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3'-(3-CYANO-4-MORPHOLINYL)-3'-DEAMINOADRIAMYCIN: A NEW ANTHRACYCLINE WITH INTENSE POTENCY

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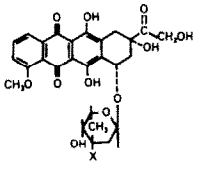
A series of anthracycline analogs has been developed recently in which the C3' amine of the daunosamine sugar has been cyclized to a morpholinyl or piperidinyl functionality (1). Although these analogs demonstrated increased potency or efficacy against P388 leukemia *in vivo* (1), they had reduced activity against the human colon carcinoma cell line HT-29 *in vitro* (2,3). Recently, the cyanomorpholino derivative of 3'-deaminoadriamycin (CMA) has been synthesized (Fig. 1). This drug is unique in that its antitumor potency is 600-fold greater than Adriamycin (ADR) against P388 leukemia in mice, although both ADR and CMA have equivalent therapeutic efficacies (4). Thus far, there has been no systematic study of the effect of CMA on cell viability and the relationship between this effect and nucleic acid synthesis.



DRUG	X
ADR	NH_2
CMA	

Fig. 1. Structures of CMA and ADR

In the present study, we wish to report the activity of CMA on the viability of the human colon carcinoma cell line HT-29 *in vitro* and the relationship of its lethal effects to the synthesis of different species of RNA.

[3]Recipient of a postdoctoral fellowship from the National Cancer Institute of Canada.

The cytocidal activities of CMA and ADR against HT-29 cells in culture are presented in Fig. 2. Following drug treatment for 2 hr, CMA was 100-fold more potent than ADR where their respective LC_{90} values were 2 x 10^{-9}M and 3 x 10^{-7}M. Prolonging drug exposure to 24 hr produced a greater enhancement in antitumor activity for CMA (50-fold) than for ADR (10-fold), and the LC_{90} for CMA and ADR was reduced to 1 x 10^{-10}M and 5 x 10^{-8}M, respectively, making CMA one of the most potent antitumor agents known. The slopes of the dose-response curves also differed in that a one and two log increase in ADR and CMA concentration, respectively, was required to reduce cell viability from 10 to 99%.

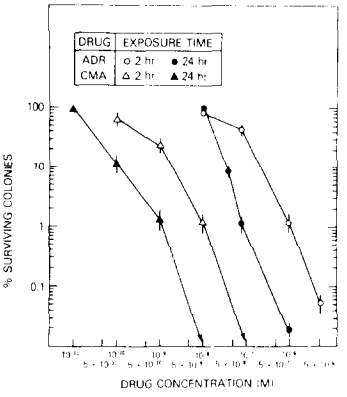


Fig. 2. Viability of HT-29 cells following exposure to CMA or ADR. HT-29 cells were treated for 2 or 24 hr with ADR or CMA, and cell viability was then determined by soft-agar cloning (2). Each value is the mean ± S.E. of 6-8 determinations.

Although the anthracyclines may produce cell death through a variety of mechanisms (3,5,6), our data indicate that the cytotoxicity of CMA is closely related to inhibition of nucleic acid synthesis (Fig. 3). Following drug treatment for 2 hr, the respective IC_{50} values for DNA and RNA syntheses were 4 x 10^{-9}M and 1 x 10^{-9}M for CMA and 5 x 10^{-7}M and 3 x 10^{-7}M for ADR (Fig. 3A). However, upon prolonging drug exposure to 24 hr, the inhibitory activity of CMA on nucleic acid synthesis was 600- to 1700-fold greater than ADR (Fig. 3B). The respective IC_{50} values for DNA and RNA syntheses were 3 x 10^{-8}M and 5 x 10^{-7}M for ADR and 5 x 11^{-11}M and 3 x 10^{-10}M for CMA.

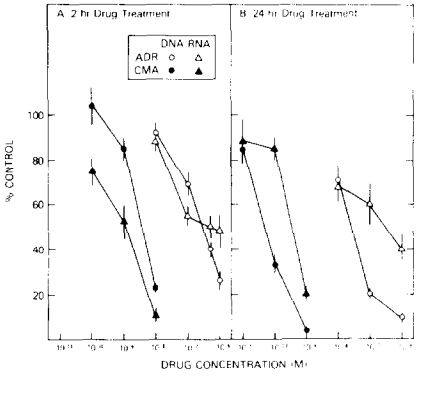


Fig. 3. Effects of CMA and ADR on DNA and RNA syntheses in HT-29 cells. HT-29 cells were exposed to ADR or CMA for either 2 or 24 hr and DNA and RNA syntheses estimated by the incorporation of [^{14}C]thymidine and [^{3}H]uridine into trichloroacetic acid (TCA)-precipitable material during the last hour of drug treatment (2). Results are expressed as the percentage of incorporation of radiolabeled precursors in drug-treated cells relative to control values. Each value is the mean ± S.E. of 6-8 determinations. Control values (dpm/10^6 cells) were: 2 hr, [^{14}C]thymidine, 44,400 ± 2,500; [^{3}H]uridine, 18,800 ± 2,200; 24 hr, [^{14}C]thymidine, 27,000 ± 2,700; [^{3}H]uridine, 15,800 ± 1,300.

It has been demonstrated previously that ADR inhibits both mRNA and rRNA syntheses (7) and that perhaps as a result of RNA binding (8) may reduce nucleocytoplasmic transport of RNA (9) as well. Thus, the effects of CMA on the synthesis of various cellular RNA fractions were examined (Table 1).

Table 1. Effects of CMA and ADR on different species of RNA*

Drug	RNA specific activity (% of control)			
	Nuclear RNA		Polysomal RNA	
	Nucleolar	Heterogeneous	Non-Poly(A)	Poly(A)
ADR, 5 x 10^{-7}M	43 ± 6	57 ± 4	62 ± 6	51 ± 3
CMA, 1 x 10^{-9}M	42 ± 7	40 ± 6	48 ± 7	33 ± 11

*Cells were prelabeled for 2 days with 25 nCi/ml of [^{14}C]uridine, chased in isotope-free medium for 1 day, and then treated with drug for 2 hr and labeled during the last hour of treatment with 1.5 μCi/ml of [^{3}H]uridine. Nuclear and polysomal RNA were isolated (10-12) and the latter RNA was fractionated on poly(U)Sepharose (12). Results are expressed as a percentage of the ratio of ^{3}H:^{14}C in RNA from drug-treated cells vs control RNA. Each value is the mean ± S.E. of 4-6 experiments. Control values (^{3}H dpm:^{14}C dpm) were: nucleolar RNA, 99,000:10,500; heterogeneous RNA, 25,500:5,300; non-poly(A)RNA, 83,000:158,000; poly(A)RNA, 7,900:3,500.

Cells were treated for 2 hr with 1 x 10^{-9}M CMA or 5 x 10^{-7}M ADR, concentrations which decreased total RNA synthesis by 50%. CMA and ADR reduced nucleolar RNA and heterogeneous nuclear RNA synthesis by 40-60%. Similarly, polysomal non-poly(A)RNA (rRNA and tRNA) was inhibited by 40-50% and poly(A)RNA (mRNA) by 50-70% by both drugs. Although CMA was a slightly more potent inhibitor of mRNA synthesis than ADR, both anthracyclines showed similar specificities for inhibiting transcription. In contrast, daunorubicin and its sugar amine analogs were found previously to have little effect on mRNA synthesis (13), suggesting that the C14 hydroxyl group is a prerequisite for inhibiting mRNA synthesis.

To examine whether CMA was a more potent inhibitor than ADR via a direct effect on DNA, transcriptional activity was assessed in a cell-free system consisting of Escherichia coli RNA polymerase and calf thymus DNA (Fig. 4).

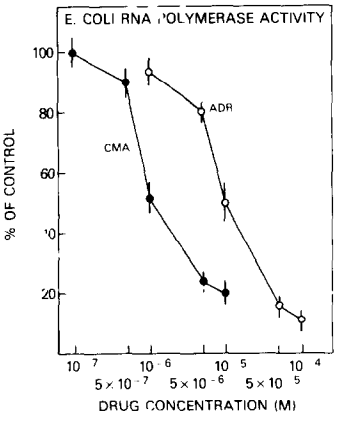


Fig. 4. Effects of ADR and CMA on E. coli RNA polymerase activity. RNA polymerase activity was measured at 30° for 10 min in a reaction (0.2 ml) which contained: 1.6 mM $MnCl_2$, 2.5 mM $MgCl_2$, 150 mM $(NH_4)_2SO_4$, 40 mM Tris-HCl (pH 7.9), 1.6 mM dithiothreitol, 100 μM each UTP, CTP, ATP and GTP, 2 μCi [5,6-^{3}H]UTP, 5 μg of calf thymus DNA, ADR or CMA dissolved in water and 0.65 units of RNA polymerase. Assays were initiated with RNA polymerase, and stopped by the addition of 10% cold TCA:2% sodium pyrophosphate, filtered onto glass fiber discs and counted. Values are presented as a percentage of control activity and represent the mean ± S.E. of 3 duplicate determinations. Control activity was 30,000 dpm per assay.

CMA was 10-fold more potent than ADR in this assay system where their respective IC_{50} values were 10^{-6}M and 10^{-5}M. Since the ΔTm of calf thymus DNA in the presence of CMA was shown previously to be 5° lower than that of ADR (4), the present experimental results suggest either an additional non-intercalative mode of binding by CMA or an altered base-pair specificity by this drug. Another possibility is the formation of an adduct between CMA and a base in DNA. Such a mechanism has been described for Safframycin A, an antibiotic similar to CMA in having a cyano group linked to a nitrogen containing heterocyclic ring which is capable of undergoing elimination and reactivity with the N2 amino group of guanine in DNA (14).

Clearly, CMA is an exciting new anthracycline both because of its extremely high potency and its clinical potential. Further studies are warranted to determine the therapeutic efficacy of CMA against human solid tumor xenografts and to determine whether its intensive potency is related to a unique interaction with chromatin.

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