Thesis Title	Characterization of Mefenamic Acid Ester Prodrugs: Physico-chemical Properties, Transepithelial and Efflux Transport Across Caco-2 Monolayers
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Abstract

A number of ester derivatives of mefenamic acid, a widely used NSAIDs in Thailand, were synthesized with the aim to suppress local gastrointestinal (GI) toxicity. The ester prodrugs were studied for their stabilities toward chemical and enzymatic hydrolysis, as well as permeability across Caco-2 monolayer. Adequate stability at various pH's were observed for the esters while hydrolysis in plasma, liver homogenate, and Caco-2 homogenate at 37^{o}C were obtained with variable rates. Transport across the Caco-2 monolayer was performed at 37^{o}C using 21-23 days grown Caco-2 cells. Apical (AP) and basolateral (BL) media adjusted to pH 6.5 and 7.4, respectively, were used in all studies and bidirectional permeabilities, i.e. AP- BL vs. BL-AP, were estimated. Compounds exhibiting efflux ratio (ER) ≥ 2 were further examined for their potential interaction to Pgp and MRP using verapamil (a Pgp inhibitor) and indomethacin (an MRP inhibitor). Among four esters examined in transport studies, morpholinoethyl ester (**3**) and pyrrolidinoethyl ester (**4**) showed ER of 3.1 (**3**) and 10.4 (**4**), suggesting the polarized transport of the esters **3** and **4** across Caco-2 monolayer. With the inclusion of verapamil in subsequent bidirectional transport studies, inhibition of **3** and **4** efflux were achieved. Indomethacin, however, completely inhibited the apical efflux of **3**, but enhanced the ER of **4**. Calcein efflux inhibition assays were additionally performed to substantiate functional activity of the efflux transporters. Ester **3** and **4** restored calcein accumulation in confluent Caco-2 monolayer, resulting in 3-4 fold increase in the fluorescence intensity over the control. Hence, results from transport experiments and calcein efflux inhibition assay demonstrated the evidences of apical efflux of some esters of mefenamic acid across

Caco-2 monolayer, which could be mediated by active membrane transporters, Pgp and MRPs. These experimental evidences suggested that secretion of **3** and **4** *in vitro* observed in this study may be the mechanisms limiting transport of these compounds across GI mucosa. The esters, however, could serve as leads in designing modulators/inhibitors of efflux transporters. Moreover, some esters showing good permeability could be further developed as prodrugs of NSAIDs with improved gastrointestinal tolerability.