Journal of Chromatography, 378 (1986) 498—502
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3103

Note

Analysis of suramin plasma levels by ion-pair high-performance liquid chromatography under isocratic conditions

RUTH M. RUPRECHT*, JON LORSCH and DOROTHY H. TRITES

Division of Cancer Pharmacology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115 (U.S.A.)

(First received November 12th, 1985; revised manuscript received January 27th, 1986)

Suramin, the hexasodium salt of 8,8'-{carbonylbis[imino-3,1-phenylene-carbonylimino-(4-methyl-3,1-phenylene) carbonylimino]}-bis-1,3,5-naphthalene trisulfonic acid, has been used for treating African trypanosomiasis and onchocerciasis since 1920 [1]. Subsequently, it was found to inhibit competitively retroviral reverse transcriptase [2], a key enzyme in the retroviral life cycle, and to suppress retroviral disease in mice [3]. Suramin is now undergoing clinical testing in patients with the acquired immune deficiency syndrome (AIDS) [4] which is causally linked to the retrovirus called either human T-lymphotropic virus type III (HTLV-III) [5] or lymphadenopathy-associated virus (LAV) [6]. Previously published methods of analysing suramin levels in plasma are either based on time-consuming colorimetric assays [7, 8] or on reversed-phase ion-paired high-performance liquid chromatography (HPLC) using gradient elution [9]. Recently, Edwards et al. [10] developed a method of analysing suramin plasma levels by HPLC using isocratic elution. However, a relatively elaborate extraction procedure led to only a 30% recovery of suramin. We report a more convenient new method to quantitate suramin in plasma based on dissociation of suramin—protein complexes with dilute sodium hydroxide and methanol followed by analysis on a reversed-phase ion-pairing HPLC system using an isocratic mobile phase.

EXPERIMENTAL

Materials

Suramin of pharmaceutical purity (Mobay Chemicals, FBA Pharmaceuticals)

0378-4347/86/$03.50

was kindly provided by Dr. S. Broder (National Cancer Institute, Bethesda, MD, U.S.A.). The internal standard *o*-tolylthiourea, 1-(2-methylphenyl)-2-thiourea, was obtained from K&K Labs (Plainview, NY, U.S.A.) and was recrystallized three times from 35% ethanol prior to use. All other chemicals and solvents were of analytical-reagent grade and were obtained from Eastman Kodak (Rochester, NY, U.S.A.).

Apparatus

Chromatographic separation and peak detection of suramin and of the internal standard *o*-tolylthiourea were carried out on a Waters Assoc. HPLC system consisting of a Model 440 fixed-wavelength detector at 280 nm set at a sensitivity of 0.01 absorbance units full scale (a.u.f.s.), a Model 6000A pump, a U6K injector and a radial compression module 100 equipped with a 10-μm Radial-Pak C_{18} cartridge and a C_{18} Guard-Pak precolumn. For automated injections, the Waters Assoc. WISP 710B injector was used.

The mobile phase consisted of 0.05 *M* ammonium acetate to which 500 μl tetrabutylammonium hydroxide (0.4 *M* in water) and 20 μl hexadecyltrimethylammonium hydroxide (0.5 *M* in water) were added per l. The pH of this solution was 7.0. Absolute ethanol was added to a final concentration of 35%. The resulting solution was degassed using a Millipore solvent clarification kit. The flow-rate was 1.8 ml/min.

Methods

Blood was collected from either human donors or from the tails of BALB/c mice into heparin-containing tubes. After centrifugation in an IEC Centra centrifuge at 4°C, the plasma was removed and frozen at −70°C until analysis. To study suramin concentration versus time in mice, groups of female BALB/c mice were injected intravenously with 40 mg/kg suramin on days 0, 1 and 7. Blood samples were collected from individual mice at various time points.

Because suramin is highly protein-bound in plasma [1], the complexes were dissociated as follows. To each 49.5 μl of plasma sample, 0.5 μl of 1 *M* sodium hydroxide and 100 μl of methanol were added. The samples were thoroughly mixed by vortexing and allowed to stand in ice for 15 min. After centrifugation in a Fisher Model 235B microcentrifuge for 15 min at 4°C, the supernatant was removed and an equal volume of extraction buffer (0.99 ml of double-distilled water, 0.01 ml of 1 *M* sodium hydroxide and 2.0 ml of methanol) was added to the pellet, which was again thoroughly vortexed, kept on ice for 15 min and centrifuged. For human samples, the pellet was extracted once more. To 96 μl of the combined supernatants, 4 μl of 1 m*M* *o*-tolylthiourea was added as an internal standard and the desired amount (5—50 μl) was injected directly onto the chromatograph. Standard curves were prepared by adding known amounts of suramin to plasma samples which were worked up as described above. The internal standard was added just prior to injection of all samples, because of the virtually complete recovery of suramin from plasma as well as the need to analyze unknown samples containing suramin within a wide range of concentrations, necessitating either injection of large volumes or further dilution. All samples were analyzed in triplicate. The results were

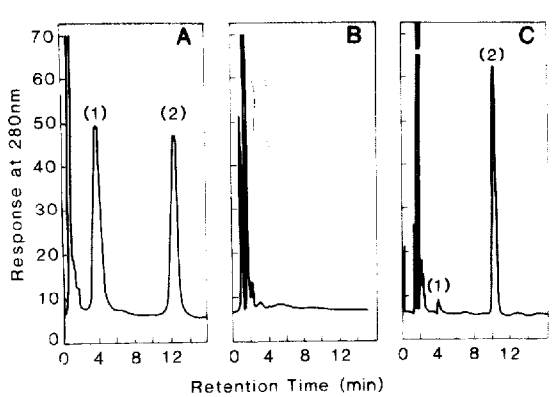
subjected to linear regression analysis using a VAX-11/750 computer according to the equation $y = ax + c$, where y stood for the peak-height ratio of suramin to *o*-tolylthiourea, a for the slope, x for the known concentrations of suramin and c for the y-intercept. The actual values obtained with mouse plasma were $a = 0.03507 \pm 0.00017$ and $c = -0.0603 \pm 0.0147$ with $p < 0.001$.

The precision of our method was tested at 15.00 μg/ml suramin ($n = 4$) and 150.00 μg/ml suramin ($n = 5$) by spiking mouse plasma samples with a standard solution of suramin.

RESULTS AND DISCUSSION

The retention times of suramin and *o*-tolylthiourea were 4 and 12.3 min, respectively (Figs. 1A and 2A). Background absorption of mouse or human plasma alone was negligible (Figs. 1B and 2B). Suramin was not degraded by exposure to the dilute alkali used in the preparation of samples as judged by adding 1 μl of 1 *M* sodium hydroxide to suramin dissolved in 100 μl of mobile phase and incubating this solution as well as a control sample not containing sodium hydroxide for 60 min in an ice bath. Subsequent HPLC analysis yielded identical peak heights and retention times for both samples.

The recovery of suramin from plasma samples was analyzed by adding known amounts of suramin to either human or mouse plasma. Each extraction step was analyzed individually by HPLC. The resulting peak areas were compared to those obtained from a standard amount of suramin added directly to the mobile phase (Table I). After only two extractions, all of the suramin was recovered from mouse plasma, whereas three extraction steps were needed for complete recovery of suramin from human plasma. As an additional control, a known amount of suramin was added to the extraction buffer and subjected to two rounds of extraction as described above. All of the suramin was recovered



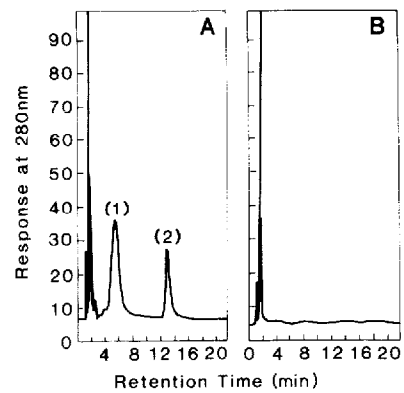


Fig. 1. HPLC analysis of (A) an experimental mouse plasma sample containing 100 μg/ml suramin and *o*-tolylthiourea as internal standard at 40 μ*M* and of (B) blank mouse plasma. (C) Representative sample of a low concentration of suramin (0.5 μg/ml). Peaks: 1 = suramin; 2 = *o*-tolylthiourea.

Fig. 2. HPLC analysis of (A) a spiked human plasma sample containing 50 μg/ml suramin (peak 1) and *o*-tolylthiourea as internal standard at 40 μ*M* (peak 2) and of (B) human plasma alone.

TABLE I

RECOVERY OF SURAMIN FROM PLASMA

Mouse or human plasma samples were spiked with a known amount of suramin to yield a concentration of 100 μg/ml. Extraction and analysis were carried out as described in the text; the peak areas were compared to those obtained when the same amount of suramin was added directly to the mobile phase.

Plasma source	Extract No.	Recovery (mean ± S.D.) (%)	Cumulative recovery (mean ± S.D.) (%)
Mouse	1	68.2 ± 4.5	
	2	30.6 ± 0.6	
	3	0	98.8 ± 3.8
Human	1	36.6 ± 5.2	
	2	51.6 ± 13.9	
	3	16.6 ± 1.9	104.8 ± 6.7

when compared to adding an equal amount of suramin directly to the mobile phase.

The standard curve was obtained by adding known amounts of suramin to plasma and using the extraction procedures described under *Methods*. The curve is essentially linear up to 200 μg/ml. The lower limit of detection is 0.5 μg/ml (Fig. 1C). A standard sample of 0.5 μg/ml suramin yielded a peak height of 3% of full scale absorption, but was nevertheless about six times above the background noise. Samples containing suramin concentrations above 200 μg/ml were diluted first for increased accuracy. The coefficient of variation ranged from 0.43 to 3.75% of individual mean peak-height ratios. Addition of the internal standard *o*-tolylthiourea either before the extraction procedure or immediately prior to injection yielded identical results as judged by working up a set of standard curves and unknown samples by either technique.

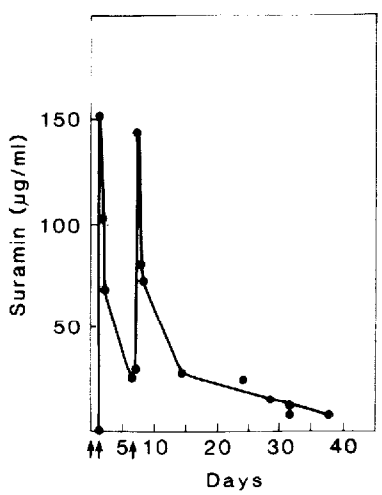


Fig. 3. Plasma levels of suramin versus time. A time profile of suramin plasma levels of mice injected at various time points with suramin (arrows) is given.

The precision of our method obtained at low and high concentrations of suramin (see *Methods*) was equally high. For an expected value of 15.00 and 150.00 μg/ml suramin, mean values of 15.10 ± 0.28 and 148.34 ± 0.39 μg/ml were obtained corresponding to a coefficient of variation of 1.85 and 0.26%, respectively.

In summary, we have developed an accurate, sensitive HPLC assay for the analysis of suramin which employs a convenient isocratic mobile phase. This method is well suited for monitoring suramin plasma levels. This is shown in Fig. 3 which demonstrates the decline of suramin as a function of time in the plasma of mice given 40 mg/kg suramin intravenously as a loading dose on day 0, followed by the same dose on days 1 and 7. Our method of analyzing suramin levels is equally sensitive and selective as that of Klecker and Collins [9], but has the added advantage of isocratic elution. Our method also compared favourably with that of Edwards et al. [10] because the simple extraction procedure not only enables direct injection of a supernatant but also results in complete recovery of suramin as well as low background absorption.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. S. Broder for supplying suramin and K. Elliott for assisting in preparing this manuscript. This work was supported in part by a contract from the State of Massachusetts and a grant from the Sandoz Foundation.

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