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STUDIES TO UNDERSTAND THE EFFECT OF CANCER ON HEPATIC CYP2C19 ACTIVITY

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Molecular Medicine, The University of Auckland.

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Inter-patient variation in effectiveness and toxicity of cancer chemotherapy may be due to differences in pharmacokinetics, influenced by genetic and environmental factors controlling the activity of hepatic drug metabolising enzymes. One such enzyme, CYP2C19, displays genetic variation; homozygous variant individuals have a poor metaboliser (PM) phenotype. Whilst this relationship is valid in healthy populations, genotype-phenotype discordance has been reported in cancer patients. The aim of this thesis was to determine if discordance occurs in a wider range of cancer patients and to elucidate mechanisms responsible for decreased CYP2C19 enzyme activity.

Two independent clinical studies were undertaken. Of 33 patients with terminal cancer, 37% were PM, significantly ($P < 0.0005$) higher than predicted from genotype. For 29 patients with colorectal carcinoma, 27% in stage IV and 14% of resected patients were PM. Although RECIST analysis of stage IV patients did not demonstrate a significant relationship between CYP2C19 activity and tumour burden, the one patient tested both before and after tumour resection, changed from a poor to an extensive metaboliser.

In patients with terminal cancer, no correlation between CYP2C19 status and inflammatory markers was observed. In contrast, PM phenotype in stage IV and resected patients was associated with elevated CRP ($P < 0.05$) and decreased serum TGF-β ($R_S = -0.5331, P < 0.005$). Interestingly, six patients changed phenotype categories over three test occasions reflected by changes in TGF-β. There was also an association between BMI and CYP2C19 activity ($R_S = 0.4953, P = 0.0063$).

NO-donor compounds reversibly inhibited CYP2C19 activity in human liver microsomes and cells over-expressing CYP2C19. In addition, 24h exposure of cells to NO-donor compounds irreversibly decreased CYP2C19 activity ($P < 0.0005$), which was blocked by MG132, an inhibitor of proteasomal degradation. However, there was no relationship between plasma nitrate/nitrite concentrations and CYP2C19 activity in the patients.

Total plasma protein and unbound drug fraction were determined for individual patients. It was demonstrated that the high drug/metabolite ratio in the PM subjects was not due to altered drug-protein binding and could only be accounted for by decreased enzyme activity (intrinsic clearance, $CL_{int}$).

In conclusion, some cancer patients have compromised CYP2C19 activity that may be due to factors including inflammation, obesity and nitrosative damage. Non-inherited variation in CYP2C19 activity may account for variable pharmacokinetics of some anticancer drugs, thus identification of phenotypic PM prior to treatment may reduce the wide variation in both toxicity and response to these agents.
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<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>5'OH OMP</td>
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<td>α-MEM</td>
<td>Alpha-minimal essential media</td>
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<td>A</td>
<td>Adenine</td>
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<td>Alanine transaminase</td>
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<td>AUC</td>
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<tr>
<td>SNAP</td>
<td>S-nitroso N-acetylpenicillamine</td>
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<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<td>Sodium nitroprusside</td>
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<td>T</td>
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<td>$t_{1/2}$</td>
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