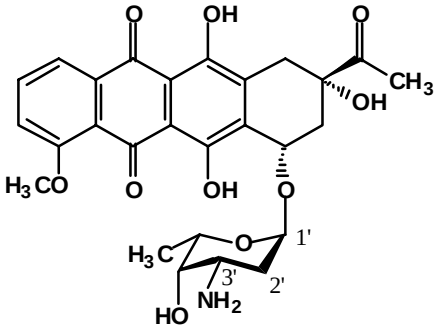
Targeting DNA with Anthracyclines: The Importance of the Sugar Moiety

Waldemar Priebe

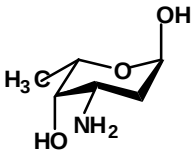
The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Our studies focusing on the role of the sugar portion in anthracycline-DNA interaction laid the foundation for the design of novel DNA interactive agents. We have designed and synthesized two new classes of such agents which: (1) bind with high affinity to specific sequences of DNA and (2) form cross-links with DNA.

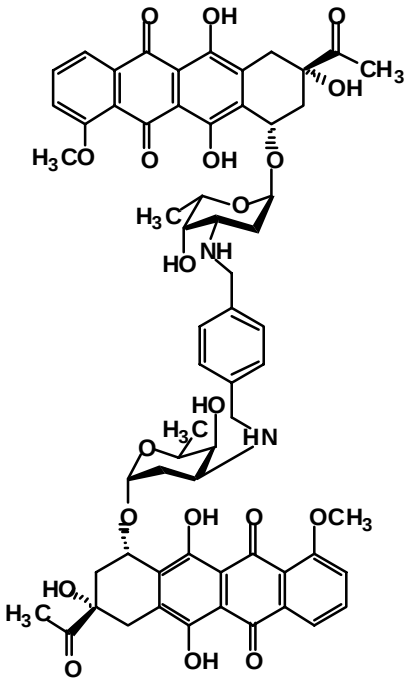
The design of agents binding with high affinity to specific sequences of DNA was based on studies of (1) the effects of a sugar portion's charge and its orientation on drug-DNA binding (daunosamine), (2) the assessment of energetic contribution to DNA binding (for daunosamine and other selected structural fragments), and (3) analysis of the crystallographic structure of the daunorubicin-DNA complex. Analysis of the structure of daunorubicin-DNA complex revealed that two daunorubicins face each other with sugar moieties and position their 3'-NH_2 groups within a distance of 6-7Å.



Daunorubicin



Daunosamine



WP631

The aglycon part serves as an intercalator, while a sugar moiety serves as a minor groove binder and is responsible for the base-pair selectivity (CGA/T). We have designed linkers to create bisintercalating, groove-binding molecules and have demonstrated that compound WP631 is, in fact, a 6-bp-recognizing agent with a DNA binding constant of $2.7x10^{11}$ M^{-1}, exceeding that of daunorubicin by a factor of 23,000. The nature of the DNA binding by selected bisanthracyclines (WP631, WP652) was confirmed by several methods including solving the x-ray structure of a complex of WP631 bound to [d(CGATCG)]2 and NMR studies of WP631 and WP652 complexes with DNA oligomers.

The bisanthracyclines exhibited unique and diverse profiles of cytotoxicity. In vitro evaluation against sensitive, MDR, and MRP-mediated multidrug resistant cells indicated that selected analogs (e.g., WP631) had unusually high activity against MRP resistant cells but remained inactive against MDR cells, while other analogs were active against both MDR and MRP cell lines. Even more surprising, the NCI's in vitro disease-oriented primary antitumor screen allowed us to identify a bisanthracycline with selective cytotoxicity against melanomas but no noticeable activity against leukemias. The cytotoxicity of WP760 against melanomas was approximately 10- to 1000-fold higher than its average cytotoxicity against other tumor cell lines.

Our other studies explored novel strategies for designing and developing selective alkylators of DNA. We have demonstrated that formaldehyde can cross-link daunorubicin with DNA in a regioselective and base-specific manner and that such a process is sequence dependent and requires the presence of an amino group at the C-3' position of the sugar moiety and occurs only with N2 of guanine. Our working hypothesis is that the process of formaldehyde-mediated cross-linking can be mimicked by introducing a sugar-based substructure into daunorubicin or doxorubicin molecules so as to allow the formation of formaldehyde-mediated alkylating intermediates without an outside source of formaldehyde. If successful, this approach could lead to a unique class of selective DNA alkylators and allow for the design of other, even more selective, anticancer drugs.

Along these lines, we have designed and synthesized two novel 3' aminoanthracycline-based compounds, WP809 and WP836, which should alkylate DNA via a base-specific process. Both compounds displayed significantly higher cytotoxicity than that of either parental daunorubicin or doxorubicin against wild-type and multidrug-resistant tumor cell lines. In brief, the compound WP836 derived from doxorubicin was 500- to more than 100,000-fold more potent than doxorubicin in in vitro tests performed in sensitive and multidrug resistant cell lines. Increased activity was also noticed for analog WP809, obtained from daunorubicin.

COMPARISON OF HCHOAND WP836 MEDIATED DNA CROSSLINKING