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DNA Damage Induced by Bleomycin in the Presence of Dibucaine Is Not Predictive of Cell Growth Inhibition†

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ABSTRACT: Growth inhibition and cell killing by bleomycin are believed to be related to the ability of this antibiotic to cleave chromosomal DNA. Because bleomycin has an intracellular site of action, its ability to cross biological membranes must be critical to its overall effectiveness as an antitumor agent. The local anesthetic dibucaine acts to enhance membrane fluidity; therefore, the reported ability of this local anesthetic to modulate bleomycin effects on KB cells was investigated. Cells were treated with various bleomycin congeners in the presence or absence of dibucaine for 24 h. Dibucaine enhanced the inhibition of cell growth mediated by bleomycin A_2, demethylbleomycin A_2, bleomycin B_2, and isobleomycin A_2. *N*-Acetylbleomycin A_2 did not inhibit cell growth in the absence of dibucaine, but it was inhibitory in the presence of dibucaine. Cells treated simultaneously for analysis of DNA breakage on alkaline sucrose gradients revealed that breakage was also enhanced in the presence of dibucaine. The degree of enhancement varied with dose and bleomycin congener. *N*-Acetylbleomycin A_2 did not induce DNA breakage in either the absence or the presence of dibucaine. While growth inhibition and net DNA breakage correlated reasonably well in the absence of dibucaine for each bleomycin analogue tested, proportionality was lost in the presence of dibucaine, and very little DNA breakage was present when growth inhibition was complete. These observations imply that, at least in the presence of dibucaine, bleomycin may mediate growth inhibition at some locus in addition to chromosomal DNA and, also, that a given net amount of bleomycin analogue induced DNA damage per se does not produce a specific degree of growth inhibition.

The antitumor antibiotic bleomycin is thought to inhibit cell growth and to cause cell death by virtue of its ability to cleave chromosomal DNA (Clarkson & Humphrey, 1976; Hittelman & Rao, 1974; Kross et al., 1982; Terasima et al., 1972; Terasima & Umezawa, 1970). Consistent with this scheme, Clarkson & Humphrey (1976) showed that treatment of Chinese hamster ovary (CHO)[1] cells with bleomycin for 30 min produced extensive DNA strand breakage and caused cells to accumulate in the G_2 phase of the cell cycle. Hittelman & Rao (1974) found that DNA damage, as assessed by premature chromatin condensation, was present in cells blocked in late G_2; others have also presented evidence that bleomycin induces a G_2 block (Barlogie et al., 1976; Barranco & Humphrey, 1971; Nagatsu et al., 1972; Watanabe et al., 1974), presumably due to DNA damage. When colony formation of bleomycin-treated cells was assayed, cell death appeared to be correlated with DNA breakage (Kohn & Ewig, 1976).

Several observations indicate that bleomycin cytotoxicity can be modulated by the state of the cell membrane. Pertinent

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[1] Abbreviations: CHO, Chinese hamster ovary; demethyl-BLM A_2, demethylbleomycin A_2; BLM A_2, bleomycin A_2; BLM B_2, bleomycin B_2; iso-BLM A_2, isobleomycin A_2; *N*-Ac-BLM A_2, *N*-acetylbleomycin A_2; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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observations include the loss of sensitivity of CHO cells to bleomycin following trypsin treatment (Barranco et al., 1980), presumably due to loss of a membrane component, and the increased sensitivity to bleomycin caused by agents that alter membrane permeability (Mizuno, 1981; Mizuno & Ishida, 1982a,b; Nakashima et al., 1974). Of particular interest in this regard was the local anesthetic dibucaine, which enhanced cell killing in a dose- and time-dependent fashion (Mizuno & Ishida, 1982a,b).

In an effort to define more clearly the mechanism(s) by which bleomycin causes growth inhibition, we have treated cultured human cells with several bleomycin congeners in the presence or absence of dibucaine and measured the effects of the congeners on DNA strand breakage and cell growth. Presently, we demonstrate that cell growth inhibition and net DNA breakage do not change in proportion for individual bleomycin congeners in the presence of dibucaine, suggesting strongly that under these conditions bleomycin can mediate growth inhibition by some mechanism in addition to DNA strand scission.

Materials and Methods

Staging and Treatment of Cell Cultures. KB cells were maintained in monolayer in Eagle's basal medium with 10% fetal bovine serum and no antibiotics. Parental DNA was labeled by incorporation of [^{14}C]thymidine (0.1 μCi/mL) followed by incubation in the absence of radiolabel for 24–48 h. Cells were then subcultured, allowed to attach, and treated with bleomycins (Berry et al., 1985). In some experiments, cells were treated with 0.2 mM dibucaine simultaneously with bleomycin. These protocols provided for simultaneous and identical treatment of cells for assay of both growth inhibition and DNA breakage (Berry et al., 1985).

Assay of Malondialdehyde Production. Reactions were conducted at room temperature in 20 mM sodium cacodylate buffer (pH 7). Reaction mixtures contained 300 μM calf thymus DNA, 100 μM $Fe(NH_4)_2(SO_4)_2$, 100 μM BLM A_2, and 0–5 mM dibucaine. Fresh solutions of BLM A_2, $Fe(NH_4)_2(SO_4)_2$, and dibucaine were prepared shortly before use. Duplicate reactions, each in a total volume of 0.2 mL, were initiated by the addition of BLM A_2 and incubated for 15 min at room temperature. After addition of 0.8 mL of 42 mM thiobarbituric acid containing 1 mM EDTA (pH 2), the reactions were incubated at 90 °C for 30 min. The cooled solutions were employed for the determination of A_{532} values. The amount of thiobarbituric acid reactive material, i.e., malondialdehyde, produced was calculated from the molar extinction coefficient, $\epsilon_{532} = 1.6 \times 10^5\ M^{-1}\ cm^{-1}$ (Waravdekar & Saslaw, 1959). This extinction coefficient agreed within 10% with an extinction coefficient calculated from standard solutions of malondialdehyde (Burger et al., 1980) prepared with each assay. Standard solutions containing 1–5 nmol of malondialdehyde were found to obey Beer's law.

Assay for Breakage of Supercoiled pBR322 DNA. Reaction of supercoiled pBR322 DNA (Clewell & Helinski, 1970) with BLM A_2 was conducted at room temperature in 50 mM sodium cacodylate buffer (pH 7). Reaction mixtures contained 30 μM pBR322 DNA (1 μg), 10 μM $Fe(NH_4)_2(SO_4)_2$, 10 nM BLM A_2, and 0–5 mM dibucaine. All solutions were freshly prepared. Reaction was initiated by the addition of BLM A_2 and, after 10 min, quenched by addition of EDTA to 50 mM final concentration. Samples were precipitated with ethanol and then dissolved in 30 μL of gel loading buffer containing 10% glycerol, 0.1% SDS, and 0.008% bromphenol blue. Electrophoresis (40 V, 16 h) was carried out at 25 °C on a 1% agarose gel in 40 mM Tris–acetate, pH 7.8, containing 20 mM NaOAc and 4 mM EDTA. Gels were stained for 1–2 h in electrophoresis buffer containing 1 μg/mL ethidium bromide. Fluorescent DNA bands were visualized on an ultraviolet light box, and densitometry was done as described by Lloyd et al. (1978).

Assay of Growth Inhibition. KB cells were plated in multiwell dishes at 2.5×10^5 cells mL^{-1} well^{-1}. After 24 h, fresh medium containing bleomycin, or bleomycin and dibucaine, was added. Twenty-four hours later, the medium was removed, and the cultures were rinsed. Following treatment of the cells with trypsin, the resulting suspension was diluted with trypan blue for counting on a hemacytometer. The number of viable cells (those excluding trypan) was used to calculate the percent growth inhibition according to the formula $100[(N_c - N_E)/(N_c - N_o)]$ where N_c is the number of cells counted in control (untreated or dibucaine treated, as appropriate) cultures, N_E is the number of cells in bleomycin-treated cultures, and N_o is the number of cells per culture at initiation of bleomycin treatment. Results are expressed as "mean ± SE".

Other Methods. The sources of bleomycins and other materials have been described (Berry et al., 1985). Methods for sucrose gradient centrifugation, calculation of DNA size, and calculation of DNA breaks were as before (Berry et al., 1985).

Results

Growth Inhibition by Bleomycins. KB cells were treated with bleomycins for 24 h. Some cultures were simultaneously exposed to 0.2 mM dibucaine. After treatment, the number of viable cells and the percent viability were determined for each culture. Dibucaine, at 0.2 mM, did not affect cell growth or viability. The number of viable cells from each culture was used to calculate the percent growth inhibition, compared to either untreated or dibucaine-treated control cultures, as appropriate. Viability, as measured by trypan exclusion, remained unchanged from control in all experiments.

In the absence of dibucaine, all of the bleomycins, with the exception of *N*-Ac-BLM A_2, caused inhibition of cell growth (Figure 1). In no case, though, was the number of viable cells after bleomycin treatment found to be less than the number of viable cells present at initiation of drug treatment. This suggested strongly that cell lysis did not occur during the 24-h treatment.

In the presence of dibucaine, all of the bleomycins, including *N*-Ac-BLM A_2, caused growth inhibition (Figure 1). Moreover, growth inhibition was markedly enhanced for all congeners except bleomycinic acid. The ID_{50}'s were estimated to be decreased approximately 10-fold in the presence of dibucaine. Again, in no case was the number of viable cells after bleomycin treatment less than the number present at initiation of drug treatment, implying that cell death did not occur during the course of the experiment.

DNA Strand Scission by Bleomycins. Bleomycin-induced growth inhibition and cell death are thought to result from DNA strand scission. Since dibucaine markedly enhanced bleomycin-induced inhibition of cell growth, cells were also assayed for DNA breakage in the presence and absence of 0.2 mM dibucaine. In order to make valid correlation possible, sister KB cells to those assayed for growth inhibition were treated identically and simultaneously for assay of DNA breakage, and cells treated with bleomycin and dibucaine were compared to cells treated with dibucaine only. Representative data from cells treated with bleomycinic acid in the presence of dibucaine are shown in Figure 2. Dibucaine alone produced no measurable DNA breakage after 24 h (Figure 2).

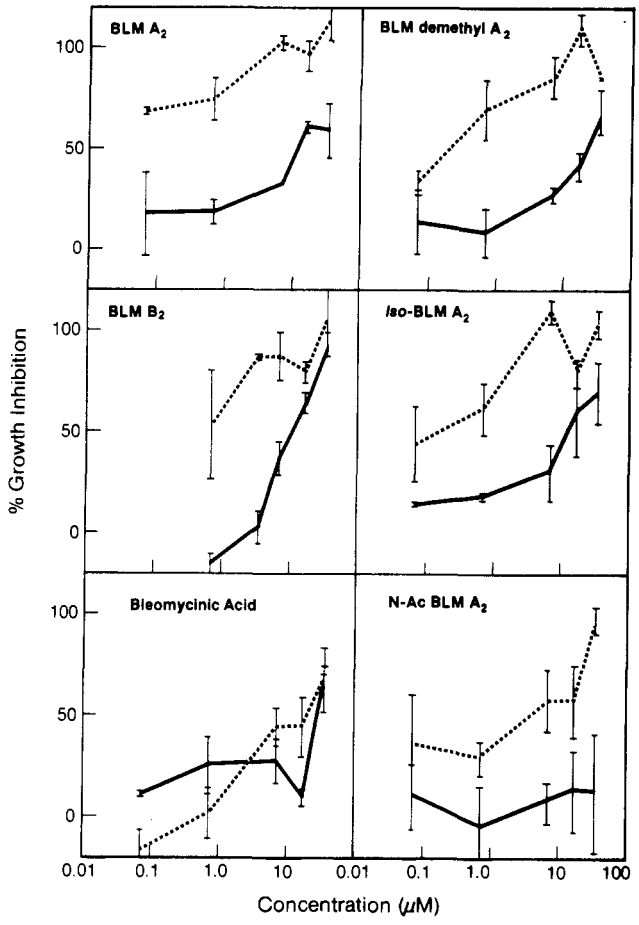

FIGURE 1: Growth inhibition by bleomycins in the presence or absence of dibucaine. KB cells were plated in multiwell dishes at 2.5×10^5 cells mL^{-1} $well^{-1}$ and incubated for 24 h. The cells were sister cells to those analyzed for DNA breakage and contained [^{14}C]thymidine-labeled DNA; they were treated with bleomycins on an equimolar basis, and some cell cultures were simultaneously treated with 0.2 mM dibucaine. After 24 h, adherent cells were trypsinized and diluted into 2.0 mL of medium, and an aliquot was diluted with trypan blue prior to counting. Growth inhibition was calculated as described under Materials and Methods. No dibucaine (—); 0.2 mM dibucaine (···).

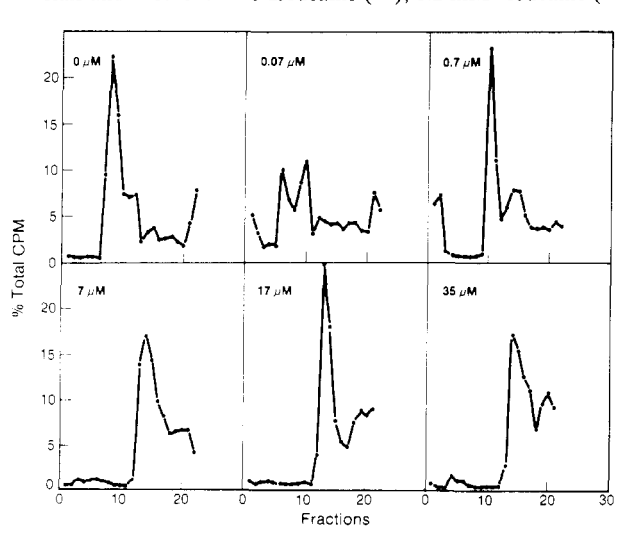

FIGURE 2: DNA breakage by bleomycinic acid in the presence of dibucaine. Parental DNA of KB cells was labeled with [^{14}C]thymidine. Cells were treated with various concentrations of bleomycinic acid for 24 h, in the presence of 0.2 mM dibucaine. The cells were layered onto 5–20% alkaline sucrose gradients, lysed, and centrifuged for 3 h at 36000g. Gradients were fractionated from the bottom, and the percent of total radioactivity in each fraction was calculated. The number of breaks per 10^8 bases was determined as described under Materials and Methods.

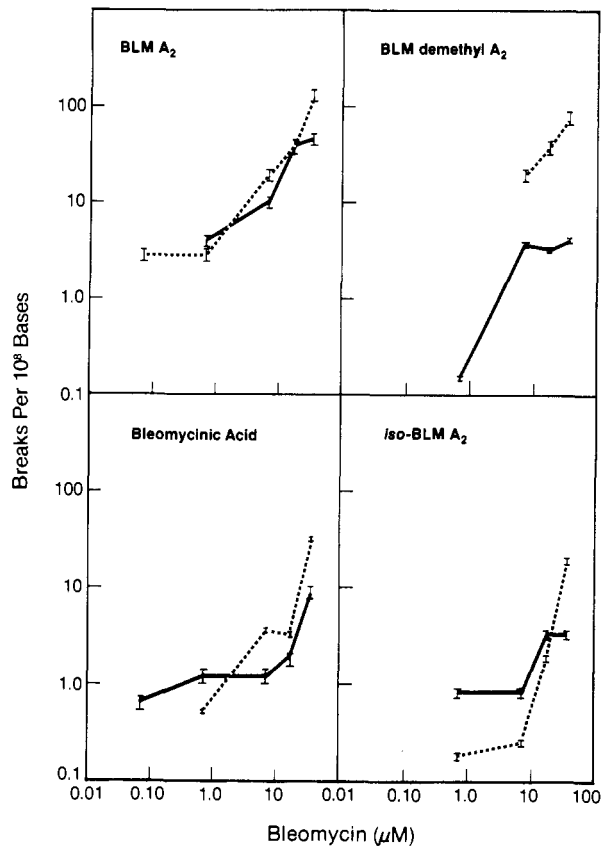

FIGURE 3: Dose–response relationships for DNA breakage induced by bleomycin congeners. The number of bleomycin-induced DNA strand breaks per 10^8 bases was plotted vs. dose for the bleomycin congeners, each of which was tested in the absence (—) or presence (···) of 0.2 mM dibucaine.

In the presence of dibucaine, net DNA breakage by demethyl-BLM A_2 was enhanced 10–20-fold (Figure 3). Breakage by the other congeners was also enhanced, but the effects were not as consistent as with demethyl-BLM A_2. Breakage by BLM A_2, for instance, was significantly increased only at 35 μM (Figure 3). Breakage by bleomycinic acid was enhanced by 2–4-fold at 7–35 μM, while breakage by iso-BLM A_2 was decreased below 10 μM but increased 6-fold at 35 μM (Figure 3). Breakage by *N*-Ac-BLM A_2 was not measurable in the presence or absence of dibucaine (e.g., 0, 0.10, 0.28, 0, and 0.15 breaks per 10^8 bases at 0.07, 0.7, 7, 17, and 35 μM, respectively, in the presence of dibucaine).

The possibility that dibucaine itself might effect DNA strand scission was also tested. DNA was treated with dibucaine in vitro in the presence and absence of BLM A_2. DNA breakage was quantified by assay of malondialdehyde[2] released from calf thymus DNA and by conversion of covalently closed circular (form I) pBR322 DNA to nicked (form II) or linear (form III) DNA. BLM A_2 induced DNA strand scission involves deoxyribose cleavage and release of malondialdehyde (precursors) (Burger et al., 1980; Giloni et al., 1981), and BLM A_2 nicks supercoiled form I DNA to yield both relaxed and linear forms (Lloyd et al., 1978; Mirabelli et al., 1980).

BLM A_2 was found to cause release of malondialdehyde in both the presence and absence of dibucaine (Figure 4). In the absence of dibucaine, BLM A_2 produced 3 nmol of thio-

[2] DNA strand scission by Fe(II)·bleomycin has been shown to produce base propenals, which can be measured conveniently as thiobarbituric acid reactive material following intentional decomposition of the base propenals to malondialdehyde and free bases.

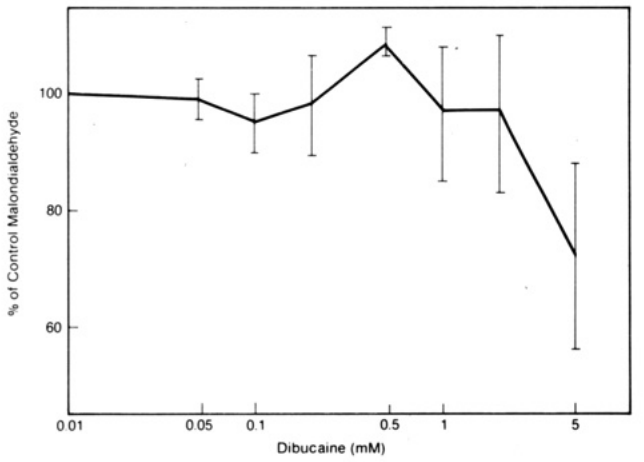

FIGURE 4: Effect of dibucaine on malondialdehyde production from DNA by bleomycin. Calf thymus DNA was treated with BLM A_2, $Fe(NH_4)_2(SO_4)_2$, and several concentrations of dibucaine at 25 °C for 15 min and then assayed for malondialdehyde production (see Materials and Methods). The amount of malondialdehyde produced in reactions containing dibucaine is expressed as a percentage of the malondialdehyde produced in the absence of dibucaine. Results are expressed as mean ± standard error for duplicate samples in three or four experiments.

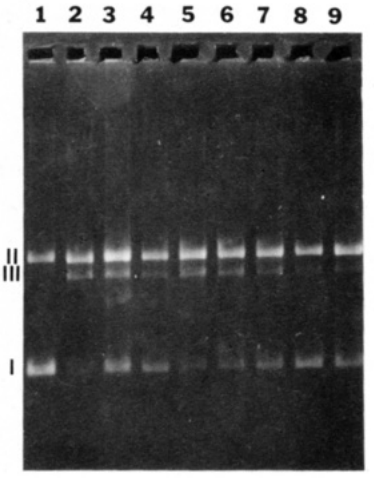

FIGURE 5: Effect of dibucaine on the cleavage of pBR322 DNA by bleomycin. Supercoiled pBR322 DNA was treated with BLM A_2, $Fe(NH_4)_2(SO_4)_2$, and several concentrations of dibucaine at 25 °C for 15 min and then analyzed by agarose gel electrophoresis (see Materials and Methods). Individual reaction mixtures contained 10 μM Fe(II), 10 nM BLM A_2, and dibucaine at concentrations of (lane 2) 0, (lane 3) 0.05, (lane 4) 0.1, (lane 5) 0.2, (lane 6) 0.5, (lane 7) 1, (lane 8) 2, and (lane 9) 5 mM. Lane 1 contained DNA and 10 μM Fe(II).

barbituric acid reactive material. Dibucaine did not affect BLM A_2 induced release at concentrations of 2 mM or less, but malondialdehyde production was somewhat decreased in the presence of 5 mM dibucaine for reasons that are uncertain, but could relate to masking of potential BLM A_2 cleavage sites on DNA by dibucaine.

A similar effect of dibucaine was observed for the BLM A_2 mediated relaxation of supercoiled pBR322 DNA (Figure 5, lane 2). The percent of DNA present as supercoils (as determined by densitometry) decreased from 70% to 10%. Dibucaine inhibited BLM A_2 induced relaxation of supercoils. Supercoiled DNA was approximately 30% of the total DNA at dibucaine concentrations ranging from 0.05 to 1 mM. At 2–5 mM, supercoiled DNA was 40–50% of total DNA (Figure 5). From the data of Figures 4 and 5, it is clear that dibucaine alone does not induce DNA breakage nor does it directly enhance bleomycin-induced DNA breakage.

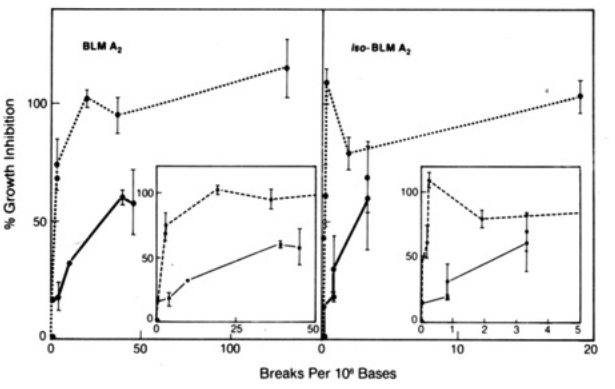

FIGURE 6: Relationship of growth inhibition to DNA damage. Growth inhibition (mean ± SE) with and without dibucaine (from Figure 1) was plotted vs. DNA breakage, with and without dibucaine (from Figure 3), for BLM A_2 and iso-BLM A_2. Similar results were obtained with demethyl-BLM A_2 and bleomycinic acid. Insets: Abscissa expanded 3-fold for BLM A_2 and 4-fold for iso-BLM A_2. Cells treated without dibucaine (—); cells treated with dibucaine (···).

Correlation between DNA Breakage and Growth Inhibition. In the belief that growth inhibition and net DNA breakage might be expected to vary in parallel for any single bleomycin congener, these two parameters were compared in the presence and absence of dibucaine. Accordingly, growth inhibition was plotted vs. the number of DNA breaks per 10^8 bases for each congener, with and without dibucaine. The data for BLM A_2 and iso-BLM A_2 are shown in Figure 6; demethyl-BLM A_2 and bleomycinic acid gave similar results (data not shown). In the absence of dibucaine, growth inhibition appeared to be linearly proportional to net DNA breakage for individual congeners ($r = 0.949$ for BLM A_2 and $r = 0.970$ for iso-BLM A_2). Complete growth inhibition was not achieved in the bleomycin concentration range tested (Figure 6). In the presence of dibucaine, on the other hand, growth inhibition and net DNA breakage were not linearly related (see Figure 6 inserts; $r = 0.613$ for BLM A_2 and $r = 0.479$ for iso-BLM A_2) over the same bleomycin concentration range. Also, in the presence of dibucaine, relatively little DNA breakage was present when growth inhibition was complete, compared to cells treated in the absence of dibucaine.

DISCUSSION

Mizuno & Ishida (1982a) observed that local anesthetics, particularly dibucaine, enhanced the cytotoxicity of bleomycin in mouse FM3A cells. Although these workers did not present evidence to establish the mechanism of enhancement, breakage of chromosomal DNA is generally accepted as the cause of bleomycin-mediated cytotoxicity, and it seemed reasonable to suppose that dibucaine acted, either directly or indirectly, to enhance DNA strand breakage. The studies described here were designed to test this assumption and, more specifically, to determine if bleomycin-induced growth inhibition was always correlated with, and hence presumably a result of, chromosomal DNA scission. Accordingly, we assayed several bleomycin congeners of varying potency for these two properties, in both the absence and presence of dibucaine. We reasoned that if growth inhibition ultimately results from DNA scission, then these two parameters should be correlated for each bleomcyin congener, regardless of the presence or absence of dibucaine.

Our data indicated that, in the absence of dibucaine, net bleomycin-induced DNA breakage and growth inhibition were linearly related for each of the congeners tested (Figure 6). In the presence of dibucaine, however, this correlation disappeared. Net DNA breakage and growth inhibition were

generally enhanced, but the effect of dibucaine was not uniform on both parameters for single congeners or among the congeners (Figures 1 and 3). Additionally, in the presence of dibucaine, very little DNA breakage was measured even when cell growth was completely inhibited (Figure 6). Loss of proportionality in the presence of dibucaine was not due to an ability of dibucaine to cause DNA breakage directly or to enhance bleomycin-mediated DNA breakage (Figures 4 and 5).

The lack of correlation, in the presence of dibucaine, between net DNA breakage and growth inhibition was most apparent with *N*-Ac-BLM A_2. This congener, which has been shown not to degrade PM-2 [^{3}H]DNA in vitro (Oppenheimer et al., 1980), had no measurable effect on KB cell chromosomal DNA, either in the presence or in the absence of dibucaine. Similarly, in the absence of dibucaine, *N*-Ac-BLM A_2 did not inhibit cell growth (Figure 1) or decrease the colony-forming ability of KB cells (Berry et al., 1985). In contrast, in the presence of dibucaine, *N*-Ac-BLM A_2 did cause growth inhibition, which was dose related and complete at 35 μM (Figure 1).

Previous results (Berry et al., 1985) indicated that when several bleomycin congeners were compared to each other, their abilities to cause DNA scission or growth inhibition varied widely. Hence, net DNA breakage per se was not correlated with growth inhibition among the group of congeners tested. Data presented here (Figure 6) indicated that when net DNA breakage and growth inhibition were compared to each other for any single congener in the absence of dibucaine, a satisfactory correlation existed. However, when such a comparison was attempted for cells treated in the presence of dibucaine, no simple (linear) correlation was evident, and the interrelationship between net DNA breakage and growth inhibition noted for individual congeners was lost. Since direct effects of bleomycin congeners on DNA could be dissociated from bleomycin congener induced growth inhibition, either by comparing different congeners in the absence of dibucaine (Berry et al., 1985) or by comparing these two parameters for any single congener in the presence of dibucaine (Figure 6), it is probable that bleomycin-induced growth inhibition can result from some action in addition to DNA strand scission. This conclusion is supported strongly by the observation that dibucaine confers upon *N*-Ac-BLM A_2 the ability to inhibit cell growth, but not the ability to cleave DNA.

Several lines of evidence suggest that biological membranes may play an important role in the action of bleomycin. This could simply involve membrane impermeability that limits bleomycin uptake (Endo et al., 1971; Moore, 1982a,b; Brabbs & Warr, 1979; Suzuki et al., 1981; Barranco & Humphrey, 1971; Clarkson & Humphrey, 1976), or it might reflect the existence of a membrane-associated site at which bleomycin can act (Barranco et al., 1980; Sugimoto et al., 1981; Uehara et al., 1982). Increased membrane permeability should enhance the activity of bleomycin at intracellular sites, but no previous attempt has been made to test this assumption directly. Agents which increase membrane permeability can also specifically increase bleomycin cytotoxicity. These include the antifungal agents pentamycin and amphotericin B (Nakashima et al., 1974) and ethanol (Mizuno, 1981). Akiyama et al. (1979) showed that some, but not all, polyene antibiotics enhance the inhibition of DNA synthesis and loss of colony-forming ability caused by BLM A_2. However, since drug exposure was for 10–18 h, these studies do not distinguish between DNA synthesis inhibition via a direct effect on nuclear DNA and an indirect effect at another locus. Certain polyenes also increase the amount of [^{14}C]BLM A_2 associated with Chinese hamster cells, but the studies reported do not distinguish between internalization of [^{14}C]BLM A_2 and adsorption to the plasma membrane.

Local anesthetics such as dibucaine may act to increase membrane fluidity and thereby membrane permeability (Papahadjopoulos et al., 1975; Seeman, 1972). Additionally, dibucaine acts to displace calcium from cellular membranes (Papahadjopoulos, 1972). Mizuno & Ishida (1982b) have shown that several membrane-active pharmacological agents which affect calcium metabolism enhance pepleomycin cytotoxicity. Enhancement is apparently due to increased intracellular Ca^{2+}. Our data obtained with *N*-Ac-BLM A_2 are in accord with this possiblity, since in the presence of dibucaine this bleomycin derivative inhibited cell growth but did not cause DNA strand scission. Further, preliminary experiments suggested that the cytotoxic effects of bleomycin can be potentiated by increased extracellular Ca^{2+} (data not shown). Finally, Chafouleas et al. (1984) have recently reported that calmodulin, a Ca^{2+} receptor that mediates many intracellular Ca^{2+}-dependent events, may play a role in repair of bleomycin-induced DNA lesions. Hence, disturbances of normal cellular Ca^{2+} metabolism by dibucaine, or other agents, may result in inhibition of DNA repair. This might explain, at least partially, the enhancement of growth inhibition we observed with several bleomycin congeners in the presence of dibucaine.

Registry No. BLM A_2, 11116-31-7; demethyl-BLM A_2, 41089-03-6; BLM B_2, 9060-10-0; iso-BLM A_2, 51041-93-1; *N*-Ac-BLM A_2, 70772-32-6; BLM, 11056-06-7; dibucaine, 85-79-0.

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Multiple Conformational States of Repair Patches in Chromatin during DNA Excision Repair†

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ABSTRACT: In mammalian cells, newly synthesized DNA repair patches are highly sensitive to digestion by staphylococcal nuclease (SN), but with time, they acquire approximately the same nuclease resistance as the DNA in bulk chromatin. We refer to the process which restores native SN sensitivity to repaired DNA as chromatin rearrangement. We find that during repair of ultraviolet damage in human fibroblasts, repair patch synthesis and ligation occur at approximately the same rate, with ligation delayed by about 4 min, but that chromatin rearrangement is only 75% as rapid. Thus, repair-incorporated nucleotides can exist in at least three distinct states: unligated/unrearranged, ligated/unrearranged, and ligated/rearranged. Inhibition of repair patch synthesis by aphidicolin or hydroxyurea results in inhibition of both patch ligation and chromatin rearrangement, confirming that repair patch completion and/or ligation are prerequisites for rearrangement. We also analyze the kinetics of SN digestion of repair-incorporated nucleotides at various extents of rearrangment and find the data to be consistent with the existence of two or more forms of unrearranged repair patch which have different sensitivities to digestion by SN. These data indicate that the chromatin rearrangement which restores native SN sensitivity to repaired DNA is a multistep process. The multiple forms of unrearranged chromatin with different SN sensitivities may include the unligated/unrearranged and ligated/unrearranged states. If so, the differences in SN sensitivity must arise from differences in chromatin structure, because SN does not differentiate between ligated and unligated repair patches in naked DNA.

Excision repair of DNA in mammalian cells involves transient changes in chromatin structure (Smerdon & Lieberman, 1978, 1980; Smerdon et al., 1979; Williams & Friedberg, 1979; Bodell & Cleaver, 1981) which have the following characteristics: repair-incorporated nucleotides are initially highly sensitive to staphylococcal nuclease (SN),[1] but with time, they acquire approximately the same degree of nuclease resistance as nucleotides in bulk chromatin; repair-incorporated nucleotides are initially underrepresented in nucleosome core-length DNA fragments produced by SN digestion, but with time, they acquire the same distribution in these fragments as nucleotides of bulk chromatin. We call the process by which repaired DNA regains the native level of SN sensitivity chromatin rearrangement. It has been suggested that the extreme SN sensitivity of newly synthesized repair patches results from an unfolding or sliding of nucleosomes required for repair enzymes to gain access to the damaged DNA and that the rearrangement process reestablishes native chromatin

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[1] Abbreviations: SN, staphylococcal nuclease; UV, ultraviolet; dThd, thymidine; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid.

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