Eur J Cancer, Vol. 27, No. 8, p. 1064, 1991.
Printed in Great Britain
0277-5379/91 $3.00 + 0.00

Conserved Cytostatic Activity of Aclarubicin in a Doxorubicin Selected Friend Leukaemia Cell Line with Multifactorial Multidrug Resistance

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IDENTIFICATION of anthracyclines which are able to overcome multidrug resistance (MDR) would be a major step to improve antileukaemic chemotherapy.

Recently it has been shown by Coley *et al.* [1] that 9-alkyl substitution of the classical anthracycline structure leads to the retention of cytostatic activity in a multidrug resistant mouse mammary tumour cell line as well as in a multidrug resistant human small cell lung cancer cell line. To confirm this important result, we investigated the cytostatic activity of several anthracyclines including aclarubicin (aclacinomycin A) as a 9-alkyl derivative in a doxorubicin resistant mouse leukaemia cell line. This cell line has been shown to be resistant by at least two different molecular mechanisms: the classical P-glycoprotein mediated MDR mechanism and by reduced DNA topoisomerase II activity.

The F4-6R cell line used has been selected by long-term exposure of the Friend mouse erythroleukaemia cell line (F4-6) to increasing concentrations of doxorubicin.

The F4-6R cell line exhibits hyperexpression of P-glycoprotein as proven by immunohistochemistry using the MDR-Ab antibody (Oncogene Science) as well as the C 219 monoclonal antibody (Centocor) [2]. Moreover, the F4-6R cell line has a reduced net uptake due to an enhanced efflux of anthracyclines as shown by the silicone oil filtration method as well as by direct immunofluorescence microscopy [3, 4].

In addition, it could be shown that the F4-6R cell line which has been selected exclusively by doxorubicin does express a resistance pattern broader than the classical MDR mediated by P-glycoprotein. This is due to a reduced DNA topoisomerase II activity compared with the wild type (F4-6) as we proved by the plasmid pBR322 relaxation assay [2].

The sensitivity of the F4-6R cell as well as of its parent line F4-6 to cytostatic substances has been tested in the 48 h proliferation assay. Resistance factors of each cytostatic drug have been determined as the ratio between the 50% inhibitory dosages (ID_{50}) for the F4-6R and the F4-6 cell lines.

As shown in Table 1, the F4-6R cell line shows an extended spectrum of crossresistance compared with classical MDR, including DNA topoisomerase II related drugs such as mitoxantrone and amsacrine. This may reflect its reduced DNA topoisomerase II activity measured by the pBR322 relaxation assay. In concordance with the recent findings of Coley *et al.* [1], aclarubicin nearly retains its activity against the doxorubicin selected F4-6R cell line in contrast to daunorubicin and idarubicin.

Table 1. Crossresistance of the F4-6R leukaemic cell line

Cytostatic drug	Resistance factor
Doxorubicin	51
Daunorubicin	35
Idarubicin	12
Vincristine	19
Etoposide	17
Mitoxantrone	16
Amsacrine	11
Aclarubicin	3

Resistance factor = ID_{50} F4-6R ÷ ID_{50} F4-6

This result gives further evidence that the 9-alkyl substitution of the anthracycline structure may provide protection against the P-glycoprotein efflux pumping system of MDR cells. In addition its special structure may improve the interaction of aclarubicin with altered DNA topoisomerase II to overcome "atypical" MDR, too. This question should be investigated further using atypically resistant P-glycoprotein negative cell lines. Those models are described by several working groups [5, 6]. Whether the trisaccharide side-chain of aclarubicin does contribute to its anti-MDR properties cannot be excluded and remains to be answered.

In conclusion our *in vitro* data confirm the results of Coley *et al.* [1] that there is a correlation between 9-alkyl substitution and cytotoxic activity of anthracyclines in P-glycoprotein positive multidrug resistant tumour cells. Moreover, it may be speculated, that the molecular structure of aclarubicin retains its cytotoxic activity against atypically MDR leukaemic cells, too.

The favourable structure activity relationship of aclarubicin in MDR resistant cells found *in vitro* seems to correlate with its appreciable antileukaemic activity in highly pretreated patients with actue myeloblastic leukaemia [7–9].

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Acknowledgements—This study was supported by Hamburger Krebsgesellschaft e.V., Fördergemeinschaft zur Erforschung und Heilung von Krebskrankheiten im Kindesalter e.V., Hamburg.