Chapter VI

Summary
Microarray based gene expression profiling has attained an important role in toxicological investigations of important therapeutic agents. Exploration of transcriptional alterations following drug treatment is helping researchers to identify adverse effects early in drug development process and in elucidating mechanisms of toxicity related to important compounds. In spite of the boom gained by high throughput expression profiling in toxicology studies, no information is available about the anti-relapse antimalarial compounds at global gene expression level. Anti-relapse antimalarials are important therapeutic agents, active against liver stages of the Plasmodium. In this study two very important anti-relapse antimalarials derived from 8-aminoquinoline, namely PQ and BQ, were investigated at global scale using DNA microarray technology in a time dependent manner to investigate important gene expression changes in normal liver tissue following acute dosing. Furthermore, gene expression profiles of PQ and BQ were compared with profiles of APAP and CCl4 to find toxicologically relevant genes. Apart from microarray studies, traditional methods of assessing hepatic stress were also evaluated following each drug treatment at different time points in order to find relative sensitivity of the methods and to anchor gene expression changes with biochemical and histological finding.

The activities of ALT and AST were not affected following administration of PQ, BQ and APAP, however, significant and progressive increase was observed after CCl4 treatment. Furthermore, histopathological lesions were absent in PQ, BQ and APAP treated animals, suggesting lack of recognizable hepatic damage, however, CCl4 treated animals showed hepatic pathological changes such as necrosis, eosinophilia and inflammation in a progressive manner. Moreover, chromosomal aberrations were absent in animals treated with PQ, BQ, APAP or CCl4. These results indicated that PQ, BQ and APAP do not produce hepatic tissue damage or affect chromosomal integrity at doses used in this study following single acute treatment; however, CCl4 administration leads to prominent biochemical and histological alterations.

Furthermore mRNA expression in mice liver was assessed with 15,247 unique probes after PQ, APAP and CCl4 treatments whereas, 22,827 unique probes were assessed after BQ exposure. PQ, affected 16 (p<0.01 and 2 fold at 6, 12 and 24) probes consisting of 11 up-regulated and 5 down-regulated probes. Further analysis of PQ data at lower stringency identified 189 probes with 2 fold differential expression. Similarly, BQ treatment resulted in the differential expression of 11 probes (p<0.01 and 2 fold at 6, 12 and 24) of which 8 were up-regulated and 3 down-regulated. Analysis at lower
stringency identified 145 probes with 2 fold differential expression following BQ administration at all time points.

Moreover, seven differentially expressed probes (p<0.01 and 2 fold at 6, 12 and 24), consisting of 3 up and 4 down-regulated probes were identified following APAP administration. Furthermore, 129 probes were detected with 2 fold differential expression following low stringency data analysis. Similarly, following CCl₄ treatment 60 probes consisting of 37 up-regulated and 23 down-regulated probes showed differential expression (p<0.01 and 2 fold at 6, 12 and 24). Low stringency data analysis revealed 362 probes with 2 fold differential expression following CCl₄ treatment.

Apart from differential expression analysis microarray data pertaining to each drug was analyzed with clustering and pathway analysis algorithms. Results of both these analyses revealed no appreciable linkage between the 8-aminoquinolines and the model hepatotoxicants. Nevertheless, important correlation was found between APAP and CCl₄ affected gene especially those with p<0.01 and 2 fold differential expression.

The identification of genes with similar expression changes (p<0.01 and 2 fold) both in direction and magnitude at all time points, particularly following administration of 8-aminoquinolines, indicates important tissue responses in the present gene expression profiles. These findings are important because no detectable damage could be recognized following 8-aminoquinoline treatment. Furthermore, the number of affected genes after each 8-aminoquinoline derivative shows that BQ affects less number of genes than PQ and thus supports previous results. However, based on present gene expression changes, no correlation could be recognized between 8-aminoquinolines and the model hepatotoxicants. This might be due to differences in the tissue response to discrete chemicals classes i.e. 8-aminoquinoline derivatives and model hepatotoxicants. Nevertheless, identification of robust gene expression changes in the absence of biochemical and histological markers advocates in favor of microarray technology as a useful tool to understand molecular events at an earliest stage.