CHAPTER V
CONCLUSION AND PERSPECTIVES

Andrographolide is a major bioactive constituent in *A. paniculata* plant, which its pharmacological activities have been well-established for a long time. CYP1A1 is a major hepatic enzyme for metabolizing a procarcinogen into its active form. In this study effect of andrographolide on CYP1A1 expression was extensively observed.

In primary mouse hepatocytes, the synergistic CYP1A1 induction was observed after co-treatment of andrographolide and a typical CYP1A1 inducer, β-NF. The results revealed a bimodal influence of andrographolide on β-NF-inducible CYP1A1 mRNA expression, namely suppression early on (9 h) and enhancement later (24 h). The synergism of CYP1A1 at 24 h using microarray analysis was studied. Beside induction of CYP1A1 mRNA, up-regulation of several metabolism/oxidation/reduction related-genes was observed by andrographolide. Interestingly, combination of andrographolide and a typical CYP1A1 inducer, β-NF, modified a large number of metabolism/oxidation/reduction related-genes (> 100 genes) including CYPs, GST, and UGT. Although we examined the effect of andrographolide and/or β-NF on the oxidative stress status in hepatocytes cultures, the relationship between oxidative stress and synergism of CYP1A1 was not found. Glutathione modulators greatly altered the effects of andrographolide on CYP1A1 expression. Therefore, investigation in the presence of glutathione modulators and other factors was carried out to enravel a mechanism of the bimodal influence of andrographolide on synergistic CYP1A1 expression by the typical CYP1A1 inducer, β-NF.

The synergism of CYP1A1 expression by andrographolide plus 3-MC in mice was collerated to those observed in primary mouse hepatocytes. Interestingly, this phenomena did not observed in an AhR-non responsive strain, DBA/2. Hence, the synergism might be regulated by AhR mediated pathway, at least in part. In addition, an AhR-responsive strain, *i.e.*, C57BL/6, might be an appropriate strain for further study. Andrographolide showed the synergism only in the males, but not in the female

mice. These observations suggested that the enhancement of CYP1A1 expression on co-treatment with andrographolide and a typical CYP1A1 inducer was gender-dependent. Orchiectomy clearly diminished the synergistic effect in the male mice, while testosterone supplement restored it in both orchiectomized and ovariectomized mice. The findings revealed that the synergistic effect of andrographolide on CYP1A1 expression by a typical CYP1A inducer occurred via the AhR-mediated pathway. In addition, a male sex hormone, testosterone, and level of glutathione play important roles in regulatory mechanism of CYP1A1 expression by andrographolide plus the typical CYP1A1 inducers, β-NF or 3-MC.

All observations suggested that the herb-drug interaction with use of andrographolide or *A. paniculata* containing health supplement products might be of concern according to potential to modify the expression of several CYPs and other metabolizing enzyme related-genes. The risk of carcinogenesis from the synergism of CYP1A1 activation by andrographolide plus a typical CYP1A1 inducer should be alerted as an important precaution for long-term use of andrographolide-containing products.