CHAPTER IV

CONCLUSIONS

In this study, a number of esters of mefenamic acid (**1**-**7**) were synthesized to reduce gastrointestinal toxicity of mefenamic acid. The esters were shown to be 10-100 folds less soluble in water than the parent mefenamic acid. Most esters were stable in buffers of various pH's, i.e. pH 2.0, 5.0, and 7.4. Ester **2** and **6** were slightly degraded in these buffers with half-lives longer than 4 hours. All esters, except ester **7**, were readily biotransformed in plasma, liver homogenate, and Caco-2 homogenate releasing parent mefenamic acid in quantitative amount. Degradation in these biological media was fit to first order kinetic with variable rate and half-lives. Chemically stable but biodegradable properties were obtained for most esters, except **7**. The esters **1**-**4** which are quite stable to hydrolysis in Caco-2 homogenate ($t_{1/2} \geq 60$ min), but exert facile cleavage in plasma could serve as prodrugs of mefenamic acid. These esters were consequently selected for permeability study using Caco-2 monolayer.

It is generally accepted that rate of transport is governed by physicochemical properties of the solute as well as the epithelium itself (Adson et al., 1995; Daugherty and Mrsny, 1999). Caco-2 cell is widely used in drug discovery setting in order to rank lead candidates with respect to their potential rate of absorption *in vivo*. Permeabilty of esters **1**-**4** were investigated across 21-26 days grown Caco-2 monolayers, and expressed as apparent permeability coefficients (P_{app}). Directional transport of the compounds was evaluated both from AP-BL and BL-AP, where asymmetric transport flux was revealed by ester **3** and **4**. The efflux ratio, determined as the ratio secretory permeability to absorptive permeability, of esters **1** and **2** were approximately unity. These esters, **1** and **2**, exhibited high P_{app} which are approximately P_{app} of the parent mefenamic acid. Low permeability, however, was observed for **3** and **4** , which also exhibited efflux ratio of 3.1 and 10, respectively. Inhibitors of efflux transporters, i.e. verapamil (a Pgp inhibitor) and indomethacin (an MRP inhibitor), were employed to confirm the apical polarized transport of esters **3** and **4**.

Apical efflux of ester **3** was strongly inhibited by either verapamil or indomethacin, though effects of indomethacin are more pronounced than those of verapamil. Verapamil partially reversed the apical efflux of **4** suggesting roles of Pgp in transport of **4**. In contrast, indomethacin not only showed no enhancing effects on absorptive permeability, but dramatically increased secretory permeability, resulting in elevated efflux ratio over the control. This could be due to the primarily roles of basolateral located MRPs, which facilitate absorptive permeability, was hampered by indomethacin.

A fluorescence based calcein efflux inhibition assay was also employed to determine activity of efflux proteins and their interaction with inhibitors. When calcein AM is co-incubated with inhibitors of efflux proteins, calcein, its hydrolyzed product, is increasingly retained within the cells and thus the fluorescence of the cells will increase. In this study, intracellular calcein could be restored, reflecting in increased fluorescence intensity, by verapamil, indomethacin, cyclosporin A, and the esters **3** and **4** over control. Verapamil demonstrated the highest response where ester **4** yield the lowest. Hence, results from bidirectional transport study in conjunction with calcein assay concurred that **3** and **4** are transported by Pgp and/or MRP.

In conclusion, the physicochemical properties, chemical and enzymatic stabilities of mefenamic acid esters were investigated. Among 4 esters selected for permeability evaluations, ester **1** showed the optimal biotransformation profiles and exhibit high permeability. It is worthwhile for further investigations of **1** regarding its gastro-protective effects, pharmacological activity and toxicity. Although ester **2** showed permeability in the same range to **1**, but instability in Caco-2 homogenate may hinder its uses as a gastro-protective NSAIDs. Interaction and translocation by Pgp and/or MRPs demonstrated by **3** and **4** as well as their low permeabilities across Caco-2 monolayer may bring up the questions regarding their oral bioavailability. These evidences might preclude these esters from being developed as gastro-protective NSAIDs. However, esters **3** and **4** could be employed as leads compounds for designing of novel inhibitors of efflux transporters.