Chromomycin A₃, an Antitumor Antibiotic: Tissue Distribution Studies in Mice as Measured by Microbial Assay

ROBERT F. PITTILLO AND CAROLYN WOOLLEY
Southern Research Institute, Birmingham, Alabama 35203

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A microbiological assay has been developed for chromomycin A₃, an antitumor antibiotic showing promise in human trials. The assay bacterium is a derived strain of Streptococcus faecalis resistant to methotrexate. Studies with mice revealed that relatively high concentrations of this antibiotic were maintained in the blood, kidneys, and liver of mice after a single-dose intraperitoneal injection of the drug.

Chromomycin A₃, an antibiotic produced by Streptomyces griseus strain 7 (9), has been reported to inhibit a variety of transplantable neoplasms in experimental animals (4, 5) and to be of some promise in inhibiting cancer in human (6, 7). The structure of this antibiotic (10) is shown in Fig. 1. Chromomycin A₃ selectively inhibits deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) synthesis by binding to cellular DNA. The mechanism of action, pharmacology, and toxicity of this antibiotic have been reported (1, 2).

The purpose of this communication is to describe a microbiological assay with sufficient sensitivity to be potentially applicable to experimental or clinical studies and to report the concentrations of chromomycin A₃ found in normal mice after a single-dose intraperitoneal injection.

MATERIALS AND METHODS

After a primary screening of approximately 100 microorganisms, a strain of Streptococcus faecalis ATCC 8043 resistant to 3 µg of methotrexate per ml (designated S. faecalis/MTX) was selected as the microorganism for the assay of chromomycin A₃. The culture was maintained on folic acid assay-agar medium (Difco) supplemented with 3 µg of methotrexate per ml and 0.5 µg of folic acid per ml. Broth cultures of S. faecalis/MTX were grown for 16 to 18 hr at 37 C in folic acid assay medium supplemented as above for the preparation of seeded agar assay plates. Cells from these cultures were collected and washed twice by centrifugation in saline (0.85% NaCl), resuspended in saline, and adjusted to 20% light transmittance (660 nm) in a Spectronic-20 colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.). A 3-ml amount of this suspension was added to 1 liter of cooled (50 C) folic acid assay-agar medium that had been adjusted to pH 6.0 and supplemented with 0.5 µg of folic acid per ml.

A stock solution of chromomycin A₃ was prepared in sterile saline and appropriately diluted. When 0.08 ml of the various dilutions was pipetted onto assay discs, the following concentrations were obtained (micrograms of chromomycin A₃ per disc): 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0. An additional stock solution of chromomycin A₃ was prepared in which the antibiotic was dissolved in freshly drawn whole mouse blood and diluted with saline.

Filter-paper assay discs (1.27 cm in diameter, no. 740-E; Schleicher and Schuell Co., Keene, N.H.) were impregnated with 0.08 ml of the respective solutions containing graded concentrations of the drug. The discs were immediately placed on the surface of each seeded agar plate and pressed down securely with flame forceps. All experimental samples and standards were tested in triplicate. Each individual plate contained two discs impregnated with either experimental sample solutions (blood, urine, or tissue homogenates) or standard curve solutions of different concentrations. In addition, a third disc containing an empirically selected concentration of 0.5 µg of chromomycin A₃ per disc allowed for the correction of plate-to-plate variation in zone size. The resulting zones of inhibition on the plates were measured and corrected as previously reported (8). The corrected mean diameters of the zones of inhibition surrounding the discs, which contained known concentrations of drug, were plotted on semilogarithmic graph paper with the zone sizes on the arithmetic scale and the drug concentrations per disc on the logarithmic scale. Standard curves were constructed through the points thus obtained by the method of least squares (8). Antimicrobial concentrations in either the experimental
blood, tissue, or urine samples were obtained by reading the concentrations on the ordinate of either the blood or saline standard curve, respectively, that corresponded to the size of the corrected zones of inhibition surrounding the discs impregnated with the tissue homogenates or body fluids. The experimental blood samples were plotted against the blood standard curve, and the experimental urine and tissue samples were plotted against the saline standard curve.

In the distribution studies, BDF mice (mixed sexes, 18 to 22 g) were used. Tissues were prepared for assay by homogenizing weighed portions (approximately 1 g) of the respective tissues in an Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.) containing 2 ml of saline.

RESULTS AND DISCUSSION

Typical standard assay curves of chromomycin A₃ in whole mouse blood and in saline are shown in Figure 2. Figure 3 presents the average concentrations of this antibiotic detected in the tissues and body fluids of mice injected intraperitoneally with a single LD₁₀ dose of chromomycin A₃ (5 mg/kg); these values represent the average concentrations obtained in five mice.

Assayable concentrations of chromomycin A₃, or cytotoxic equivalents, were found in the blood, liver, urine, and kidneys of mice. Approximately 7.8 µg of chromomycin A₃ per ml of blood (6.5 to 9.4 µg/ml) was detected 15 min postinjection and remained present through 1 hr. Maximum concentrations in the liver and kidney tissues were found at 60 min after drug administration and averaged approximately 5.6 µg/g (4.4 to 6.5 µg/g) and 4.7 µg/g (4.1 to 5.8 µg/g), respectively. Urinary excretion reached an average peak value of 3.5 µg/ml (3.1 to 4.5 µg/ml) in 15 min.
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FIG. 3. Concentrations of chromomycin A3 detected in the tissues and body fluids of mice by microbiological assay. Assay microorganism: Streptococcus faecalis ATCC 8043/methotrexate. Mice were injected with a single LD50 intraperitoneal dose (5 mg/kg) of drug.