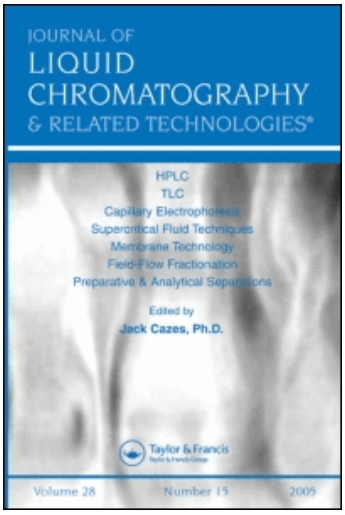

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THERAPEUTIC DRUG MONITORING OF SURAMIN AND PROTEIN BINDING

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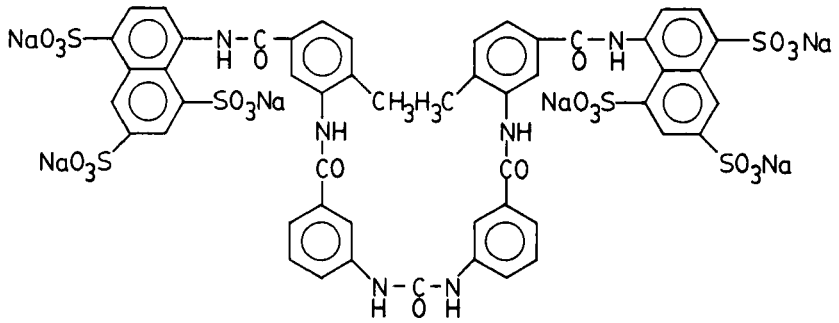
ABSTRACT

A liquid chromatographic method of suramin has been used for the determination of the polysulfonated naphtylurea in both plasma and plasma filtrate from prostate cancer patients treated with the drug. The chromatographic system is based on the use of tetrabutylammonium bromide as an ion-pairing agent, while UV detection at 237 nm and 313 nm 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. 20 ng/mL at 237 nm, in plasma filtrate 10 ng/mL at 237 nm.
The method was routinely applied in plasma level guided treatment of prostate cancer patients with suramin, as well as in protein binding studies. The data of the study demonstrated the necessity of therapeutic drug monitoring in suramin treatment: development of severe, irreversible toxicity could be prevented owing to timed withdrawal of suramin administration when total drug levels were beyond 300 μg/mL. Data of protein binding studies explained in part the development of severe toxicity associated with plasma levels beyond 300-350 μg/mL: at that point free fraction of suramin sharply increases from 500 ng/mL at a total plasma level of 500 μg/mL to 10 μg/mL at a total plasma concentration of 1000 μg/mL, which corresponds with a twenty-fold dose increase (2000% !).



1. Structure of suramin.

INTRODUCTION

The polysulphonated naphtylurea suramin (Fig. 1) has been discovered recently as a potent anticancer agent (1), possibly owing to its growth factor inhibitory activity (anti PDGF, anti TGF-ß and anti EGF) (2-4) as well as to its influence on glycosaminoglycans (5), wich influence tumour angiogenesis (6) and differentiation (7). It was demonstrated recently that inhibition of DNA polymerases in HeLa cells contributed to affection of DNA sysnthesis by suramin (8).

Suramin activity in the treatment of prostate cancer has been documented by Myers *et al* (9) and their preliminary data could be established in a Phase I/II study (10). Frequent monitoring of suramin in patients under continuous treatment has been suggested in order to maintain non-toxic, active levels of 200-300 µg/mL (9,10).

Several assays are available now to fullfil to the demand described above and exploit either gradient or isocratic elution in reversed-phase ion-pair HPLC (11-14). We recently described a liquid chromatographic method that can be used for the determination of suramin in plasma samples from cancer patients treated with the drug (15).

The chromatographic system is based on the use of tetrabutylammonium bromide as an ion-pair agent, while the polysulphonated naphtylurea is detected at 313 nm. Detection at 237 nm ($^{-}$=126000 *vs* $^{-}$=28000 at 313 nm) seems very attractive when low detection limits are required as in determination of unbound suramin. Detection at 237 nm however was seriously hampered by interfering compounds originating from the plasma matrix in several patient samples (15).

The present paper reports on application of our method in routine suramin monitoring and on determination of free suramin fractions at different total plasma concentrations using a slightly modified assay. Anticipating the need for monitoring total drug levels of suramin both in plasma in a range of 10-500 μg/mL and unbound levels at 50-1000 ng/mL in different laboratories with varied experience in liquid chromatography, efforts were directed to optimize accuracy and speed of measurements.

EXPERIMENTAL

Chemicals

Tetrabutylammonium bromide (TBABr) was supplied by Fluka (Buchs, Switzerland) and Suramin (Germanin) by Bayer (Leverkusen, F.R.G.). Throughout the study deionized water (Sation 9000/Aqualab 30, VEL, Leuven, Belgium) was used. The phosphate buffer was a mixture of 10 mM Na_2HPO_4 and 10 mM NaH_2PO_4. These reagents and all other chemicals were of analytical grade and were used as such.

Apparatus

Chromatography was performed using a model 110B pump (Beckman Instruments, Berkeley, CA, USA), a manual injector (Altex 210A) equipped with a 100 μL loop, and a UV absorbance detector (Beckman 163 variable wavelength detector) containing a dual path

14 μL flow cell (path length 10 mm). Detection of plasma samples and ultrafiltrate was performed at 237 nm and 313 nm. The detector signal was monitored by a Spectra Physics SP 4290 integrator (Spectra Physics, San Jose, CA, USA).

Chromatography

The analytical column was a stainless steel (250 mm x 4.6 mm I.D.) Ultrasphere C_{18} (5 μm) (Beckman, Altex Div., San Ramon, CA) packed column. A guard column (10 mm x 2 mm I.D.) (Chrompack, Middelburg, The Netherlands) was slurry handpacked with Polygosil 60 C_{18} 40-63 μm (Macherey & Nagel, Düren, F.R.G.) and was installed in series with the analytical column. The mobile phase was composed of methanol-10 mM phosphate buffer (pH 7.5) and 5 mM TBABr (62.5 : 37.5, v/v). Chromatography was performed at a flow rate of 1 mL/min. The eluent and the column were thermostated at 25°C.

Sample preparation and storage

Blood samples were collected in heparin polyethylene tubes at appropriate time intervals for pharmacokinetic interpretation immediately before, after starting and during treatment with suramin. The samples were immediately centrifuged at 4°C at 1000 g for 10 min, decanted and stored at -30°C until thawing for subsequent analysis.

Sample pretreatment

Aliquots of 0.25 mL of thawed plasma were mixed in a polyethylene Eppendorf cup with 0.2 mL of freshly prepared 1 M TBABr during 30 s. Then 0.5 mL of methanol was added, and the solution was mixed for 30 s. After stabilization for 30 min at 4°C, the tubes were centrifugated 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.

For specific questions 2-naphtol was applied as internal standard (16). However, the use of an internal standard is not essential in routine analysis of suramin, owing to the accuracy of the present assay.

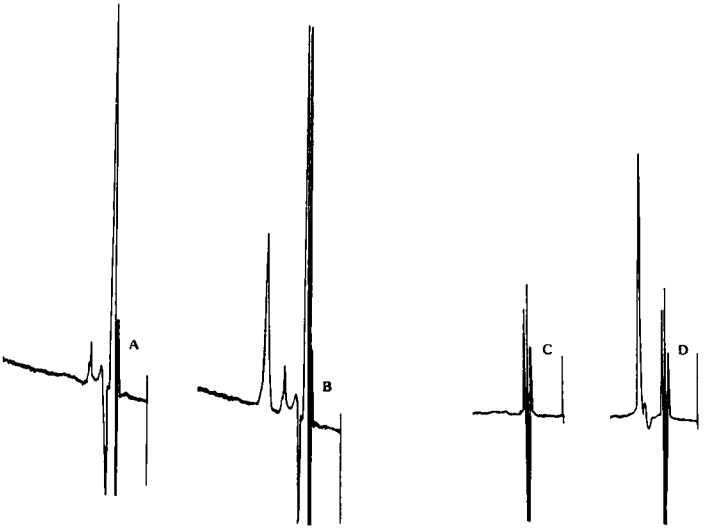
Protein binding studies

Blood samples of 1 mL were spiked with suramin at concentrations of 100, 200, 500, 660, 1000, 1320 and 2000 μg/mL and incubated overnight at 37°C. The samples were then installed in the sample reservoir of an ultrafiltrate system, (micropartition system MPS-1) containing a YMT membrane cut-off value of 30.000 (Amicon, Oosterhout, The Netherlands). The whole device was centrifuged for 15 min at 2000 g at 25°C using a fixed-angle (34°) rotor. The resulting filtrate was analysed with the LC system described using both 237 and 313 nm as detection wavelengths.

RESULTS AND DISCUSSION

Chromatography

Methanol was used as modifier owing to its use in sample pretreatment. TBABr appeared to be easy in its use as ion-pairing agent with methanol as modifier. It should be noted however that the capacity factor is markedly influenced by the methanol content in the mobile phase: a difference of only 1% methanol can result in a dramatic change of the capacity ratio. In routine analysis of patient plasma samples, we used 5 mM TBABr in a 62.5% (v/v) methanol-10 mM phosphate buffer (pH 7.5) as mobile phase in combination with a C_{18} stationary phase. Column regeneration included 12 h pumping of 50% methanol in water and subsequent 5 h equilibration once a week. When plasma filtrate was monitored, regeneration intervals could be extended. Representative chromatograms of patient blank plasma, patient plasma with suramin, blank plasma filtrate and plasma filtrate containing suramin are presented in Fig. 2A-D.



2. Chromatograms of blank plasma (a), plasma with suramin (90 μg/mL) (b), blank plasma filtrate (c) and plasma filtrate with suramin (90 μg/mL) (d) detected at 313 nm. Plasma samples were obtained from a patient before and during suramin treatment and demonstrated interfering peaks of plasma matrix compounds when measured at 237 nm, even when pretreatments were carried out according to ref. 16. In ultrafiltrate suramin could be detected at 237 nm at the low ng/mL level.

Sample pretreatment

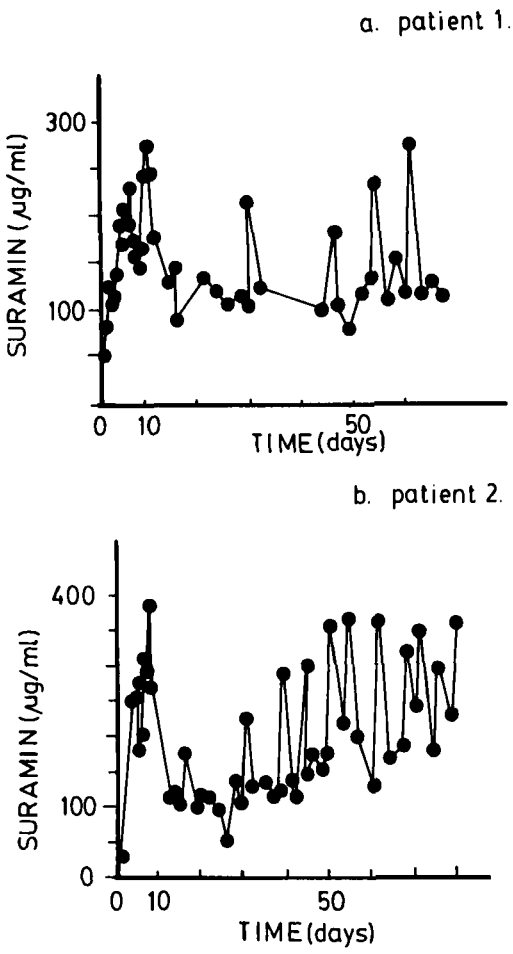
The sample pretreatment procedure described here proved to be simple, non-labourious and suitable for routine analysis. Almost complete recovery could be obtained in a single step extraction by addition of an elecrolyte to the deproteinating methanol. A disadvantage of the denaturation step, i.e. excess of counterions which cause gradual increase of the peak width within one series of plasma samples, could be overcome by dilution with water. This resulted in a similar ion-pair concentration as the mobile phase.

Calibration curves were obtained by spiking blank citrate human plasma with suramin; plasma samples were subsequently equilibrated for complete protein binding during 1 h at ambient temperature. Calibration curve samples could be either used directly or after storage at -30°C, since no differences between series was noted in earlier experiments (15). Stability of frozen suramin was demonstrated up to 3 months; significant differences between suramin contents after 3 months storage at -30°C and the content of immediately prepared suramin plasma samples could not be demonstrated. The linearity of calibration curves obtained with spiked plasma was good ($r : 0.9994$; $y = 3.15(\pm 0.04)x - 4.2(\pm 9.2)$) whereas recovery was calculated to be above 95%. Quality of calibration curves for plasma filtrate were comparable with those for plasma. Data of within-assay precision and limits of determination (lambda: 237 nm, when no interferences of plasma matrix compounds were present) in plasma (20 ng/mL) and plasma filtrate (10 ng/mL) allowed sensitive and selective measurements of suramin in therapeutic drug monitoring sessions and protein binding studies.

Therapeutic drug monitoring (TDM)

Plasma concentration time-profiles of suramin in 7 patients over different periods of time are depicted in Fig. 3.

The time periods of TDM ranged from 16 (patient 5) - 90 days (patient 7). In some patients (e.g. patient 6) suramin administration was discontinued owing to development of (reversible) side-effects which were -in some cases- related with plasma levels of 300 μg/mL. However, other patients also demonstrated levels at or beyond 300 μg/mL without development of toxicity (patient 2) while toxicity was observed also at levels between 150 and 250 μg/mL. Thus, we have not been able to demonstrate a clear relation between toxicity of suramin and plasma levels, in contrast to other reports (17). TDM of suramin can be recommended taken the large inter- and intra individual variation of plasma levels into account (patient 1 - patient 7). Furthermore we felt that development of severe, irreversible



3 a-g. Suramin plasma levels in 7 patients over prolonged periods of time ranging from 16 days (patient 5, Fig. 3 e) to 90 days (patient 7, Fig. 3 g). In some patients (e.g. patient 6, Fig. 3f) suramin administration was discontinued owing to development of reversible side-effects. In comparing the data of patient 1 to 7 it can be concluded that large inter-individual differences between suramin handling occur. Furthermore suramin levels drop from 300 μg/mL to 150 μg/mL in a manner which is subjected to intra-individual differences and which can not be predicted from loading dose and test dose pharmacokinetics (E.A. de Bruijn, *in press*). (continued)

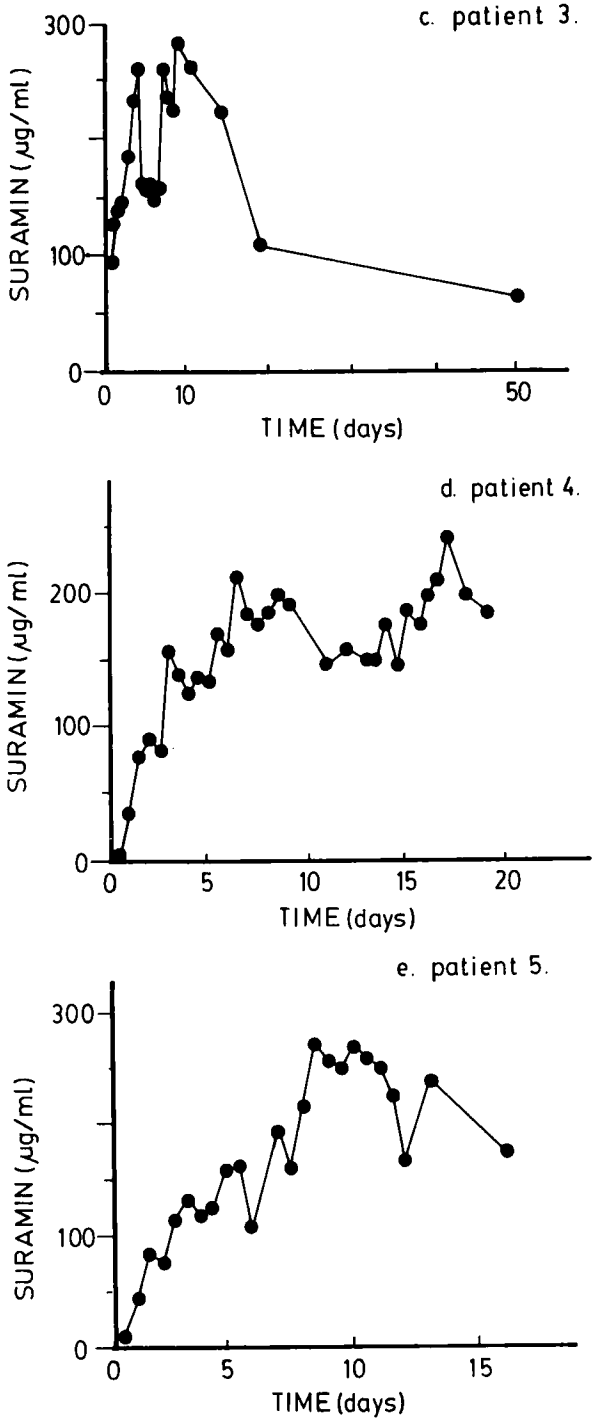
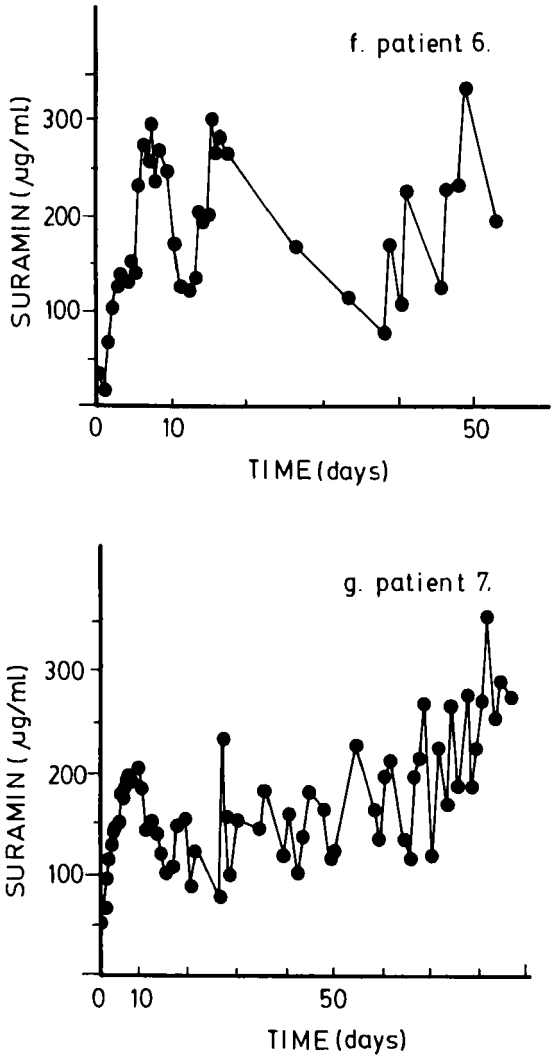


Fig. 3 (continued)



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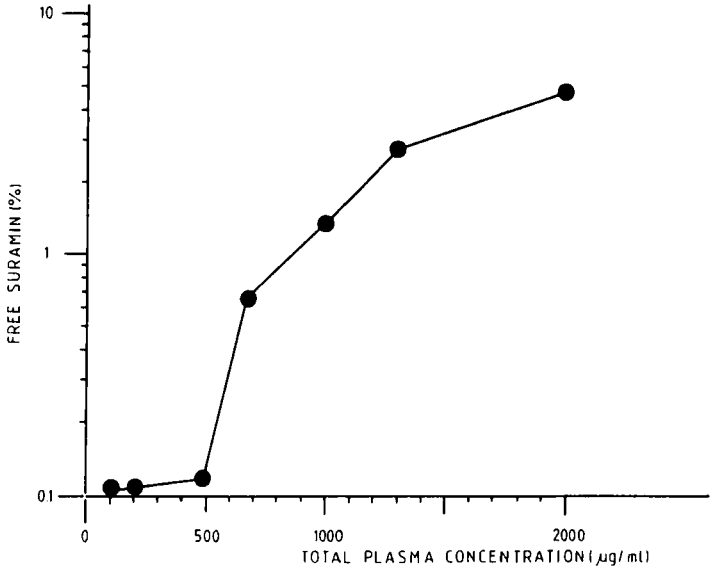

4. The saturation of suramin binding to plasma components.

toxicity has been prevented since suramin treatment was withdrawn when levels reached 300-350 µg/mL. The frequently observed sharp decrease of suramin levels as well as the increase of concentrations upon start of treatment could not be predicted by single dose, iv suramin pharmacokinetic data (18). This also warrants frequent monitoring in continuous suramin treatment.

Drug-protein binding

Plasma of spiked blood samples was separated by centrifugation and free drug fraction was subsequently separated from protein bound fraction by ultrafiltration. The micropartition is based on filtration of the free analyte through an anisotropic, hydrophylic membrane. This

ultrafiltration is performed by centrifugation. The free fractions were collected in a polyethylene vial and were directly analysed on the LC system. Adsorption of suramin on either the membrane or on polyethylene vials was judged to be negligible in the concentration range studied.

The unbound fraction of suramin appeared to be plasma concentration dependent and sharply increased beyond total plasma concentrations of 500 μg/mL (Fig. 4).

The free fraction amounted 0.11% up to 500 μg/mL; at 2000 μg/mL however free fraction percentage was above 5%. The level of drug-protein binding of 99.89% observed at 500 μg/mL was reported earlier (15,19). The sharp increase noted at 660 μg/mL found in the present study might in part explain toxicity observed at levels beyond 300 μg/mL. As has been discussed excellently by Koch-Weser and Sellers (21, 22), it is the free fraction of high protein bound drugs which determines therapeutic outcome, including toxicity. The increase of free suramin in a range of 500-1000 μg/mL total plasma suramin should be interpreted as a dose increase far beyond the two-fold, i. e. a 10-fold. This might explain in part the small window of therapeutic levels (200-300 μg/mL) in suramin treatment, which necessitates TDM of suramin (10,17-19). Furthermore it should be stressed that the point of saturation of protein (albumin) binding and binding to other blood constituents such as low density proteins, might be strongly subjected to large interindividual differences (18). The sharp increase of free suramin might therefore be found also at lower total plasma concentrations, especially in patients selected for suramin treatment: (prostate) cancer and AIDS. Ongoing studies in our laboratory will reveal the importance of free suramin levels in cancer patients treated with the drug. Therefore, we conclude that suramin treatment should include frequent monitoring of both total and free plasma levels in order to optimize treatment with the polysulphonated naphtylurea.

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REFERENCES

1. Z. Spigelman, A. Dowers, S. Kennedy, D. Disorbo, M. O'Brien, R. Barr and R. McCaffrey, Antiproliferative Effects of Suramin of Lymphoid Cells. Cancer Res. 47, 4694-4698, 1987.

2. J. Garrett, S. Coughlin, H.L. Niman, P.M. Tremble, G.M. Giels and L.T. Williams, Blockade of Autocrine Stimulation in Simian Sarcoma Virus Transformed Cell Reverses Down-regulation of Platelet-derived Growth Factor Receptors. Proc. Natl. Acad. Sci. U.S.A. 81, 7466-7470, 1984..

3. C. Betsholtz, A. Johnsson, C. Heldin and B. Westermark, Efficient reversion of simian sarcoma virus-transformation and inhibition of growth factor-induced mitogenesis by suramin. Proc. Natl. Acad. Sci. U.S.A. 83, 6440-6444, 1986.

4. R.J. Coffey, E.B. Leof, G.D. Shipley and H.L. Moses, Suramin inhibition of growth factor receptor binding and mitogenicity in AKR-2B cells. J. cell. Phys. 132 143-148, 1987.

5. G. Constantopoulos, S. Rees, B. Cragg, J.A. Barranger and R.O. Brady, Experimental animal model for mucopolysaccharidosis: Suramin-induced glycosaminoglycan and sphingolipid accumulation in the rat. Proc. Natl. Acad. Sci. U.S.A. 77, 3700-3704, 1980.

6. J. Folkman and M. Klagsbrun, Angiogenic Factors. Science 235, 442-447, 1987.

7. M. Fujita, D. Spray and H. Choi, Glycosaminoglycans and proteoglycans induce gap junction expression and restore transcription of tissue-specific mRNA's in primary liver cultures. Hepatology 7, 1S-9S, 1987.

8. M.K. Jindal, C.W. Anderson, R.G. Davis and J.K. Vishwanatha, Suramin Affects DNA Synthesis in HeLa Cells by Inhibition of DNA Polymerases. Cancer Res. 50, 7754-7757, 1990.

9. C. Myers, C. Stein, R. La Rocca and M. Cooper, In: Abstracts of the Sixth NCI-EORTC Symposium on New Drugs in Cancer Therapy, p. 152, 1989.

10. A.T. van Oosterom, Eric A. De Smedt, Louis J. Denis, Ernst A. de Bruijn and C. Mahler, Suramin for Prostatic Cancer: a Phase I/II Study in Advanced Extensively Pretreated Disease. Eur. J. Cancer 26, 422, 1990.

11. R.W. Klecker and J.M. Collins, Quantification of Suramin by Reverse-phase Ion Pairing High-Performance Liquid Chromatography. J. Liq. Chromatogr. 8, 1685-1996, 1985.

12. G. Edwards, G.L. Rodick and S.A. Ward, Determination of suramin in plasma by High-Performance Liquid Chromatography. J. Chromatogr. 343, 224-228, 1985.

13. R.M. Ruprecht, J. Lorsch and D.H. Trites, Analysis of Suramin Plasma Levels by ion-pair high-performance liquid chromatography under isocratic conditions. J. Chromatogr. 378, 498-502, 1986.

14. O. Teirlynck, M.G. Bogaert, P. Demedts and H. Taelman, Rapid high-performance liquid chromatographic determination of suramin in plasma of patients with acquired immune deficiency syndrome (AIDS) or AIDS-related complex (ARC). J. Pharm. Biomed. Anal. 7, 123-126, 1989.

15. U.R. Tjaden, H.J.E.M. Reeuwijk, J. van der Greef, G. Pattyn, E.A. de Bruijn and

A.T. van Oosterom, Bioanalysis of Suramin in Human Plasma by Ion-Pair High Performance Liquid Chromatography. J. Chromatogr. 525, 141-149, 1990.

16. J.G. Supko and L. Malspeis, A rapid isocratic HPLC assay of Suramin (NSC 34936) in human plasma. J. Liq. Chromaogr. 13(4), 727-741, 1990

17. B. Allolio, M. Reincke, W. Arlt, U. Deuss, W. Winkelmann and L. Siekmann, Suramin for Treatment of Adrenocortical Carcinoma. Lancet II, 277, 1989.

18. E.A. de Bruijn, Clinical Pharmacokinetics of suramin and influence of Protein Binding. Submitted for publication, 1991.

19. J.M. Collins, R.W. Klecker, R. Yarchoan, H. C. Lane, A.S. Fauci, R.R. Redfield, S. B. Broder and C.E. Myers, Clinical Pharmacokinetics of Suramin in Patients with HTLV..III/LAV Infection. J. Clin. Pharmacol. 26, 22-26, 1986.

20. P. Feuillan, M. Raffeld, C.A. Stein, N. Lipford, D. Rehnquist, C.E. Myers, R.V. La Rocca and G.P. Chrousos, Effects of Suramin on the Function and Structure of the Adrenal Cortex in the Cynomolgus Monkey. J. Endocrin. Metab. 65, 153-158, 1987.

21. J. Koch-Weser and E.M. Sellers, Binding of Drugs to Serum Albumin (First of Two Parts). N. Engl. J. Med. 294, 311-316, 1976.

22. J. Koch-Weser and E.M. Sellers, Binding of Drugs to Serum Albumin (Second of Two Parts). N. Engl. J. Med. 294, 526-531, 1976.

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