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DNA CROSSLINKING BY 3'-(3-CYANO-4-MORPHOLINYL)-3'-DEAMINOADRIAMYCIN IN HT-29 HUMAN COLON CARCINOMA CELLS in VITRO

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SUMMARY: The Adriamycin analog, 3'-(3-cyano-4-morpholinyl)-3'-deamino-adriamycin, is 100- to 1000-fold more active than Adriamycin as an anti-tumor agent in vivo and in vitro. The interaction of 3'-(3-cyano-4-morpholinyl)-3'deaminoadriamycin and Adriamycin with DNA has been studied in HT-29 human colon carcinoma cells in vitro. The Adriamycin analog produced significent DNA-DNA crosslinking at a drug concentration range that resulted in from 1 to 4 logs of cell kill, but did not produce any DNA-protein crosslinks. Equitoxic concentrations of Adriamycin produced no significent levels of DNA-DNA crosslinks. The formation of DNA-DNA crosslinks by the Adriamycin derivative is an additional mechanism of action that may account for the high potency of this agent.

The Adriamycin analog, CMA, is the most potent anthracycline prepared to date. Its antitumor potency is 100- to 1000-fold greater than ADR against P388 leukemia in mice (1) and 100- to 500-fold greater than the parent drug against HT-29 human colon carcinoma cells in vitro (2). Although the cause of the enhanced potency of CMA is not known, it may be related to the greater cellular uptake and slower efflux of the drug in HT-29 cells and to the more potent inhibition of both DNA and RNA synthesis in these cells compared to ADR (3). The cyanide group and an intact quinone ring are essential structural features for the enhanced activity of CMA (3) and it has been suggested that, as with saframycin A (4), CMA may bind covalently to DNA following elimination of the cyanide group (2). To examine this possibility, we have studied the interaction of CMA with DNA in HT-29 cells using alkaline elution assays (5).

Abbreviations: CMA, 3'-(3-cyano-4-morpholinyl)-3'-deaminoadriamycin; ADR, Adriamycin.

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MATERIALS AND METHODS

CMA [3'-(3-cyano-4-morpholinyl)-3'-deaminoadriamycin] and Adriamycin were gifts from Dr. E.M. Acton, Stanford Research Institute International, Menlo Park, CA. [^{14}C] Thymidine (specific activity, 50 mCi/mmol) and [^{3}H]thymidine (specific activity, 50 to 80 Ci/mmol) were obtained from New England Nuclear, Boston, MA. Proteinase K was from E. Merck, Darmstadt, West Germany and tetrapropylammonium hydroxide was from Eastman Kodak Co., Rochester, NY. Polyvinylchloride filters (2μm) were obtained from Millipore Corp., Bedford, MA. and polycarbonate filters (0.8 μm) were obtained from Nucleopore Corp., Pleasanton, CA.

Elution assays for measuring DNA-DNA crosslinking and DNA-protein crosslinking were carried out as described previously (5). HT-29 cells labelled with [^{14}C]thymidine were treated with drugs in RPMI 1640 media containing 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY) and 0.1% dimethyl formamide. The level of crosslinking was calculated as described previously (5).

RESULTS AND DISCUSSION

HT-29 cells were incubated for 2 hr at 37 with concentrations of CMA ranging from 5 to 40 nM and then assayed for crosslinks using the alkaline elution assay with proteinase K (5). Similar drug treatment has previously been shown to produce from 1 to 4 logs of cell kill in this cell line (2). The level of crosslinking showed a significent correlation with increasing drug concentration ($p < 0.001$) reaching a maximum of 150 rad equivalents (Fig. 1). In contrast, when HT-29 cells were incubated with

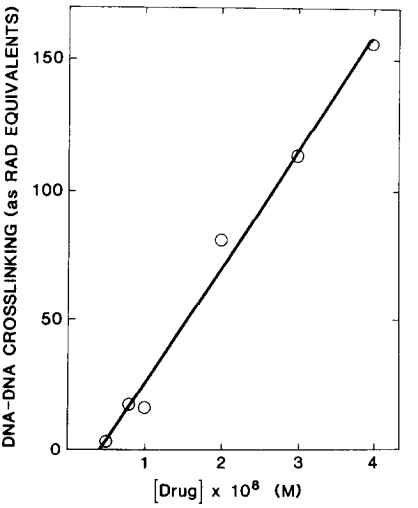

Figure 1. DNA-DNA crosslinking produced by CMA in HT-29 human colon carcinoma cells.
HT-29 cells were incubated with CMA at 37 for 2 hr. at the concentrations shown. DNA-DNA crosslinks were determined by the alkaline elution assay with proteinase K as described previously (5). The number of crosslinks, expressed as rad equivalents, is plotted against the concentration of drug used. The curve was determined by linear regression analysis.

equitoxic concentrations of ADR, no significent levels of DNA-DNA cross-linking were found.

In order to determine if CMA is able to produce additional DNA-protein crosslinks, HT-29 cells treated with CMA were assayed for crosslinks using the alkaline elution procedure without proteinase K (5). This assay measures both DNA-DNA and DNA-protein crosslinks. CMA produced no additional crosslinks with this assay, indicating that this drug does not cause DNA-protein crosslinks.

ADR and other anthracyclines have previously been shown to form DNA-protein crosslinks (6-8); however, only Konopa has reported the formation of DNA-DNA crosslinks with these agents (9). Thus, the observation that CMA can form DNA-DNA crosslinks, but not DNA-protein crosslinks may explain its high potency and provides support for the proposal that this agent acts through a different mechanism than ADR. Studies investigating the role of the cyanide group and the quinone moiety in the crosslinking activity of CMA are presently being carried out.

ACKNOWLEDGEMENTS

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