Characterization of the Association of Radiolabeled Bleomycin A2 with HeLa Cells

Samar N. Roy and Susan Band Horwitz

Departments of Molecular Pharmacology [S. N. R., S. B. H.] and Cell Biology [S. B. H.], Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT

The association of [3H]bleomycin A2 and Cu(II):[3H]bleomycin A2 with HeLa cells has been characterized. Under the conditions of our experiments, approximately 0.1% of the total drug in the medium associates with HeLa cells. Both forms of the drug bind to HeLa cells in a specific and saturable manner, with a Kd of 20 μM and a Vmax of 2.5 pmol/min/10^6 cells. Scatchard analysis of the specific binding data demonstrates a single set of high-affinity binding sites. Cytotoxic activities of both forms of the drug are similar, with a 50% lethal dose of 0.5 μM at 48 hr. The specific binding in HeLa cells of either the labeled metal-free drug or its copper complex is reversible by a 100-fold excess of either unlabeled drug. Interaction of the drug with cells is temperature sensitive but is unaffected by metabolic poisons, suggesting that this process is not energy dependent. Isolation of DNA from HeLa cells incubated with the drug indicates that 1 mol of either [3H]bleomycin A2 or Cu(II):[3H]bleomycin A2 binds per 10^8 nucleotides. Further studies with the radiolabeled drug are required to define precisely the mechanisms involved in bleomycin uptake and compartmentalization within the cell.

INTRODUCTION

The bleomycins are a family of glycopeptide antibiotics that were originally isolated as copper complexes from cultures of Streptomyces verticillus (24). The drug contains both a binding site for DNA (7) and a binding site for metals (4, 11). The major activity of the bleomycins, which are used in the treatment of selected neoplastic disease in humans (9, 25), is the degradation of cellular DNA (16, 21). Both Cu(II):bleomycin and metal-free bleomycin are equal in their ability to inhibit the growth of tumors selected neoplastic disease in humans (4, 11), information on the mechanism by which this complex degrades DNA in vitro is developing (4, 11), information on the interaction of bleomycin with cells is meager. This is partly because of the general unavailability of radiolabeled bleomycin. We have recently prepared pure radiolabeled BLMA2 and Cu(II):BLMA2 (18), and we report here our initial studies, in which we have examined the interaction of these compounds with HeLa cells.

MATERIALS AND METHODS

Materials

Bleomycin sulfate (Blenoxane, Lot E 90X3) was a gift from Bristol Laboratories, Syracuse, NY. Calf thymus DNA was purchased from Worthington Biochemical Corp., Freehold, NJ. Spectrapor dialysis tubing (pore diameter 4.8 mm; flat width, 3.0 cm) and all other chemicals and solvents were from Fisher Scientific Co., Pittsburgh, PA. Tissue culture medium and supplies were obtained from Grand Island Biological Co., Grand Island, NY.

Methods

Preparation of Radiolabeled BLMA2. [3H]BLMA2 and Cu(II):[3H]-BLMA2 (2.5 Ci/mmol) were prepared and analyzed as described previously (18). Twenty μl of Cu(II):[3H]BLMA2 (3 mg) were purified (Chart 1) on an Altex Ultrasphere ODS (C18) reversed-phase column (4.5 x 150 mm; particle size, 5 μm) with an Altex Model 100 metering pump. A guard column (3.2 x 45; particle size, 10 μm) was used. Fractions of 1.0 ml were eluted at room temperature by a solvent consisting of 1% CH3COONH4:CH3OH (75:25, v/v), pH 6.4, for the initial 100 min. The CH3OH was increased to 40% at 100 min and to 100% at 130 min. Absorbance in each fraction was monitored at 292 nm. The retention time of Cu(II):[3H]BLMA2 was approximately 20 min, and it was eluted between Fractions 9 and 15. These fractions, containing a minimum of 90% of the radioactivity applied to the column, were evaporated and dissolved in H2O. On thin-layer chromatography, a single spot identical to that of Cu(II):[3H]BLMA2 was detected (18). Ten % of the radioactivity had a retention time of approximately 123 min, and by thin-layer chromatography, it was identified as Cu(II):bleomycin dimethylether A2. The latter compound was formed apparently from Cu(II):[3H]BLMA2 during its preparation.

Cells. HeLa cells (S3) were maintained as monolayers on 100-mm tissue culture dishes in complete medium consisting of DME supplemented with penicillin, streptomycin, 1% glutamine, and 10% fetal calf serum. This supplemented DME is referred to as complete medium. The cell cultures grown at 37° in a humid incubator with an air mixture containing 8% CO2. The cells were trypsinized with 2 ml of a 0.25% trypsin:EDTA solution. For growth curves, cells (4 x 10^5) were plated in complete medium plus the indicated concentrations of BLMA2 in 60-mm tissue culture dishes in a final volume of 4 ml. At appropriate time intervals, the medium was removed, and the cells were suspended with the aid of a rubber policeman in 2 ml of DME containing 0.02% EDTA. The cells were counted with a hemocytometer.

Interaction of [3H]BLMA2 and Cu(II):[3H]BLMA2 with HeLa Cells. Confluent 35-mm tissue culture dishes were used to measure the interaction of labeled BLMA2 and Cu(II):BLMA2 with cells. Each dish contained

---

1This work was supported in part by USPHS Grants CA 17514 and GM 29042 and American Cancer Society Grant CH-86.
2To whom requests for reprints should be addressed.

Received May 20, 1983; accepted December 6, 1983.

APRIL 1984

1541
approximately $10^8$ cells. Two ml of DME containing the appropriate concentration of labeled drug were added to each dish, and the cells were incubated for 30 min at 37° in a CO2 incubator. The cells were washed 3 times with 1 ml of ice-cold phosphate-buffered saline (5 mM Na2HPO4, 1.8 mM KH2PO4, 0.037 mM KCl, 0.17 mM NaCl, pH 7.4); further washing did not alter the amount of label in the cells. The cells were lysed with 1 ml of 0.2 N NaOH for 16 hr at 25°. An aliquot of the cell lysate was neutralized with an equal volume of glacial acetic acid. Radioactivity was determined in 10 ml of ACS II liquid scintillation fluid (Amersham/Seartec Corp., Arlington Heights, IL).

Nonspecific uptake was measured as the amount of labeled drug taken up in the presence of a 100-fold excess of unlabeled drug. This nonspecific uptake may include some low-affinity binding sites for the drug. Specific uptake was calculated as the difference between binding of labeled drug in the presence and absence of a 100-fold excess of unlabeled drug.

Binding of $[^3H]$BLMA2 and Cu(II):$[^3H]$BLMA2 in HeLa Cell Nuclei. Confluent 60-mm tissue culture dishes were incubated for the indicated times with either 100 nM $[^3H]$BLMA2 or Cu(II):$[^3H]$BLMA2 in 4 ml of DME at 37° in a CO2 incubator. Nuclei were isolated by homogenization according to the modified method of Chauveau et al. (6) and lysed with 0.2 N NaOH as described above for whole cells. Radioactivity in the nuclear and cytosol fractions was measured. Both total and specific uptake were determined in whole cells and in their cytosol and nuclei.

The binding of labeled BLMA2 and Cu(II):BLMA2 to DNA in the nucleus was examined by neutral CsCl equilibrium centrifugation (13, 15). Nuclei (2.4 x 10^7) were isolated from HeLa cells that had been incubated with either 100 nM $[^3H]$BLMA2 or Cu(II):$[^3H]$BLMA2 in 4 ml of DME for 2 hr at 37° in a CO2 incubator. The nuclei were lysed at 37° for 30 min in 1 ml of lysing buffer consisting of 10 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1% sodium lauryl sulfate, 50 μg of pancreatic RNase, and 150 μg of pronase (self-digested for 2 hr at 37°). The nuclear lysate was homogenized with 7 ml of CsCl, and the density was adjusted to 1.70 before centrifugation at 45,000 rpm for 48 hr at 25° in a fixed-angle Type 50 rotor. Approximately 25 fractions were collected from the bottom of each gradient. Absorbance at 260 nm and radioactivity were determined for each fraction. The amount of drug bound to DNA was calculated from the radioactivity coincident with the peak of DNA ($\sim 260$ nm $= 66,000$ M$^{-1}$ nucleotides).

Dissociation of DNA:Bleomycin Complexes in Vitro. The rate of dissociation of $[^3H]$BLMA2 and Cu(II):$[^3H]$BLMA2 from calf thymus DNA was measured by the continuous flow equilibrium dialysis method described by Colowick and Womack (8). The upper chamber of the dialysis cell (2.5 ml) contained $2 \times 10^{-4}$ M calf thymus DNA plus $1.5 \times 10^{-5}$ M labeled drug in 2.0 ml of 2.5 mM sodium phosphate buffer, pH 7.0. This solution was separated by a membrane (flat width, 3.0 cm; prepared from dialysis tubing) from a lower chamber (2.8 ml) through which the buffer was pumped at a constant rate of 5 ml/min. Radioactivity was measured in the effluent that was collected as 2.5-ml fractions. After each 5 fractions were collected, small increments of unlabeled drug (5 to 10 μl) were added to the upper chamber until all of the labeled ligand was removed from the DNA.

RESULTS

Interaction of $[^3H]$BLMA2 and Cu(II):$[^3H]$BLMA2 with HeLa Cells. The specific binding of both $[^3H]$BLMA2 and Cu(II):$[^3H]$BLMA2 saturates at approximately 60 pmol/10^6 cells (Chart 2). In these experiments, specific binding is defined as that fraction of the total binding which is inhibited by a 100-fold excess of the appropriate unlabeled drug, either the metal-free or Cu(II) form. All experiments were performed in the absence of serum, although serum does not influence specific or nonspecific binding.

Double reciprocal plot analysis of the specific binding data for both forms of the drug produced a calculated $K_d$ of 20 μM and a $V_{max}$ of 2.5 pmol/min/10^6 cells (Chart 3A). Scatchard analysis
of the specific binding data produces a linear plot, suggesting a single set of high-affinity binding sites with a dissociation constant of 12 μM (Chart 3B).

The nonspecific component of uptake, which is defined as binding that cannot be diluted with a 100-fold excess of unlabeled BLMA₂ or Cu(II):BLMA₂, increases linearly in a concentration-dependent manner (Chart 2). With [³H]BLMA₂, nonspecific binding ranges from 22% of total binding at 2 μM of drug to 74% at 200 μM. With Cu(II):[³H]BLMA₂, nonspecific binding ranges from 19% of total binding at 2 μM to 68% of total binding at 200 μM.

The total association of [³H]BLMA₂ with HeLa cells was greater than that of Cu(II):[³H]BLMA₂ as the concentration of added ligand increased beyond 80 μM. At 200 μM, the total uptake of [³H]BLMA₂ was 223 pmol/10⁶ cells compared to 160 pmol/10⁶ cells for Cu(II):[³H]BLMA₂ (Chart 2). The total binding of Cu(II):[³H]BLMA₂ reaches a steady state within 10 min at 37° and remains constant for 110 min (Chart 4). Although the major portion of total binding of [³H]BLMA₂ is completed within 10 min, there is a small but continual increase in uptake. The specific uptake saturates by 30 min with both forms of the drug (Chart 4).

In preliminary experiments, radiolabeled BLMA₂ was isolated from the cytosol of cells that had been incubated with [³H]BLMA₂ for 2 hr at 37°. Analysis by high-performance liquid chromatography indicated that approximately 85% of the drug was in its original form.

The concentration of drug that produced maximal binding resulted in the most significant inhibition of growth (Chart 5). The LD₅₀ for both forms of bleomycin was measured after a 24-, 48-, and 72-hr incubation with HeLa cells (Table 1). At 24 hr, the LD₅₀ for BLMA₂ was 2.6 μM, and for Cu(II):BLMA₂, it was 2.4 μM, indicating that the cytotoxicity of both forms of the drug is similar.

To determine if specific binding of labeled drug could be diluted with excess unlabeled drug, cells were incubated with 40 μM of either [³H]BLMA₂ or Cu(II):[³H]BLMA₂ for 30 min. On addition of

<table>
<thead>
<tr>
<th>Drug</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLMA₂</td>
<td>2.55 ± 0.15*</td>
<td>0.51 ± 0.06</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>Cu(II):BLMA₂</td>
<td>2.35 ± 0.13</td>
<td>0.44 ± 0.05</td>
<td>0.25 ± 0.05</td>
</tr>
</tbody>
</table>

* Mean ± S.D. of 2 determinations.

Chart 5. Relationship between the inhibition of growth of HeLa cells and specific uptake of labeled drug. A growth curve of HeLa cells incubated for 24 hr with various concentrations of drug was performed as described in "Materials and Methods." The specific uptake of [³H]BLMA₂ by HeLa cells at 30 min was measured according to the procedure described in "Materials and Methods." Each point represents the average of 2 determinations. O, specific uptake; ◦, percentage of control growth.

Chart 4. Kinetics of total and specific uptake of [³H]BLMA₂ and Cu(II):[³H]BLMA₂ in HeLa cells. Total uptake was measured by incubating cells with DME containing 40 μM of either [³H]BLMA₂ (A) or Cu(II):[³H]BLMA₂ (B) for 30 min. Non specific uptake was determined by subtracting the nonspecific uptake from total uptake. Each point represents the average of 4 determinations. O, total; ◦, specific.

Chart 6. Reversibility of uptake of labeled drug in HeLa cells. Cells were incubated with DME containing 40 μM of either [³H]BLMA₂ (A) or Cu(II):[³H]BLMA₂ (B), and total uptake was measured (△). Reversibility was determined by the addition (↓) of a 100-fold excess of either BLMA₂ (O) or Cu(II):BLMA₂ (○). The level of drug in the cells was followed for an additional 120 min in the absence (——) and presence (---) of excess drug. Each point represents the average of 4 determinations.

A 100-fold excess of either form of the unlabeled drug, there was a rapid decrease in the amount of bound label to the level of nonspecific binding (Chart 6). The latter binding remained constant for 120 min. It is not clear if all or part of this nonspecific binding represents free bleomycin.
Association of $[^3H]BLMA_2$ and Cu(II):$[^3H]BLMA_2$ with Cell Fractions. The total interaction of $[^3H]BLMA_2$ in cells was greater than that of Cu(II):$[^3H]BLMA_2$ (Chart 7). For both drugs, approximately twice as much of the total bound drug was found in the cytosol compared to the nuclei. However, the specific uptake of both forms of drug in whole cells and nuclei was similar. It has been suggested that bleomycin in its copper form interacts with specific cytoplasmic proteins that are involved in the reduction of Cu(II) and its removal from the drug. The metal-free drug could then be activated with Fe(II) and oxygen to degrade DNA (10, 22).

The binding of both $[^3H]BLMA_2$ and Cu(II):$[^3H]BLMA_2$ to nuclei was further characterized by equilibrium centrifugation in CsCl (Chart 8). The peak of radioactivity coincided with the peak of absorbance at 260 nm at a density of 1.705. Under our conditions, the DNA from untreated nuclei sedimented at a density of 1.723. A decrease in the density of DNA when drug binding occurs has been reported for a number of drugs, including actinomycin (5). Approximately 0.1% of both forms of the labeled drug in the medium was taken up by the cells, and 20% of this labeled drug was found in the nuclei. The binding ratio was estimated as 1 mol of $[^3H]BLMA_2$/1.1 x $10^6$ nucleotides and 1 mol of Cu(II):$[^3H]BLMA_2$/0.99 x $10^6$ nucleotides in HeLa cell nuclear DNA (Table 2). These differences were not significant.

Effect of 4°, Sodium Azide, and Sodium Cyanide on the Association of Bleomycin with Cells. To examine the effect of temperature on drug transport, cells were maintained at 4° in 35-mm tissue culture dishes for 30 min. the medium was removed, and 1 ml of DME containing 40 /mM of either form of labeled drug was added. Incubation continued at 4° for 30 min, and specific uptake was determined. Under these conditions, specific uptake was reduced to less than 5% of that observed at 37° (Table 3).

To determine the effect of metabolic poisons on specific binding, cells were preincubated with 10 mM of either NaCN or NaN_3 in 1.0 ml of phosphate-buffered saline for 60 min at 37°. $[^3H]BLMA_2$ or Cu(II):$[^3H]BLMA_2$ was added at a final concentration of 40 /mM, and incubation continued for 30 min at 37°. Essentially, no change was seen in the specific binding of either form of BLMA_2 (Table 3).

Dissociation of DNA:bleomycin Complexes in Vitro. The upper chamber of the equilibrium dialysis apparatus contained 2 x $10^{-4}$ m calf thymus DNA and 1.5 x $10^{-5}$ m of either $[^3H]BLMA_2$ or Cu(II):$[^3H]BLMA_2$. Experiments were done at 4° in 2.5 mM sodium phosphate buffer, pH 7.0. A steady-state equilibrium was observed after 12.5 ml of buffer were pumped through the lower chamber. Small increments of the corresponding unlabeled drug were added to the upper chamber after 5 fractions (each 2.5 ml) were collected. This procedure was continued until all of the labeled drug had been displaced from the DNA. The amount of labeled drug released was identical to that found in the effluent of samples to which no DNA had been added. With each incre-
Association of \( ^{3}H \)BLMA\(_2 \) with Cells

In vitro the interaction of the drug with calf thymus DNA and synthetic nucleic acids. Binding data obtained with calf thymus DNA by gel filtration revealed an apparent equilibrium constant for \( ^{3}H \)BLMA\(_2 \) of \( 5.7 \times 10^5 \) mol/l and, for Cu(II):\( ^{3}H \)BLMA\(_2 \), \( 3.9 \times 10^5 \) mol (18).

In the present paper, we have examined the association of both forms of the drug with HeLa cells and their interaction with cellular DNA. As has been reported by Miyake et al. (15) in rat ascites hepatoma cells, we also found, under our experimental conditions, that approximately 0.1% of the total radioactivity in the medium was bound to HeLa cells. The interaction of radiolabeled drug with our cells was the same in the absence and presence of 10% fetal calf serum. Although all experiments were done in the absence of serum, no further attempt was made to eliminate copper from the medium. In animals, both Cu(II):bleomycin and metal-free bleomycin exhibit antitumor activity (16, 17, 26). Bleomycin is an excellent chelator of Cu(II) and is known to sequester Cu(II) from amino acid and peptide complexes (10, 22). Evidence suggests that when metal-free bleomycin is administered to animals, it chelates Cu(II) (14). Uehara et al. (23) have reported that, in L5178Y cells, cobalt ions enhance the uptake of pepleomycin, a member of the group of bleomycin-like antibiotics. However, a complex of bleomycin and cobalt has no antitumor activity in animals (16). These authors observed no effect of copper on pepleomycin uptake.

Our experiments suggest a single set of high-affinity binding sites for the saturable component of both forms of the drug in HeLa cells. The saturation of specific binding is observed at approximately 60 pmol of drug/10\(^6\) cells. The \( LD_{50} \) for both forms of the drug at 24 hr was very similar.

Neither Na\(_3\) nor NaCN altered the association of either form of BLMA\(_2 \) with cells, indicating that this was not an energy-dependent process. However, association examined at 4\( ^\circ \) was drastically reduced, suggesting that the low temperature may be inducing membrane changes that result in inhibition of uptake of BLMA\(_2 \). In contrast to specific binding, all of our experiments demonstrated that the total uptake of BLMA\(_2 \) was greater than that of Cu(II):BLMA\(_2 \), indicating that there is a greater association of the metal-free form than the copper complex of the drug with cells. The absence of Cu(II) may result in more available binding sites in the metal-free drug.

The specific binding of both forms of the drug to HeLa cells is reversible. An excess of either form of unlabeled drug immediately results in a level of binding equivalent to that of nonspecific binding that may include free BLMA\(_2 \). These data correlate with our experiments done \textit{in vitro} with calf thymus DNA and radiolabeled BLMA\(_2 \), which indicate that the drug:DNA complex can be dissociated with unlabeled drug. One mol of BLMA\(_2 \) binds for every 3.1 base pairs in DNA and that 1 mol of Cu(II):\( ^{3}H \)BLMA\(_2 \) binds for every 2.3 base pairs in DNA. The same results were observed when this experiment was done at 25\( ^\circ \).

DISCUSSION

Although the interaction of bleomycin with DNA has been studied extensively in cell-free systems, very little information is available concerning the association of bleomycin with cells. This is because radiolabeled bleomycin has not been readily available, and in the few cell systems that have been studied, a very small percentage of the bleomycin present in the medium is taken up into cells. Akiyama et al. (1) have indicated that the permeation of bleomycin in cultured cells can be enhanced by polyene antibiotics.

A procedure for preparing biologically active radiolabeled BLMA\(_2 \) and Cu(II):BLMA\(_2 \) was developed in our laboratory (18). The availability of radiolabeled BLMA\(_2 \) allowed us to examine in vitro the interaction of the drug with calf thymus DNA and synthetic nucleic acids. Binding data obtained with calf thymus DNA by gel filtration revealed an apparent equilibrium constant for \( ^{3}H \)BLMA\(_2 \) of \( 5.7 \times 10^5 \) mol/l and, for Cu(II):\( ^{3}H \)BLMA\(_2 \), \( 3.9 \times 10^5 \) mol (18).

In the present paper, we have examined the association of both forms of the drug with HeLa cells and their interaction with cellular DNA. As has been reported by Miyake et al. (15) in rat ascites hepatoma cells, we also found, under our experimental conditions, that approximately 0.1% of the total radioactivity in the medium was bound to HeLa cells. The interaction of radiolabeled drug with our cells was the same in the absence and presence of 10% fetal calf serum. Although all experiments were done in the absence of serum, no further attempt was made to eliminate copper from the medium. In animals, both Cu(II):bleomycin and metal-free bleomycin exhibit antitumor activity (16, 17, 26). Bleomycin is an excellent chelator of Cu(II) and is known to sequester Cu(II) from amino acid and peptide complexes (10, 22). Evidence suggests that when metal-free bleomycin is administered to animals, it chelates Cu(II) (14). Uehara et al. (23) have reported that, in L5178Y cells, cobalt ions enhance the uptake of pepleomycin, a member of the group of bleomycin-like antibiotics. However, a complex of bleomycin and cobalt has no antitumor activity in animals (16). These authors observed no effect of copper on pepleomycin uptake.

Our experiments suggest a single set of high-affinity binding sites for the saturable component of both forms of the drug in HeLa cells. The saturation of specific binding is observed at approximately 60 pmol of drug/10\(^6\) cells. The \( LD_{50} \) for both forms of the drug at 24 hr was very similar.

Neither Na\(_3\) nor NaCN altered the association of either form of BLMA\(_2 \) with cells, indicating that this was not an energy-dependent process. However, association examined at 4\( ^\circ \) was drastically reduced, suggesting that the low temperature may be inducing membrane changes that result in inhibition of uptake of BLMA\(_2 \). In contrast to specific binding, all of our experiments demonstrated that the total uptake of BLMA\(_2 \) was greater than that of Cu(II):BLMA\(_2 \), indicating that there is a greater association of the metal-free form than the copper complex of the drug with cells. The absence of Cu(II) may result in more available binding sites in the metal-free drug.

The specific binding of both forms of the drug to HeLa cells is reversible. An excess of either form of unlabeled drug immediately results in a level of binding equivalent to that of nonspecific binding that may include free BLMA\(_2 \). These data correlate with our experiments done \textit{in vitro} with calf thymus DNA and radiolabeled BLMA\(_2 \), which indicate that the drug:DNA complex can be dissociated with unlabeled drug. One mol of BLMA\(_2 \) binds for every 3.1 base pairs in DNA and that 1 mol of Cu(II):\( ^{3}H \)BLMA\(_2 \) binds for every 2.3 base pairs in DNA. The same results were observed when this experiment was done at 25\( ^\circ \).

DISCUSSION

Although the interaction of bleomycin with DNA has been studied extensively in cell-free systems, very little information is available concerning the association of bleomycin with cells. This is because radiolabeled bleomycin has not been readily available, and in the few cell systems that have been studied, a very small percentage of the bleomycin present in the medium is taken up into cells. Akiyama et al. (1) have indicated that the permeation of bleomycin in cultured cells can be enhanced by polyene antibiotics.

A procedure for preparing biologically active radiolabeled BLMA\(_2 \) and Cu(II):BLMA\(_2 \) was developed in our laboratory (18). The availability of radiolabeled BLMA\(_2 \) allowed us to examine in
inhibit cell growth similarly, and replication is almost completely inhibited at the concentration of drug that is saturating for specific uptake. The total uptake of metal-free drug is greater than that of the copper complex. Since there is less nonspecific uptake of the copper complex, it is possible that this form of the drug, if compared to metal-free bleomycin, would exhibit fewer side effects in the treatment of malignant disease.

An initial study on the association of radiolabeled bleomycin with HeLa cells is presented. Further studies are required to define precisely the mechanisms involved in bleomycin uptake, metabolism, compartmentalization, and efflux in cells.

REFERENCES

Characterization of the Association of Radiolabeled Bleomycin A₂ with HeLa Cells

Samar N. Roy and Susan Band Horwitz