Characterization of the DNA-DNA Cross-Linking Activity of 3′-(3-Cyano-4-morpholinyl)-3′-deaminoadriamycin

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ABSTRACT

3′-(3-Cyano-4-morpholinyl)-3′-deaminoadriamycin (CMA) is a highly potent analogue of the antitumor agent, Adriamycin (ADR), being up to 1500 times more cytotoxic both in vivo and in vitro. In contrast to ADR, CMA, and 5-imino-3′-(3-cyano-4-morpholinyl)-3′-deaminoadriamycin (ICMA) have been shown to possess alkylating activity, as seen by their ability to produce DNA-DNA cross-links in human and murine tumor cells and in isolated λ-phage DNA. We have compared the pharmacological activities of CMA, ICMA, and the alkylating agent, chlorambucil (CHL), in order to determine the roles of intercalation, the quinone ring, and DNA base composition, in cross-linking by CMA. CMA was 27- and 1000-fold more active than ICMA and CHL, respectively, in cross-linking DNA in L5178Y cells. In addition, the maximum level of cross-linking in L5178Y cells was reached more rapidly with CMA than with CHL, and the CMA cross-links were removed faster and more efficiently by these cells. CMA was 26- and 450-fold more active than ICMA and CHL, respectively, in producing DNA cross-links in isolated λ-phage DNA. In contrast, the alkylating activity of CMA was only 6-fold greater than CHL, as measured by the ability of the drugs to bind to the nucleophile, p-nitrobenzyl pyridine. CMA was a better DNA intercalator than ICMA, whereas CHL did not intercalate. In addition, the intercalating agent, ethidium bromide, inhibited the cross-linking activity of CMA, but not that of CHL, suggesting that intercalation contributed to the cross-linking activity of CMA. CMA produced an increasing level of cross-linking, but showed no difference in intercalation, with isolated DNA of increasing G-C content, suggesting a preference for alkylating G-C bases. Both the cross-linking and intercalating, but not the alkylating, activities of CMA and ICMA were decreased by the reducing agent, sodium borohydride, providing additional evidence that the intercalative interaction of the ADR analogues with DNA contributes to their DNA cross-linking activity. Thus, alterations to the quinone group may effect the intercalating activity of these analogues and may contribute to the difference in cross-linking activity between CMA and ICMA.

INTRODUCTION

The anthracycline antibiotic, ADR,1 is one of the most important agents used for the chemotherapy of cancer, due to its wide spectrum of antitumor activity (1, 2). However, the clinical usefulness of ADR is limited by its unfavorable side-effects, which include chronic cardiotoxicity (3) and myelosuppression (4), and by its inactivity against some tumors (1, 2). In an attempt to enhance the antitumor activity of ADR, analogues in which the C3′ amine was replaced with a morpholinyl or a substituted-morpholinyl ring have been synthesized (5). The potent of these analogues, CMA (Fig. 1), is 100–1500-fold more cytotoxic than ADR in vivo (5) and in vitro (6–9). As well, at concentrations which produce an antitumor effect in mice and rats, CMA is noncardiotoxic (5, 8), and ADR-resistant human ovarian sarcoma or P388 leukemia cells are not cross-resistant to the analogue in vitro (8, 10, 11). These findings indicate that CMA may have a different mechanism of antitumor action than ADR.

Previous studies have shown that CMA can act as an alkylating agent producing DNA-DNA cross-links in a number of tumor cell lines (9, 10, 12–15), and in isolated DNA (9, 10). This activity has not been observed with ADR, or other anthracyclines which do not contain a cyanomorpholinyl group, and may account for the enhanced potency of CMA. ICMA, the 5-imino derivative of CMA (Fig. 1), has also been shown to cross-link DNA (10), but its cytotoxic and cross-linking activities are less than those observed with CMA (5, 9, 16). These studies indicated that the cyanide group is essential for cross-linking and that the quinone ring is also involved in this activity. As cross-linking could occur in isolated DNA, metabolic activation of the agents is not required for this effect.

Since the anthracyclines are known to be good intercalators (1, 17), and there is evidence that 5-imino-substituted anthracyclines intercalate less than their parent compounds (5), it is possible that prior intercalation into DNA could potentiate the cross-linking activity of CMA and might explain its enhanced potency compared to ICMA. Thus, in the present study we have compared the alkylating and intercalating activities of CMA to those of ICMA and the clinically used alkylating agent, CHL (Fig. 1), in an attempt to determine the roles of intercalation, the quinone ring, and DNA base composition in the cross-linking activity of CMA.

MATERIALS AND METHODS

Materials. CMA and ICMA were kindly supplied by Dr. Edward M. Acton of the NIH, Bethesda, MD, dissolved in N,N-dimethyl formamide and stored in the dark at −20°C. CHL, ethidium bromide, acridine orange, NBP, sodium borohydride, and ultrapure Clostridium perfringens, Escherichia coli, and calf thymus DNAs were obtained from Sigma Chemical Co., St. Louis, MO. CHL was freshly prepared in 1% acetic acid ethanol. Isolated λ-phage DNA was from Boehringer Mannheim, Dorval, Quebec, Canada and had an approximate molecular weight of 32,000,000. Fischer’s medium and horse serum were obtained from Gibco Laboratories, Grand Island, NY. [3H]Thymidine (specific activity, 50 mCi/mmole) and [3H]Thymidine (specific activity, 50–80 CI/mmole) were obtained from New England Nuclear, Boston, MA. Protease K was from E. Merck, Darmstadt, West Germany, tetrapyrrolylammonium hydroxide was from Eastman Kodak Co., Rochester, NY, and the 0.8-μm polycarbonate filters were from Nucleopore Corp., Pleasanton, CA.

Tissue Culture. L5178Y murine lymphoma cells were grown in suspension culture at 37°C, in Fischer’s medium supplemented with 12% horse serum. Log-phase cells had a doubling time of 12 h (18). DNA Cross-Linking in L5178Y Cells. Cells were incubated in vitro at 37°C with various concentrations of the drugs for 2 h and then analyzed for DNA-DNA cross-links by the alkaline elution assay, as previously described (19, 20). The level of cross-linking was calculated as described by Kohn et al. (19), and expressed as rad equivalents.

To study the removal of DNA cross-links, L5178Y cells were incu-
CHARACTERIZATION OF DNA CROSS-LINKING BY CMA

bated with CMA (60 nM) or CHL (120 µM) for 2 h at 37°C and following removal of the drug the cells were again incubated at 37°C. At various timed intervals cell samples were analyzed for DNA-DNA cross-links by alkaline elution assay.

DNA Cross-Linking in Isolated λ-Phage DNA. Isolated λ-phage DNA (2.5 units/ml) was incubated in vitro for 100 min at 37°C with various concentrations of CMA, ICMA, or CHL, in the presence or absence of the reducing agent, sodium borohydride. For each drug, the concentration of reducing agent used was 10 times that of the highest drug concentration. To confirm that the drug was reduced, the absorbance of sodium borohydride treated CMA was measured, on a Beckman DU-8 spectrophotometer, using a wavelength scan from 220 to 600 nm. Cross-linking was measured, by the ethidium bromide fluorescence assay at pH 11.8 (21), using a Gilson Spectra/Glo Filter Fluorimeter, and expressed as percentage cross-linking (percentage of return of fluorescence after heat denaturation). A time course of cross-linking by each drug indicated that maximum cross-linking was reached at approximately 100 min. Inhibition of CMA cross-linking was determined by the addition of various concentrations of ethidium bromide to the incubation mixture. The increase in fluorescence caused by the inhibitor was corrected for by controls at each ethidium bromide concentration. A control study was carried out with the nonintercalating alkylating agent, CHL, to determine if intercalation of ethidium bromide would result in the distortion of DNA and interfere with cross-linking.

Effect of DNA Base Content on Cross-Linking. CMA cross-linking was measured, as described above, in ultrapure isolated DNAs containing different percentages of G+C residues. Clostridium perfringens, calf thymus, and E. coli DNA (5 units/ml) contained 26.5, 42, and 50% G+C, respectively, and each had a molecular weight of approximately 16,000,000.

Alkylation of NBP. The alkylating activities of CMA and CHL, in the presence or absence of sodium borohydride, were determined, as described by Linford (22), using a colorimetric measurement of the alkylation product of the drug with the nucleophile, NBP. The absorbance spectrum was recorded using a Beckman DU-8 spectrophotometer and the absorbance peaks for the alkylation products from both drugs were at 578–594 nm.

Displacement of Acridine Orange. Various concentrations of CMA, ICMA, or CHL, in the presence or absence of sodium borohydride, were added to 10 mM sodium cacodylic acid buffer (pH 6.7) containing 0.5 µM of the intercalating agent, acridine orange. Using a Gilson Spectra/Glo Filter Fluorimeter with an excitation wavelength of 480 nm and an emission wavelength of 520 nm, the fluorescence of the samples were determined both before and after the addition of 0.05 units calf thymus, C. perfringens, or E. coli DNA. Acridine orange displacement was determined, as described previously (24, 25), and the change in fluorescence after the addition of DNA was expressed as a percentage of control. For each drug, the IC50 (concentration of drug required to reduce the change in fluorescence to 50% of the control) was determined.

RESULTS

DNA Cross-Linking in L5178Y Cells. L5178Y cells were incubated in vitro for 2 h at 37°C with CMA, ICMA, or CHL, and the cells were then analyzed for DNA cross-links by the alkaline elution assay using proteinase K (Fig. 2). All three drugs produced concentration-dependent DNA-DNA cross-linking, with cross-linking reaching a level of 280 rad equivalents at 100 nM CMA, a level of 262 rad equivalents at 1.5 µM ICMA, and a level of 638 rad equivalents at 200 µM CHL. A comparison of the slopes of the concentration-response curves showed that CMA was 27-fold more active than ICMA and 1000-fold more active than CHL in producing DNA cross-links.

Cross-linking by CMA began to decrease immediately following drug removal and by 4 h cross-linking had been reduced to 22% of the maximum level (Fig. 3). In contrast, CHL cross-linking continued to increase after drug removal reaching a maximum after an additional 8 h, and then began to decrease reaching a level of 48% of maximum after 18 h.

DNA Cross-Linking in Isolated λ-Phage DNA. Isolated λ-phage DNA was incubated with CMA, ICMA, or CHL at 37°C in the presence or absence of a 10-fold excess of sodium borohydride. The broad absorbance peak of the quinone group, between 450 and 550 nm, was lost upon the addition of sodium borohydride, whereas, dithiothreitol had no effect on this peak. DNA cross-linking was measured by the ethidium bromide fluorescence assay (Fig. 4). All three drugs produced concentration-dependent cross-linking. In the absence of the reducing agent, CMA produced a level of 68% cross-linking at 7 µM, ICMA a level of 76% cross-linking at 200 µM CHL. A comparison of the slopes of the concentration-response curves showed that CMA was 26-
CHARACTERIZATION OF DNA CROSS-LINKING BY CMA

Fig. 3. Formation and removal of DNA cross-links by CMA and CHL in L5178Y cells. L5178Y cells were incubated for 2 h at 37°C with 60 nM CMA (□) or 120 nM CHL (○). The drug was removed and cells were incubated at 37°C for the time intervals shown. Cross-linking at each time point was measured by alkaline elution as described in “Materials and Methods” and expressed as rad equivalents. Points, mean of four to six determinations; bars, standard error; arrow, time when drug was removed.

Fig. 4. Cross-linking by CMA, ICMA, and CHL, in λ-phage DNA. λ-Phage DNA was incubated at 37°C with CMA, ICMA, or CHL, in the presence (□) or absence (○) of 70 mM, 2 mM, or 25 mM sodium borohydride, respectively. Cross-linking was measured using the ethidium bromide fluorescence assay at pH 11.8 (21), and expressed as percentage cross-linking (% return of fluorescence after heat denaturation). Points, mean of six determinations; bars, standard error; curves, linear regression of concentration versus cross-linking. On occasion the confidence intervals were too small to be shown.

Fig. 5. Inhibition of CMA cross-linking by ethidium bromide. λ-Phage DNA was incubated at 37°C with 5 μM CMA, and ethidium bromide at the concentrations shown. Cross-linking was measured, using the ethidium bromide fluorescence assay at pH 11.8 (21), and expressed as percentage cross-linking (% return of fluorescence after heat denaturation). Points, mean of seven determinations; bars, standard error. On occasion the confidence intervals were too small to be shown.

Fig. 6. Effect of DNA base content on cross-linking by CMA. Isolated DNA from C. perfringens (26.5% G+C), calf thymus (42% G+C), or E. coli (50% G+C), was incubated at 37°C with 30 μM (○) or 50 μM (□) CMA. Cross-linking was measured, using the ethidium bromide fluorescence assay at pH 11.8 (21), and expressed as percentage cross-linking (% return of fluorescence after heat denaturation). Points, mean of four determinations; bars, standard error.

fold more potent that ICMA and 453-fold more potent than CHL in producing cross-links in isolated DNA. The addition of sodium borohydride to the reaction mixture reduced CMA cross-linking by 55%, ICMA cross-linking by 41%, but had no significant effect on the level of CHL cross-linking.

CMA cross-linking in λ-phase DNA was inhibited by the addition of ethidium bromide (Fig. 5). Cross-linking produced by 5 μM CMA was reduced to 27% of control by 8 μM of the intercalating dye, ethidium bromide. Concentrations of ethidium bromide as high as 80 μM did not inhibit cross-linking by 1.5 mM CHL suggesting that intercalation by the dye did not decrease cross-linking by blocking alkylating sites or by distortion of the DNA.

Effect of DNA Base Content on Cross-Linking. Cross-linking by CMA was measured, using the ethidium bromide fluorescence assay, in isolated DNAs having different percentages of G+C residues (Fig. 6). A concentration of 30 μM CMA produced 32, 53, and 75% cross-linking in DNAs having 26.5, 42, and 50% G+C, respectively. At 50 μM, CMA produced 40, 71, and 90% cross-linking, respectively, in the same DNAs.

Alkylation of NBP. The alkylating activities of CMA, with or without sodium borohydride, and of CHL, were determined by measuring the ability of the drugs to bind to the nucleophile, NBP (Fig. 7). Both drugs produced concentration-dependent alkylation. A comparison of the concentration versus NBP-binding curves showed that CMA produced a 6-fold higher level of alkylation than did CHL. When CMA was reduced, there
concentrations as high as 750 \text{nM}. In the presence of sodium borohydride, displacement of acridine orange by CMA was determined immediately as described in "Materials and Methods." Points, mean of three to four determinations; bars, standard error; curves, linear regression of concentration versus NBP alkylation.

<table>
<thead>
<tr>
<th>Drug</th>
<th>NaBH₄</th>
<th>5 \text{µM}</th>
<th>10 \text{µM}</th>
<th>15 \text{µM}</th>
<th>Relative intercalating activity</th>
<th>Acridine orange displacement/IC₅₀ (µM)</th>
<th>Relative intercalating activity*</th>
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<tr>
<td>CMA</td>
<td>–</td>
<td>4</td>
<td>8.3</td>
<td>12</td>
<td>1.00</td>
<td>0.18</td>
<td>1.00</td>
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<tr>
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<td>2</td>
<td>4.3</td>
<td>5.5</td>
<td>0.47</td>
<td>0.65</td>
<td>0.28</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;750</td>
<td>1.06</td>
<td>0.17</td>
</tr>
<tr>
<td>CHL</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Drug was added to calf thymus DNA (100 µg/ml) and the change in Tm was determined immediately as described in "Materials and Methods." Numbers, means of two to four determinations.

The relative intercalating activity was obtained from the ratio of the slope of the change in Tm versus concentration curve of the drug, to that of CMA.

The ability of the drug to displace acridine orange from calf thymus DNA was determined, as described in "Materials and Methods." The IC₅₀ is the drug concentration required to reduce acridine orange-DNA binding to 50% of the control. IC₅₀ values were obtained from Fig. 8.

The relative intercalating activity was obtained from the ratio of the IC₅₀ of CMA, to that of the drug.

was little or no change in the level of alkylation.

DNA Intercalation. CMA, ICMA, or CHL was added to calf thymus DNA and the Tm of the DNA was determined (Table 1). In the absence of drug the Tm of calf thymus DNA was 64°C. CMA caused a dose-dependent increase in the Tm, reaching a maximum of 76°C at 15 µM, for a change in Tm of 12°C. At the same concentration, ICMA produced a change of 5.5°C, whereas, CHL produced no change in Tm. In a control study, CMA (15 µM) was shown to produce 18% cross-linking of calf thymus DNA after 20 min of incubation, the time required to carry out the Tm measurement. Mechloethamine (250 µM), a nitrogen mustard cross-linking agent, produced a similar level of cross-linking in calf thymus DNA after 20 min of incubation; however, this concentration of mechloethamine produced no change in Tm of the DNA.

CMA, ICMA, and CHL, in the presence or absence of sodium borohydride were examined for their ability to displace acridine orange from intercalation sites in calf thymus DNA (Table 1 and Fig. 8). In the absence of sodium borohydride, CMA produced a dose-dependent displacement of acridine orange with an IC₅₀ of 0.18 µM, while ICMA had an IC₅₀ of 0.65 µM. CHL did not cause any displacement of acridine orange at concentrations as high as 750 µM. In the presence of sodium borohydride, displacement of acridine orange by CMA was decreased 63% with the IC₅₀ increasing to 0.49 µM, while displacement of acridine orange by ICMA was 39% lower with the IC₅₀ increasing to 1.06 µM.

In a control study to determine if ethidium bromide and CMA can compete for DNA intercalation sites, it was found that 10 µM CMA produced 55% displacement of 8 µM ethidium bromide from intercalation sites in calf thymus DNA.

Effect of DNA Base Content on Displacement of Acrdine Orange. The ability of CMA to displace acridine orange from calf thymus, *C. perfringens* or *E. coli* DNA was measured (Fig. 9). There was no significant difference in the acridine orange-displacing activity of CMA in the three DNAs at drug concentrations of 125 or 250 nm.

**DISCUSSION**

It has been suggested that the potent antitumor activity of CMA may be related to its ability to produce DNA-DNA cross-links (9, 10, 12). Only cyanomorpholino-containing analogues of the anthracyclines have been shown to possess this activity (9, 13), and as the cyanide group of CMA can act as a leaving group (26, 27), alkylation may occur by displacement of the cyanide. However, other structural requirements for cross-linking, and the role of this activity in the cytotoxic action of CMA, are still unclear. We have previously observed that both the cytotoxic and cross-linking activities of ICMA, the 5-imino cyanide, could be reduced compared to CMA (9), suggesting that the quinone group may play a role in the cross-linking action of these analogues. We have therefore compared the interaction of CMA, ICMA, and CHL with DNA, in order to characterize CMA cross-linking and to determine the role of the quinone group, and of intercalation, in this activity.

Similar to previous findings with HT-29 human colon carcinoma cells (9), the potency of DNA cross-linking activity in L5178Y cells was in the order of CMA > ICMA > CHL (Fig. 2). In addition, the rates of formation and removal of DNA cross-links were considerably faster with CMA compared to CHL (Fig. 3). In isolated λ-phage DNA, CMA was 26-fold more active than ICMA, and 453-fold more active than CHL, in forming DNA cross-links (Fig. 4). In contrast, CMA was only 6-fold more potent than CHL in its ability to alkylate the
ALKYLATING ACTIVITY OF ICMA BECAUSE THE VISIBLE ABSORBANCE OF
CALCIUM CONTRIBUTES TO THE FORMATION OF CROSS-LINKS BY CMA,
CMA BEING 2-3-FOLD MORE POTENT THAN ITS 5-IMINO ANALOGUE,
THE ANALOGUE INTERFERED WITH THE NBP ASSAY. BOTH CMA AND
NUCLEOPHILE, NBP (FIG. 7). WE WERE UNABLE TO DETERMINE THE
ALKYLATING ACTIVITY OF ICMA BECAUSE THE VISIBLE ABSORBANCE OF
THE ANALOGUE INTERFERED WITH THE NBP ASSAY. BOTH CMA AND
ICMA, BUT NOT CHL, WERE ABLE TO INTERCALATE INTO DNA, WITH
CMA BEING 2-3-FOLD MORE POTENT THAN ITS 5-IMINO ANALOGUE,
AS MEASURED BY BOTH THE DISPLACEMENT OF ACIDINE ORANGE AND
THE CHANGE IN Tm OF CALF THYMUS DNA (TABLE 1 AND FIG. 8).
ADDITIONALLY, THE INTERCALATING AGENT, ETHIDIUM BROMIDE,
INHIBITED THE CROSS-LINKING ACTIVITY OF CMA (FIG. 5), BUT DID NOT
INHIBIT CROSS-LINKING BY CHL. THESE FINDINGS SUGGEST THAT INTER-
CALATION CONTRIBUTES TO THE FORMATION OF CROSS-LINKS BY CMA,
AND THAT THE DECREASED INTERCALATING ACTIVITY OF ICMA COMPARED
TO CMA MAY ACCOUNT FOR ITS LESSER CROSS-LINKING ACTIVITY.

IN A PREVIOUS STUDY (9), WE SHOWN THAT THE REDUCING AGENT,
DIETHIORETHIOL, PRODUCED NO CHANGE IN THE CROSS-LINKING ACTIVITY
OF EITHER CMA OR ICMA IN ISOLATED DNA. HOWEVER, IN THE PRESENT
STUDY SPECTROPHOTOMETRIC ANALYSIS SUGGESTED THAT THIS
RESULT WAS DUE TO LACK OF REDUCTION OF THE QUINONE GROUP. IN
CONTRAST, THE STRONGER REDUCING AGENT, SODIUM BOROHYDRIDE,
PRODUCED NEARLY COMPLETE REDUCTION OF THE QUINONE GROUP
OF CMA, AND DECREASED THE CROSS-LINKING ACTIVITY OF CMA AND
ICMA BY 55 AND 41%, RESPECTIVELY (FIG. 4). AS WELL, SODIUM
BOROHYDRIDE PRODUCED A CORRESPONDING DECREASE IN THE INTER-
CALATING ACTIVITY OF CMA AND ICMA, BUT HAD LITTLE OR NO EFFECT
ON THE ALKYLATING ACTIVITY OF CMA (FIG. 7). SODIUM BOROHYDRIDE
REDUCTION OF CMA AND ICMA LIKELY RESULTS IN REDUCTION OF BOTH
THE QUINONE OR IMINO-QUINONE GROUP AND THE C13 CARBONYL
GROUP. THE C13 HYDROXYL DERIVATIVES OF BOTH CMA AND ICMA
HAVE PREVIOUSLY BEEN SHOWN TO BE POORER DNA INTERCALATORS
THAN THE PARENT AGENTS (5). THESE RESULTS CONFIRM THE CONTRIBUTION OF INTERCALATION TO THE
CROSS-LINKING ACTIVITY OF CMA, AND SUGGEST THAT BOTH THE C13
CARBONYL GROUP AND THE QUINONE RING MAY PLAY AN IMPORTANT,
BUT INDIRECT, ROLE IN REGULATING THE ACTIVITY OF THE DRUG, BY
INFLUENCING ITS ABILITY TO INTERCALATE. PREVIOUS STUDIES (28, 29),
HAVE SHOWN THAT THE ANTHRACYCLINE CHROMOPHORE, WHICH
CONTAINS THE QUINONE GROUP, IS RESPONSIBLE FOR THE INTERCALATIVE
NATURE OF THESE AGENTS. DURING THE INTERCALATION PROCESS, THE
QUINONE AND DIHYDROQUINONE RINGS OF THE CHROMOPHORE HYDRO-
GEN-BOND WITH ADJACENT BASE PAIRS OF DNA. THE REDUCTION OF
THE QUINONE GROUP, OR A 5-IMINO SUBSTITUTION, MAY INTERFERE
WITH THE ABILITY OF THE CHROMOPHORE TO FORM SUCH BONDS, AND
THUS DECREASE INTERCALATION.

INCREASING THE G-C CONTENT OF DNA RESULTED IN AN INCREASE
IN CROSS-LINKING BY CMA (FIG. 6), WHEREAS, IT HAD NO EFFECT ON
THE ABILITY OF THE DRUG TO INTERCALATE INTO DNA (FIG. 9). THIS
FINDING INDICATED THAT THE ENHANCED CROSS-LINKING OF CMA TO
G-C BASES IS DUE TO PREFERENTIAL ALKYLLATION OF THESE BASES RATHER
THAN TO ALTERED INTERCALATION.

IN SUMMARY, THIS STUDY HAS PROVIDED EVIDENCE THAT CMA
PREFERENTIALLY CROSS-LINKS G-C-RICH DNA, AND THIS ACTIVITY IS
POTENTIATED BY THE ABILITY OF THE DRUG TO INTERCALATE INTO DNA.
THE QUINONE RING AND THE C13 CARBONYL GROUP DO NOT APPEAR TO
DIRECTLY INFLUENCE ALKYLLATION BUT MAY PLAY AN IMPORTANT,
ALTHOUGH INDIRECT, ROLE IN THE CROSS-LINKING ACTIVITY OF CMA
BY MODULATING THE ABILITY OF THE DRUG TO INTERCALATE. ADDITIONAL
STUDIES ARE REQUIRED TO DETERMINE IF THERE IS A CORRELATION
BETWEEN THE CROSS-LINKING ACTIVITY OF CMA AND THE HIGHLY POTENT
ANTITUMOR ACTIVITY OF THE DRUG.

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