CHAPTER V

DISCUSSION AND CONCLUSION

The result of HPLC method has shown that 6-MMP was well separated and there was no interfering substances at retention time of 6-MMP. A linear relationship between the peak areas and 6-MMP concentration over the range of 50 -1000 ng/ml was observed. The limit of detection, the limit of quantification, the inter-assay %RSD, the intra-assay %RSD, the accuracy and the recovery of this method were in the acceptable ranges. These results confirmed the suitability of HPLC method using for detection of 6-MMP formation in RBC after incubation with 6-MP and SAM.

TPMT is a cytoplasmic enzyme widely distributed in RBC, lymphocytes, platelets, kidney, and liver cells. There is a correlation between TPMT activity in RBC and other tissues. TPMT can catalyze the methylation of aromatic thiol compounds, including thiopurine drugs. Population studies in Caucasian have shown a trimodal distribution which have low (0.3%), intermediate (11.1%), and high (88.6%) activities. The range of TPMT activity in this study was 5.54-67.36 units/ml pRBC/hr. The cut off point was measured by probit analysis at ~15 units/ml pRBC/hr. In our study, the TPMT activity has higher values than other laboratory using HPLC method. This may be explained by the differences of equipment, substances, procedure and researcher. This finding disagrees with a previous study (Parida, 1996 and Hongeng et al, 2000) in that the distribution of TPMT activity was bimodal, intermediate (6.67%) and high (93.33%) activities. The result is more consistent with the study in Chinese (Lee and Kalow., 1993) and Korean population (Park-Hab et al., 1996) which also found the bimodal distribution of TPMT activity. In agreement with Lee (1993) this study also demonstrated the no effect of gender on TPMT activity. This finding agrees with the pervious research (Mcleod et al., 1995) erythrocyte TPMT activity in ALL children was increased during the continuation of chemotherapy (6-MP) and after the cessation of therapy. The genotype-phenotype correlation was shown in 84 patients with high acticity

group (>15 units/ml pRBC/hr) who had wild type of TPMT*3C, TPMT*3A and TPMT*2. The rest were six patients with intermediate activity (5-15 units/ml pRBC/hr). Two of these intermediate metabolizers were TPMT*3C but four were not TPMT*3C, TPMT*3A and TPMT*2. This data confirms that TPMT*3C is the predominant mutant allele in Asian population which has allele frequency ~2.5%, whereas TPMT*3A is rare (Kham et al., 2002). Among four of the undetectable genotype, the TPMT activities were 5.54, 9.81, 14.45 and 14.88 units/ml pRBC/hr. The values 14.45 and 14.88 units/ml pRBC/hr were very close to the cut off point of the intermediate subgroup. The other two patients with enzyme activity of 5.54 and 9.81units/ml pRBC/hr were considered decreasing as the result from 6-MP dose due to their suffering in myelosuppression. The possibility for detection of other mutant alleles needs to be considered because there are now eight mutant TPMT alleles. Besides, some patients with high activity offered the toxicity but some patients with intermediate activity had no toxic effect from 6-MP. As in McLeod and Siva., 2002 some individuals with a heterozygous genotype exhibit high activity whereas some homozygous wild type subjects exhibit an intermediate phenotype. Some patients have high activity but are suffered with toxicity. Such discrepancies are due to the fact that the SNPs discussed so far are not the only factors regulating catalytic activity. Population genetic studies have shown the genotype at the "major locus", which regulates TPMT activity accounted for only approximately two-thirds of the total variance in the level of RBC enzyme activity. Other factors such as promoter polymorphism, drug interaction, diagnosis, environment and etc, could also play a role in causing the differences but the answer is still unclear.

Suggestion

1. Detection of all mutant TPMT alleles to find genotype responsible for TPMT intermediate activities.
2. Increase the number of ALL children studied to provide more exact frequency distribution of TPMT activity.
3. Genotype-phenotype correlation is related in patient treated with 6-MP. Phenotype and DNA based diagnosis should be performed in order to adjust dose of 6-MP.