Biological Assay of Streptonigrin (NSC 45383) in Body Fluids and Tissues of Mice

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Received for publication 13 September 1973

Streptonigrin, a quinone antitumor antibiotic, has been reported to be effective in human trials. A sensitive and precise microbiological assay for the determination of distribution and concentrations of streptonigrin in the body fluids and tissues of treated mice has been developed in an attempt to supplement successful clinical application of this drug.

Streptonigrin, an antibiotic produced by cultures of Streptomyces flocculus (15), inhibits a variety of experimental neoplasms including CA755, Wagner and Ridgway osteogenic sarcoma, the transplantable mouse adenocarcinoma EO771, and Walker carcinosarcoma 256 in rats (16). Inhibition of human cell tumors HS no. 1 and HEp no. 3 in conditioned rats (11, 19) and in Swiss mice (8) by streptonigrin also has been observed. Additionally, streptonigrin was found to possess antiviral activity against the Ruscher virus in mice (6). In human trials, significant, objective responses have been observed in a wide range of cases of advanced neoplastic disease treated with streptonigrin (17).

This antibiotic has also been shown to be effective in at least short-term regression of advanced cancer, including malignant lymphomas (2) as well as lymphosarcoma, adenocarcinomas, and Hodgkins disease (1, 3, 10, 22), although severe toxic effects with single daily injection have been noted. However, Sullivan et al. (18) concluded that significant tumor regression and associated clinical benefit occurred in advanced cancer patients when toxic effects were diminished as a result of prolonging the period of streptonigrin administration without concomitant loss of antitumor effects.

Streptonigrin has been reported to inhibit cell division as well as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis in mammalian cell culture (9). In metabolic studies employing monolayers of HeLa cells, streptonigrin was found to inhibit incorporation of RNA, DNA, and protein, all to an equal degree (23). Streptonigrin was also reported to preferentially inhibit DNA synthesis in a number of strains of Salmonella typhimurium and Escherichia coli and to be an excellent inducer of phage production in inducible lysogenic bacteria (5).

The chemistry of streptonigrin has recently been reviewed (14, 21). Its empirical formula is C₉₆H₁₂₂O₄N₄ and its structural formula (14) is as follows:

\[
\text{CH₃O} \
\text{H₂N} \
\text{O} \
\text{N} \
\text{COOH} \
\text{HO} \
\text{CHO} \
\text{OCH₃}
\]

Although a microbiological assay employing Micrococcus pyogenes var. aureus was apparently used in the initial isolation of streptonigrin, no details of the procedure are available (15).

MATERIALS AND METHODS

Streptonigrin was tested against approximately 100 strains of microorganisms. Previously described methods were used to find an appropriate microorganism for the assay of streptonigrin (4, 12). Bacillus subtilis ATCC 6633 was selected as the assay microorganism because of its unique sensitivity to streptonigrin. The culture is maintained on tryptic soy agar slants (Difco). For the assay, broth cultures of B. subtilis were grown for 16 to 18 h at 37°C in tryptic soy broth. Cells from these cultures were collected and washed 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 0.5-ml
amount of this cell suspension was added to 1 liter of cooled (50 C) tryptic soy agar medium adjusted to pH 6.3, and a 6-ml amount of the inoculated agar was dispensed into assay plates (100 by 15 mm, Lab-Tex Products).

Stock solutions of streptonigrin prepared in sterile saline (0.85% NaCl), in freshly collected mouse blood and in serum, were diluted to yield appropriate concentrations when a 0.08-ml amount was pipetted onto filter paper disks (1.27 cm in diameter, no. 740-E, Schleicher & Schuell Co., Keene, N.H.). Concentrations obtained were: 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 µg per disk of streptonigrin. The saturated filter paper disks were placed on the surface of each seeded agar plate and were pressed down securely with sterile forceps. All plates and drug concentrations per disk were prepared in triplicate. Each individual plate contained a maximum of three disks. Two disks contained, individually, either experimental samples (tissue homogenates or body fluids) or standard solutions of different concentrations. A third control disk, containing an empirically selected concentration (0.2 µg of streptonigrin per disk), was added to each plate, which allowed for the correction of plate-to-plate variation in zone sizes. All plates with disks were held for 24 h at 4 C prior to incubation at 30 C for 18 to 22 h. The resulting zones of inhibition on the plates were measured and corrected as previously reported (13). The corrected mean diameters of the zones of inhibition surrounding the disks, which contained known concentrations of drug, were plotted on semilogarithmic graph paper, the zone sizes on the arithmetic scale, and drug concentrations per disk on the logarithmic scale. Standard curves were constructed through the points thus obtained by the method of least squares. Drug concentrations in the experimental samples were obtained by reading the drug concentrations on the ordinate of the blood standard curve, which corresponded to the size of the corrected zones of inhibition surrounding the disks impregnated with tissue preparations or body fluids.

BDF, mice (mixed sexes, 18 to 22 g) were used in all experiments. Blood samples were obtained by cardiac puncture of sacrificed mice. Urine samples were collected directly from the bladder of sacrificed mice. Care was taken to remove excess blood from the extracted tissues by blotting with absorbent tissues. All tissues and body fluids were frozen and stored overnight. The samples were then thawed and prepared for assaying by homogenizing weighed tissues in an Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.) containing 1 ml of saline. The individual tissues were pooled before assaying, with the exception of blood and urine, which were assayed individually.

RESULTS AND DISCUSSION

Representative standard assay curves of streptonigrin in mouse blood, saline, and in serum are graphically illustrated in Fig. 1. Estimated concentrations of this antibiotic detected in the tissues and body fluids of mice injected intravenously (i.v.) with a single 1.5 mg/kg or 0.75 mg/kg mouse lethal dose (LD) of streptonigrin are shown in Fig. 2 (LD10 or 0.5 LD50, respectively).

After i.v. injection of a single (1.5 mg/kg) dose of streptonigrin, a peak blood level of 7.25 µg/ml (6.25 to 10.75) was observed at 10 min. This level declined to 1.38 µg/ml (1.05 to 2.15) in 30 min. Lower levels of drug were observed in the serum. A peak serum level of 2.05 µg/ml was observed immediately after injection and de-

![Fig. 1. Standard assay curves (constructed by method of least squares) for the logarithmic-ratio microbiological assay of streptonigrin in the tissues and body fluids of mice. Assay microorganism: Bacillus subtilis ATCC 6633. A, Blood standard curve (index of precision, 0.026). B, Saline standard curve (index of precision, 0.028). C, Serum standard curve (index of precision, 0.022).](http://aac.asm.org/Downloaded-from)
clined to 1.25 µg/ml at 10 min. The lung displayed the highest concentration of drug. Immediately after drug administration, a peak level of 51.62 µg/g of lung tissue was detected which declined to 3.47 µg/g at 20 min.

In mice injected with a single i.v. dose of 0.75 mg of streptonigrin per kg, the initial blood level was 7.5 µg/ml (6.25 to 9.75) which declined to 3.75 µg/ml (3.5 to 4.25) in 20 min. A peak tissue level of 6.23 µg/g was observed in the lung immediately after injection. This level declined to 3.38 µg/g at 20 min.

No drug was detected in the brain, spleen, liver, kidney, or urine at either dose under these conditions of drug administration. Failure to detect streptonigrin in urine or homogenized organs such as the spleen, liver, and kidney may be due either to the fact that streptonigrin is converted to some other compound or to the presence of inhibition-reversing metabolites. Studies to clarify this question are in progress.

ACKNOWLEDGMENTS

The advice and support of J. A. R. Mead and L. B. Mellett are gratefully acknowledged. The technical assistance of Diane B. Graveman and Sherry Burris is appreciatively noted.

These investigations were supported by Public Health Service contract PH43-65-654 from the National Cancer Institute.

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