Journal of Chromatography, 343 (1985) 224—228
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2676

Note

Determination of suramin in plasma by high-performance liquid chromatography

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(First received February 5th, 1985; revised manuscript received March 29th, 1985)

Suramin was introduced in 1920 for the treatment of African trypanosomiasis and in 1947 for the treatment of onchocerciasis. Little information is available concerning its clinical pharmacology due principally to an absence of suitably selective methods of analysis. One method published in 1938 relies on exhaustive hydrolysis of suramin, and presumably any metabolites, to amines which may then be determined colorimetrically following reaction with compounds such as methyl-N-(1-naphthyl)ethylenediamine [1]. A similar procedure has recently been reported by Fairlamb and Bowman [2]. Since suramin is extensively bound to plasma proteins [3] this poses difficulties with its extraction from plasma. We wish to report a method for the extraction of suramin from plasma and analysis by high-performance liquid

0378-4347/85/$03.30

chromatography (HPLC), taking into account this problem of extensive binding.

EXPERIMENTAL

Chemicals

Suramin sodium, hexasodium 3,3′-ureylene-bis[8-(3-benzamido-*p*-toluido)]-1,3,5-naphthalene sulphonate, was supplied by Bayer (U.K.) (Newbury, U.K.) and the internal standard naproxen, (+)-6-methoxy-α-methyl-2-naphthyl acetic acid, was a gift from Syntex Pharmaceuticals. Tetrabutyl ammonium hydroxide (40%, w/v as aqueous solution) was supplied by Fisons (Loughborough U.K.). Solvents were of HPLC grade and were obtained from the same source. All other reagents were of analytical grade and were obtained from British Drug Houses (Poole, U.K.). Glass culture (Sovirel) tubes and poly-tetrafluoroethylene (PTFE) lined screw-caps were supplied by V.A. Howe (London, U.K.).

Chromatography

The method was developed on a Spectra-Physics liquid chromatograph. The system consisted of an SP 8770 solvent delivery system with an SP 8750 organiser module equipped with a Rheodyne valve injection system and coupled to a Pye Unicam LC871 variable-wavelength UV absorbance detector operating at 325 nm. The separation was carried out at room temperature on a LiChrosorb RP-18 (10 μm particle size) reversed-phase column (20 cm × 0.2 cm I.D., Technicol, Macclesfield, U.K.). The mobile phase consisted of methanol—0.05 *M* disodium hydrogen phosphate (60:40, v/v) containing the ion-pairing reagent tetrabutyl ammonium hydroxide (0.005 *M*) buffered to pH 7.5 with orthophosphoric acid. The flow-rate was 1.5 ml min^{-1}.

Extraction procedure

The extraction was carried out in 10-ml capacity glass culture (Sovirel) tubes treated with dimethyldichlorosilane (5%, v/v in toluene) to minimise adsorption. To samples of plasma (0.1—0.5 ml) containing the internal standard, acetonitrile (1.0 ml) and water (1.0 ml) were added. The contents were vortexed for 1 min in order to precipitate plasma proteins. Tetrabutyl ammonium hydroxide (0.025 ml) was added as an ion-pairing reagent followed by ethyl acetate (5.0 ml). The mixture was extracted by mechanical tumbling for 10 min and after centrifugation (500 *g*, 5 min) the organic phase was removed and retained. To the aqueous phase was added a second aliquot of ethyl acetate (5.0 ml) and the extraction procedure was repeated. The organic phases were combined and evaporated to dryness under nitrogen (37°C). The residue was then dissolved in water (40—50 μl), and an aliquot of 10–20 μl was injected on to the column.

Standard curves

Standard curves were prepared by adding known quantities of suramin as an aqueous solution (0—15 μg) to a fixed quantity of internal standard (3 μg) in drug-free plasma (0.1—0.5 ml). Samples were analysed as described above and

the peak height ratio of suramin to internal standard was plotted against the weight ratio of suramin to internal standard. Peak height ratios of unknown samples were measured and the suramin concentration determined by reference to the standard curve. The extraction efficiencies of suramin and the internal standard were estimated by comparison of the peak height obtained from an extracted plasma sample with that from an aqueous solution containing the same amount of each compound. The intra- and inter-assay precision data were determined by replicate assay of the same sample.

Clinical study

Six Ghanaian patients (aged 30 to 40 years) and with moderate to severe *O. volvulus* infections were studied at the Onchocerciasis Chemotherapeutic Research Centre (Tamale Hospital, Tamale, Ghana). Each subject received an initial dose of suramin (200 mg) by intravenous (i.v.) bolus injection. This was followed by subsequent doses of 400, 600, 800 and 1000 mg suramin at weekly intervals. Venous blood samples were drawn pre-dose and at frequent intervals following the initial dose. Blood was collected into heparinised tubes, centrifuged (1000 *g*, 15 min) and the plasma transferred to plain plastic vials and stored at −20° C until assayed.

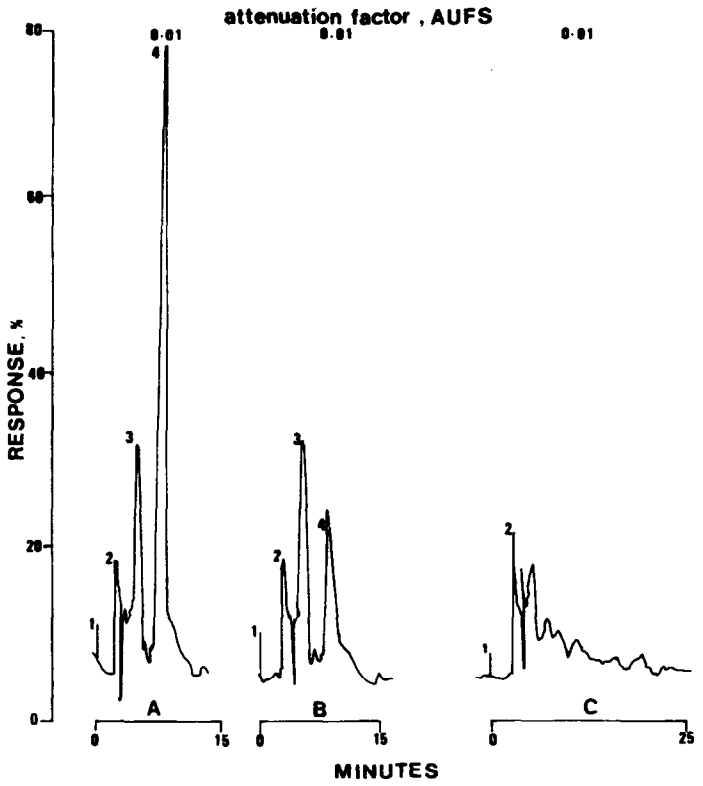

Fig. 1. High-performance liquid chromatograms for (A) a spiked plasma (0.5 ml) extract (suramin concentration 30 μg/ml); (B) an extract of a plasma sample (0.5 ml) taken from a patient who received 200 mg suramin i.v. (suramin concentration 11.2 μg/ml); and (C) a blank plasma (0.5 ml) extract. Peaks: 1, injection event; 2, solvent front; 3, internal standard, naproxen; and 4, suramin.

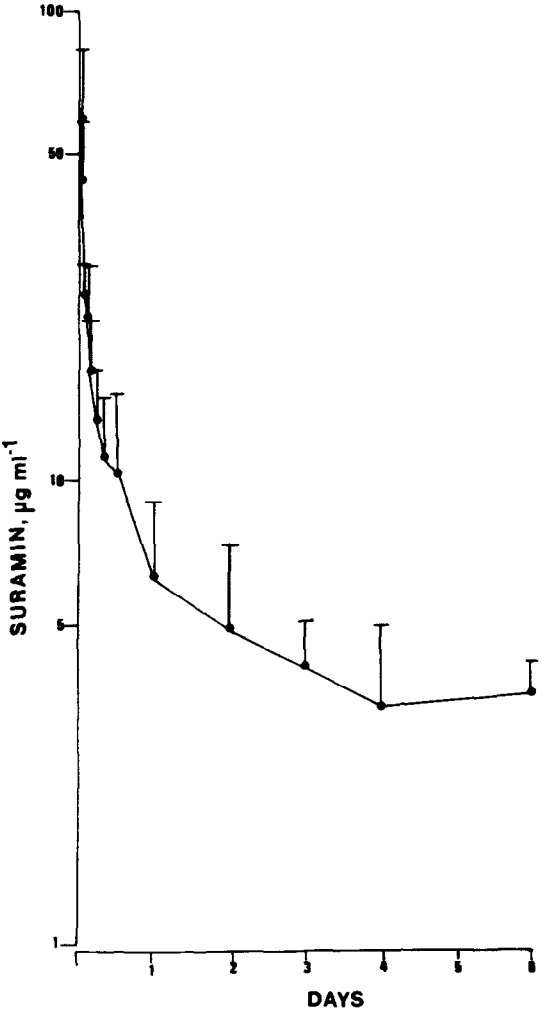

Fig. 2. Semi-logarithmic plot (mean ± S.D.) of plasma concentrations of suramin against time obtained from six patients who received suramin (200 mg i.v.).

RESULTS AND DISCUSSION

Chromatograms of an extract of a pre-dose plasma sample, spiked plasma (0.5 ml) and a plasma extract (0.5 ml) obtained after an i.v. dose (200 mg) of suramin are shown in Fig. 1. The plasma extract from both the patient receiving suramin and the spiked sample showed a distinct peak with a retention time of 8 min corresponding to suramin. This peak was completely resolved from that of that of the internal standard, retention time 4.5 min. The extraction method, employing an ion-pairing reagent, produced minimal extraction of endogenous material. Indeed suramin could not be extracted from plasma in the absence of this reagent. Further, extraction of suramin could not be achieved without prior denaturation of plasma proteins. Calibration curves were linear in the range 0–15 μg (r = 0.99). The analytical recoveries

from plasma were 30% for suramin and 47% for the internal standard. The minimum detectable quantity of suramin extracted from plasma which gave a peak three times baseline noise at the highest detector sensitivity (0.005 a.u.f.s.) was 200 ng. The intra- and inter-assay variation of spiked plasma samples was 3.6% (n = 5) and 8.1% (n = 5) respectively at 20 μg/ml, and 5.7% (n = 3) and 9.2% (n = 9) respectively at 2.5 μg/ml.

This assay was used to determine the plasma concentration versus time profiles of suramin following intravenous administration of the initial dose of 200 mg to Ghanaian patients under treatment for onchocerciasis. Suramin plasma concentrations declined in a polyexponential fashion in all subjects (Fig. 2).

The advantages of this method over earlier techniques are enhanced selectivity and a simplified assay procedure. It is sufficiently sensitive to be applicable to the analysis of plasma samples derived from field studies of the clinical pharmacology of suramin.

ACKNOWLEDGEMENTS

This work was supported by the Wolfson Foundation (GE) and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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