INDUCTION OF DRUG RESISTANCE AND DIFFERENTIATION IN HUMAN LEUKAEMIA CELL LINES

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ABSTRACT

The ability of low, clinically relevant levels of the chemotherapeutic drugs epirubicin and vinblastine to induce drug resistance was examined in the K562. U937, KG-1a and HEL human leukaemia cell lines. Treatment with epirubicin and vinblastine induced the MDR phenotype and P-glycoprotein expression in K562 and U937 cells. However this treatment had no effect on drug resistance in the P-glycoprotein expressing KG-1a and HEL cells. In the U937 cells, drug resistant cells were not only MDR but were also resistant to other drugs including cisplatinum and chlorambucil which are not normally associated with MDR. The drug resistant U937 sublines were also sensitised to doxorubicin, cisplatinum and chlorambucil by buthionine sulphoximine (BSO), suggesting that glutathione-related mechanisms also contributed to resistance in these sublines. The U937 sublines also had an increased DNA content and an increased ability to recover from DNA damage, as determined by cell cycle analysis, indicating that the broad cross-resistance exhibited by these cells was due to the co-existence of multiple resistance mechanisms. Drug treatment induced changes in expression of differentiation associated antigens in all four cell lines.

Treatment with inducers of differentiation (TPA, sodium butyrate, granulocyte-macrophage colony-stimulating factor; GM-CSF). Treatment of K562 and K562/E15B cells with TPA induced megakaryocytic differentiation, with increases in drug resistance, and increased P-glycoprotein expression in the K562/E15B subline. TPA induced monocytic differentiation in the U937 cells but not the U937/E15 subline, with increased P-glycoprotein expression and function in the U937/E15 cells but not the U937 cells. Staurosporine, an inhibitor of PKC, inhibited differentiation in these cell lines, but did not inhibit increases in P-glycoprotein expression, suggesting drug resistance was not mediated by PKC.

Sodium butyrate induced erythroid differentiation, and increased P-glycoprotein expression in the K562/E15B cells. However at a higher concentration (15 mM) this was not accompanied by increased drug resistance. Granulocyte monocyte colony stimulating factor (GM-CSF) did not induce differentiation in the K562 cells or K562/E15B subline, although the K562/E15B cells became more drug resistant after treatment with GM-CSF. Treatment with GM-CSF induced differentiation in the U937/E15 subline but did not change drug resistance in either the U937 cells or the U937/E15 subline.

Therefore the P-glycoprotein expressing K562/E15B and U937/E15 sublines were more responsive to inducers of differentiation than the parental cell lines. Induction of differentiation therefore induced increases in P-glycoprotein expression and drug resistance, suggesting that expression of P-glycoprotein or a multidrug resistance phenotype was associated with differentiation.

Abbreviations

Abbreviation	Full Name
AML	acute myeloid leukaemia
ALL	acute lymphocytic leukaemia
APAAP	alkaline phosphatase anti-alkaline phosphatase
BCIP	5-bromo,4-chloro,3-indolyphosphate
BSO	buthionine sulphoximine
CML	chronic myeloid leukaemia
CLL	chronic lymphocytic leukaemia
COL	colchicine
DMSO	dimethylsulfoxide
DNR	daunorubicin
DOX	doxorubicin
DTT	dithiothreitol
EDTA	ethylene diamine triacetic acid
EPR	epirubicin
GM-CSF	granulocyte-macrophage colony-stimulating factor
GSH	glutathione (reduced)
GST	glutathione-S-Transferase
h	hour
IDA	idarubicin
MDR	multidrug resistance
min	minutes
MTT	3-4,5-dimethylthiazol-2,5 diphenyl tetrazolium bromide
NBT	nitro blue-tetrazolium
PAGE	polyacrylamide gel electrophoresis
PI	propidium iodide
PBS	phosphate buffered saline
PKC	protein kinase C
Rh123	rhodamine 123
SDS	sodium dodecyl sulphate
STP	staurosporine
TBS	tris buffered saline
TEMED	tetramethylethylenediamine
topo II	topoisomerase II

TPA	12-O-tetradecanoylphorbol-13-acetate
VCR	vincristine
VER	verapamil
VLB	vinblastine
VP-16	etoposide

PUBLICATIONS

1. Denese C. Marks, Larissa Belov, Mary W. Davey, Ross A. Davey and Antony D. Kidman. (1992) The MTT cell viability assay for cytotoxicity testing in multidrug resistant human leukemic cells. *Leukemia Research*, **16**;1165-1173.

2. Denese C. Marks, Mary W. Davey, Ross A. Davey and Antony D. Kidman. (1993) Differentiation and multidrug resistance in response to drug treatment in the K562 human leukaemia cell line. *British Journal of Haematology*, **84**;.83-89.

ABSTRACTS AND PRESENTATIONS

1. Poster presentation entitled "The establishment of a colorimetric (MTT) assay for cytotoxicity testing" at the Australian Society for Biochemistry and Molecular Biology. Sydney, Australia. September, 1990.

2. Poster presentation entitled "Development and characterisation of drug resistant K562 cell lines" at the American Association for Cancer Research Special Conference: Membrane transport in multidrug resistance, development and disease. Banff, Alberta, Canada. March, 1991.

3. Poster presentation entitled "Is P-glycoprotien clinically relevant" at the Australian Society for Medical Research. Canberra, Australia. December, 1991.

4. Poster presentation entitled "Induction of haematopoietic differentiation and multidrug resistance in human leukaemic cell lines"at the Australian Society for Medical Research conference. Brisbane, Australia. December, 1992.

5. Poster presentation entitled "Induction of haematopoietic differentiation and multidrug resistance in human leukaemic cell lines" at the General Motors Cancer Research Foundation International Symposium "Resistance against anticancer drugs: Molecular mechanisms and clinical opportunities". Toronto, Canada. May, 1993.

6. Oral presentation entitled "Induction of megakaryocytic differentiation and drug resistance in human leukaemic K562 cell lines" at the Royal North Shore Hospital/University of Technology, Sydney Annual Scientific Meeting. Sydney, Australia. November, 1993.

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RESULTS AND DISCUSSION