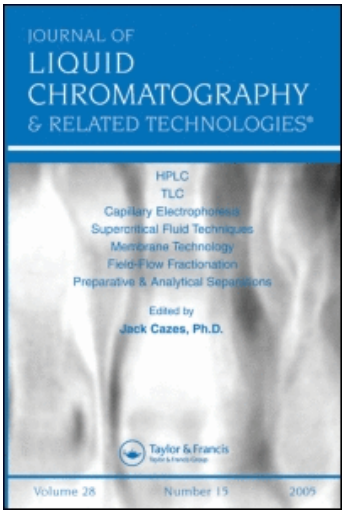

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A TLC-HPLC METHOD FOR DETERMINATION OF TIAZOFURIN (NSC 286193) IN SERUM

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INTRODUCTION

The nucleoside compound 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC 286193; tiazofurin; see Fig.1) has recently been shown to possess significant antitumor activity against L1210 and P388 murine leukemias (1,2). Its demonstrated antitumor efficacy in treating mice with Lewis lung carcinoma (2), a tumor refractory to many chemotherapeutic drugs, is of considerable potential significance. Anabolism of the compound is required for its antitumor activity and involves the production of a state of guanine nucleotide depletion secondary to inhibition

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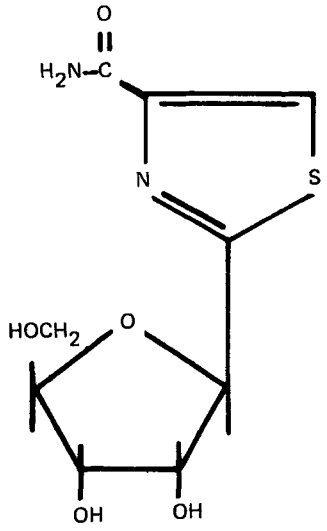

FIGURE 1

Structure of 2-β-D ribofuranosylthiazide-4-carboxamide (tiazofurin).

of inosine monophosphate (IMP) dehydrogenase (3). Thiazolecarboxamide adenine dinucleotide is formed in the metabolism of tiazofurin and appears to be responsible for the inhibition of IMP dehydrogenase (4,5).

The National Cancer Institute will soon commence with clinical Phase I trials of tiazofurin. The present study describes a useful and sensitive procedure for the determination of tiazofurin concentrations in biological fluids through a combination of thin layer (TLC) and high pressure liquid chromatographic (HPLC) techniques. The method has been applied for the measurement of serum

tiazofurin levels in mice and demonstrates the utility of this method in anticipated pharmacokinetic studies soon to begin in humans.

MATERIALS AND METHODS

Standards and Reagents

Tiazofurin (NSC 286193) was obtained from the Investigational Drug Branch, National Cancer Institute, National Institutes of Health (Bethesda, MD, U.S.A.). 2-propanol, sodium acetate and HPLC grade water and methanol were purchased from J.T. Baker (Phillipsburg, N.J., U.S.A.). Porcine serum was obtained from Pel-Freeze (Rogers, AR, U.S.A.) and was routinely used in the development of analytical methodology.

Chromatographic Apparatus and Conditions

All analyses were performed with a Spectra-Physics Model 8000 liquid chromatograph equipped with a Schoeffel Model 770 variable-wavelength U.V. detector set at 235 nm. The mobile phase consisted of 96% 0.02M sodium acetate pH 4.5 and 4% methanol which was used with an analytical reverse-phase C_{18} μ-Bondapak column (Waters Assoc. 3mm ID x 30cm, 10μ particle). A flow rate of 2.0 ml/min was used. A guard column (Brownlee Labs, 10μ RP-18 LiChrosorb, 4.6mm ID x 3cm) was installed between the injector valve and the

main column for extended column life. Avicel-F (250μ particle size) TLC plates were purchased from Analtech (Newark, DE, U.S.A.).

Sample Preparation and Drug Analyses

For the determination of drug recovery, serum aliquots were spiked with known amounts of tiazofurin. Protein was precipitated from the spiked serum samples by addition of 5 volumes (V/V) of acetonitrile. Samples were centrifuged (600g, 10 min., room temp.) and the supernatant solution decanted and evaporated to dryness at 60°C with air purging in a Fisher IMD sample concentrator (Fisher Scientific, Pittsburg, PA, U.S.A.). The residue was reconstituted with 0.4ml HPLC grade water and an aliquot (50ul) of this suspension was spotted manually along with appropriate tiazofurin standards onto TLC plates.

Initial studies of the serum elimination of tiazofurin were performed using male CD_1 mice (Charles Rivers Canada, St. Constant, Quebec, Canada). Serum was obtained by retro-orbital puncture at specified times (0, 5, 10, 15, 30, 45, 60, 90 min.; 2, 3, 4, 5, 6, 7, 8 and 24 hrs.) after the intravenous administration of 250 mg/kg (750 mg/m^2 body surface area) of tiazofurin. There was sufficient tiazofurin concentration in the murine blood to permit direct spotting of small aliquots of plasma (10 ul) onto the TLC plate thereby eliminating the need for the deproteinization and concentration steps.

All TLC plates were developed over a six-hour period in a solvent system of 2-propanol:water (70:30, V/V) in a closed humidified chamber at room temperature. In this system tiazofurin migrated with an RF of 0.61. The area of the chromatogram corresponding to tiazofurin (identified under U.V. light at 254 nm) was excised and eluted into 0.3 to 0.5 ml of HPLC grade water. This suspension was sonicated, centrifuged (600g., 3 min.) and 0.1 ml of the supernatant solution was injected onto the HPLC column. The resulting peak was integrated by the preset program in the Spectra-Physics data system using peak area to determine drug concentration.

Results and Discussion

The analytical methodology for the determination of the thiazole derivative, tiazofurin, described in this paper is closely analogous to the combined TLC-HPLC approach recently reported by our group for the determination of a related thiadiazole derivative (6). A typical standard curve of tiazofurin extracted from spiked serum samples is shown in Fig. 2. Drug levels from HPLC injected samples are linear over at least one hundredfold concentration (10 ng to 1000 ng) with an average correlation coefficient of 0.995 as calculated by the least squares regression method. The average inter-day recovery of tiazofurin from serum is 88.4±2.4% despite the various chromatographic procedures to which the sample is subjected.

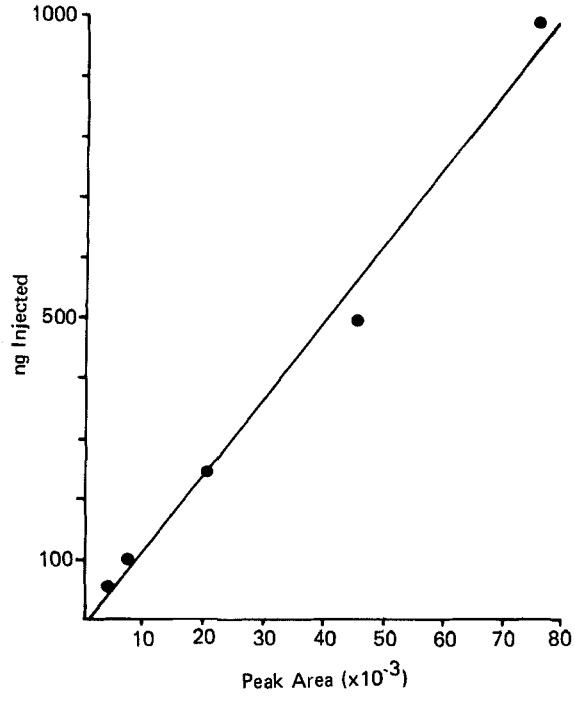

FIGURE 2

Standard curve of tiazofurin extracted from serum.

A representative chromatograph of the TLC-processed serum sample is shown in Fig. 3. This method, to the best of our knowledge, is the first reported for quantitation of tiazofurin in serum samples. Serum levels of tiazofurin as low as 3.2 ug/ml can be accurately and reproducibly determined.

In an effort to determine the applicability of this method for the measurement of murine serum drug pharmaco-

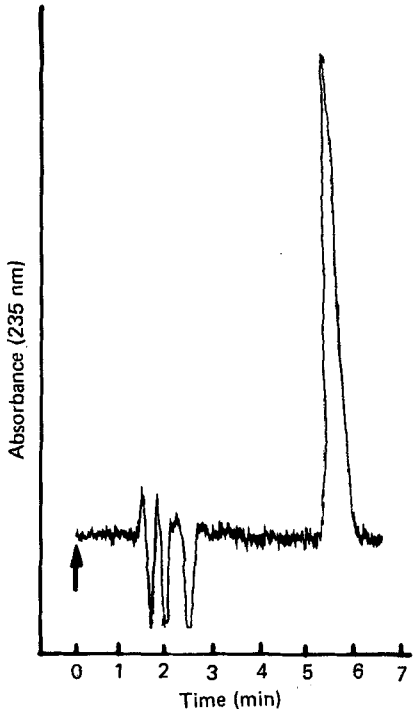

FIGURE 3

Representative chromatograph of TLC-processed mouse serum sample obtained 30 minutes following I.V. injection with tiazofurin. Arrow indicates point of injection.

kinetics, mice were administered 250 mg/kg tiazofurin which is one-tenth the LD_{10} (dose producing 10% mortality) for mice as reported by Southern Research Institute (7). The mouse LD_{10} dose ($MELD_{10}$) is typically ten times the initial drug dose administered to humans in a Phase I clinical trial of experimental cancer chemotherapeutic agents. Since serum drug levels were easily detected as late as 7 hr. after drug

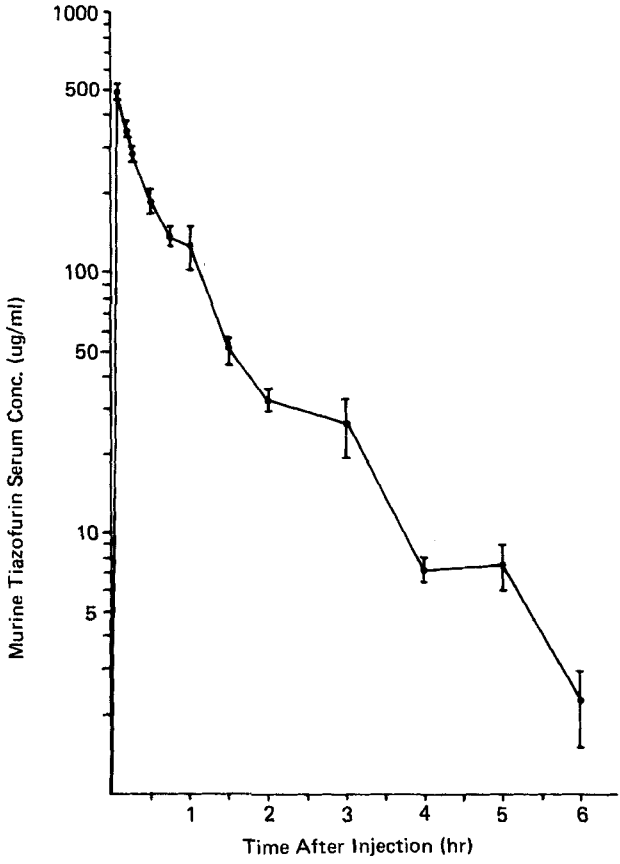

FIGURE 4

Serum drug concentrations from mice administered 250 mg/kg (I.V.) of tiazofurin. Data are presented as mean ± S.E. of 8 mice per time point.

administration, it is clear that this method will be suitable for establishing pharmacokinetic parameters in humans. As seen in Fig. 4, a terminal drug elimination phase of 60 to 72 min. can be calculated from the murine serum drug levels; more

extensive studies will obviously be required for complete kinetic analysis in mice and in anticipated Phase I studies in man.

ACKNOWLEDGEMENTS

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