CHAPTER I

INTRODUCTION

Phyllanthin is a major active lignan found in *Phyllanthus amarus* Schum. et Thonn. and *Phyllanthus urinaria* (family Euphorbiaceae) (Calixto J.B., Santos A.R.S. et al. 1998). This lignan has been demonstrated its broad spectrum of pharmacological activities such as hepatoprotective, antioxidative, antihyperuricemic, and antibacterial actions (Mazumder A., Mahato A. et al. 2006, Krithika R., Mohankumar R. et al. 2009, Chirdchupunseree H. and Pramyothin P. 2010). In addition, phyllanthin was able to potentiate daunorubicin-induced cell death in human leukemic Lucina-1 cells (Leite D.F., Kassuya C.A. et al. 2006). Hence, this lignan might be able to enhance the chemosensitivity of multidrug-resistant cancerous cells toward cytotoxic drugs (Somanabandhu A., Nitayangkura S. et al. 1993, Leite D.F., Kassuya C.A. et al. 2006).

Considering its potential therapeutic values, phyllanthin can be further developed into a new oral administered medicine. The major concerns of oral route of administration are drug interaction potential and drug bioavailability. The USFDA recommends that drug interaction potential of a new molecular entity should be assessed early in the drug development process (US Department of Health and Human Services Food and Drug Administration 2000, Huang S.M., Strong J.M. et al. 2008). P-glycoprotein (P-gp) is the most studied drug efflux pump in the transporter-based drug interactions. This transporter has a significant role in limiting the translocation of certain drugs through restrictive barriers such as intestinal absorptive barrier and blood brain barrier (Terao T., Hisanaga E. et al. 1996, Fromm M.F. 2004. Murakami T. and Takano M. 2008). Interference on P-gp function may affect

absorption, distribution and elimination of its drug substrates, leading to changes in drug bioavailability (Fromm M.F. 2003, Zhou 2008, Staud F., Ceckova M. et al. 2010).

Intestinal absorption is the primary process influencing oral bioavailability (El-Kattan A. and Varma M. 2012). The expression of drug efflux transporters including P-gp on the apical side of the enterocytes may hinder the transcellular drug transport from intestinal lumen into the systemic circulation (Pal D. and Mitra A.K. 2006, Staud F., Ceckova M. et al. 2010). Recently, phyllanthin was shown to directly inhibit P-gp function in the Caco-2 intestinal cells (Sukhaphirom N., Vardhanabhuti N. et al. 2012). However, it is unclear whether phyllanthin-mediated P-gp inhibition significantly affects the rate and extent of its intestinal absorption.

Drug absorption across the intestinal barrier can be influenced by aqueous solubility, stability and permeability (Ungell A-L. and Abrahamsson B. 2009). The high permeability drugs (Biopharmaceutics Classification System (BCS) class I and class II) are good candidates for the oral route of drug delivery (Dahan A., Miller J.M. et al. 2009, Ungell A-L. and Abrahamsson B. 2009). However, if the drug is a substrate of efflux transporters, solubility can be a significant limiting factor for the absorption. High solubilization allows the high amount of drug to be available in the solution. Consequently, the drug concentration at the enterocytes will be high enough to overcome the effects of transporters. Thus, transporter effects on drug transcellular transport will be minimal for the BCS Class I compounds, but predominant for the BCS Class II compounds (Wu C.Y. and Benet L.Z. 2005). Classification of phyllanthin according to the BCS would help to predict its absorption and oral bioavailability.

The in vitro Caco-2 cell monolayers is an established model for permeability study (US Department of Health and Human Services Food and Drug Administration

2000). A number of studies showed a good correlation between the permeability across the Caco-2 cell monolayers and the absorbed fraction through human GI tract (Artursson P., Palm K. et al. 2001, Ungell A.L. and Karlsson J. 2003). The Caco-2 cells is a human epithelial colon adenocarcinoma cell line which consists of several important drug transporters such as H^+/di-tripeptide transporter (PEPT1), organic anion-transporting polypeptide 2B1 (OATP-B), monocarboxylic acid transporter 1 (MCT1), organic cation/carnitine transporter (OCTN2), P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP) (Taipalensuu J., Törnblom H. et al. 2001, Xia C.Q., Liu N. et al. 2005, Sun H., Chow E.C. et al. 2008). The presence of the influx and efflux transporters along with tight junction allows the Caco-2 model to be suitable for the study of passive and active transport mechanisms (Hubatsch I., Ragnarsson E.G. et al. 2007).

The aims of this study were to determine the permeability of phyllanthin across the cultured intestinal absorptive cells. The polarized permeation study was conducted under the pH gradient condition across the Caco-2 cell monolayers as recommended by the USFDA. The influence of P-gp drug efflux pump on phyllanthin permeability was also evaluated. In addition, the stability and aqueous solubility of this lignan were determined.

Hypothesis

It was possible that both permeability and solubility were major determinants of phyllanthin permeation across the intestinal absorptive barrier. Moreover, P-gp might have influence on phyllanthin absorption.

Objectives

The objectives of this study were:

1. To determine the permeability of phyllanthin across the Caco-2 cell monolayers.

2. To determine the stability and aqueous solubility of phyllanthin.

3. To determine the potential effect of P-gp efflux transporter on phyllanthin permeability.

Scope of the study

According to the USFDA, phyllanthin would be determined its permeability under the pH gradient condition across the polarized Caco-2 cell monolayers. In this study, the Caco-2 cell monolayers was cultured for 21 days in transwell for the transport studies. The development of absorptive barrier was verified with the use of TEER values and leakage of lucifer yellow. The transport of phyllanthin across the Caco-2 monolayers was determined along with antipyrine, theophylline and furosemide. These three compounds are permeability markers recommended by USFDA for the permeability study. Moreover, the short term stability and aqueous solubility of phyllanthin were also investigated.

Scope of the study