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Morpholinyl Anthracyclines: Option for Reversal of Anthracycline Resistance

ANTHRACYCLINES are potent chemotherapeutic drugs whose mechanisms of action are still intriguing. The most widely used are doxorubicin and daunorubicin. Intrinsic or acquired anthracycline resistance in tumours hampers their clinical effectiveness. Efficacy, toxicity and drug resistance may be related to different parts of the anthracycline molecule. Helen Coley and her colleagues (p. 665) add to our knowledge of newly synthesized anthracycline derivatives which have several attractive features.

Anthracyclines may be cytotoxic to cells at the outer cell membrane [1]. In general, however, to be cytotoxic, the drug has to enter the cell. One of the earliest proposed mechanisms is DNA intercalation. The anthracycline chromophore fits stereometrically between two base-pairs, preventing replication and transcription and leading to cell death [2]. Another possible mechanism is the ability to turn topoisomerases into cellular toxins [3]. This, also, is not a full explanation because the *in vitro* capacity to inhibit topoisomerase II is not linearly related to cytotoxicity [3]. Anthracyclines can induce DNA damage. After exposure to anthracyclines, DNA–DNA crosslinks, DNA–protein crosslinks, and single-stranded and double-stranded breaks can be detected. This could, at least in part, be due to the generation of free radicals by the quinone moiety [4]. None of these mechanisms can be seen as the principal cause of cytotoxicity; all probably participate to varying extents depending on the structure of the anthracycline.

The cell membrane plays, in contrast to its limited role in cytotoxicity, an important part as a first-line defence in drug resistance. The most extensively studied mechanism is multidrug resistance (MDR) mediated by P-glycoprotein. The P-glycoprotein mediated membrane pump removes anthracyclines that have entered the cells by diffusion through the lipid compartment of the membrane [5]. The result is decreased intracellular drug concentrations. Other membrane pumps have also been described [5]. Alternative mechanisms of anthracycline resistance include decreased topoisomerase II activity and enhanced detoxifying enzyme activities, including raised glutathione peroxidase and glutathione S-transferase [5–8].

The ideal anthracycline should have several properties. It should have at least the same anti-tumour activity as the parent compound. Therefore, all the supposed mechanisms of action should be present and additional mechanisms would be welcome. In addition, the compound should not be affected by anthracycline resistance mechanisms. Several drugs partly fulfil these requirements. In *in vitro* systems, these drugs have good anti-tumour activity and also show activity in cell lines with various resistance mechanisms [9]. Their greater effectiveness is primarily due to increased lipophilicity, itself leading to higher intracellular drug levels. Some of these drugs have been tested in clinical trials. Examples include idarubicin, detorubicin and esorubicin. However, the advantage of the analogues over doxorubicin is limited [10].

Interesting analogues are the 3′ *N*-morpholinyl substituents. These drugs have several of the required properties. Morpholinyl anthracyclines are highly lipophilic, diffuse rapidly through the cell membrane, and reach high intracellular levels [11].

Intercalation in DNA occurs in the same way as for doxorubicin. In particular, cyanomorpholinyl substituted anthracyclines can be activated, after which they bind covalently to DNA [11–13]. This mechanism could contribute to cytotoxicity and is a new finding compared with doxorubicin. The binding is very rapid—the drug is bound to DNA before it can be pumped out by P-glycoprotein or other membrane pumps [11, 12]. *In vitro* and *in vivo* evidence from Coley and colleagues and others [11, 12, 14, 15] suggests that these drugs can overcome resistance. Doxorubicin-resistant cell lines with resistance due to an altered topoisomerase II were not cross-resistant to morpholinyl anthracyclines [14, 16]. These drugs also show promise for the treatment of topoisomerase II related resistance. The morpholinyl anthracyclines can generate free radicals. In human ovarian carcinoma cells made resistant to cyanomorpholino-doxorubicin, an increased free radical scavenging system was found [17]. Potential cardiotoxicity due to free radicals will not be of clinical importance because morpholinyl anthracyclines are 100–1000 fold more active than the parent drug and will therefore be administered at lower dose. The morpholinyl anthracycline structure can possibly be further improved by substitution of other parts of the molecule. Coley and colleagues describe 9-alkyl substitutions that circumvent resistance. Two phase I studies with the morpholinyl anthracyline MX2 have been reported: haematological toxicity was dose-limiting but no cardiac toxicity occurred [18, 19].

MDR associated with P-glycoprotein expression is the best studied resistance mechanism [5, 6]. P-Glycoprotein is expressed in several normal tissues as well as in various tumours [20]. There are, however, many tumours that show intrinsic or acquired doxorubicin resistance mostly without P-glycoprotein expression. The frequency of other mechanisms, such as topoisomerase II related anthracycline resistance, in human tumours is not yet clear. To date, clinical trials with anthracyclines and modulators that block the P-glycoprotein pump have had limited success. This is partly because full use of some modulators is hampered by side-effects and it is often not known whether the resistant tumours are actually P-glycoprotein positive.

Resistance mechanisms may vary between tumour types. Heterogeneity may also occur within a tumour and various mechanisms may even play a role within one tumour cell. Therefore, new anthracycline analogues with activity against P-glycoprotein MDR cells as well as against cells with atypical (topoisomerase II) mediated MDR are extremely interesting clinically. Whether the new morpholinyl analogues will have good anti-tumour activity in the clinic remains to be seen.

Anthracyclines have impressive clinical activity, although the exact mechanism of action is not known and the drugs are subject to several cellular resistance mechanisms. Based on factors known to determine cytotoxicity, improved anthracycline structures can be synthesized with high activity, resistance-modifying effects, and decreased cumulative cardiotoxicity.

Elisabeth G.E. de Vries
Jan G. Zijlstra
Division of Medical Oncology
Department of Internal Medicine
University Hospital Groningen
The Netherlands

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