CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Discussion

In this study, the pharmacokinetics of plasma daidzein and genistein were evaluated in 12 Thai postmenopausal women. Since the study design of this study was similar to that of the bioequivalence testing, 12 subjects were enrolled to this study according to the minimum number of subjects stipulated by the Canadian and European guidelines for bioequivalence testing (272). The results of the present study showed wide variation of plasma concentrations of daidzein and genistein after taking various dosage regimens of soy extract were large among both intra-individual and inter-individual subjects. The plasma concentration-time profiles of daidzein and genistein were typically biphasic in every individual regardless of soy extract regimens taken. The first and second peak concentrations of both aglycone forms were attained at 2-4 h and 6-8 h, respectively. The second peak concentrations of both isoflavones were generally higher than the first peak concentrations. Only T_{max} of daidzein and genistein after "single ISO + D_3-calcium" or "continuous D_3-calcium/single ISO" were significantly longer than "single ISO". Other pharmacokinetic parameters of daidzein and genistein after 3 different treatments were not different significantly. This is the first study to investigate the interaction between vitamin D and calcium with soy isoflavones.

Theoretically, the isoflavones in non-fermented soy food appear mostly as the glycoside conjugates, whereas the aglycones dominate in fermented soy products

(37). Since the amounts of daidzein and genistein detected in the Flava Soy® capsule were negligible (data not shown), it was postulated that the predominant forms of isoflavones existing in the preparation were glycoside conjugates (i.e., daidzin, genistin). However, because human intestinal or gut microfloral glucosidases seemingly cleave these moieties before these isoflavones can be absorbed, therefore, most of the absorbed isoflavones are the aglycones (10). The absorbed aglycones are then largely converted to their β-glucuronides by enzymes in the gut wall, which may be a major site of glucuronidation, and also in the liver after they reach systemic circulation. Isoflavones are predominantly conjugated with glucuronic acid and to a lesser extent, with sulfate (54). The purpose of using a mixture of β-glucuronidase/sulfatase in quantification of plasma isoflavone concentrations in this study was to hydrolyze glucuronide and sulfate conjugates to aglycones. Thus, plasma concentrations of daidzein and genistein were determined instead of their glucuronide and sulfate conjugates.

In this study, the biphasic pattern observed from concentration-time curve of both aglycones indicates an entero-hepatic recirculation as already suggested by several authors (56, 273, 274). The first peak corresponds to absorption occurring readily in small intestine (56, 273, 275) that the glycoside forms of isoflavones are hydrolyzed by glucosidases to the aglycone forms (45). The second peak possibly results from enterohepatic recirculation of the glucuronide and sulfate conjugates of isoflavones excreted in bile (273). In this study, the second peak concentrations of both isoflavones were attained approximally at 2 h after lunch in most subjects, this finding supports the existence of enterhepatic recirculation. Additionally, the long transit time of unabsorbed glycosides reaching the colon and subsequently cleaved by

micofloral glucosidases may also help explaining the second surge of plasma daidzein and genistein concentrations (276).

The mean T_{max} of daidzein (5.50 $\pm$ 2.11 h) and genistein (4.58 $\pm$ 2.11 h) was quite shorter than those of 8-11 h after ingestion of isoflavone conjugates reported in previous studies (55, 272). This discrepancy might be the result from such factors as race, uptake rates, and rapidity of hydrolysis of glycosides by gut bacteria or gut wall enzymes. However, the difference in the rate of cleavage of these glycosides by gut wall enzymes and bacteria among the difference population seem to be a more plausible explanation since the T_{max} of these aglycones of the present study was more comparable to another study done in Thai population (277).

"Single ISO + D_3-calcium" or "continuous D_3-calcium/single ISO" significantly delayed T_{max} of both daidzein and genistein compared to "single ISO", of which "continuous D_3-calcium/single ISO" delayed the T_{max} the most. The reason behind the delay in T_{max} by D_3-calcium is unknown. Since aglycones are absorbed by passive diffusion (278), and there is no evidence for facilitated or active transport of isoflavones, it seems unlikely that D_3-calcium exerts this interference via involvement of a carrier or saturation process. However, this delayed rate of absorption might be the results from at least 3 possibilities: Firstly, since it has been shown that the rate-limiting step for absorption is initial hydrolysis of sugar moiety (44), therefore, either vitamin D or calcium (or both) possibly and transiently inhibits glucosidase activity leading to the delayed cleavage of these glycoside conjugates into aglycones and thus increases in their T_{max}. Secondly, calcium carbonate in Caltrate 600 + D® may cause delayed gastric emptying time leading to the delayed T_{max} (279).

Thirdly, some inactive ingredients in Caltrate 600 + D® might alter the bioavailability of aglycones because it has been shown that fiber and carbohydrate are associated with differences in absorption of aglycones (52, 271, 280). Among these possibilities, the latter appears unlikely because the amount of inactive ingredients would be too little to cause such significance and that in the continuous D_3-calcium treatment regimen, no direct contact between D_3-calcium and soy extract exist.

Although "Single ISO + D_3-calcium" or "continuous D_3-calcium/single ISO" significantly delayed the T_{max} of both isoflavones, however, both treatments did not significantly affect other pharmacokinetic characteristics of daidzein and genistein since C_{max}, AUC and $t_{1/2}$ of these isoflavones were not significantly different among the three treatment regimens. Therefore, D_3-calcium only delayed the absorption of isoflavones under study without significant effects on the amount of isoflavones absorbed or on their metabolism. However, since the kinetics of isoflavones were complex and exhibited marked intra-individual and inter-individual variation coupled with small sample size could undermine the true significant difference of pharmacokinetic parameters under study, if it truly exists. More subjects in future study may help revealing this controversy.

Although our study has demonstrated that both single-dose and multiple-dose administrations of D_3-calcium delayed rates of isoflavone absorption, we postulate that these drug interactions cause negligible effect on isoflavones' therapeutic outcomes because reduced absorption rate is only important if a rapid pharmacological effect is sought which depends on attaining a high peak concentration (e.g., with antibiotics and analgesics) (273). Therefore, co-administration of D_3-calcium and isoflavones seems to be the simple method to

enhance drug compliance, especially in the elderly patients taking several medications, and this advantage might outweigh minor effect of D_3-calcium on isoflavones' pharmacokinetics. In deed, the D_3-calcium-isoflavones should be encouraged for treatment of some indications (such as in post-menopausal osteoporosis) and might become an appropriate alternative food supplementation to HRT in Thai postmenopausal women in the near future. Nevertheless, the clinical studies in this aspect should be further investigated.

The major limitation of this study was the lack of quantification of daidzin and genistin in the soy preparation used; the variations in amount per capsule may contribute to the remarkably high variations in C_{max} and AUC of aglycones among the three phases in some or even in the same subjects. Another limitation was the lack of verification of dissolution of the soy formulation, yielding the unanswered question whether delayed absorption of aglycones was due to different dissolution rate or drug interaction. The small sample size also contributes to marked variation encountered in this study. Future study should be designed to overcome these limitations by determination of isoflavone contents existing in the study preparation as well as their dissolution profiles and using larger sample size.

4.2 Conclusion

Single-dose and multiple-dose of D_3-calcium delayed T_{max} of daidzein and genistein without significant alteration in C_{max}, AUC_{0-32}, $AUC_{0-\infty}$ and $t_{1/2}$ of both aglycones.