INHIBITION OF BLEOMYCIN-INDUCED [^{3}H] THYMIDINE 5′-TRIPHOSPHATE INCORPORATION INTO LIVER AND HEPATOMA NUCLEI BY N-ETHYL MALEIMIDE AND DAUNOMYCIN

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The addition of bleomycin to a nuclear incorporating system results in an increased incorporation of ^{3}H-thymidine 5′-triphosphate (^{3}H-TTP) into the DNA of liver and hepatoma nuclei. Bleomycin added to the nuclear incorporating system also produces scissions of DNA as determined by sucrose density gradient centrifugation of the extracted DNA. The action of bleomycin is dependent on the presence of sulfhydryl agents in the incubation mixture.

Two compounds, N-ethyl maleimide and daunomycin, inhibit the bleomycin-induced incorporation of ^{3}H-TTP preferentially. N-Ethyl maleimide inhibits bleomycin-induced activity in liver and hepatoma 7777 nuclei equally. Lower levels of daunomycin inhibit the bleomycin-induced activity in the hepatoma 7777 nuclei than are required to inhibit the activity in liver nuclei. The two compounds inhibit the bleomycin effect by different mechanisms. The addition of N-ethyl maleimide to bleomycin in the incubation system prevents bleomycin from causing breaks in the DNA. The addition of daunomycin, despite inhibition of bleomycin-induced ^{3}H-TTP incorporation, does not affect the bleomycin-produced breaks in the DNA. N-Ethyl maleimide acts by binding to the DNA and by competing with a sulfhydryl agent for bleomycin-sensitive sites on the DNA. Daunomycin apparently inhibits a repair enzyme that is responsible for the increased incorporation following bleomycin treatment.

Numerous studies on the antitumor antibiotic, bleomycin, have been reported.[1~6] Various effects of bleomycin on biological systems have been shown. Bleomycin caused an inhibition of growth of HeLa cells in culture and of Sarcoma 180 cells in mice.[7] It has been shown to inhibit DNA synthesis and, to a lesser extent, protein synthesis in *Escherichia coli* and EHRLICH carcinoma cells.[8] The incorporation of ^{3}H-thymidine into phytohemagglutinin-stimulated lymphocytes was inhibited by bleomycin.[9] *In vitro*, the antitumor antibiotic has been shown to cause a decrease in the melting temperature of DNA,[10,11] single strand scissions of DNA,[4,10] and double-strand breaks of DNA in cultured mouse fibroblasts.[12,13] Repair of such scissions or breaks presumably leads to increased incorporation of ^{3}H-thymidine or ^{3}H-TTP in several systems. A dramatic increase in incorporation of ^{3}H-TTP into bleomycin-treated isolated nuclei from normal rat liver or liver after partial hepatectomy has been reported.[9,14] Incorporation into normal liver nuclei or nuclei from partially hepatectomized animals in the absence of bleomycin was inhibited by cytosine arabinonucleoside-triphosphate. The activity due to stimulation by bleomycin on the other hand was not inhibited by cytosine arabinonucleoside-triphosphate.[14] The stimulation of ^{3}H-thymidine incorporation into nuclei from various mammalian cell lines exposed to phleomycin has also been reported.[15] We found that nuclei from several MORRIS hepatomas incorporated increased amounts of ^{3}H-TTP in the presence of bleomycin. Bleomycin-induced activity in the faster growing hepatomas was much less than in rat liver nuclei and in nuclei from a very slow growing hepatoma.[16]

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In this paper we report on the inhibition of the bleomycin-induced activity in rat liver and hepatoma 7777 nuclei.

Materials and Methods

Materials and Animals.

^{3}H-TTP was obtained from Amersham/Searle Corporation, Arlington Heights, Ill., U.S.A. The unlabeled deoxyribonucleotides and NEM* were from Sigma Chemical Company, St. Louis, Mo. Blenoxane (bleomycin sulfate) was from Bristol Laboratories, Syracuse, N.Y. and daunomycin (daunorubicin hydrochloride) from Ben Venue Laboratories Inc., Bedford, Ohio.

Male Buffalo rats bearing MORRIS hepatomas 16, 7800, and 7777 were used. Transplants were made routinely in our laboratory, bilaterally in the thigh muscle, and animals were fed *ad libitum*. A transplanting schedule of 4 weeks was maintained for hepatoma 7777, of 7 weeks for hepatoma 7800, and of 6 months for hepatoma 16. Animals bearing hepatoma 7777 between 115 and 125 generations, hepatoma 7800 between 76 and 80 and hepatoma 16 after 13 generations were used. The biology and growth properties of these hepatomas have been described[17].

Isolation of Nuclei.

Nuclei from liver and MORRIS hepatomas were isolated as previously described[18,19]. The washed nuclei were resuspended in 0.25 M sucrose: TKMM** and were used immediately for assay. Nuclei that were to be assayed without 2-mercaptoethanol in the reaction mixture were washed with TKMM from which 2-mercaptoethanol had been omitted. Aliquots of the nuclear preparations were removed for comparative purity analyses of DNA[20], RNA[21] and protein[22] as previously described[23]. As further criteria for purity, we have reported[24] specific activity ratios, in the original homogenate and in the final nuclear suspension, of cytochrome c oxidase, glucose-6-phosphatase, and 5′-nucleotidase.

Incorporation of ^{3}H-TTP by Isolated Nuclei.

The regular reaction mixture (0.5 ml final volume) contained 50 μmoles Tris-HCl, pH 7.5; 2 μmoles $MgCl_2$; 2 μmoles 2-mercaptoethanol; 80 μmoles KCl, 1 μmole ATP, 0.04 μmole each dATP, dCTP, and dGTP; and 0.02 μmole ^{3}H-TTP (50 μCi/μmole). The reaction mixture was incubated for 2 minutes at 37°C before the addition of nuclei (75~100 μg of DNA). The reaction was stopped by the addition of 1 ml 1 M NaOH, and radioactivity was determined as previously described[18,19].

DNA Extraction and Sucrose Density Gradient Centrifugation.

Nuclei were incubated in groups of 10 tubes (150~200 μg DNA in 1.0 ml of reaction mixture) in the presence or absence of the drugs. Each set of 10 tubes was combined and centrifuged at $1,000 \times g$, and the nuclear DNA was extracted according to ZAMENHOF[25].

Neutral and alkaline sucrose gradients (5~20%) were prepared according to SAITO and ANDOH[12]. Three to 4 absorbance units of DNA were layered on the gradient. For the analysis of denatured DNA on alkaline sucrose gradients, the DNA sample was brought to 1.0 M NaOH and allowed to stand at 0° for 20 minutes before layering on the gradient. The gradients were centrifuged in a SW50L rotor at 25,000 rpm for 18.5 hours. Fractions were collected from the bottom of the tube and brought to 1.0 ml with H_2O and absorbance at 260 nm read in a Hitachi Perkin-Elmer spectrophotometer. ^{14}C-Labeled ΦX 174 DNA (23 S), β-galactosidase (16 S), and lactate dehydrogenase (7.0 S) were used as markers. The ΦX DNA sedimented in fraction 2 in both neutral and alkaline gradients. The β-galactosidase sedimented in fraction 5 in neutral and in fraction 7 in alkaline gradients. Lactate dehydrogenase sediment ed in fraction 9 and fraction 10 in neutral and alkaline sucrose gradients, respectively.

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* Abbreviation used is NEM, N-ethyl maleimide.

** Abbreviation used is: TKMM, 50 mM Tris (pH 7.5); 25 mM KCl; 5 mM $MgCl_2$; 1 mM 2-mercaptoethanol.

Table 1. Stimulation of [^{3}H]TTP incorporation by bleomycin

The assay procedure has been described in "Materials and Methods." Incubation was for 30 minutes at 37°C. Where indicated, the bleomycin concentration was 100 μg/ml. The values are the averages of the number of experiments indicated in parentheses±S.D.

Nuclei	[^{3}H]TTP incorporation (pmoles/mg DNA)			
	Control	Bleomycin	Bleomycin/Control	Bleomycin no MSH*
Normal rat liver (4)	14.5±1.5	171.1±7.8	11.8	16.4±1.6
Host liver (12)	13.9±1.3	169.5±15.6	12.2	14.2±0.8
Hepatoma 16 (2)	13.4	154.9	11.5	15.9±2.1
Hepatoma 7800 (4)	29.2±6.2	122.6±30.6	4.2	29.8±3.9
Hepatoma 7777 (8)	69.7±9.1	174.3±18.2	2.5	72.2±8.2

* 2-mercaptoethanol

Table 2. Inhibition of bleomycin-induced repair activity by NEM

The assay procedure has been described in "Materials and Methods." Incubation was for 30 minutes at 37°C. Where indicated, the bleomycin concentration was 100 μg/ml reaction mixture. Bleomycin-induced activity was determined by subtracting incorporation without bleomycin from incorporation with bleomycin for each concentration of NEM. Numbers are from at least 3 different determinations±S.D.

NEM (μmoles)	[^{3}H]TTP incorporation (pmoles/mg DNA)									
	Host liver nuclei					Hepatoma 7777 nuclei				
	− Bleomycin	% inhibition	+ Bleomycin	Bleomycin-induced activity	% inhibition	− Bleomycin	% inhibition	+ Bleomycin	Bleomycin induced activity	% inhibition
0	14.1±2.1	0	169.2±13.2	155.1	0	74.3±9.1	0	178.3±21.2	104.0	0
1	11.8±1.9	16	112.6±15.1	100.8	35	63.9±4.1	14	134.6±18.1	70.7	32
2	10.3±2.3	27	87.8±6.2	77.5	50	51.3±2.1	31	98.1±0.9	46.8	55
5	8.7±0.8	38	10.3±1.1	1.6	99	43.1±1.2	42	42.8±3.1	0	100
10	5.9±1.6	58	9.0±0.4	3.1	98	28.2±3.8	62	29.2±4.2	1.0	99

Results

The incorporation of ^{3}H-TTP in the presence and absence of bleomycin into isolated nuclei from normal rat liver, host liver, hepatoma 16, hepatoma 7800 and hepatoma 7777 is shown in Table 1. No difference in the incorporation was found between normal liver, host liver and hepatoma 16 nuclei. The addition of bleomycin (50 μg/0.5 ml of reaction mixture) caused a 12-fold increase in ^{3}H-TTP incorporation in the three nuclear preparations. As reported previously,[18,19,23] nuclei from hepatoma 7800 and hepatoma 7777 incorporated more label than liver nuclei. Stimulation of incorporation by bleomycin, however, was only 4.2-fold for hepatoma 7800 and 2.5-fold for hepatoma 7777 nuclei. It can also be seen that 2-mercaptoethanol was essential for the bleomycin-induced incorporation. Not shown here and, to be published in a separate communication, are results that indicate that 2-mercaptoethanol could be replaced by other reducing agents such as dithiothreitol or 2,3-dimercapto-1-propanol (BAL). Omission of 2-mercaptoethanol from the reaction mixture without bleomycin reduced incorporation by only 10~15%.

The remaining studies were done only with host liver and hepatoma 7777 nuclei since host liver was readily available any time a hepatoma-bearing animal was used and hepatoma 7777 nuclei showed the smallest response to bleomycin.

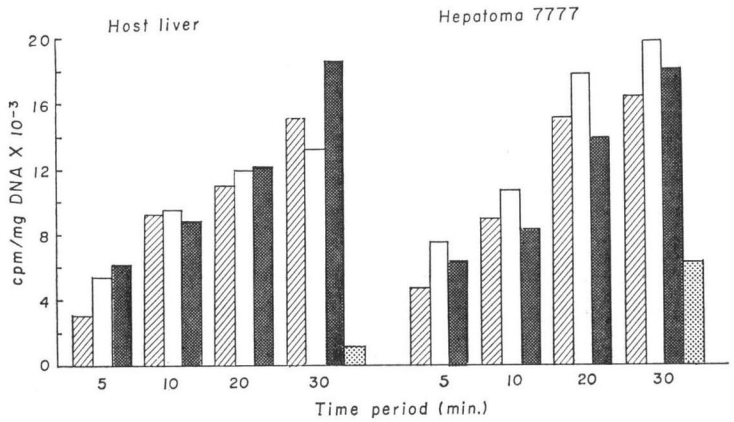
Table 3. Inhibition of bleomycin induced [^{3}H]TTP incorporation by daunomycin

Assay procedures were as for Table 2. Bleomycin-induced activity was determined by subtracting incorporation without bleomycin from incorporation in the presence of bleomycin for each concentration of daunomycin. Numbers are from at least 3 determinations±S.D.

Daunomycin (pmoles)	[^{3}H]TTP incorporation (pmoles/mg DNA)									
	Host liver nuclei					Hepatoma 7777 nuclei				
	− Bleomycin	% inhibition	+ Bleomycin	Bleomycin-induced activity	% inhibition	− Bleomycin	% inhibition	+ Bleomycin	Bleomycin-induced activity	% inhibition
0	13.8±2.1	0	167.0±11.2	153.2	0	76.2±4.1	0	181.2±12.7	105	0
20	11.7±0.8	15	141.9±6.4	130.2	15	69.3±6.6	9	120.8±12.0	51.5	51
50	6.6±0.6	52	74.0±7.1	67.4	56	38.9±2.5	49	58.8±7.1	19.9	81
100	5.2±1.1	62	19.0±2.2	13.8	91	32.8±1.9	57	31.9±1.2	0	100

Fig. 1. Addition of NEM or daunomycin at different times of incubation.

^{3}H-TTP incorporation without bleomycin is indicated by the bars with dots. Incorporation in the presence of 50 μg bleomycin in 0.5 ml of reaction mixture for the indicated times is indicated by the bars with diagonal lines. The time of incubation for samples indicated by open and solid bars was 30 minutes. The samples, all in 0.5 ml, received bleomycin at zero time and 5 μmoles NEM (open bars) or 100 pmoles daunomycin (solid bars) at the times indicated on the abscissa. The reaction mixture has been described in "Materials and Methods." Bleomycin means bleomycin plus 2-mercaptoethanol unless indicated otherwise. The values shown are from one experiment only. Several additional experiments gave similar results.



The results in Table 2 show that NEM preferentially inhibited the bleomycin-induced incorporation. Five μmoles added to 0.5 ml of reaction mixture inhibited the bleomycin-induced activity completely, in both host liver and hepatoma 7777 nuclei. Incorporation in the absence of bleomycin was inhibited only by about 40% in both host liver and hepatoma 7777 nuclei. The concentration of 2-mercaptoethanol was 4 mM in our reaction mixture. Higher levels of NEM were required to inhibit the bleomycin-induced incorporation when the 2-mercaptoethanol concentration in the reaction mixture was increased. With a concentration of 8 mM 2-mercaptoethanol in the reaction mixture the stimulation of ^{3}H-TTP incorporation by bleomycin was not significantly enhanced, but 10 μmoles of NEM were needed to inhibit the bleomycin-induced activity completely.

Fig. 2. Neutral sucrose gradients of DNA extracted from liver nuclei.

The assay, extraction and gradient procedures have been described in "Materials and Methods." Bleomycin means bleomycin plus 2-mercaptoethanol unless indicated otherwise. For all sucrose gradient experiments shown the amounts added were bleomycin; 50 μg, NEM; 5 μmoles, daunomycin; 100 pmoles, in 0.5 ml of reaction mixture. A, (1) incubated for 30 minutes in the presence of bleomycin; (2) incubated for 10 minutes in the presence of bleomycin; (3) incubated for 30 minutes in the presence of bleomycin and NEM. B, (4) incubated for 30 minutes in the presence of bleomycin with 2-mercaptoethanol omitted; (5) incubated for 30 minutes in the presence of NEM; (6) incubated for 30 minutes in the presence of bleomycin with NEM added after 10 minutes of incubation.

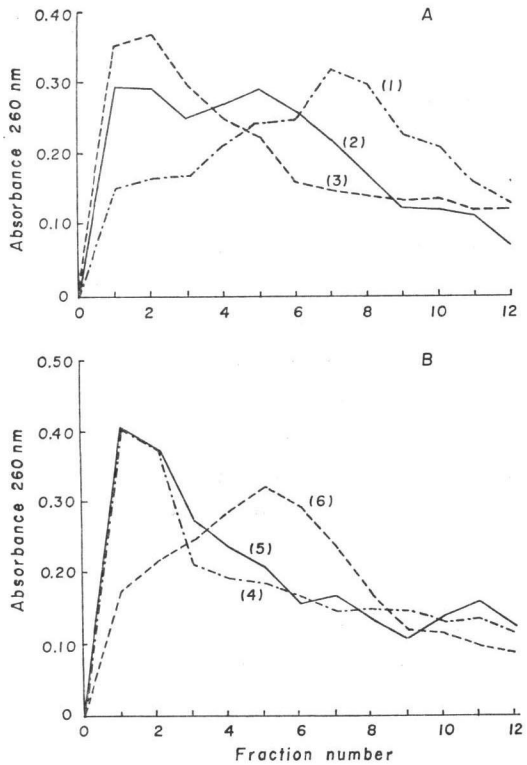


Fig. 3. Neutral sucrose gradients of DNA extracted from hepatoma 7777 nuclei.

Procedures were as for Fig. 2. (1) incubated for 30 minutes plus bleomycin; (2) incubated for 30 minutes plus daunomycin; (3) incubated for 30 minutes plus bleomycin and daunomycin.

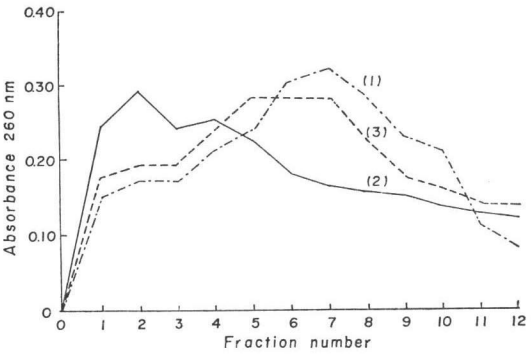
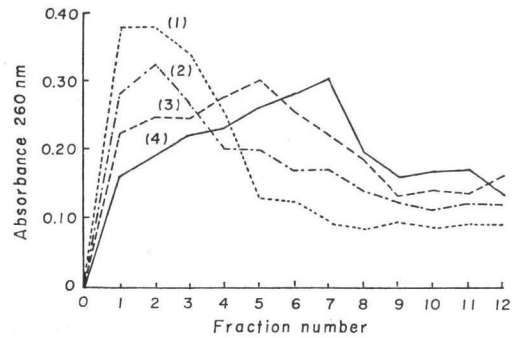


Fig. 4. Neutral sucrose gradients of DNA extracted from hepatoma 7777 and liver nuclei.

Procedures were as for Fig. 2. Incubation was for 30 minutes (1) liver nuclei plus bleomycin and NEM; (2) hepatoma nuclei plus bleomycin and NEM; (3) liver nuclei plus bleomycin and daunomycin; (4) hepatoma nuclei plus bleomycin and daunomycin.



Inhibition of bleomycin-induced activity by daunomycin is shown in Table 3. Daunomycin inhibited bleomycin-induced activity in hepatoma 7777 nuclei much more readily than in host liver nuclei. Fifty pmoles inhibited the activity by 80% in the hepatoma nuclei but only by 56% in host liver nuclei. A similar difference was found with 20 pmoles of daunomycin, but 100 pmoles inhibited almost completely in both nuclear preparations. Inhibition of incorporation by daunomycin in the absence of bleomycin was much less and was similar in the two nuclear preparations. Unlike NEM, the levels of daunomycin needed for inhibition of bleomycin-induced activity were not dependent on the level of 2-mercaptoethanol.

Fig. 5. Alkaline sucrose gradients of DNA extracted from liver nuclei.

Procedures have been described in "Materials and Methods." Levels of bleomycin and NEM, were as indicated for Fig. 2. (1) incubation for 30 minutes plus bleomycin; (2) incubation for 30 minutes plus bleomycin and NEM; (3) incubation for 30 minutes plus bleomycin with 2-mercaptoethanol omitted.

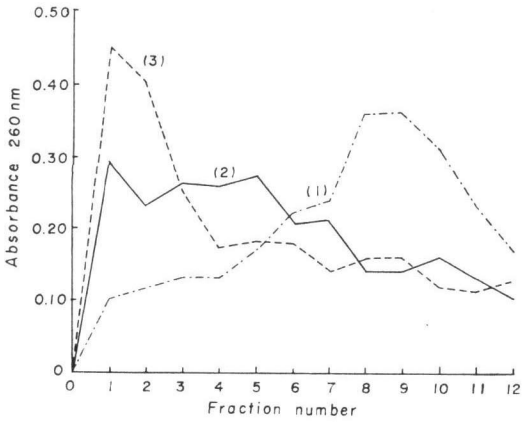
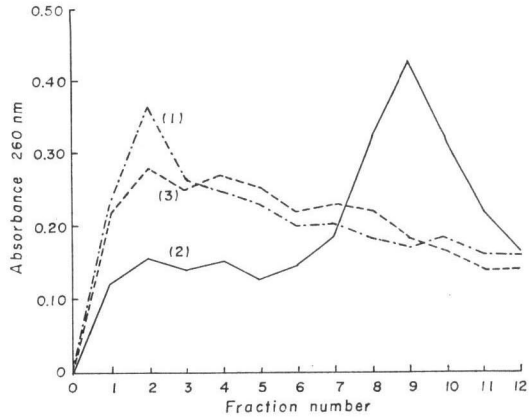


Fig. 6. Alkaline sucrose gradients of DNA extracted from hepatoma 7777 nuclei.

Procedures have been described in "Materials and Methods." Levels of bleomycin, NEM, and daunomycin were as indicated for Fig. 2. (1) incubated for 30 minutes plus daunomycin; (2) incubated for 30 minutes plus bleomycin and daunomycin; (3) incubated for 30 minutes plus bleomycin and NEM.



In Fig. 1 we show results that indicate that the addition of NEM or daunomycin at indicated times of a 30-minute total incubation period inhibits further bleomycin-induced activity. Incorporation without bleomycin is shown only for the 30-minute incubation period. We have shown previously that incorporation into nuclei in the absence of bleomycin was linear for only 10 minutes.[18,19] In the presence of bleomycin, incorporation was linear for at least 30 minutes. We could calculate that the addition of NEM at 5, 10, 20 and 30 minutes resulted in a 1.8, 1.0, 1.1 and 0.9-fold increase in incorporation in the presence of bleomycin alone, for the indicated times with host liver nuclei. With daunomycin the numbers were 2.1, 1.0, 1.1 and 1.2 for 5, 10, 20 and 30 minutes respectively. With hepatoma 7777 nuclei the numbers were 1.7, 1.2, 1.2 and 1.2 (NEM) and 1.3, 0.9, 0.9, and 1.1 (daunomycin) for addition at 5, 10, 20 and 30 minutes respectively.

We had shown previously that bleomycin caused both single and double-stranded breaks in the DNA of treated nuclei.[16] To get some insight into the mode of the inhibition of the bleomycin-induced activity by the two compounds, we analyzed the DNA on sucrose gradients. The results of the neutral and alkaline sucrose gradient analyses are shown in Figs. 2 to 6.

It is apparent from the gradient profiles in Fig. 2A that a 30-minute incubation with bleomycin resulted in double-stranded breaks. A 10-minute incubation with bleomycin also caused breaks, but to a lesser extent. The presence of NEM during the 30-minute incubation with bleomycin prevented DNA breakage.

In Fig. 2B it can be seen that NEM alone did not affect the gradient profile of DNA. The addition of bleomycin did not result in DNA breaks when 2-mercaptoethanol was omitted from the incubation. These two profiles were the same as were found when no additions were made to nuclei incubated for 30 minutes in the regular incubation mixture. This profile is not shown in the Fig. 2B. The third profile

in Fig. 2B shows that a 10-minute incubation in the presence of bleomycin, followed by a 20-minute incubation in the presence of both bleomycin and NEM did not cause more breaks than a 10-minute incubation in the presence of bleomycin alone, as shown in Fig. 2A. This is in agreement with incorporation results shown in Fig. 1. The results in Fig. 2 were obtained with host liver nuclei. Hepatoma 7777 nuclei gave identical results. Despite a reduced bleomycin-induced incorporation in hepatoma 7777 nuclei, sucrose gradient profiles were the same. Similar findings were published previously[16] and were confirmed in Fig. 3, which shows sucrose gradient profiles of DNA extracted from hepatoma 7777 nuclei. Incubation in the presence of bleomycin resulted in a similar profile as shown in Fig. 2A for DNA from host liver nuclei. It can further be seen in Fig. 3 that daunomycin, unlike NEM, does not prevent bleomycin-caused breaks, despite a complete inhibition of bleomycin-induced incorporation. Daunomycin alone on the other hand does not cause breaks.

In Fig. 4 we compared DNA extracted from host liver nuclei and from hepatoma 7777 nuclei incubated in the presence of bleomycin plus NEM or daunomycin. There was no significant difference in the sucrose gradient profiles when NEM was added. DNA breakage was prevented in both nuclear preparations. When daunomycin was added, however, more breaks were apparent in DNA extracted from hepatoma 7777 nuclei than in DNA extracted from host liver nuclei. This finding was consistent with incorporation studies shown in Table 3. Daunomycin inhibited bleomycin-induced incorporation more readily in hepatoma nuclei than in host liver nuclei.

Alkaline sucrose gradient profiles are shown in Figs. 5 and 6. In Fig. 5 it can be seen that the absence of 2-mercaptoethanol prevented bleomycin-induced breaks completely. The presence of bleomycin with 2-mercaptoethanol present in the incubation mixture resulted in a shift of the absorbance towards the top of the gradient as compared to neutral gradients, indicating additional single strand breaks. It is also apparent that the addition of NEM to the bleomycin-treated nuclei did not prevent some single-strand breaks whereas it inhibited double-stand breaks completely.

Alkaline sucrose gradient profiles for hepatoma 7777 DNA are shown in Fig. 6. Again the presence of NEM and bleomycin in the incubation mixture allowed some single-strand breaks. Daunomycin alone did not cause single-strand breaks and resulted in profiles similar to NEM alone (not shown) or the omission of 2-mercaptoethanol in the presence of bleomycin. The addition of daunomycin to bleomycin during the incubation did not diminish the breaks caused by bleomycin alone. On alkaline sucrose gradients there was no difference in profiles obtained from DNA extracted from host liver or hepatoma nuclei in the presence of both bleomycin and daunomycin.

Discussion

It is well established and has been confirmed by our results that bleomycin causes breaks in DNA[10,12,13,26]. Partial repair of such breaks seems to be the reason for increased incorporation of ^{3}H-TTP in our system with isolated nuclei[16]. Increased incorporation of deoxyribonucleotides into DNA exposed to bleomycin or phleomycin has been reported in several systems[9,14,15]. We have shown that bleomycin-induced activity is greater in host liver, normal liver, and slow growing hepatoma nuclei than in nuclei from faster growing hepatomas[16].

Our results show that this bleomycin-induced incorporation of ^{3}H-TTP can be inhibited by NEM and daunomycin. The mechanisms by which these two compounds inhibit, however, are different. The major effect of NEM might be to compete for 2-mercaptoethanol or to inhibit a deoxyribonuclease that might be necessary for extensive DNA breakage. Daunomycin seems to inhibit a repair enzyme.

Bleomycin did not cause breaks in the DNA nor did it stimulate ^{3}H-TTP incorporation in the

absence of 2-mercaptoethanol or another sulfhydryl compound. At present we do not know why the effect of bleomycin is dependent on a sulfhydryl-reducing agent. It has been shown that sulfhydryl agents were needed for bleomycin to decrease the melting temperature of DNA[11] and to bind and produce strand scissions in DNA[5,10]. The need for sulfhydryl compounds for phleomycin-induced cleavage of DNA has been shown[27]. That bleomycin releases free bases, preferentially thymine from DNA with subsequent spontaneous cleavage of the phosphodiester backbone and that this action is aided by sulfhydryl compounds has been shown.[28,29]

The effect of bleomycin with 2-mercaptoethanol present in the system can be inhibited by the addition of NEM. Both the bleomycin-induced incorporation and the formation of breaks in the DNA are inhibited by NEM. Some single-stranded breaks may occur in the presence of NEM as judged from alkaline sucrose gradient profiles, but these breaks are not extensive. The results suggested that NEM might compete for the reducing agent and thus prevent the action of bleomycin.

Daunomycin inhibits the bleomycin-induced incorporation by a different mechanism, most likely by inhibiting the repair process. The addition of daunomycin did not affect the bleomycin-caused single- or double-stranded breaks despite inhibiting incorporation of ^{3}H-TTP completely. That the inhibitory effect of daunomycin appears to be on the repair process is further indicated by the different sensitivity of the bleomycin-induced incorporation to daunomycin in host liver and hepatoma 7777 nuclei. We have suggested previously that hepatoma nuclei have less repair enzyme or are deficient in their repair ability[16]. If there is less repair activity in hepatoma nuclei, lower levels of daunomycin would be sufficient to inhibit this repair.

Our findings should have applications for a basic understanding of DNA synthesis in mammalian cells and for chemotherapy. We know that extracted DNA can be treated with bleomycin and becomes a more efficient template for a relatively crude DNA polymerase preparation[16]. It will be possible with the use of bleomycin and daunomycin to identify which of the several mammalian DNA polymerases[19,30~33] is primarily responsible for repair activity.

In chemotherapy the effect of bleomycin may be enhanced by the addition of daunomycin. It has been shown by several investigators that bleomycin-caused breaks in DNA are repaired in mammalian cells[12,13,34]. The addition of daunomycin would prevent such repair. Since hepatoma nuclei are more sensitive to repair inhibition by daunomycin, it should be possible to use levels that might not inhibit repair in the host cells thus causing a more selective effect of bleomycin on hepatoma cells.

Acknowledgement

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