DAUNORUBICIN KINETICS AND DRUG RESISTANCE IN LEUKAEMIA

By

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B. Sc. (Hons)

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I would also like to thank the entire staff of both the Haematology and Clinical Pharmacology Departments at Royal North Shore Hospital for their help and support throughout my years of study.

Finally, I express my gratitude to John Burnside and Brownyn Williams for their moral support through all the good and bad times during my years at Royal North Shore Hospital.
Abstract

The aims of this thesis were to examine: (1) plasma and cellular pharmacokinetics of daunorubicin and its major metabolite daunorubicinol in patients with acute leukaemia, and the relationships between pharmacokinetics, patient response and the presence of P glycoprotein; (2) actions of the multidrug resistance reversing agents cyclosporin A and trifluoperazine, at clinically achievable concentrations, on daunorubicin accumulation and retention in human leukaemia cell lines and patients with acute leukaemia; and (3) effect of daunorubicin on the cell membrane of both sensitive and resistant cell lines, with and without the multidrug resistance reversing agents.

Twenty-seven patients with acute leukaemia received daunorubicin as part of induction therapy. The plasma and cellular levels of daunorubicin and its metabolite daunorubicinol were determined using HPLC. There were no significant differences between patients who went into complete remission (12/23) compared to those who did not respond for any of the plasma pharmacokinetic parameters. There was a significant difference in the cellular daunorubicin and daunorubicinol area under the concentration-time curve between responders and non responders (p < 0.02), as well as in cellular Cmax, cellular clearance and cellular volume of distribution. Eleven patients were P glycoprotein positive and 10 P glycoprotein negative (no sample available for 2 patients). There was no correlation between patient response and the presence of P glycoprotein; nor a correlation between the cellular concentration of daunorubicin or daunorubicinol and P glycoprotein. Patients responding to chemotherapy had higher cellular daunorubicin and daunorubicinol compared to non responders. In contrast to in vitro studies, overexpression of P glycoprotein was not the reason for the lower cellular daunorubicin levels.

Cyclosporin A was capable of increasing both cellular accumulation and retention in the drug resistant CEM/MLB and HL 60/ADR cell lines, but not in the drug sensitive CEM and HL 60 cell lines. Trifluoperazine had no effect in any of the four cell lines. In contrast to the cell line findings, only the combination of cyclosporin A and trifluoperazine were able to increase both accumulation and retention in the blast cells of patients at initial presentation. The multidrug resistant reversing agents alone had no effect in increasing accumulation or retention in the blast cells of P glycoprotein positive patients, nor patients in relapse. The cell line studies show that at clinically relevant concentrations only cyclosporin A is capable of increasing daunorubicin accumulation in both the drug resistant P glycoprotein positive (MLB) and P glycoprotein negative (ADR) cell lines. Thus, cyclosporin A does not work only by inhibiting the actions of P glycoprotein. Trifluoperazine
was unable to reverse drug resistance at clinically relevant concentrations in either cell lines or patient blast cells. However, the combination of cyclosporin A and trifluoperazine increased accumulation in patient blast cells at initial presentation, suggesting that these agents may be more useful in patients at initial presentation than relapse.

Daunorubicin was immobilised by linking it to poly vinyl alcohol and the effect of immobilised-daunorubicin was studied on the four cell lines above. The immobilised-daunorubicin was able to decrease cell growth in the drug sensitive HL 60 cell line but not in the drug resistant VLB or ADR cell lines. Poly vinyl alcohol itself was cytotoxic to the CEM cell line. The multidrug resistance reversing agents cyclosporin A and trifluoperazine were only capable of increasing cytotoxicity in the HL 60 cell line, with no effect in the drug resistant VLB or ADR cell lines.
Publications supporting this thesis


Publications in preparation


In addition, some of the work contained in this thesis has been presented at Scientific Meetings as follows:


on cell lines and patient leukaemic cells. Vth World Conference on Clinical Pharmacology and Therapeutics, Yokohama.


Preface

The work described in this thesis was carried out in the Departments of Haematology and Clinical Pharmacology, Royal North Shore Hospital, under the supervision of Dr David Ma, Dr John Boutagy and Dr Anita Piper. This thesis has not been submitted for a degree at any other university. Full acknowledgement has been made where the work of others has been cited and used. A list of publications in support of this thesis is included.

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## Glossary of Abbreviations

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<tr>
<td>ACDA</td>
<td>acid citrate dextrose A</td>
</tr>
<tr>
<td>ADR</td>
<td>HL 60/ADR, doxorubicin resistant HL 60 subline</td>
</tr>
<tr>
<td>ALL</td>
<td>acute lymphocytic leukaemia</td>
</tr>
<tr>
<td>AML</td>
<td>acute myeloid leukaemia</td>
</tr>
<tr>
<td>ANLL</td>
<td>acute nonlymphocytic leukaemia</td>
</tr>
<tr>
<td>Ara C</td>
<td>cytosine arabinoside</td>
</tr>
<tr>
<td>at-MDR</td>
<td>atypical multidrug resistance</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>AUMC</td>
<td>area under the first moment curve</td>
</tr>
<tr>
<td>CEM</td>
<td>T cell lymphoblastic leukaemia cell line</td>
</tr>
<tr>
<td>CL</td>
<td>clearance</td>
</tr>
<tr>
<td>CL-PVA</td>
<td>cross linked polyvinyl alcohol</td>
</tr>
<tr>
<td>Cmax</td>
<td>maximum drug concentration</td>
</tr>
<tr>
<td>CR</td>
<td>complete remission</td>
</tr>
<tr>
<td>Cy A</td>
<td>cyclosporin A</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNR</td>
<td>daunorubicin</td>
</tr>
<tr>
<td>DOL</td>
<td>daunorubicinol</td>
</tr>
<tr>
<td>DOX</td>
<td>doxorubicin</td>
</tr>
<tr>
<td>DSIM</td>
<td>double strength iscoves medium</td>
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<td>EPI</td>
<td>epirubicin</td>
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FCS  foetal calf serum
FE   Fisher exact test
Fr   Friedman two-way analysis of variance
GSH  glutathione
HL 60 acute myeloid leukaemia cell line
HPLC high performance liquid chromatography
IC 50 inhibitory dose at 50% cell death
IDA  idarubicin
Imm-DNR immobilized-daunorubicin
KW   Kruskal-Wallis one way analysis of variance
MDR  multidrug resistance
mdr1 multidrug resistance gene
mRNA messenger ribonucleic acid
MRP  multidrug resistance-associated protein
MRT  mean residence time
MTT  3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
MW   Mann-Whitney U test
n    number
NR   non responders
PBS  phosphate buffered saline
PCR  polymerase chain reaction
Pgp  P-glycoprotein
PKC  protein kinase C
PR   partial remission

xii
<table>
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<td>PVA</td>
<td>polyvinyl alcohol</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>Tm</td>
<td>transition temperature</td>
</tr>
<tr>
<td>Tmax</td>
<td>time at maximum drug concentration</td>
</tr>
<tr>
<td>topo II</td>
<td>topoisomerase II</td>
</tr>
<tr>
<td>Tri</td>
<td>trifluoperazine</td>
</tr>
<tr>
<td>Vd</td>
<td>volume of distribution</td>
</tr>
<tr>
<td>VLB</td>
<td>VLB 100, drug resistant CEM subline</td>
</tr>
<tr>
<td>W</td>
<td>Wilcoxon signed rank test</td>
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