THE ROLE OF TOPOISOMERASE II IN AMSACRINE RESISTANCE

SANDRA J. RATTRAY (neé DARKIN)

Department of Cellular and Molecular Biology

University of Auckland.

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FRONTISPIECE

There is no end, indeed, to making books or experiments, or, to travel, or to gathering wealth. Problem gives rise to problem. We may study for ever, and we are never as learned as we would. We have never made a statue worthy of our dreams. And when we have discovered a continent, or crossed a chain of mountains, it is only to find another ocean or another plain upon the farther side. In the infinite universe there is room for our swiftest diligence and to spare. It is not like the works of Carlyle, which can be read to an end. Even in a corner of it, in a private park, or in the neighbourhood of a single hamlet, the weather and the seasons keep so deftly changing that although we walk there for a lifetime there will be always something to startle and delight us.

Robert Louis Stevenson
(out of El dorado)

ABSTRACT

1. Antitumour agents from many different chemical classes including acridines, have been shown to induce topoisomerase II-associated DNA breaks both in cultured mammalian cells and in vitro in the presence of purified mammalian DNA topoisomerase II. However, mechanisms linking these protein-associated DNA breaks with drug cytotoxicity are poorly understood. Whether this type of DNA damage is responsible for drug cytotoxicty was investigated using a number of acridine derivatives belonging to the same chemical class but with varying potency in vivo. The modulation of topoisomerase II-mediated DNA breaks in whole L1210 cells, isolated nuclei and nuclear extract systems correlated with drug-induced cytotoxicity, at least for the drugs belonging to the amsacrine lineage. In contrast, there was no direct relationship between the inhibition of topoisomerase II strand-passing activity and the cytotoxic action of topoisomerase II-specific anticancer drugs.

It was concluded that drug-induced formation and stabilization of the topoisomerase II-DNA cleavable complex, rather than inhibition of its formation or of strand-passing functions, is responsible for DNA strand breakage and cell death caused by antitumour acridines such as amsacrine.

2. Two tissue culture systems (CHO-AA8 cells or P815 cell cycle mutants) were used as models to study the involvement of topoisomerase II in the resistance of non-cycling cells to amsacrine.

Plateau-phase CHO-AA8 cells with a G0/G1 DNA content are resistant to amsacrine and contain fewer DNA breaks than log-phase cells after drug treatment (Robbie et al., 1988). The observations of Robbie et al. (1988) were further investigated in this thesis. The phage P4 DNA

unknotting activity in CHO-AA8 cell nuclear extracts decreased 2-fold when the cells entered plateau-phase, but there was no difference in the sensitivity of unknotting to amsacrine between log- and plateau-phase nuclear extracts. Furthermore, drug stimulation of protein-DNA complex formation was similar in whole cells, isolated nuclei and nuclear extracts from either log- or plateau-phase cells. However, stimulation of complex formation in cells, nuclei or nuclear extracts was approximately 4-fold lower in plateau-phase than in log-phase. These results suggested that drug-enzyme interaction was altered in plateau-phase and there was a good correlation between the proliferative state of cells, amsacrine sensitivity, DNA breakage and topoisomerase II-DNA complex formation.

The second system used to study the effect of amsacrine was the cold-sensitive (proliferating at 39.5^{o}C; reversibly arrested in G1-phase at 33^{o}C) cell-cycle mutant 21-Fb of the murine mastocytoma cell line, P815. The sensitivity of arrested 21-FB cells to amsacrine decreased less than two-fold in cell survival experiments when compared to proliferating cells. In contrast, DNA breakage and stimulation of protein-DNA complex formation in intact cells, lysed cells or isolated nuclei was reduced approximately 10-fold in amsacrine-treated arrested cells and DNA-topoisomerase II activity in arrested cells was only 5 % of the activity in proliferating cells. Thus in contrast to the CHO-AA8 cell system, there was no correlation between cell survival and DNA damage or DNA topisomerase II activity in drug treated 21-Fb cells.

The exact reasons for the differences in amsacrine sensitivity in growth arrested CHO and 21-Fb cells were not resolved. However, it was concluded that a complex relationship exists between amsacrine-induced DNA breakage, topoisomerase II-DNA complex formation, topoisomerase activity and drug cytotoxicity. Moreover, a very complex set of parameters can

influence drug-induced topoisomerase II-mediated lesions and cytotoxicity in different cells or even a single type of cell under different growth conditions.

3. Extracts of K21 murine mastocytoma cells were found to contain a factor that enhances formation of amsacrine-induced topoisomerase II-DNA complexes (PDC) when added to isolated K21 nuclei. The PDC enhancing activity was reduced in extracts from 2 or 6 h cycloheximide- or cordycepin-treated cells, implying that continuous protein synthesis is required to maintain the factor. Preliminary characterization showed that the factor was heat labile and proteinase-sensitive suggesting the factor was a labile protein which was distinct from the two known classes of topoisomerase.

The protein factor was present in at least four other cell lines and was substantially reduced in cells induced into a G1 state by temperature arrest or serum deprivation. Human Jurkat cells selected for resistance to amsacrine and displaying cross-resistance to other topoisomerase II-targeted drugs also exhibited significantly reduced PDC enhancing activity. These results suggested a contributing role for the PDC enhancing factor in mediation of drug resistance.

Fractionation of mouse mastocytoma cell cytoplasmic extracts by salt precipitation, DEAE-cellulose chromatography and SDS-PAGE resulted in a 3571-fold purification of the PDC enhancing activity. The PDC enhancing activity was shown to reside in a 70 kDa protein kinase with specificity for a casein kinase II substrate and sensitive to heparin and anti-casein kinase II antiserum.

This appears to be the first direct evidence of a protein factor that modulates amsacrine induced topoisomerase II action possibly by

phosphorylating topoisomerase II or proteins associated with topoisomerase II.

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ABBREVIATIONS

9-AA	*9-aminoacridine*
ALL	*acute lymphoblastic leukemia*
α-MEM	*alpha modified minimum essential medium*
AMSA	*4'-(9-acridinylanine)methanesulphonanilide*
[γ-^{32}P]-dATP	*deoxyadenoside 5'-[γ-^{32}P] triphosphate*
ATP	*adenosine 5'-triphosphate*
bisacrylamide	*N,N'-methylenebisacrylamide*
BSA	*bovine serum albumin*
cDNA	*complementary DNA*
CHO	*chinese hamster ovary cells*
CI-921	*N,5'-dimethyl-9-[(2-methoxy-4-methyl-sulphonylamine)-phenylamino]-4-acridine carboxamide.*
DAPI	*4',6'-diamino-2-phenylindole*
dATP	*2'-deoxyadenosine 5'-triphosphate*
ddH2O	*double distilled water*
dGTP	*2'-deoxyguanosine 5'-triphosphate*
DMSO	*dimethyl sulphoxide*
DSB	*(DNA) double-strand break*
DTT	*1,4-dithiothreitol*
dTTP	*2'-deoxythymidine 5'-triphosphate*
EDTA	*ethylene diamine tetraacetic acid*
EGTA	*ethylene gycol bis(β-aminoethylether)-N,N'-tetraacetic acid*
FADU	*fluorescence assay for DNA unwinding*
FCS	*fetal calf serum*
FPLC	*fast pressure liquid chromatography*
HMG	*high mobility group (protein)*
HPLC	*high pressure liquid chromatography*
hsp	*heat shock protein*
m-AMSA	*4'-(9-acridinylamine)methanesulphon-m-anisidide*
o-AMSA	*4'-(9-acridinylamine)methanesulphon-o-anisidide*
PBS	*phosphate-buffered saline*
PDC	*protein-DNA-complex*
POPOP	*1,4-bis-[2-(5-phenyloxazolyl)] benzene*
PPO	*2,5-diphenyloxazole*
QSAR	*quantitative structure-activity relationship*
SCE	*sister chromatid exchange*
SD	*standard deviation*
SDS	*sodium dodecyl sulphate*
SE	*standard error*
TBS	*Tris buffered saline*
TCA	*trichloroacetic acid*
TEMED	*N,N,N',N'-tetramethylenediamine*
Tris	*tris(hydroxymethyl)aminomethane*