University of Macau

Institute of Chinese Medical Sciences

ABSTRACT

In-Vitro Studies on the Intestinal Absorption Mechanisms of Flavonoids in Herba Epimedii

Ao Hei Sio

Thesis Supervisor: Dr. Ying ZHENG

Purpose: *Herba Epimedii* is a Traditional Chinese Medicine used to treat cardiovascular diseases and other chronic illness, such as infertility, amnesia, neurasthenia, impotence and senile functional diseases in China for thousands of years. The major active constituents of *Herba Epimedii* are flavonoids and more than 60 kinds of flavonoids have been identified. Among them, Epimedin A, Epimedin B, Epimedin C, Icariin and Icariside II are considered as the major bioactive flavonoids in *Herba Epimedii*. Although *in vitro* and *in vivo* studies have demonstrated that these flavonoids have many beneficial effects, especially potential biological activity against osteoporosis, the extent to which flavonoids are absorbed and the mechanisms involved are not known. This study aimed to investigate the intestinal transport mechanisms of Epimedin A, Epimedin B, Epimedin C, Icariin and Icariside II in different dosing formulations, by a well validated Caco-2 monolayer model *in vitro*. In addition, the impact of efflux transporters including P-glycoprotein and multidrug-resistance associated proteins on these flavonoids absorption was also studied.

Methods: The transport of the five flavonoids from both apical to basolateral (AP-BL) and basolateral to apical (BL-AP) directions, in the form of individual pure

compounds, pure compounds mixture and total flavonoids extract, were studied in the Caco-2 cell monolayer. The bi-directional transport studies were performed in the presence or absence of EGTA which disrupts tight junctions by its Ca^{2+} chelating effect, in the presence or absence of cyclosporine, ketoconazole or verapamil which is inhibitor of the P-glycoprotein (P-gp), and in the presence or absence of MK571 which is inhibitor of the multidrug-resistance associated protein (MRP). In addition, the expression and localization of P-gp and MRP2 on Caco-2 cell monolayer were examined by western blot and fluorescent microscopy.

Results: In the form of individual pure compounds, no flavonoids could be detected from both directions for Epimedin A, Epimedin B and Epimedin C. A small P_{app} value ($0.89\pm0.07\times10^{-6}$ cm/s) was obtained for the secretion transport of Icariin from basolateral to apical side (BL-AP) and no Icariin could be detected for the absorption transport (apical to basolateral side, AP-BL). On the other hand, Icariside II has a high Papp values ($5.66\pm0.40\times10^{-6}$ cm/s) from BL to AP, but no Icariside II could be detected from AP to BL. In the presence of EGTA; P_{app} values of Epimedin A, Epimedin B, Epimedin C and Icariin markedly increased to 10^{-6} cm/sec in both directions. Furthermore, the Papp value of Icariin from the BL to AP significantly decreased ($P<0.05$) in the presence of MK571 but did not affected by cyclosporine. On the other hand, in the case of Icariside II, still no flavonoid could be detected from the AP to BL direction while the Papp value significantly decreased ($P<0.05$) from the BL to AP direction in the presence of EGTA. However, the Papp values of Icariside II from AP to BL significantly increased to $0.68\pm0.28\times10^{-6}$ cm/s, $0.64\pm0.07\times10^{-6}$ cm/s and $2.56\pm028\times10^{-6}$ cm/s in the presence of 50 μM varapamil, 50 μM ketoconazole or 10 μM cyslosporin, respectively, while the P_{app} value decreased significantly ($P<0.05$) from BL to AP. Moreover, P_{app} values of Icariside II from AP to BL increased to $1.12\pm0.06\times10^{-6}$ cm/s and $0.90\pm0.13\times10^{-6}$ cm/s in the presence of 50 μM MK571 while the P_{app} value from BL to AP decreased significantly ($P<0.05$).

For Epimedin A, Epimedin B and Epimedin C, no flavonoid could be detected in both directions neither in the form of pure individual compounds, pure compound mixture

nor total flavonoids extracts. For Icariin, the P_{app} values from BL to AP decreased significantly ($P<0.05$) either in the mixture or extract form while still no flavonoid could be detected from AP to BL in any forms. In the case of Icariside II, P_{app} values from AP to BL increased to more than 10^{-6} cm/sec while the P_{app} values from BL to AP did not significantly changed in the mixture or extract form. In addition, P-gp and MRP2 were expressed on Caco-2 cell monolayer validated by western blot and fluorescent microscopy.

Conclusion: The above results suggest that the absorption and secretion transport of Epimedin A, Epimedin B and Epimedin C and Icariin are mainly via paracellular pathway. Meanwhile, MRP efflux systems maybe involved in the secretion transport of Icariin. It is expected these four flavonoids are poorly absorbed *in vivo*. For Icariside II, its transport involves interaction with the P-gp and MRP efflux systems but not via the paracellular pathway. The results that Icariside II would be detected in the form of pure compound mixture and total flavonoids extract but not in the form of individual pure compound suggested that the co-occurring of Icariside II and Icariin or other flavonoids may saturate the efflux transporters, and/or these efflux transporters maybe partly inhibited in the mixture or extract form.

Keywords: *Herba Epimedii*, Icariin; Icariside II; Epimedin A, B, C; Caco-2 cell absorption model; P-glycoprotein (P-gp) ; Multidrug Resistance Associated Protein (MRP)