Platform-Technology Based on Prodrug/Enzyme Systems for Rapid Absorption of Hydrophobic Drugs: Toward Intranasal Treatment of Seizure Emergencies

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ABSTRACT SUMMARY

Poor water solubility of novel drugs is a key challenge in drug discovery and development as it results in low drug bioavailability upon local or systemic administration. The prodrug approach is commonly utilized to enhance solubility of hydrophobic drugs. However, for rapid drug absorption, supersaturated solutions need to be employed. In this work, a platform technology based on prodrug/enzyme systems was developed wherein prodrug and enzyme are co-administered at the point of absorption (e.g. nasal cavity) to form in situ supersaturated drug solutions for enhanced bioavailability. This accelerated absorption was demonstrated by developing prodrug/enzyme systems for two seizure emergency drugs - phenytoin (fosphenytoin/alkaline phosphatase) and diazepam (avizafone/A.O. protease). Enzyme kinetics was performed in assay buffer pH 7.4 at 32°C. In vitro permeation studies were performed using Madin Darby canine kidney II-wildtype (MDCKII-wt) monolayers as a nasal epithelia model. Results indicated that drug absorption with prodrug/enzyme system increased proportionately to the degree of supersaturation; this flux was 1.5 to 6-fold greater and 2-17.6 fold greater compared to saturated phenytoin and diazepam, respectively. The experimental data fitted reasonably well to a two compartment pharmacokinetic model with first order conversion of prodrug to drug. The developed prodrug/enzyme systems remarkably enhanced drug (phenytoin, diazepam) transport across model membrane. This strategy could be particularly useful when fast action is required, for example for intranasal therapy of seizure emergencies.

INTRODUCTION

Potent anti-epileptic drugs suffer from low aqueous solubility, limiting their bioavailability when administered intravenously, orally, or by other parenteral routes. On the other hand, low aqueous solubility is often correlated with high potency at the site of action and with the ability of the drug to cross lipidd membranes. Prodrug approaches, where the native hydrophobic drug is derivatized to a bioavailable hydrophilic form that can be converted by endogenous enzymes to the native drug, have been utilized in attempts to “rescue” water insoluble drug candidates or to enhance the usefulness of established drugs [1]. Examples of such prodrugs include fosphenytoin, a phosphate derivative of phenytoin, and avizafone, a dipeptide derivative of diazepam.

In the present work, we provide in vitro data suggesting a new drug delivery strategy based on prodrug conversion, in which water soluble prodrug and its converting enzyme are co-delivered at a parenteral point of administration such as the nasal mucosa. Enzymatic conversion produces drug in concentrations exceeding the drug’s thermodynamic solubility, or saturation level. Provided drug is absorbed faster than it crystallizes, such supersaturated solutions could lead to rapid drug absorption. This principle has been demonstrated by using prodrug/enzyme systems for two drugs for seizure emergencies cure – diazepam (first choice) and phenytoin (second choice) [2].

EXPERIMENTAL METHODS

Fosphenytoin/alkaline phosphatase and avizafone/A.O. protease (from Aspergillus Oryzae) were used as prodrug/enzyme systems for phenytoin and diazepam, respectively. Prodrug conversion kinetics was evaluated with various prodrug/enzyme ratios at pH 7.4 and 32°C. The prodrug was co-delivered with the converting enzyme apically onto Madin-Darby Canine Kidney II-wild type (MDCKII-wt, a nasal epithelium model) monolayers and drug permeation rates were determined at various degrees of supersaturation (S=0.8-6.1 for phenytoin, S=0.7-10.2 for diazepam). The degree of supersaturation was defined as $S = \frac{c_p(0)}{c_{d,sat}}$ where $c_p(0)$ is the initial molar concentration of prodrug and $c_{d,sat}$ is molar concentration of saturated drug solution.
Membrane intactness was confirmed by measuring trans-epithelial electrical resistance (TEER) and lucifer yellow permeability. Prodrug and drug concentrations were analyzed using HPLC. An in-vitro pharmacokinetic model was developed and utilized to predict prodrug conversion kinetics upstream (monolayer apical side) and drug transport kinetics downstream (basal side).

RESULTS AND DISCUSSION

Enzyme kinetics measured in the fosphenytoin/alkaline phosphatase system revealed $K_M = 827.8 \pm 81.6$ µM and $V_{max} = 51.1 \pm 1.8$ µM/min. For the avizafone/A.O. protease system, $K_M = 1496 \pm 229.9$ µM and $V_{max} = 22.8 \pm 1.6$ µM/min. Conversion of the prodrug (fosphenytoin or avizafone) in the absence of activating enzyme on the apical side of MDCKII-wt monolayer was less than 30% after 2 h. With enzyme, 80% conversion occurred in 2 h, establishing the importance of exogenous enzyme for rapid drug formation. Monolayers were intact with supersaturated solutions, as indicated by TEER values. No drug precipitates were observed upstream/downstream, at any supersaturation level, indicating feasibility of the prodrug-enzyme system to form supersaturated solutions without precipitation (Figure 2). Drug permeation rate across monolayers increased proportionately with increasing degree of supersaturation; this flux was 1.5-6 fold greater than saturated phenytoin (at $S=1$) [3] and 2-17.6 fold greater than saturated diazepam (at $S=0.7$) (Figure 1). The experimental data fitted well to a two compartment pharmacokinetic model.

**Figure 1:** Transport flux of phenytoin and diazepam into the basal side of MDCKII-wt monolayers, when their respective prodrug-enzyme mixtures prepared at various degree of supersaturation were introduced apically onto the membranes for 2 h at 32°C in assay buffer, pH 7.4. Flux of saturated phenytoin (at $S=1.1$): 0.028±0.003 µg/cm².min. Flux of saturated diazepam (at $S=0.7$): 0.027±0.0042 µg/cm².min.

**Figure 2:** Concentration-time profile for (a) fosphenytoin-alkaline phosphatase ($S=6.1$, $c_{sat}=0.6$ U/mL), (b) avizafone-protease reaction ($S=5.6$, $c_{sat}=4$ U/mL), on the apical side of MDCKII-wt membrane. Horizontal line represents drug saturation level ($C_{d,sat}$).

CONCLUSIONS

The developed prodrug-enzyme systems remarkably enhanced drug transport across model membranes. If this principle carries over to nasal delivery, such systems could prove useful for treating neurological emergencies, including seizures. The pharmacokinetic models can predict conversion and transport kinetics of similar prodrug-enzyme systems.

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