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Microbiological Assay and Tissue Distribution of β-Thioguanine Deoxyriboside in Mice[1]

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A sensitive, precise microbiological assay has been developed for the determination of tissue distribution of β-thioguanine deoxyriboside, a new antitumor agent.

β-Thioguanine deoxyriboside (2-amino-9-[2-deoxy - β - D - erythro - pento - furanosyl]9*H*-purine-6-thiol, monohydrate), NSC 71261, has been reported to inhibit L1210 leukemia in mice and Walker 256 carcino-sarcoma in rats and is being considered for chemotherapeutic trials in humans (2). The structure of β-thioguanine deoxyriboside is shown in Fig. 1.

The successful development of a potentially useful chemotherapeutic agent is sometimes expedited by the availability of a sensitive and precise assay procedure for measuring tissue distribution and concentration of the new drug. We have developed a microbiological assay for the determination of the distribution of β-thioguanine deoxyriboside in the tissues of mice which have been injected with therapeutically realistic doses of the drug.

Previously described methods were used to select an appropriate microorganism for the assay of β-thioguanine deoxyriboside (3). A strain of *Escherichia coli* derived from *E. coli* ATCC 9637 as resistant to 6-diazo-5-oxo-L-norleucine (DON) and designated *E. coli*/DON was selected as the assay organism because of its sensitivity to the compound. A simple glucose-salts medium was used for culture maintenance, for inocula preparation, and for the preparation of the assay plates. It consists of 1% NH_4Cl, 0.73% K_2HPO_4, 0.3% KH_2PO_4, 0.012% $MgSO_4$, and 2% glucose (prepared separately and added aseptically). When needed, 1.5% agar was added.

For the assay, stationary overnight cultures of *E. coli*/DON, grown in the simple glucose-salts medium at 37 C, were used. After washing, the cells were collected, suspended in saline, and adjusted to 20% light transmission in a Spectronic-20 colorimeter at 660 nm (Bausch & Lomb, Inc., Rochester, N.Y.). This suspension was diluted 1:200 in saline.

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Base plates were prepared by dispensing 8 ml of the glucose-salts agar medium into leveled petri plates. After the base layer congealed, the plates were overlaid with 5 ml of the same agar seeded with 10 ml of the diluted cell suspension per liter of agar.

β-Thioguanine deoxyriboside was diluted in physiological saline or in heparinized mouse blood according to the needs of the experiments.

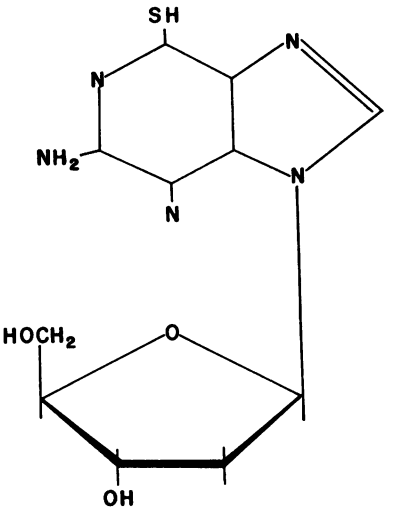

FIG. 1. *Structure of β-thioguanine deoxyriboside (NSC 71261).*

A 0.08-ml amount of the respective drug concentrations, in solution, was pipetted onto filter-paper discs (1.27 cm in diameter, no. 740-E, Schleicher and Schuell Co., Keene, N.H.). The moist discs were placed on the surface of each seeded agar plate and pressed down securely with flamed forceps. All samples and standards were run in triplicate. Each individual plate contained a maximum of three discs; two discs contained

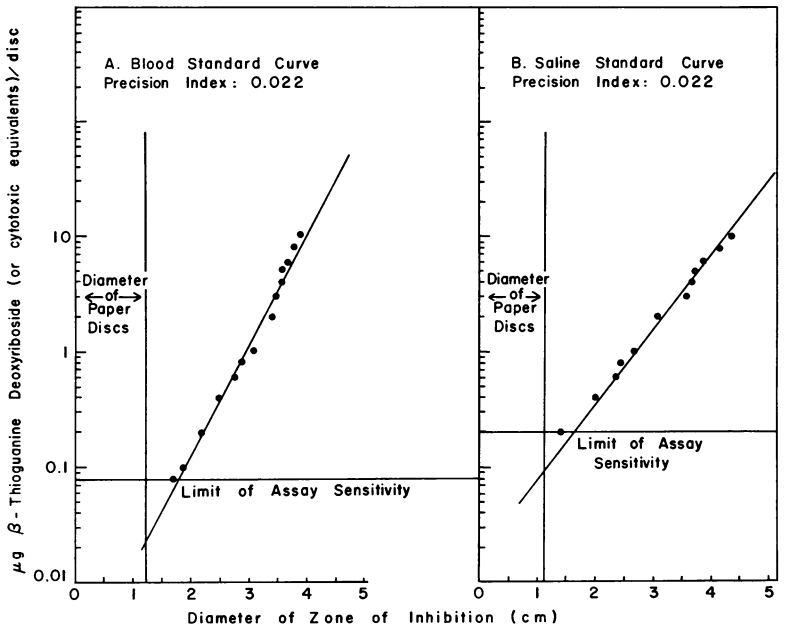

FIG. 2. *Standard assay curves for the logarithmic-ratio microbiological assay of β-thioguanine deoxyriboside in tissues of mice. The assay microorganism was Escherichia coli ATCC 9637/DON. (A) Blood standard curve; index of precision, 0.022. (B) Saline standard curve; index of precision, 0.022.*

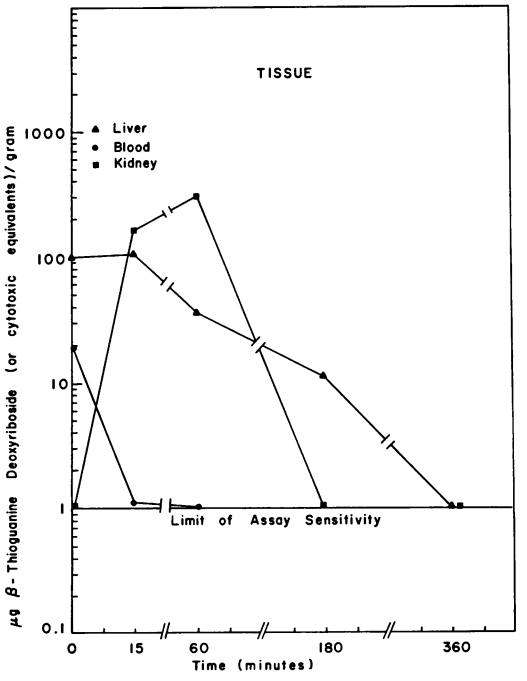

FIG. 3. *Concentrations of β-thioguanine deoxyriboside detected in tissues of mice by microbiological assay. Each point represents the mean drug level of five mice. The assay microorganism was Escherichia coli ATCC 9637/DON. Animals were injected with a single intraperitoneal dose (100 mg/kg) of drug.*

either experimental samples or standard curve solutions of different concentrations. A third control disc, containing an empirically selected concentration of drug (1.0 μg/disc), was added to each plate to allow for correction of plate-to-plate variation in zone sizes. All plates were simultaneously incubated at 30 C for 18 to 22 hr with plates which contained discs impregnated with blood from animals which had received β-thioguanine deoxyriboside. The resulting zones of inhibition on the triplicate standard plates were measured and corrected as follows. If the mean diameter of all of the control disc zones was greater than that of an individual control disc zone, the difference was added to all of the zones on that plate. Conversely, if the average diameter of the control disc zones was less than that of an individual control disc, the difference was subtracted from all the zones on that plate. The mean diameter of these corrected zones was determined for each drug concentration. Standard curves were constructed through the points thus obtained by the principle of least squares.

In all in vivo experiments, BDF_1 mice (mixed sexes, 18 to 22 g in weight) were used. Drug was administered in 0.85% NaCl intraperitoneally. Blood samples from treated animals were secured by cardiac puncture of sacrificed mice.

Typical standard inhibition curves obtained with β-thioguanine deoxyriboside, dissolved in saline or in heparinized whole mouse blood, are

shown in Fig. 2. The indexes of precision (1) are 0.022 in both cases (a value of < 0.1 is considered indicative of precision). Concentrations as low as 0.1 μg/disc could be detected reproducibly. The concentration of β-thioguanine deoxyriboside detected in tissues of mice which were injected intraperitoneally with 100 mg of β-thioguanine deoxyriboside (the estimated LD_{10} dose) is shown in Fig. 3.

Assayable concentrations of β-thioguanine deoxyriboside, or cytotoxic equivalents, were detected in the blood, liver, and kidneys of the mice. Blood levels peaked quickly but fell rapidly, and no β-thioguanine deoxyriboside was detected in the blood 1 hr after injection. The highest concentrations of β-thioguanine deoxyriboside detected were in the kidneys, 150 to 300 μg/g of tissue 15 to 60 min after injection. Assayable levels of β-thioguanine deoxyriboside were found in the liver immediately after injection (ca. 100 μg/g) and diminished gradually over a 6-hr period.

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