

Use of PM-2 DNA Degradation as a Pharmacokinetic Assay for Bleomycin¹

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ABSTRACT

The PM-2 DNA fluorescence assay has been shown to be a rapid, sensitive, and reproducible assay for bleomycin biochemical activity. The assay can detect bleomycin in human serum in the nmol range. The method measures DNA degradative activity of bleomycin and could be used to determine activity of bleomycin analogs and metabolites. The usefulness of the assay to perform bleomycin pharmacokinetic studies in cancer patients has been demonstrated. Linear regression analyses of parallel bleomycin assays with the radioimmunoassay gave a coefficient of correlation of 0.98 to 0.78 with trichloroacetic acid-treated serum. These results indicate excellent agreement between the two assays.

INTRODUCTION

Bleomycin is a glycopeptide antitumor antibiotic extracted from *Streptomyces verticillus* (16) with significant clinical activity against germ cell tumors of the testes, lymphomas, and squamous cell carcinomas of various sites (1, 5). Although it induces minimum bone marrow toxicity, administration of bleomycin is associated with significant pulmonary and mucocutaneous toxicities (4, 5).

Several methods have been used to study the clinical pharmacology of bleomycin. Initial studies were performed using a microbiological assay (9, 12). The microbiological assay was of limited utility because of its relatively low sensitivity and lack of reproducibility. Subsequent studies used isotopically labeled bleomycin (10). The obvious difficulties associated with administration of radioactive compounds to humans are compounded by the fact that the isotopes used have been ¹¹¹In or ⁵⁷Co which may dissociate from bleomycin or alter the pharmacokinetics of bleomycin.

More recently, 2 radioimmunoassays have been developed and contributed significantly to the understanding of the clinical pharmacology of bleomycin (2, 6, 8). However, both radioimmunoassays have been shown to cross-react with a variety of bleomycin analogs, some of which are significantly less active than the bleomycin analogs in the clinically used mixture (14). Moreover, although the detoxification of bleomycin is effected primarily by renal excretion (13), studies on the pharmacokinetics of long-term i.v. infusions have suggested that in some circumstances bleomycin may be metabolized to products which are inactive but which cross-react with antibleomycin antibody (13).

Thus, a rapid, sensitive, reproducible assay for bleomycin activity is needed. The results obtained with such an assay

should be compared to results obtained with the radioimmunoassay in patients who have poor renal function and/or are receiving long-term infusions of bleomycin.

Since the mechanism of action of bleomycin appears to be related to its ability to degrade DNA (5), methods which determine the extent of DNA degradation are appropriate estimates of antitumor activity. Recently, the decrease in binding of ethidium bromide to covalently closed circular PM-2 DNA induced by bleomycin has been used to determine the extent of degradation induced by bleomycin (11, 15). We have adapted this rapid, sensitive, reproducible method to be used as a pharmacokinetic assay.

MATERIALS AND METHODS

Assay Procedures. The radioimmunoassay of bleomycin was performed as previously described using a competitive protein-binding technique using ¹²⁵I-labeled and unlabeled bleomycin (2, 6). The fluorometric assay used to determine PM-2 DNA degradation was performed as previously described (15). Analyses were performed by adding drug or serum (90 μ l) to 50 μ g PM-2 DNA in buffer (0.015 M NaCl:0.05 M sodium borate, pH 9.5) to give a final total volume of 500 μ l. Incubations were carried out for 60 min at room temperature. Triplicate aliquots (0.1 ml) of the assay solution were then placed in 0.9 ml denaturation buffer followed by the addition of 0.1 ml ethidium bromide (Sigma Chemical Co., St. Louis, Mo.; 22 μ g/ml in denaturation buffer).

Serum Sampling and Assay Methods. Serum samples were obtained from adult males with Stage III or IV testicular cancer who received bleomycin in combination with *cis*-platinum and vinblastine. Previous publications have described the regimen, sampling methods, therapeutic, toxicological, and pharmacokinetic results (6, 13). Blood specimens were collected in nonheparinized tubes, refrigerated, centrifuged, and stored frozen (-70°) until analyzed. All patients gave informed consent.

Since during preliminary studies a protein which binds to PM-2 DNA and inhibits bleomycin degradation of PM-2 DNA was discovered in the serum of all subjects studied, all serum samples were treated with TCA⁴ prior to performing either assay. To each serum sample, an equal volume of 10% TCA was added, and the sample was centrifuged in an RC 3 refrigerated centrifuge (Sorvall, Inc., Newtown, Conn.) at 2000 rpm for 15 min. The supernatant was then neutralized with 0.5 N NaOH. The characteristics of the serum bleomycin-inhibiting protein are the subject of a separate report.

RESULTS

Preliminary experiments demonstrated that a protein present

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⁴ The abbreviation used is: TCA, trichloroacetic acid.

in the serum inhibited the degradation of PM-2 DNA by bleomycin. Chart 1 shows the concentrations of bleomycin in buffer, serum, and TCA-treated buffer and serum as determined by the PM-2 DNA assay, and shows that in untreated serum bleomycin was inhibited by 100%. Studies demonstrated that a unique protein which binds to PM-2 DNA was responsible for the inhibition of bleomycin degradation of PM-2 DNA. However, after treatment of serum or buffer with 5% TCA, the PM-2 DNA assay gave accurate and reproducible results in a range of concentrations of 10 to 60 μ units bleomycin per ml under the conditions used in this study (Chart 2). The range of concen-

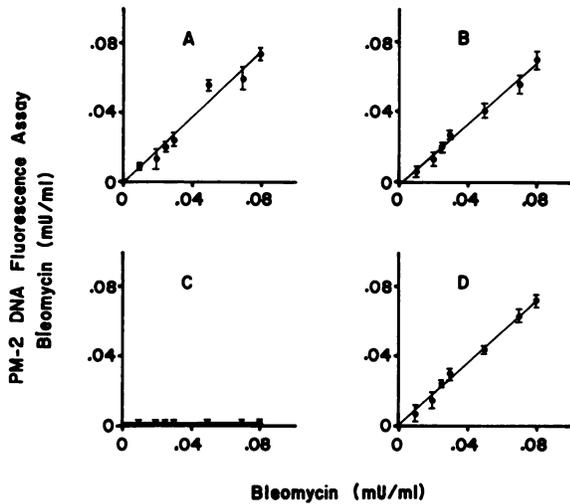


Chart 1. Bleomycin concentrations in buffer (A), 5% TCA-treated buffer (B), serum (C), and 5% TCA-treated serum (D) as determined by the PM-2 DNA assay. Varying amounts of bleomycin were added to 1-ml samples of serum, buffer, or TCA-treated serum or buffer, then the bleomycin concentration was determined by the PM-2 DNA assay, and the results were compared to the amount of bleomycin added. Bars, S.D.; mU, milliunit of activity.

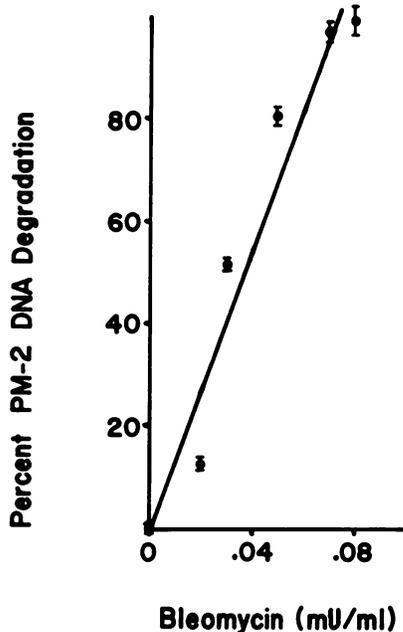


Chart 2. The standard curve for bleomycin in serum treated with 5% TCA. Known amounts of bleomycin were added to human serum. The serum was then treated with 5% TCA and assayed as described in "Materials and Methods." Bars, S.D.; mU, milliunit of activity.

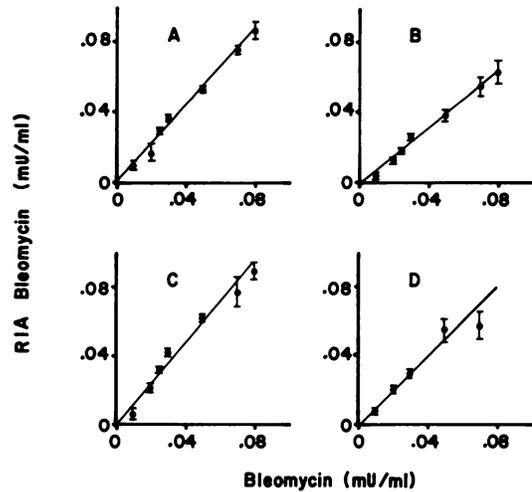


Chart 3. Bleomycin concentrations in buffer (A), 5% TCA-treated buffer (B), serum (C), or 5% TCA-treated serum (D) as determined by the radioimmunoassay. Procedures were as indicated for Chart 1. S.D.; mU, milliunit of activity; RIA, radioimmunoassay.

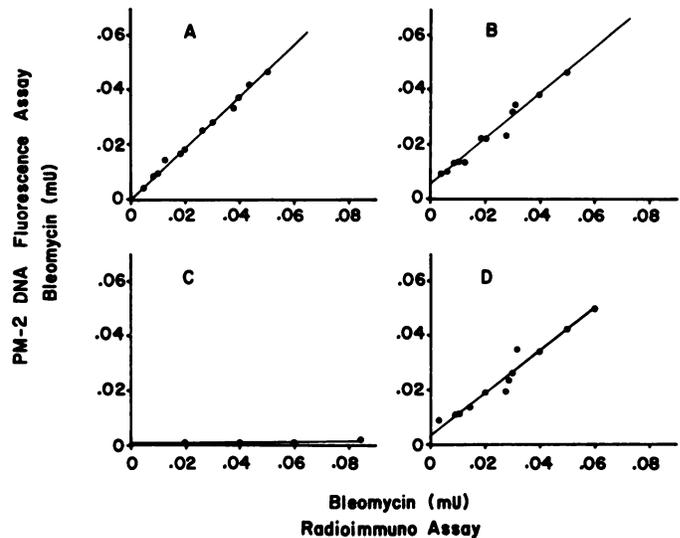


Chart 4. Comparison of bleomycin concentrations in buffer and serum samples obtained from patients treated with bleomycin as determined by the PM-2 DNA and radioimmunoassays. Serum samples were obtained and handled as described in "Materials and Methods." The chart was constructed by plotting the PM-2 DNA results on the ordinate, and the radioimmunoassay results on the abscissa. The points shown are the points at which perpendiculars drawn to the data obtained with each assay intersected. The lines were drawn by the method of least squares and linear regression analysis gave values of coefficient or correlation, the intercept, and the slope. The correlation in bleomycin concentrations in buffer (A), buffer treated with 5% TCA (B), serum (C), and serum treated with 5% TCA (D) as determined by the 2 assays are shown. mU, milliunit of activity.

trations for which the assay gives linear results can be varied by diluting the serum (high concentrations) or increasing the incubation time (low concentrations).

Chart 3 shows that the radioimmunoassay gave accurate and reproducible results in buffer and serum, and the linearity of the results in both buffer and serum were unaffected by TCA treatment, although a slight reduction in recovery in buffer was noted.

Chart 4 compares the results obtained using the PM-2 DNA assay to those obtained with the radioimmunoassay when serum samples of patients receiving bleomycin were assayed.

The coefficient of correlation was 0.98 and 0.95, the slope was 1.03 and 0.83, and the intercept was -0.001 and 0.005 in buffer and buffer treated with 5% TCA. The coefficient of correlation was 0.91, the slope was 0.78, and the intercept was 0.003 in serum treated with 5% TCA, indicating good agreement between the 2 assays in serum treated with 5% TCA. In serum which was not treated with 5% TCA, no degradation induced by bleomycin was detected at any concentration. Chart 5 shows the serum concentration curve of bleomycin as determined by both assays in 2 representative patients. In Chart 5A, the results obtained in sera from a patient with normal renal function (creatinine clearance, 185 ml/min; elimination half-life, 108 min) are shown. In Chart 5B, results obtained in sera from a patient with renal dysfunction (creatinine clearance, <10 ml/min; elimination half-life, 24 hr) are shown. In the patient, whose results are shown in Chart 5A, both assays provided comparable data. However, for the patient with severe renal dysfunction, the results of the 2 assays were slightly different resulting in different elimination half-lives (PM-2 DNA, 18 hr; radioimmunoassay, 24 hr). The greater

differences observed between the results of the 2 assays in the patient with compromised renal function may reflect metabolic differences, and this is currently being investigated.

DISCUSSION

Although the radioimmunoassay used is a rapid, accurate, and sensitive assay for bleomycin, it measures immunochemically intact bleomycin rather than bleomycin activity. Thus, a sensitive reproducible assay of bleomycin activity, such as the PM-2 DNA assay, may allow further definition of the extent and significance of the contribution of metabolism to the clearance of bleomycin, particularly in patients with compromised renal function and those being treated with long-term infusions.

The results presented in this report demonstrate that the PM-2 DNA assay is sensitive and reproducible, and in patients with normal renal function gave results comparable to those obtained with the radioimmunoassay. The sensitivity of the assay is adequate for routine pharmacokinetic studies but can be increased by simply increasing the duration of incubation of the serum samples with PM-2 DNA. Similarly, the range of concentrations at which the PM-2 DNA assay is linear can be shifted by variations in either the time of incubation, concentration of PM-2 DNA, or the dilution of serum used. For example, by increasing the duration of incubation from 60 to 120 min, the minimum reproducibly detectable concentration was decreased to less than $1.0 \mu\text{unit/ml}$. Under these conditions, the assay results were linear from 1.0 to $15 \mu\text{units/ml}$. Moreover, preliminary studies in lung and liver homogenates demonstrate that the PM-2 DNA assay is as sensitive and useful for tissue homogenates as it is for serum determinations. Studies using this assay and comparing the results obtained to those with the radioimmunoassay in a variety of patients are in progress and should help to further delineate the clinical pharmacology of bleomycin.

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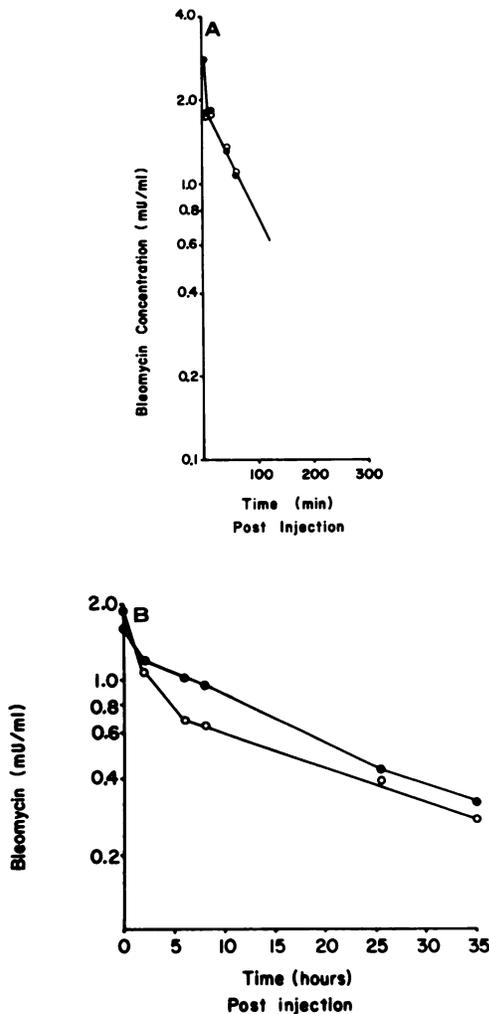


Chart 5. Bleomycin serum concentrations in a patient treated with 15 units bleomycin as an i.v. bolus. Patient treatment and serum sampling were as described in "Materials and Methods." O, PM-2 DNA results; ●, radioimmunoassay results. A, results from a patient with normal renal function; B, results from a patient with compromised renal function. mU, milliunit of activity.

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