61

DNA AMPLIFICATION AND ANTIMETABOLITE RESISTANCE

A.H. Calvert

62

CELLULAR RESISTANCE TO DNA TOPOISOMERASE II INHIBITORS.

A. Jacquemin-Sablon, M.R. Casabianca-Pignède, S. Crémier, C. Delaporte, T. Khelifa, A.K. Larsen, B. René and J.M. Saucier. U140 INSERM - URA147 CNRS, Unité de Biochimie, Institut Gustave-Roussy, 94805 Villejuif, France.

Chinese hamster lung cells resistant to 9-OH-ellipticine (DC-3F/9-OH-E) exhibit the following phenotypic changes :

- DC-3F/9-OH-E cells, about 150-fold resistant to 9-OH-ellipticine, are cross-resistant to other topoisomerase II inhibitors such as m-AMSA and VP-16. Furthermore, these cells are cross-resistant to suramine which also inhibits topoisomerase II activity but does not stabilize the DNA-enzyme cleavable complex and inhibits the m-AMSA induced stabilization of this complex. Finally, through the overexpression of the MDR system, DC-3F/9-OH-E displays variable levels of cross-resistance to drugs like actinomycin D, vincristine and taxol.
- DC-3F/9-OH-E cells exhibit a decreased topoisomerase II activity as demonstrated by determination of the enzyme activity (kinetoplastic DNA decatenation), Northern blot analysis of the enzyme transcripts and Western blot analysis .
- Finally DC-3F/9-OH-E cells injected to nude mice display a loss of tumorigenicity. The myc gene is about 10-fold amplified and 30-fold overexpressed in the parental DC-3F cells. Both amplification and overexpression are lost in the resistant cells. Transfection of the myc gene in the DC-3F/9-OH-E cells did not restore the tumorigenicity and did not alter the resistance to topoisomerase II inhibitors. However, the MDR phenotype was reversed, roughly in proportion of the myc gene expression. The mechanism of this reversion will be discussed.

63

MULTIDRUG RESISTANCE AND KINETICS OF DRUG TRANSPORT.

Skovsgaard, T., Department of Oncology, University Hospital of Herlev, Denmark

The membrane transport is an essential component of the mechanism of action and the mechanism of resistance of cytostatics. In the case of the anthracyclines and vincaalkaloids reduced drug accumulation is the most common finding in resistant cells. This change is narrowly related to occurence of the glycoprotein PgP in the membrane. Several studies support that drug uptake may be described by a "leak and pump" model i.e. a passive influx of the uncharged form of the molecule together with an active drug efflux. However a direct proof for active efflux implies measurement of the free cytoplasmatic drug concentration, which is technically questionable. Using three different experimental models, indirect information about the kinetic of efflux of daunorubicin will be presented. In all cases Ehrlich ascites tumor cells with classical characteristics of MDR were used. In model I the determination of efflux is based on data obtained in the steady-state situation which involves that influx is equal to efflux. The influx function is determined in separate experiments. In model II the cells are loaded with drug after depletion of ATP and subsequently the efflux is started by addition of glucose. As the extracellular medium in this case contains drug, the efflux must be corrected for the influx which takes place simultaneously. In model III cells are loaded with drug in standard medium. After washing the initial efflux is measured in a drug-free medium (unidirectional efflux). In all cases the efflux is a function of the free intracellular drug concentration. This value is estimated from drug binding studies of whole cells at equilibrium. This condition is obtained by elimination of the active efflux process by inhibition with sodium azide or verapamil. The kinetic of efflux obtained by the three different methods are compared and discussed.

64

MULTIDRUG RESISTANCE IN HUMAN LUNG CANCER

Baas, F[1,2]., Eijdems, E.W.H.M.[2], de Haas, M.[2], Borst, P[2].
[1,2]Dept. Neurology, AMC and [2]The Netherlands Cancer Institute, 1066CX, Amsterdam, The Netherlands.

Exposure of cancer cells to a single drug may induce resistance to a wide variety of anti-cancer agents. This phenomenon is called multidrug resistance (mdr) and in some cases due to overproduction of the mdr1 P-glycoprotein. Overexpression of mdr1, however, cannot explain all forms of mdr. To obtain more insight in mechanisms for resistance, we have generated a series of doxorubicin resistant cell lines by selecting the drug sensitive human lung cancer cell line SW-1573 with low dosis of doxorubicin. Analysis of the resistant cell lines showed that: 1) almost all resistant cell lines were also resistant to vincristine, indicating that mdr was induced; 2) the mdr cell lines have a decreased accumulation of drugs, but seldom overproduce mdr1 Pgp mRNA; 3) the majority of the resistant cell lines showed a decrease of topoisomerase II mRNA; 4) only high levels of dox selection induced P-glycoprotein mediated MDR.

65

STRUCTURE ACTIVITY RELATION OF ANTHRACYCLINES (AC) IN MULTIDRUG RESISTANCE

R. Erttmann, S. Bielack, K. Kallenbach, K. Beer, *F. Gieseler, G. Looft, K. Winkler
Dep. Ped. Hemat. Oncol. University of Hamburg GFR
*Med. Poliklinik University of Würzburg GFR

Structurally modified ACs are beeing evaluated against p-glykoprotein and topoisomerase-II related multidrug resistance using a doxorubicin-selected F4-6/F4-6R Friend erythroleukemia cell system. In comparison with the classical ACs doxorubicin and daunorubicin 50% inhibitory concentrations in the sensitive (ID 50-S) and in the resistant cell line (ID 50-R) as well as the resistance factors (ID 50-R/ID 50-S) could be changed remarkably by structural modifications of the anthracyclines:

Anthracycline	ID50-S ng/ml	ID50-R ng/ml	RF
Doxorubicin	8.7	1540	177
Daunorubicin	8.0	680	85
Idarubicin	3.6	31	8.6
Aclacinomycin A	5.9	29	4.9
Morpholino-DOX	9.3	39	4.2
Cyanomorpholino-DOX	0.015	0.052	3.5
Methoxymorpholino-DOX	1.9	5.5	2.9
MX2	7.8	12.1	1.6

In our model MDR could be almost completely overcome by the N-morpholinyl substituted agents methoxymorpholino-DOX and MX2.

Support.by:Fördergemeinschaft Kinderkrebszentrum Hamburg
Werner-Otto-Stiftung Hamburg

66

STRUCTURE ACTIVITY RELATIONSHIP OF MULTIDRUG REVERSAL

Ramu A & Ramu N
Dept. of Oncology, Hadassah University Hospital.
P.O. Box 12000, Jerusalem 91120, Israel

The multidrug resistance (MDR)-reversal activity of 573 compounds was tested in MDR P388 cells in vitro. Such an activity was found among compounds exhibiting 2-3 ring structures, such as phenyl (but not hydroxy- or amino-phenyl), or compounds where one phenyl was substituted by cyclopentyl, cyclohexyl, thienyl, 5-norbornen, indole, 3,4-dihydrocarbostryl, quinazoline, phthalazine or benzodioxazole.

These rings are linked by 1-2 bridges of a variety of types, to a secondary or tertiary amine groups.

The most active MDR-reversing compounds possess 2-3 phenyls linked by 1-2 bridges to a cyclic secondary amine or to cyclic or non-cyclic tertiary amine group. They also possess 1-2 carbonyls and/or 3,4-dimethoxy-phenyl residues.