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Enhancement and in vitro evaluation of amifostine permeation through artificial membrane (PAMPA) via ion pairing approach and mechanistic selection of its optimal counter ion

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ABSTRACT

This study presents the results of in vitro evaluation of a series of organic counter ions that form ion pairs with amifostine. The selected counter ions have different lipophilicity, shape and flexibility. Intrinsic octanol buffer partition coefficient and binding constant of the ion pairs were calculated using quasi-equilibrium analysis. Permeation through hydrophobic PAMPA membranes of amifostine and its ion pairs with different counter ions was studied. Three counter ions, succinic acid, benzoic acid and phthalic acid demonstrated an increase in the apparent partition coefficient of amifostine in n-octanol. These counter ions were selected for permeability experiments in PAMPA membranes and an increase of the apparent permeability value P_{app} (cm/s) was also observed as a function of the counter ion concentration. Phthalic acid produced 1.6-fold increase of log P_{AB} while for benzoic acid and succinic acid the values were 1.2 and 0.75-fold respectively. PAMPA permeability of amifostine significantly increased in the presence of phthalic acid (42-fold), benzoic acid (37-fold) and succinic acid (10.5-fold). This study showed that the permeability of amifostine across a lipophilic membrane was enhanced in the presence of counter-ions resulting ion pair formation.

1. Introduction

Oral delivery of hydrophilic, ionizable drugs remains a major challenge in drug delivery and development. Amifostine is a cytoprotective agent in a broad range of normal tissues preserving the cells from the toxic effects of chemotherapy and radiotherapy without attenuating tumor response. This selective protection is due to the greater conversion and uptake of the active metabolite, WR-1065, in normal versus neoplastic tissues. Currently amifostine must be administered by intravenous or subcutaneous route as no oral formulation is available. These routes of administration are invasive, require special nursing procedures, and are difficult to use in routine clinical settings. Because of the obvious drawback of drug delivery by injections the development of alternative formulation with enhanced oral bioavailability is receiving much attention in pharmaceutical research. In order to achieve effective systemic absorption of a drug through the intestine, the drug moiety must have good solubility and lipophilicity. Unfortunately, amifostine is a very soluble and highly polar drug and its zwitterion structure at physiological pH as well as its degradation in lumen leads to very poor absorption via the oral route (van der Vijgh and Korst, 1996).

Ion pairing is defined as the formation of neutral species formed by electro statistic interaction between oppositely charged ions in solutions. Ion pair formation improves partitioning into non polar solvents and also in lipophilic membranes. This pair of oppositely charged ions is held together by coulombic attraction without formation of a covalent bond. They behave like a single unit. This strategy involves co administering an excess concentration of a counter ion. In theory, an ionized drug and a counter ion associate as a lipophilic ion pair and then partition into the membrane easily. The pair dissociates when diluted or displaced after absorption.

The approach is simple, in principle, and eliminates the need for prodrug uptake by transporters and activation by specific enzymes (Miller, 2009; Suresh, 2011).

Moreover, the approach should not rely on disrupting membrane integrity to facilitate absorption. A potential disadvantage of the ion-pairing approach is that the ionic bonding and other non-covalent interactions (i.e. hydrogen bonding) may be too weak in solution to facilitate membrane permeation.

Assays that predict passive absorption of orally administered drugs have become increasingly important in the drug discovery process. The ability of a molecule to be orally absorbed is one of

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the most important aspects in deciding whether the molecule is a potential lead candidate. Kansy and Faller have addressed PAMPA as one of the assays providing rapid, low cost and easy measures of passive permeability (Faller, 2008; Kansy et al., 2004).

In this study, we reported an investigation on ion pair partitioning of amifostine in octanol–buffer using traditional shake-flask method. Lipophilicity and binding constant of ion pairs were characterized by quasi equilibrium analysis. Permeation of amifostine in the presence of organic counter ions was determined in PAMPA. The counter ions used in this study vary in their lipophilicity, shape and flexibility to explore the relationship between counter ion structure and ion pair permeation and partition. Some of the used counter ions are human endogenous compounds like maleic acid, fumaric acid, tartaric acid and citric acid and they have the advantage of not being toxic like surfactant which may affect membrane integrity.

2. Material and method

2.1. Materials

Amifostine was obtained from Ziboponix Company (Shanghai, China), benzoic acid, phthalic acid, citric acid, p-toluene sulfonic acid, tartaric acid, succinic acid, maleic acid and sodium tetra borate were obtained from Merck company (Berlin, Germany). Lecithin was purchased from Sigma (Berlin, Germany) and n-octanol and dodecan were obtained from Merck Company (Berlin, Germany). Ortho-phthaladehyde (OPA) and 2-mercaptoethanol were purchased from Sigma–Aldrich (Berlin, Germany). All other chemicals were HPLC or analytical grade and used as received. Deionized water was used in preparation of all solutions and mobile phases. Hydrophobic micro filter membranes (polyvinylidene fluoride, 0.45 mm) were used as the donor compartment and were purchased from Millipore Corporation as well as the acceptor plates for PAMPA experiments (Bedford, MA, USA).

2.2. Analytical methodology

Amifostine was quantitatively analyzed after pre-column derivatization with OPA/2-mercaptoethanol in the presence of borate buffer pH 9.5 by a Kanuer 1000 series HPLC system and a model PDA detector 2800, with a symmetric C18 column (25 cm, 100 μm, 4.6 mm Kanuer (Berlin, Germany). The mobile phase consisted of a mixture of methanol and phosphate buffer 30 mM (40/60 v/v, respectively) at pH 3. The flow rate was 1.5 mL/min and detection wavelength was set at 340 nm. Retention time of amifostine was 7.5 min and limit of detection in HPLC assay was 10 ng/ml.

2.3. Ion pair mediated octanol–buffer partitioning (pH 6.5)

The formation of an ion pair in aqueous solution and its partition into an immiscible organic solvent (like octanol) are described by quasi-equilibrium analysis as reported by Miller et al. (2009). In this study amifostine was selected as Basic compound, where [B] represent concentration of basic drug in aqueous phase. Several acidic counter ions were used as ion pairing agents, then [A] represents the concentration of acidic counter ion in aqueous phase.

$$[B^+]_{aq} + [A^-]_{aq} \leftrightarrow [B^+A^-]_{aq} \quad (1)$$

$$[B^+A^-]_{aq} \leftrightarrow [B^+A^-]_{oct}$$

In the equation above, B^+_{aq}, A^-_{aq}, represent basic drug and acidic counter ion in aqueous phase and $\{B^+A^-\}_{aq}, \{B^+A^-\}_{oct}$ represent ion pair concentration in aqueous and octanol phase respectively. The partition of the ion pairs formed between the aqueous and the organic phase will decrease its initial concentration in the aqueous phase.

The formation (stability) constant of ion pair K_{ip} is defined by Eq. (2). The distinct and thermodynamically stable formed species $[AB]_{aq}$ partition between the aqueous and organic phases (Miller, 2009; Miller et al., 2009, 2010).

$[AB]_{aq}$, $[A]_{aq}$, $[B]_{aq}$ are aqueous phase concentration of ion pair, counter ion and basic drug respectively.

$$K_{ip(aq)} = \frac{[AB]_{aq}}{[A]_{aq}[B]_{aq}} \quad (2)$$

The intrinsic octanolbuffer partition coefficient of ion pair P_{AB} can be defined as:

$$P_{AB} = \frac{[AB]_{oc}}{[AB]_{aq}} \quad (3)$$

Assuming the total amount of basic drug in the octanol phase $[B]_{oc}$, exists only as ion pair. $[B]_{oc} \sim [AB]_{oc}$, and apparent octanol–buffer distribution coefficient is expressed as:

$$D_B = \frac{[AB]_{oc}}{[B]_{aq} \times [AB]_{aq}} \quad (4)$$

Combining the Eqs. (1)–(3) gives the following equation:

$$\frac{1}{D_B} = \frac{1}{K_{ip(aq)} \times P_{AB} \times [A]} + \frac{1}{P_{AB}} \quad (5)$$

Thus the plot of $1/D_B$ versus $1/[A]$ will yield a straight line with y-intercept $1/P_{AB}$ and slope $1/K_{ip}P_{AB}$ from which K_{ip} and P_{AB} may be ascertained.

The K_{ip} in octanol phase can be expressed as Eq. (6):

$$K_{ip(oc)} = \frac{[AB]_{oc}}{[A]_{oc} \times [B]_{oc}} \quad (6)$$

In this equation $[A]_{oc}$, $[B]_{oc}$ and $[AB]_{oc}$ are the octanol phase concentration of organic acidic and basic drug and the ion pair, respectively. K_{ip} can be obtained from Eq. (7):

$$K_{ip(oc)} = \frac{P_{AB} \times K_{ip(aq)}}{D_A \times D_B} \quad (7)$$

In Eq. (7) D_A represents the apparent octanol/aqueous distribution of organic acid. Where [A] can calculated by $[A] \approx \frac{[Ai]_{aq}}{1+DA}$ and [Ai] is initial organic acid concentration before equilibration with octanol.

Octanolbuffer partitioning studies were performed using solutions of known amount of amifostine (50 μm) that were prepared in octanol saturated-sodium phosphate buffer (50 Mm, pH 6.4) with molar excess of counter ions. These solutions then equilibrated at 30 °C with equivalent amount of buffer saturated-octanol using magnetic stirrer at 500 rpm for 2.5 h. Three replicates of each counter ion were carried out to assess reproducibility. The octanol and buffer phases were separated by centrifugation. The total drug concentration in aqueous phase was determined by HPLC. A solution depletion technique was employed for determination of the partition coefficient based on following equation:

$$P = (A_0 - A_\infty)/A_\infty$$