DAUNORUBICIN KINETICS AND DRUG RESISTANCE IN LEUKAEMIA

By

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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/VLB 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 (VLB) 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

1. P. Galettis, J. Boutagy and D.D.F. Ma. (1994) Daunorubicin pharmacokinetics and the correlation with p-glycoprotein and treatment response in patients with acute leukaemia. *Br. J. Cancer* **70**, 324 - 329

Publications in preparation

1. P. Galettis, J. Boutagy and D.D.F. Ma. (1996) Effects of the MDR reversing agents, cyclosporin A and trifluoperazine, on daunorubicin accumulation and retention in patient leukaemic cells.

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

1. P. Galettis, D.D.F. Ma and J. Boutagy. (1990) Pharmacokinetics of daunorubicin and correlation with treatment outcome in acute leukaemia. Annual Scientific Meeting of the Haematology Society of Australia, Christchurch.

2. P. Galettis, D.D.F. Ma and J. Boutagy. (1990) Accumulation and retention of daunorubicin in the blasts from patients with leukaemia. Annual Scientific Meeting of the Haematology Society of Australia, Christchurch.

3. P. Galettis, D.D.F. Ma and J. Boutagy. (1991) Pharmacokinetics of daunorubicin and correlation with treatment outcome in acute leukaemia. AACR Special Conference in Cancer Research. Membrane Transport in Multidrug Resistance, Development and Disease, Banff.

4. D.D.F. Ma, P. Galettis, J. McLachlan and J. Boutagy. (1991) Clinical significance of in-vitro daunorubicin accumulation and retention in human leukaemias. VII Congress, Asian-Pacific Division, International Society of Haematology.

5. P. Galettis, J. Boutagy and D.D.F. Ma. (1992) Effects of MDR reversing agents

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

6. J. Boutagy, P. Galettis and D.D.F. Ma. (1992) Relationship between treatment outcome, p-glycoprotein and daunorubicin pharmacokinetics. Vth World Conference on Clinical Pharmacology and Therapeutics, Yokohama.

7. P. Galettis, J. Boutagy and D.D.F. Ma. (1992) Effects of MDR reversing agents on cell lines and patient leukaemic cells. Annual Scientific Meeting of the Australasian Society of Clinical and Experimental Pharmacology and Toxicology, Sydney.

8. P. Galettis, J. Boutagy and D.D.F. Ma. (1993) Reversal of drug resistance by cyclosporin A via a non p-glycoprotein mechanism. Annual Scientific Meeting of the Australasian Society of Clinical and Experimental Pharmacology and Toxicology, Brisbane.

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.

Peter Galettis

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Glossary of Abbreviations

ACDA	acid citrate dextrose A
ADR	HL 60/ADR, doxorubicin resistant HL 60 subline
ALL	acute lymphocytic leukaemia
AML	acute myeloid leukaemia
ANLL	acute nonlymphocytic leukaemia
Ara C	cytosine arabinoside
at-MDR	atypical multidrug resistance
AUC	area under the curve
AUMC	area under the first moment curve
CEM	T cell lymphoblastic leukaemia cell line
CL	clearance
CL-PVA	cross linked polyvinyl alcohol
Cmax	maximum drug concentration
CR	complete remission
Cy A	cyclosporin A
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNR	daunorubicin
DOL	daunorubicinol
DOX	doxorubicin
DSIM	double strength iscoves medium
EPI	epirubicin

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

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