibitors and B-lactam antibiotics. Similarly, monocarboxylic acid transporter (MCT)-1 also known as SLC16A1 was believed to play an essential role in the rapid transport of monocarboxylates. Recent studies by Kobayashi et al. (2003) identified another super family of organic anion transporting polypeptide (OATP)-B expressed in the apical membrane of the small intestine with a specific role for the transport of anionic molecules across the gut.

Many techniques have been employed in order to improve drug uptake via intestinal membranes. Prodrugs were commonly used for this purpose and achieved great success by using groups of compounds such as carboxylic acid esters and amino acids esters (Weller et al., 1993). Other studies suggested that ion-pairing (neutral species formed by electrostatic attraction between oppositely charged ions in solution) can be used to improve drug permeability. The interaction between the two opposite charges results in burying of the charge in the drug molecule and hence alters its physical properties. A study conducted by Irwin et al. (1969) demonstrated that the rate and efficacy of an ionic drug isopropamide was improved upon ion-pairing with an exogenous counter ion such as trichloroacetate. Another study concluded that ion-pairing of methotrexate with L-arginine enhances the nasal permeability of the drug 24 times when compared to the drug alone (Ivaturi and Kim, 2009).

In a study by Miller et al. (2010), a FDA-approved compound called 1-hydroxy-2-naphthoic acid (HNAP) (Cazzola et al., 2002) was used to improve the permeability of two antiviral drugs; zanamivir heptyl ester (ZHE) and guanidino oseltamivir (GO). The lipophilic counterion improved the Log*P* values of the two polar drugs by 3.7 log units resulting in better partitioning. The study also concluded that ZHE-HNAP is less prone to dissociation and was able to remain intact during membrane permeation (Miller et al., 2010). Similar approach was reported by Samiei and coworkers as the low lipophilicity of amifostine was amended using Succinic acid, phthalic acid and benzoic acid. Drug bioavailability was the highest upon ion pairing with succinic followed by phthalic acid which demonstrated 20 and 10-fold increase respectively (Samiei et al., 2013). The degree of enhancement of amifostine lipophilicity and permeation was not influenced solely by the lipophilicity of the organic counter ion, but also by the extent of intermolecular interaction and its ability to reduce the charge over the drug molecule (Samiei et al., 2014).

Miller et al., developed a quasi-equilibrium transport models to describe influence of lipophilic counter ions on the permeation and apparent Log*D* values of basic drugs. The model was used to calculate $K11_{aq}$, $K11_{oct}$, and $Log P_{AB}$ by plotting a double reciprocal plot of *D* against the counterion concentration (Miller et al., 2009). It is believed that high $K11_{aq}$ values are required in order to enable the ion paired formulation to compete with endogenous ions such as phosphatidylserine, phosphatidylinositol, sialic acid and bile acids. Besides, for a sufficient lipophilicity $Log P_{AB}$ of the ion paired formulations should be between 2 and 5 (Miller et al., 2009).

However, the mechanism of ion-pairing is still not clear and the permeability increase is attributed to the direct effect of the counter ion i.e. binding to mucosal membrane, lowering the interfacial tension of the gut wall and causing mucosal erosion (Quintanar-Guerrero et al., 1997).

The current investigation focuses on the permeability profile of the weakly acidic model drug indomethacin (IND) upon increasing the concentration of the counter ion. IND is a non steroidal anti-inflammatory drug which is used for the treatment of rheumatoid arthritis. IND is a weak monocarboxylic acid ($pK_a = 4.5$) and has poor solubility because of its hydrophobic nature ($Log P = 3.1$). For instance, a recent paper by Samiei et al. (2014) has stated that forces such as electrostatic interactions and hydrogen bonding that operate during ion pair formation may not facilitate drug permeation. Nevertheless, lipophilic nature of IND enables the molecule to diffuse quickly and to get absorbed completely through the intestinal membrane after oral ingestion and therefore is classified as a class II drug under the biopharmaceutical classification system (BCS) (ElShaer et al., 2011).

IND was selected for this study as it is metabolically stable at intestinal pH and our previous studies (ElShaer et al., 2011) showed a substantial improvement in solubility and dissolution upon addition of cationic amino acids (lysine and arginine). The primary hypothesis of this study was to explore the role of ion-pairing IND with cationic amino acids (arginine and lysine) on drug uptake across Caco-2 cells and determine whether the ion-paired molecules would decrease the uptake of IND because of increasing its degree on ionisation. The current study builds on previous work that investigated the impact of inclusion of charged amino acids on solubility and dissolution (ElShaer et al., 2011) with a view to understanding their effect on drug permeability which would enable the selection of optimal concentration of counter ion to develop a two pronged approach by addressing both solubility and evaluating permeability. Arginine and lysine were selected for this study because they carry positive charge upon ionisation which could interact electrostatically with the anionic carboxylic group of IND to form ion-pairs.

Caco-2 cells are human colorectal carcinoma cell lines and upon culturing, they differentiate continuously and produce the structural and functional patterns of enterocytes (Pinto et al., 1983). Caco-2 cells can reach confluence after 3–6 days and become completely differentiated within 20 days (Pinto et al., 1983). After differentiation, the cells express many enzymes such as alkaline phosphatase, sucrase isomaltase and aminopeptidase which are characteristic for enterocyte brush border microvilli. Caco-2 cells also express a lot of transporter protein and therefore they have been used successively to evaluate carrier mediated uptake of B-lactam antibiotics (Inui et al., 1988), amino acids (Thwaites et al., 1995), amino acids analogue (Hu and Borchardt, 1990) and efflux transport (Eneroth et al., 2001).

2. Materials and methods

2.1. Materials

Indomethacin (TLC ⩾ 99%), L-arginine (non-animal source), L-lysine, 1-octanol (ACS spectrophotometric grade ⩾99%) and ninhydrin reagent (2% solution) were purchased from Sigma Aldrich, UK.

Acetonitrile, glacial acetic acid and absolute ethanol were purchased from Fisher Scientific UK.

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Nonessential amino acids (NEAA), 1% pencillin–streptomycin, 2 mM glutamine and Hank's balanced salt solution (HBSS) were purchased from Bio Sera, UK. 1% Trypsin–EDTA was obtained from Gibco Lab. UK.

2.2. Methods

2.2.1. Preparation of pre-saturated solution of 1-octanol and de-ionised water

1000 ml of de-ionised water was added to 4 mL of 1-octanol. The mixture was shaken for few minutes and left overnight. The de-ionised water was separated from 1-octanol using 1000 mL separating funnel.