CHAPTER V

GENERAL DISCUSSION AND CONCLUSION

5.1 Differences in mitochondrial and cellular energetic state of drug-sensitive and -resistant cells

Recently, it have been demonstrated that cancer cells exhibited an abnormal energy metabolism compared with normal mammalian cells which derive most of their energy supplies from oxidative phosphorylation. On the basis of ^{1}H–NMR finding, since glutamate was found 2.61 ± 0.8 fold for K562 and 1.31 ± 0.16 fold for GLC4 cells higher than lactate signifies that both of drug–sensitive cells produce energy via oxidative metabolism. However, the two drug–sensitive cells produced the lactate in high amounts, particularly GLC4 two–fold higher than K562 cells (P<0.01), indicating that these cells can maintain an increased rate of glucose utilization and sustain high rates of glycolysis. The rate of glucose utilization via glycolysis significant increased when the similar series of experiments were performed using their corresponding drug-resistant cells. The ratios of glutamate to lactate was equal to 0.74 ± 0.14 for K562/*adr* and 0.73 ± 0.03 for GLC4 cells where no-significant difference of lactate was found in both MDR cells (lactate = 0.59 ± 0.17 for K562/*adr* and 0.68 ± 0.15 for GLC4/*adr*, P< 0.56). Both drug-resistant cells significantly produced lactate higher than K562 cells (P< 0.01) but no–significant difference to GLC4 (P < 0.16).

These results were along with the mitochondrial membrane potential that reflect to the mitochondrial function; the $|\Delta\Psi_m|$ in the four cell lines were -160 ± 4 mV for K562 cell, -146 ± 6 mV for K562/*adr* cell, -161 ± 10 mV for GLC4 cell and -168 ± 2 mV for GLC4/*adr* cell. All cell lines, except K562/*adr* cell were found similar mitochondrial energetic state. Both drug–resistant cell lines consisted about 25 % ATP contents higher degree than their corresponding drug-sensitive cells. These results suggest that the MDR cells produce an addition energy supplies via glycolysis pathway. In fact, the differences in production energy supplies between the drug–sensitive and drug–resistant cells can be used as drug targets for overcoming cancers, particularly MDR phenomenon in cancer chemotherapy.

5.2 Anticancer activities of artemisinin and its derivatives

According to the results through out my thesis, artemisinin, artesunate and dihydroartemisinin, the new class of antimalarial drugs could be also considered as anticancer drugs particularly against MDR cells. The drugs efficiently inhibited cancer cell growth; IC_{50} values equal to 15 ± 5 μM for K562, 29 ± 9 μM for K562/*adr*, 34 ± 6 μM for GLC4 and 31 ± 5 μM for GLC4/*adr* cells for artemisinin; 1 ± 0.3 μM for K562, 1 ± 0.3 μM for K562/*adr*, 0.6 ± 0.3 μM for GLC4 and 0.9 ± 0.2 μM for GLC4/*adr* cells for artesunate; 1 ± 0.2 μM for K562, 1.4 ± 0.3 μM for K562/*adr*, 0.5 ± 0.2 μM for GLC4 and 0.5 ± 0.1 μM for GLC4/*adr* cells for dihydroartemisinin. The results demonstrated that artemisinin, artesunate and dihydroartemisinin poorly inhibited P–glycoprotein and did not inhibit MRP1 protein function in multidrug resistant K562/*adr* cells, in overexpression P–glycoprotein, or in GLC4/*adr* cells overexpression of MRP1–protein, respectively. However, they

increased in cytotoxic effect induced by pirarubicin or doxorubicin only in MDR cell lines. We also demonstrated that these qinghaosu modulate mitochondrial function, leading to a decrease in intracellular ATP content in all cell lines tested.

^{1}H–NMR spectra obtained from the treatment series revealed that artemisinin, artesunate and dihydroartemisinin affected the metabolism these cell lines. In drug-sensitive cells, they inhibited the oxidative metabolism. In drug–resistant cells, the drugs inhibited the anerobic but stimulate the oxidative metabolic of these MDR cells. These should be resulted in a decrease in global cellular ATP contents. However, an abnormal oxidative metabolism was observed in K562/*adr* cells and a decrease in glutamate is not accompanied by any increase in aspartate, signifies that an abnormal mitochondrial function of the cells, probably the original of a lower mitochondrial energetic state which can be measured by the $\Delta\Psi$m compared with K562 cells. These results suggest that altered mitochondrial dynamics is nonrandom in MDR cells; thus, the mitochondria may be involved in the production of a pleiotropic MDR phenotype. It was proposed that rapid overexpression of MDR1/P-glycoprotein was associated with a decrease of $\Delta\Psi$m. An increase or a decrease in $\Delta\Psi$m consequently followed by increase or a decrease in cellular ATP content, respectively.

We have also demonstrated that at lower to 15 μM, even through cells were exposed to drugs varied from 0 to 72 h, and only 3 % total apoptosis were determined. These are along with the ^{1}H–NMR studies which found that there was no significant changes of mobile lipids. The significant apoptosis-inducing activity of drugs were found when the concentrations used are higher than 15 μM and the maximal apoptosis-inducing activity was about 20% even the concentration of drugs increase up to 50 μM.

In conclusion, this thesis had studied anticancer activities and potential of mechanisms of artemisinin, artesunate and dihydroartemisinin. We have demonstrated that the drugs poorly inhibited P–glycoprotein and did not inhibit MRP1 protein function in multidrug resistant K562/*adr* cells, overexpression P–glycoprotein, or in GLC4/*adr* cells overexpression of MRP1–protein, respectively. However, they increased in cytotoxic effect induced by pirarubicin or doxorubicin only in MDR cell lines (Reungpatthanaphong & Mankhetkorn, 2002). Indeed, The drugs inhibited the oxidative metabolism of K562 but not of GLC4 cells, inhibited the anaerobic and stimulated the oxidative metabolism of the MDR cells. An abnormal oxidative metabolism was observed in K562/*adr* cells; as a decrease in glutamate is not accompanied by any increase in aspartate. The change in metabolic patterns was accompanied by an intracellular acidification and an induction of both necrosis and apoptosis.

Our results clearly show for the first time that artemisinin and its derivatives are very potent anticancer drugs and can be used in combination with anticancer drugs to overcome MDR phenomena. Further studies are needed to explain its mechanism of anticancer and investigate the pharmacokinetics and dynamic of artemisinin, artesunate and dihydroartemisinin in *in vivo*.