CHAPTER IV

DISCUSSIONS AND CONCLUSIONS

Quercetin and its glycoside derivatives present great interest as potential anticancer and anti-carcinogenic molecules, unfortunately its very poor solubility and unstable in an aqueous solution that hampers the progression in clinical trials. In fact the anticancer drug development process of these compounds needs a lot of data base such is a lacking of the physicochemical property and molecular based mechanism of these compounds. Recently, Tungjai et al. reported the speciation of the molecule in physiological like conditions and determined the log P value as well as the mean rate passive diffusion through the lipid bilayer membrane (1). These findings suggested that quercetin was passively diffused throughout the plasma membrane into the cytosolic site and the available cytosolic concentration of quercetin should be responsible for its specific anticancer action.

During my long years of my thesis, we have found that BSA can considerably improve the dissolubility of quercetin and its glycoside derivatives. In particularly these flavonoids have high affinity to BSA. The complexation of BSA-flavonoid was rigorously studied by using FRET. The microscopic and macroscopic binding rate constant was determined. The ratio of complex was 1 mole of BSA to 2 moles of quercetin or quercetrin. At pH 7.3, the macroscopic dissociation rate constant was 1.37×10^5 M^{-2} for BSA-quercetrin and 1.68×10^5 M^{-2} for BSA-quercetin. It should be noted that the ratio of BSA-rutin complexation was 1: 1. Moreover, the binding constant of the BSA-rutin was equal to 5.0×10^4 M^{-2}. In fact among the compounds studied, the BSA-rutin is the most

stable and the cell-rutin concentration is very low compared with quercetin and quercetrin. The subsititution of rhamnoside and rutinoside at C3 particular rutinoside yields an increase in complex stability by ~29-fold compared with quercetin. The macroscopic dissociation rate constant was directly influenced on the cellular uptake of molecules since we have found that in the presence or the absence of BSA, quercetin was similarly incorporated into cells. These results suggested that the binding of quercetin with BSA did not affect the cellular uptake while the BSA-quercetin complexation can increase in the solubility of quercetin. Indeed, the study reported for the first time the suitable range of dissociation rate constant of the carrier and drug by which the carrier can be useful for increasing the solubility of drug and also spontaneously release the drug into the solution and cells. As a consequence the complexation did not change in the mode of action of molecule. Contrary, an increase in complex stability by 29-fold caused dramatically decreased in the cellular concentration such as the case of rutin. It should be expected that the available cytosolic concentration might not be enough to mediate any biological effects in cells. These results suggested that the BSA-quercetrin and BSA-rutin complexes might play role and change on the mode of action. We hypothesized that both BSA-quercetrin and BSA-rutin complexes might mediated anticancer action via an interaction with the extrinsic pathway, activated by pro-apoptotic receptor signals at the cellular surface.

However, huge reports proposed that quercetin and its glycoside derivatives mediated anticancer action via different molecular based pathways. All researchers considered the cellular biochemistry as a function to the total concentration of molecule addition into their suspension of cells. Moreover, none of these molecular biological

results reported emphasizing the interaction of molecule with targeted genes and proteins in the international literature never shown the percentage of cells that responded to their treatment. It should be noted that the molecular biological methods are very high sensitivity, only 1-5% of 2 million cells is high enough to be measured, in our opinion it cannot be represented the cells. The different observation should be due to the different experimental conditions. Our results can clarify the different observation made among research groups and teach us that we cannot compare the cytotoxicity and mechanisms of molecules without concerning the experimental conditions.

The results of this thesis also revealed that in the cytotoxicity assay conditions, the cellular quercetin and IC_{50} values were similarly found in both drug-sensitive and drug-resistant cells. These signified that the MDR transport proteins cannot maintain the gradient, high at extracellular and low at intracellular site. There were two possibilities for explaining the mechanism of quercetin action; one is the mean rate influx of quercetin should be very fast compared with the mean rate active efflux of MDR transporters and the other is that quercetin is not substrate of these MDR transporters, thus it did not pump out of MDR cells. Of relevance to their use as anticancer agents alone or in combination with other agents may be their interaction with the MDR transporters including P-glycoprotein and MRP1 protein which frequently expressed in human cancerous tissues. This study rigorously analyzed the kinetic parameters of P-glycoprotein- and MRP1-mediated efflux of pirarubicin in the presence of quercetin, quercetrin and rutin in living K562/adr and GLC4/adr cells. We thus firstly demonstrated that the treatment using quercetin, quercetrin and rutin alone efficiently inhibited cancer cell proliferation; particularly quercetin has almost the same activity in drug-sensitive and drug-resistant

cells while the differential sensitivity was observed for quercetrin and rutin. However, among flavonoids studied, rutin was the most potent following quercetrin then quercetin. The results suggested that an esterification of rhamnoside particular the rutinoside at C3 position of quercetin enormously increased in their cytotoxicity in drug-sensitive but differential sensitivity in drug-resistant cells.

This study also shows that quercetin, quercetrin and rutin can increase in cytotoxicity of pirarubicin but not doxorubicin signified that the nature of the anticancer molecule use should play important role. A combination treatment, the nature of anticancer drugs and flavonoids should be token account. Except quercetin, the compounds studied exhibited more MDR reversing efficacy in MRP1 compared with P-glycoprotein phenotype. We also demonstrated that the functional P-glycoprotein and MRP1 protein was localized on the plasma membrane of K562/adr and GLC4/adr cells, respectively. In fact, both doxorubicin and pirarubicin are substrates of P-glycoprotein and MRP1 proteins and were pumped out of cells by using energy from ATP hydrolysis. If these flavonoids inhibited the ATPase activity of the two transporters, the kinetics parameters of transport of the two drugs should be found in similar way. The results suggested that quercetin, quercetrin, rutin may directly interact with substrate-binding sites of P-glycoprotein and MRP1 protein. This suggestion is along with Hooijberg et al. (2) who stated that some isoflavonoids such as genistin did not inhibit ATPase activity but some such as genistein, kaempferol, flavopiridol affected the ATPase activity of GLC4/adr cells. These authors also mentioned that the inhibition of MRP-mediated efflux of anticancer drug by flavonoids is due to a direct interaction with MRP. In addition, the

direct interaction of some flavonoids with recombinant nucleotide binding domain (NBDs) of human P-glycoprotei and MRP1 were reported (3).

Many research groups play attention to the interaction of flavonoids with MDR transporters especially P-glycoprotein and MRP1 protein (2,4,5,6). Among flavonoids, genestein is the first flavonoid that was found to be a potent inhibitor of MRP1 (2, 7). Our results clearly show that quercetin, quercetrin except rutin non-competitively inhibited the function of P-glycoprotein in K562/adr and MRP1 in GLC4/adr cells. The determined K_I value of P-glycoprotein was equal to 0.33 μM for quercetin and 1 μM for quercetrin and K_I value of MRP1 was equal to 0.45 μM for quercetin and 0.5 μM for quercetrin. However, from these data it is difficult to distinguish between a purely non-competitive and mixed-type of inhibition. The results suggested that an esterification of rutinoside at C3 position of quercetin might change the transport behavior of the molecule (8).

Let consider the interaction of quercetin series as inhibitors on the substrate-binding sites for pirarubicin of P-glycoprotein and MRP1; (1) pirarubicin was 2 mole pumped out per turnover by P-glycoprotein and MRP1 signified that there are two binding-sites for pirarubicin; (2) flavonoids should be recognized and bind on the two proteins at different binding-sites of those pirarubicin; (3) An esterification of rhamnoside at C3 position slightly increase in the affinity of molecule on both P-glycoprotein and MRP1 protein; and (4) an esterification of rutinoside at C3 position probably the rutinoside affected the molecular steric of quercetin thus changes the behavior of interaction of rutin with substrate binding sites.