Pharmacokinetics of Bleomycin in Man

III. Bleomycin $^{57}$Co Vs Bleomycin

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Summary. Of all the bleomycin-containing radiopharmaceuticals, bleomycin $^{57}$Co has proven the most useful whole-body tumor-imaging agent. We have studied its in vitro physicochemical properties and in vivo disposition in animals and man to optimize its use as a scanning agent. High-pressure liquid chromatographic analysis of the standard bleomycin $^{57}$Co preparation (1 unit bleomycin plus 1 mCi chloride $^{57}$Co showed it to contain 1% free chloride $^{57}$Co. Dialysis experiments showed that bleomycin $^{57}$Co does not dissociate as it diffuses through a dialysis membrane. In nine patients, bleomycin $^{57}$Co had a t½ of 3.4 h, a t½ of 45.8 h, a Vₐ of 12.1 liters/m² and a 24-h urinary excretion of 82.1% of the administered dose. In comparison, bleomycin, assayed by radioimmunoassay, had a terminal phase plasma t½ of only 4.0 h, a similar Vₐ (17.3 liters/m²), and a 24-h urinary excretion of only 44.8%. Bleomycin $^{57}$Co tumor-to-plasma concentration ratios ranged from 14.1-23.8 at 1 day to 5.4 at 2 days after administration. Our finding that tumor imaging with bleomycin $^{57}$Co is best achieved at 24 h is well explained by its almost complete urinary elimination in the first few hours after administration and the peak tumor-to-plasma ratio achieved at 24 h. One disadvantage of bleomycin $^{57}$Co as a scanning agent is its very extended plasma t½. In rabbits chloride $^{57}$Co has the same prolonged plasma terminal elimination phase (t½) as our standard bleomycin $^{57}$Co preparation, which contains chloride $^{57}$Co as a 1% impurity. Removal of this impurity prior to scanning or use of cold cobalt chloride to help eliminate it from the plasma might result in a shortened bleomycin $^{57}$Co plasma t½.

Introduction

Bleomycin $^{57}$Co is a useful whole-body tumor imaging agent in the evaluation of cancer [4, 6, 9–12]. It is one of a group of scanning agents formed by chelating the antitumor antibiotic bleomycin with a radionuclide label [5, 9]. Several investigators [3, 5, 18] have shown that bleomycin $^{57}$Co has the highest tumor-to-nontumor tissue distribution ratios of all radiolabeled bleomycins. Furthermore, bleomycin $^{57}$Co chelate may concentrate in tumor to a higher degree than free bleomycin [5]. Although there are published disposition data for bleomycin $^{57}$Co in animals and man there has been no direct comparison between bleomycin and its $^{57}$Co chelate in patients. We have studied the disposition of both bleomycin and bleomycin $^{57}$Co in nine cancer patients in an attempt at a fuller understanding of the tumor-imaging properties of bleomycin $^{57}$Co. Control studies were conducted in vitro and in animals to determine the stability of the chelate and the pharmacokinetics of bleomycin $^{57}$Co so that effects of dissociation and/or metabolism could be determined.

Materials and Methods

Bleomycin $^{57}$Co in Preparation and Tumor Imaging

Bleomycin $^{57}$Co was prepared by diluting $^{57}$Co in 0.5 N HCl to 0.1 N with normal saline, reconstituting bleomycin (Blenoxane, Bristol Laboratories, Syracuse, N.Y.) with normal saline, and combining $^{57}$Co and bleomycin to achieve an activity of 1 mCi $^{57}$Co per unit of bleomycin. The pH of the complex was adjusted to 6.0 with sodium acetate, and the volume was adjusted with normal saline to yield an activity of 1 mCi per milliliter. The final solution was filtered through a 0.22-μm sterile Millipore filter. Tests were performed to monitor sterility, apyrogenicity, and radiochemical and radionuclide purity. Imaging studies were performed with the aid of a gamma camera with whole-body imaging table (Searle Radiographics Pho/Gamma IV) at 6 and 24 h after injection in all patients, and again at 48 h in 5 patients. Whole-body images and spot views of the anatomic areas of concern were obtained in all patients.

HPLC Analysis of Bleomycin $^{57}$Co

The purity of bleomycin $^{57}$Co was analyzed by high-pressure liquid chromatography (HPLC). Freshly prepared bleomycin $^{57}$Co was di-
rectly injected onto a reverse phase column (4 mm × 25 cm μC18, Bondapak, Waters Assoc., Milford, Mass.) and the inorganic $^{57}$Co separated from the bleomycin $^{57}$Co. The column was equilibrated with 10% acetonitrile in 0.01 N ammonium acetate (pH 4.5). Upon injection of the sample the solvent was programmed to 35% acetonitrile in 0.01 N ammonium acetate (pH 4.5). The flow rate was maintained at 2 ml per min and each 1-ml fraction was separately assayed for $^{57}$Co content.

**Dialysis of Bleomycin $^{57}$Co**

Back dialysis experiments were done to determine whether the $^{57}$Co label remained in a chelated form as bleomycin $^{57}$Co diffused through a dialysis membrane. Thirty 1-ml dialysis bags containing human plasma were placed into a 500-ml beaker containing 200 μCi bleomycin $^{57}$Co plus 27 U bleomycin in phosphate-buffered saline (PBS) at 37°C. Twenty four hours later the dialysis bags were removed and placed in 500 ml fresh PBS. At 0, 0.5, 1, 2, 3, 5, 6, 8, 16 and 24 h, three dialysis bags were removed and their contents assayed for $^{57}$Co by gamma counting and for bleomycin by radioimmunoassay (RIA).

**Blood, Urine, and Tumor Sampling**

Blood samples (10 ml) were obtained from patients through a heparin lock and collected in tubes containing 100 IU heparin. Blood samples were taken just prior to the start of bleomycin therapy and at 5, 10, 15, 30, 45, 60 min and 2, 3, 4, 6, 8, 16 and 24 h after drug administration. Fractional urine collections were taken for the first 8 h after drug injection and then at varying intervals for at least 24 h. Urine samples were stored in sterile containers at 4°C. Tumor biopsies were obtained for diagnostic purposes in four head and neck cancer patients between 24 h and 6 days after radionuclide administration. The tissue samples were weighed and then assayed for $^{57}$Co activity in a well scintillation counter.

**Assay Procedure**

Blood samples were centrifuged at 4°C (2,000 rpm for 10 min). The resulting plasma was frozen at -20°C. The bleomycin concentrations in plasma and urine were determined by means of the antiserum and radioimmunoassay (RIA) technique developed by Broughton and Strong [2]. Bleomycin was labeled for RIA with $^{125}$Iodine; $^{57}$Co and $^{131}$I were counted simultaneously in a Hewlett Packard well scintillation counter with appropriate energy discrimination and correction for cross-talk between channels.

**Plasma Disposition of Bleomycin $^{57}$Co and Chloride $^{57}$Co in Rabbits**

Female New Zealand white rabbits (Blue Ribbon Rabbit Tree, Tucson, AZ) weighing 4 kg and maintained on normal laboratory rabbit chow were given 0.2 ml bleomycin $^{57}$Co (200 μCi $^{57}$Co) as IV bolus injections. Blood samples were collected from a heparinized, indwelling jugular catheter at 5, 15, 30, 60 min and 2, 4, 8, 12, 24, and 48 h after drug administration. The rabbits were kept in metabolic cages for collection of 24-h urine samples.

**Patients and Treatment**

Patient characteristics for the nine study patients are summarized in Table 1. Informed consent was obtained from each patient prior to study. All patients had advanced cancer and received bleomycin for therapeutic purposes. All patients but one (EL) received bleomycin and bleomycin $^{57}$Co simultaneously by IV bolus injection. The bleomycin dose varied between 13.7 and 19.9 (mean 15.4) U/m² body surface area. All patients received 1 mCi bleomycin $^{57}$Co.

None of the patients received other anticancer drugs within 3 weeks of the bleomycin pharmacokinetic studies. An attempt was made to stop all drugs at least 3 days prior to the bleomycin disposition studies; however, it was necessary to continue analgesic medication in four of the nine patients (Table 1).

Since bleomycin is eliminated mainly through the kidney, we studied patients with normal renal function. Serum creatinine concentrations were normal (0.7–1.3 mg%) for all nine patients.

**Data Analysis**

Bleomycin $^{57}$Co concentration versus time data obtained from each patient and the composite data for all patients were fitted to a multi-exponential equation, using NONLIN [7]. Preliminary parameter estimates were obtained from a recently published method CSTRIP [15]. Except for one patient (MR), the equation used was

$$\ln C = \ln \left( A_1 e^{-\alpha t} + A_2 e^{-\beta t} + A_3 e^{-\gamma t} \right),$$

where C is the bleomycin $^{57}$Co plasma concentration at time t after drug administration, $A_{1,2,3}$ are coefficients, and $\alpha$, $\beta$, and $\gamma$ are first-order elimination rate constants. For patient MR a bi-exponential

<table>
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<th>Patient</th>
<th>Tumor type</th>
<th>Sex</th>
<th>Age (year)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BSA (m²)</th>
<th>Serum creatinine (mg-%)</th>
<th>Bleomycin IV dose (U)</th>
<th>Bleomycin IV dose (U/m²)</th>
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