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BIOANALYSIS OF SURAMIN IN HUMAN PLASMA BY ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A liquid chromatographic method is described that can be used for the determination of suramin in plasma samples from cancer patients treated with this drug. The chromatographic system is based on the use of tetrabutylammonium bromide as an ion-pairing agent, while ultraviolet detection is applied. The sample pretreatment is a simple deproteination step by an organic solvent. The same counter-ion as used in the phase system is added in order to increase the recovery of the almost complete protein-bound suramin. The minimum detectable concentration in plasma is ca. 0.1 μg/ml, thus allowing the monitoring of patients treated with this drug. One example of a plasma concentration–time course after administration of suramin is given.

INTRODUCTION

Suramin is a polysulphonated naphthylurea (Fig. 1) that has been used for Rhodesian and Gambian trypanosomiasis and Onchocerca volvules since the early 1920s [1]. Subsequent to the observation that suramin was a potent reverse transcriptase inhibitor and exhibited anti-human immunodeficiency virus (anti-HIV) activity in vitro [2], clinical trials were initiated to test its

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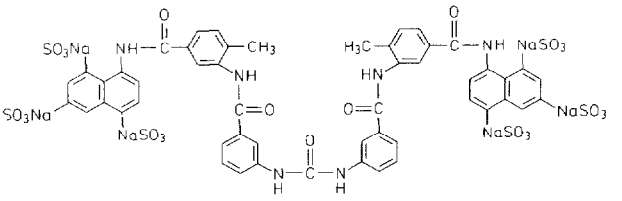

Fig. 1. Structure of suramin.

efficiency in the treatment of acquired immunodeficiency syndrome (AIDS) [3]. The AIDS trials indicated anticancer activity as a response was noted in an HIV-associated Kaposi's sarcoma, and suramin also demonstrated cancer cell growth inhibition in vitro [4]. Inhibition of growth factor activity has been shown for suramin with respect to platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β) and epidermal growth factor (EGF) [5–7]. These growth factors have important effects on tumour biology, and suramin antitumour activity might be based on inhibition of these factors.

Data from animal studies demonstrated the influence of suramin on the concentration of glycosaminoglycans in tissues [8]. Glycosaminoglycans have important effects on tumour biology, including differentiation [9] and tumour angiogenesis antagonism [10]. This represents an alternative explanation for suramin anticancer activity.

Recently suramin activity in prostate cancer treatment was highlighted, together with the necessity for suramin monitoring in patients under treatment in order to generate non-toxic, active levels of 300 μg/ml in the steady state [11]. Frequent monitoring of suramin requires a rapid, selective and sensitive assay.

Recently, analyses based on high-performance liquid chromatography (HPLC) have been described for the high plasma protein-bound naphthylurea derivative [12–15]. The four assays are based on reversed-phase ion-pair HPLC. One includes gradient elution [12] and the other three exploit an isocratic mobile phase. Ruprecht et al. [14] and Teirlynck et al. [15] reported more convenient procedures but reported linearity up to 200 μg/ml. In clinical settings, however, suramin levels can exceed 200 μg/ml, either at steady-state conditions (up to 300 μg/ml) or after bolus injections preceding steady-state conditions; the latter is mandatory in cancer therapy with suramin in order to screen for development of allergy.

This paper reports a rapid and sensitive method for the determination of suramin in plasma of cancer patients by HPLC with a linearity from 5 to 500 μg/ml using a one-step sample pretreatment method. Data on plasma protein binding are also given.

EXPERIMENTAL

Chemicals

Suramin (Germanin) was supplied by Bayer (Leverkusen, F.R.G.) and the tetrabutylammonium bromide (TBABr) must be of high purity (Fluka, Buchs, Switzerland).

The phosphate buffer was a mixture of 10 m*M* disodium hydrogenphosphate and 10 m*M* sodium dihydrogenphosphate. These reagents and all other chemicals were of analytical grade and were used as such. Throughout the study deionized water (Milli Q water purification system, Millipore, Bedford, MA, U.S.A.) was used.

Apparatus

The mobile phase was delivered by a Spectra Physics Model Isochrom pump (Spectra Physics, San Jose, CA, U.S.A.). Sample injection was performed with a manual Model 7125 loop injector (Rheodyne, Berkeley, CA, U.S.A.) equipped with a 100-μl loop. Detection was performed with a Spectra Physics 100 UV–VIS detector used at a wavelength of 313 nm. The detector signal was monitored by either a Shimadzu CR-3A integrator (Shimadzu, Kyoto, Japan) or a Model BD8 flat-bed recorder (Kipp & Zonen, Delft, The Netherlands).

Chromatography

The stainless-steel column was a 100 mm$\times$3.0 mm I.D., Nucleosil 100-5C_{18} (Macherey & Nagel, Düren, F.R.G.), packed in the laboratory with a pressurized slurry technique. A guard column (10 mm$\times$2 mm I.D.) (Chrompack, Middelburg, The Netherlands), installed in series with the analytical column, was slurry handpacked with Polygosil 60 C_{18} 40–63 μm (Macherey & Nagel).

The eluent for the routine analysis of suramin was composed of methanol–10 m*M* phosphate buffer (pH 7.5) and 5 m*M* TBABr (57.5:42.5, v/v). A flow-rate of 0.5 ml/min was maintained. The eluent and the column were thermostated at 298 K.

Determination of protein binding

Citrate plasma samples of 1 ml, used for protein binding studies, were spiked with 100, 200 or 500 μg/ml suramin and incubated overnight at 310 K. After incubation, the plasma samples were transferred to the sample reservoir of an ultrafiltration system, the micropartition system (MPS-1) containing a YMT membrane with cut-off value of 30,000 (Amicon, Oosterhout, The Netherlands). The whole device was centrifuged for 15 min at 298 K at 2000 *g* using a fixed-angle (34°) rotor. Subsequently, 50 μl of the resulting filtrate were analysed with the LC system described.

Sample preparation and storage

Blood samples were taken by venepuncture at defined times during 48 h after the administration of suramin and collected in heparin polyethylene tubes. The tubes were centrifuged immediately at 277 K at 1000 *g* for 10 min, and stored at 243 K until analysis.

Sample pretreatment

Aliquots of 0.25 ml of defrosted plasma samples were mixed in a polyethylene Eppendorf cup with 0.2 ml of freshly prepared 1 *M* TBABr for 30 s. Then 0.5 ml of methanol was added, and this solution was mixed for 30 s. After stabilization for 30 min at 277 K, the tubes were centrifuged for 10 min at 1000 *g*, and 0.1 ml of the clear supernatant was diluted with 4.0 ml water. An aliquot of 0.1 ml was injected into the LC system.

RESULTS AND DISCUSSION

Chromatography

In the development of a suitable phase system for suramin, the possible use of the cetrimonium ion as ion-pairing agent was investigated. However, it appears that the range of the modifier content was rather limited. A variation of only 1% in methanol content resulted in a considerable shift in retention, and the peak efficiency in this phase system appeared to be extremely poor. An endogenous compound at 45 min was also observed, which prolonged the analysis considerably. Applying a backflush system [16] can overcome this drawback, but we preferred to use a phase system based on another ion-pairing agent.

TBABr is easier to use as ion-pairing agent than cetrimonium bromide, but the capacity ratio is still strongly influenced by the methanol content in the mobile phase (Fig. 2). Fig. 2, which represents the relationship between the capacity ratio of suramin and the methanol content of the mobile phase, shows

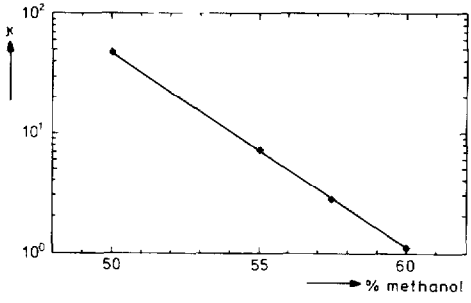

Fig. 2. Influence of the methanol content in the mobile phase on the capacity ratio of suramin. Column, 100 mm×3 mm I.D. Nucleosil 100-5C_{18}; eluent, methanol in 10 m*M* phosphate buffer (pH 7.5).

that the retention is strongly dependent on the modifier content. The use of this method of sample pretreatment led to the choice of methanol as modifier.

Changing the ion-pair concentration influenced the retention of the analyte (Fig. 3). Since the column material plays only a minor role in ion-pair chromatography, we used C_{18} material as stationary phase, as previously described [12–15]. The use of 5 m*M* TBABr in a 57.5% (v/v) methanol–10 m*M* phosphate buffer (pH 7.5) mobile phase in combination with a C_{18} stationary phase gives a reliable system for routine analysis. With this phase system it appeared that a time-consuming regeneration of the column every day, as described previously [15], is not needed: such a regeneration procedure takes at least 4–6 h. The column was satisfactorily regenerated by pumping through a mobile phase of 50% methanol in water once a week. After such a procedure an equilibration time of at least 5 h is needed.

The column effluent can be monitored at different wavelengths. Suramin has two pronounced absorption maxima, at 237 nm ($\epsilon = 126\,000$) and at 313 nm ($\epsilon = 28\,000$). Although detection at 237 nm seems very attractive, we preferred to use the longer wavelength, because at 237 nm the determination is

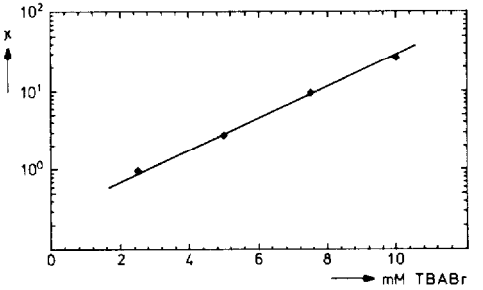

Fig. 3. Relationship between the capacity factor of suramin and the counter-ion concentration in the mobile phase. Column, 100 mm × 3 mm I.D. Nucleosil 100-5C_{18}; eluent, 57.5% methanol in 10 m*M* phosphate buffer (pH 7.5) containing TBABr.

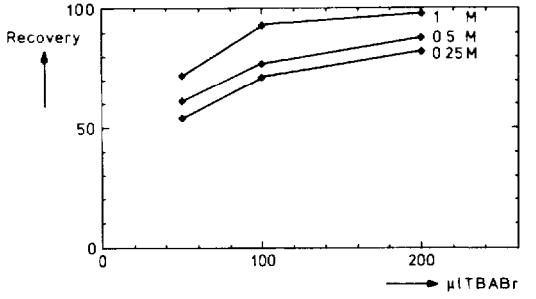

Fig. 4. Influence of the amount of TBABr, added to the extraction solvent, on the recovery of suramin.

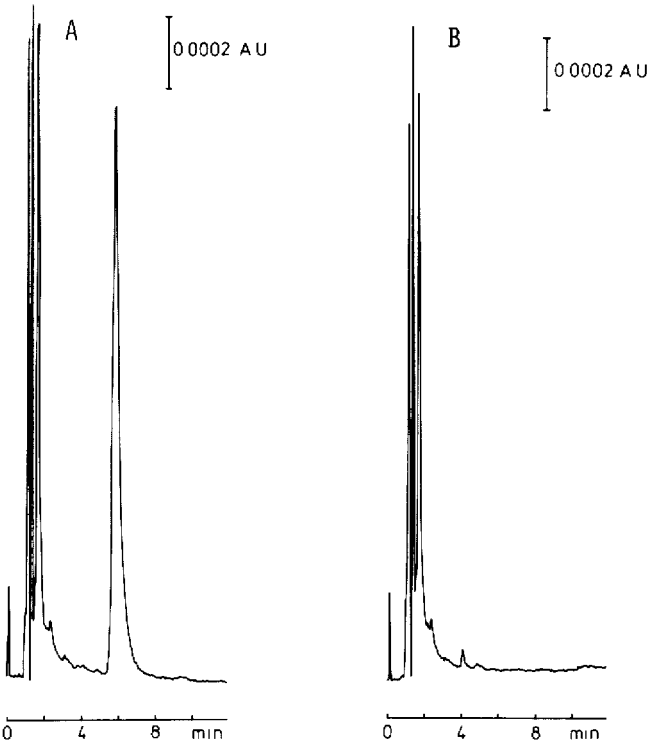

Fig. 5. (A) Chromatogram of an extract of plasma spiked with 50.5 μg/ml suramin. (B) Chromatogram of an extract of blank plasma. For conditions see text.

seriously hampered by interfering compounds. These interferences are not detected at 313 nm. Nevertheless, it must be stated that, when lower limits of determination are needed, a more extensive sample pretreatment is mandatory in order to allow detection at 237 nm.

Sample pretreatment

The sample pretreatment of plasma is based on deproteination by adding methanol containing the counter-ion TBABr. Complete recovery is obtained in a single step. In the literature different procedures have been described. Complete recovery has been obtained using a triple liquid–liquid extraction [12,14]; a double liquid–liquid extraction [13] resulted in 30% recovery and less than 10% recovery in one-step extraction [15].

The procedure described here is rather simple, non-laborious and suitable for routine analysis. By adding an electrolyte to the deproteinating methanol, the yield of extraction can be improved from initially ca. 25% to complete recovery. We investigated the possibilities of ammonium sulphate, since this salt causes a reversible denaturation of proteins. This implies that owing to

locally high concentrations of salt the risk of loss caused by inclusion of the analyte is avoided. Unfortunately, use of ammonium sulphate (and also of sodium chloride) leads to a negative peak at the time that the analyte is eluted. By replacing ammonium sulphate by the counter-ion TBABr, this phenomenon is prevented and the recovery is improved (see Fig. 4), although a more careful addition of the methanol is mandatory.

A disadvantage of such a denaturation step is the excess of counter-ions, which is needed in order to obtain high recoveries. Direct injection of the supernatant leads to a gradual increase of the peak width within one series of plasma samples. Dilution with water in order to achieve a similar ion-pair concentration as the mobile phase circumvents this problem.

The calibration curve was obtained by spiking blank citrate human plasma with suramin. After spiking, the plasma samples were equilibrated to get complete protein binding for 1 h at ambient temperature. Then two series were incubated overnight at 277 and 310 K, respectively. Analysis of the two series did not show any difference, implying that complete equilibration was obtained when the calibration samples were prepared the day before analysis.

Drug–protein binding

For the determination of the protein binding, spiked plasma samples were separated in the free drug fraction and the protein-bound fraction by ultrafiltration. The principle of this micropartition technique is based on filtration of the free analyte through an anisotropic, hydrophylic membrane [17]. This ultrafiltration is performed by centrifugation. The free fraction is collected in a polyethylene vial so that it can be analysed directly on the LC system. No adsorption of suramin on either the membrane or the polyethylene vials has been detected.

The free suramin fraction for the samples spiked with 500 μg/ml amounted to 0.11% ($n=2$). Suramin could be detected in samples spiked with lower levels (200 μg/ml), but quantitative data are not given because the suramin peak only just exceeds a signal-to-noise ratio of 3. For samples spiked with 100 μg/ml no free suramin could be observed in the ultrafiltrate.

The level of drug–protein binding (99.89%) observed at 500 μg/ml does not significantly differ from values in the literature [18,19].

Quantitative aspects

In order to investigate the linearity of the method, different amounts of suramin were added to blank citrate plasma samples and analysed according to the developed method. Ten plasma samples of 1 ml were spiked with concentrations ranging from 5 to 500 μg/ml. These samples were incubated overnight at 310 K to ensure complete protein binding.

The calibration curve could be characterized by the equation $y=3.15(\pm 0.04)x-4.2(\pm 9.2)$, where x is the concentration in μg/ml and y is

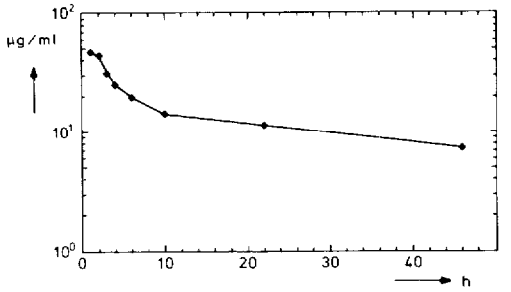

Fig. 6. Plasma concentration–time course of a patient after intravenous infusion of 200 mg of suramin in 1 h.

the peak area. The linearity appeared to be good, as demonstrated by the correlation coefficient (r) of 0.9994.

The recovery of the drug from plasma was determined by assaying ten samples spiked with concentrations ranging from 5 to 500 μg/ml (see Fig. 5). From comparison with the results obtained with aqueous samples containing the same concentrations of suramin, subjected to the same procedure, the recovery was calculated to be 95.9%.

The within-assay precision for the analysis of suramin in plasma samples was determined for six samples at two different concentrations: at 50.5 μg/ml the accuracy was $104.5 \pm 2.4\%$ and at 202 μg/ml it was $95.5 \pm 4.2\%$ (mean $\pm$ S.D.). The minimum detectable concentration in plasma with a signal-to-noise ratio of 3 is ca. 0.1 μg/ml.

Drug monitoring

An example of plasma concentration–time curve after infusion of suramin during 1 h and a total dose of 200 mg is presented in Fig. 6. After distribution half-lives of several hours, extremely long elimination half-lives were found in all patients monitored up to days or even weeks. This observation is in accordance with literature data [12–14], and reinforces the necessity for early drug monitoring in patients treated under continuous administration conditions [11].

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