Biochemical Pharmacology, Vol. 35, No. 19, pp. 3389–3392, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

THE DISPOSITION OF SURAMIN IN THE ISOLATED PERFUSED RAT LIVER

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(*Received* 13 *March* 1986; *accepted* 6 *May* 1986)

Abstract—We have investigated the disposition of suramin in the isolated perfused rat liver preparation (IPRL) after the administration of suramin (18 mg, 8 μCi). At 30 min post drug administration, almost 100% of the [^{14}C]radioactivity and unchanged suramin were located in the perfusate plasma. During the course of the study, the elimination of suramin from the IPRL was barely perceptible. The AUC_{0-5} hr of suramin (730.6 ± 86.2 μg hr/ml) corresponded to that of [^{14}C] radioactivity (815.1 ± 105.5 μg ml/hr) at 5 hr, indicating a lack of perfusate suramin metabolites. At 5 hr only a small proportion of [^{14}C] radioactivity was recovered from the livers (2.5 ± 1.1%). Subsequent HPLC analysis of the liver tissue indicated this to be unchanged suramin. Sub-cellular fractionation of the homogenised livers revealed suramin to be distributed in the liposomal rich tissue fractions (10,000 *g* pellet, 1.6 ± 0.8%; 105,000 *g* supernatant, 1.1 ± 0.35%). Biliary excretion of [^{14}C] radioactivity was low (2.1 ± 0.7%), however, none could be accounted for as unchanged suramin. Previously undetected metabolites of suramin may have accounted for the unidentified biliary radioactivity.

Suramin has been widely used in the therapy of trypanosomasis and is the only effective macrofilaricide in onchocerciasis. Recent studies have also shown suramin to have a virostatic action on human T-cell lymphotropic virus type III [1, 2]. However, due to analytical limitations, little is known of the pharmacokinetics and metabolic fate of suramin. The recent development of a selective and sensitive HPLC assay in our department [3] has enabled a number of aspects of this drug's disposition to be investigated.

In the following study, we have investigated the hepatic disposition of suramin in the isolated perfused rat liver preparation (IPRL). We have chosen the IPRL as an experimental model, as this preparation allows the hepatic component of drug elimination to be examined in a whole organ system, excluding the influences of other routes of elimination present in the intact animal. This experimental model has been of value in studies of the hepatic disposition of a number of drugs, e.g. cimetidine and propranolol, and the antimalarials pyrimethamine and primaquine [4–7].

MATERIALS AND METHODS

Reagents. 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-naphythyl acetic acid was a gift from Syntex Pharmaceuticals. [^{14}C]Suramin (specific activity: 45.7 mCi/mmol) was supplied by New England Nuclear (Boston, U.S.A.) and was naphthalene ring labelled. N.C.S. tissue solubiliser and hydrogen peroxide (30% w/v) were supplied by B.D.H. Chemicals Ltd. (Poole, Dorset, U.K.). Emulsifying liquid scintillant (ES 299) was obtained from the Packard Instrument Company Ltd. (Caversham, Reading, U.K.). All other reagents were of HPLC or analytical grade (Fisons, Loughborough, U.K.).

Animals. Male Wistar rats (200–250 g) were housed in well-ventilated cages and kept at a room temperature of 24°. They were allowed to feed *ad libitum* on pelleted food (Oxoid Breeding Diet, Oxoid Ltd., London, U.K.) and tap water.

Isolated perfused rat livers (IPRL). Rats were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.), their livers isolated using standard techniques and then perfused in a constant flow (15 ml/min) recirculating system at 37°, as previously described [4]. The perfusate (100 ml) comprised 10% washed human red cells 1% w/v bovine serum albumin (Sigma, Poole, U.K.) and 0.1% glucose in a standard electrolyte solution (Krone *et al.*, 1974). The principal indices of liver viability were steady oxygen consumption (1.5–2 μmol/g liver/min), sustained bile flow (0.2–0.6 ml/hr) constant perfusion pressure (6–8 cm H_2O) reproducible liver function tests (i.e. determination of perfusate sodium, potassium total protein, alanine amino transferase and gamma-glutamyl transferase concentrations) and normal visual appearance.

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PROTOCOL

The disposition of suramin in the IPRL was studied over 5 hr following an 18 mg bolus dose of suramin containing 8 μCi of [^{14}C]-labelled drug (N = 5). Suramin was added as a solution in 0.9% saline (100 μl) directly into the reservoir thereby simulating systemic dosage. Samples (1 ml) were removed from the perfusate reservoir pre dose, and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 hr post dosage. After centrifugation (1100 g, 2 min), the perfusate plasma was removed and stored at −20° until assayed for suramin and radioactivity. An equal volume of fresh perfusate was added to the reservoir to replace that removed by sampling. Bile was collected at hourly intervals into pre-weighed vials, and the bile volume determined by weight (assuming 1 ml bile = 1 g) before freezing at −20°. Perfusate gases were measured and liver function tests performed before and after each experiment to assess liver viability. The sub-cellular localisation of suramin and [^{14}C] radioactivity was determined for each liver at the end of each experiment (5 hr) as described below.

Preparation of liver tissue fractions

At the conclusion of each experiment, the livers were flushed with 0.9% saline, weighed and then homogenised in three times the liver weight of ice-cold 0.067 M phosphate buffer (pH 7.5 containing 1.5% KCl) using a Teflon-in-glass homogeniser. The 25% homogenate was centrifuged at 10,000 g for 20 min at 4°. The resulting supernatant was decanted without disturbing the pellet and centrifuged at 105,000 g for 60 min at 4°. The 105,000 g and 10,000 g pellets were then resuspended in three times their weight of phosphate buffer. Prior to HPLC analysis of suramin in the various liver fractions, separate suramin standard curves were prepared in drug-free whole liver homogenate, 10,000 g pellet (resuspended), 10,000 g supernatant, 105,000 g pellet (resuspended) and 105,000 g supernatant.

Analytical procedures

Perfusate plasma suramin levels were determined by HPLC as previously described [3]. [^{14}C] Radioactivity was measured using a Packard Tri-Carb 4034 liquid scintillation spectrometer. Duplicate samples of perfusate plasma (10 μl) and bile (5 μl) were assayed directly for [^{14}C] radioactivity after the addition of scintillation fluid (5 ml). Samples of whole liver homogenate and the various liver tissue fractions were solubilised and decolourised as previously described [6] before assay for [^{14}C] radioactivity levels.

Analysis of bile

In order to prepare a representative aliquot of the total bile production of each IPRL preparation, 40% of each hourly bile sample was removed and combined. An aliquot (5 μl) of this pooled bile obtained from each IPRL was used to determine the biliary levels of [^{14}C] radioactivity. A further aliquot (500 μl) removed from the pooled bile, and was subjected to enzyme hydrolysis (pH 5.0, 37°, 6 hr) with H 1 hydrolase preparation obtained from *H. pomatia* (Sigma, Poole, Dorset, U.K.) 100 units of enzyme activity per 100 μl of bile. This enzyme preparation contains both arylsulphatase and β-glucuronidase activity. Control incubations contained 0.1 M sodium acetate buffer (pH 5.0). Prior to hydrolysis of the pooled bile samples, suramin was found to be stable to hydrolysis by this enzyme preparation. A 10 μl aliquot from each pooled bile sample was injected directly onto the HPLC. Effluent fractions corresponding to the void volume, (0–4.5 min) void volume to suramin, (5–7.5 min) and suramin (7.5–8.5 min) were collected and assayed for [^{14}C] radioactivity after the addition of liquid scintillant.

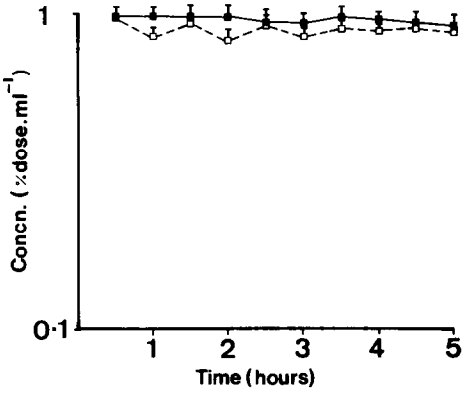

Fig. 1. Perfusate concentrations (% dose/ml) of suramin (□—□) and [^{14}C] radioactivity (■—■) after systemic administration of suramin sodium (18 mg, 8 μCi) to the IPRL (perfusate volume 100 ml).

Pharmacokinetic calculations and statistical analysis

The area under the perfusate/concentration time curve for suramin and [^{14}C]radioactivity from time = 0 to time = 5 hr (AUC_{0-5hr}) was calculated by the trapezoidal rule [8]. The statistical comparisons between two groups were made using Student's unpaired t-test. Data were tabulated as mean ± S.D. and presented graphically as mean ± S.E.M. Statistical significance was accepted when $P \leq 0.05$.

RESULTS

Perfusate disposition of suramin and [^{14}C] radioactivity

At 30 min post dose, almost 100% of [^{14}C]radioactivity and unchanged suramin were located in the perfusate plasma. However, it is clear from Fig. 1 that during the 5 hr of the study, elimination of suramin from the IPRL was barely perceptable. The

Table 1. Massed balance: % suramin and [^{14}C]radioactivity at 5 hr post administration of suramin (18 mg, 8 μCi) to the IPRL (N = 5)

Compound	Liver	Bile	Perfusate	Total recovery
Suramin	3.6 ± 0.7	n.d.	97.8 ± 20.1	101.2 ± 19.6
[^{14}C]Radioactivity	2.5 ± 1.1	2.1 ± 0.7	95.1 ± 14.7	99.5 ± 14.7

n.d. = not detectable.

Table 2. The percentage of [^{14}C] radioactivity and suramin present in whole liver and fractions at 5 hr post administration of suramin (18 mg. 8 μCi) to the IPRL (N = 5)

Tissue	Recovery (%)	
	[^{14}C] Radioactivity	Suramin
Whole liver	2.5 ± 1.1	3.6 ± 0.7
10,000 *g* Supernatant	0.96 ± 0.23	1.1 ± 0.32
10,000 *g* Pellet	1.62 ± 0.74	1.6 ± 0.8
105,000 *g* Supernatant	0.6 ± 0.07	1.1 ± 0.35
105,000 *g* Pellet	0.3 ± 0.12	0.1 ± 0.05

AUC_{0-5hr} of suramin (730.6 ± 86.2 μg hr/ml) corresponded to the AUC_{0-5hr} for [^{14}C] radioactivity (815.1 ± 105.5 μg hr/ml), indicating a lack of perfusate suramin metabolites in the perfusate at 5 hr. A summary of the mass balance data is shown in Table 1. Over 5 hr, only a small quantity (2.1 ± 0.7%) of [^{14}C] radioactivity was eliminated in bile. At the conclusion of each experiment, the vast majority of the [^{14}C] radioactivity and unchanged suramin (Table 2) were located in the perfusate while only a small quantity (≃3%) was recovered from the liver. Total recovery of both suramin and [^{14}C] radioactivity was virtually 100%.

Liver fractionation studies

HPLC analysis of the whole liver homogenates revealed that the [^{14}C] radioactivity found in the livers consisted entirely of unchanged suramin (Table 1). There was no significant difference in the distribution of suramin and [^{14}C] radioactivity between either the 10,000 *g* pellet or 10,000 *g* supernatant (Table 2). However, significantly more suramin and [^{14}C] radioactivity were localised in the 105,000 *g* supernatant in comparison with the 105,000 *g* pellet.

Analysis of biliary metabolites

Although only 2.1 ± 0.7% of the dose of [^{14}C] radioactivity was excreted in the bile over 5 hr, none could be accounted for as unchanged suramin. Most of the biliary radioactivity (1.31 ± 0.25%) was recovered in the void volume, i.e. as polar suramin derivatives. The remainder of the unidentified radioactivity (0.86 ± 0.3%) was recovered from the fraction which eluted between the void volume and suramin. Flushing the HPLC system with methanol at the end of each sample run accounted for a negligible quantity of radioactivity (0.01%).

The disposition of [^{14}C] radioactivity was not affected by subjecting the bile to deconjugating enzymes, nor was unchanged suramin detected in these samples. Suramin is known to be extensively protein bound [9] hence aliquots of the pooled bile samples and also blank bile samples spiked with [^{14}C]suramin were extracted and chromatographed both before and after deconjugation, in identical fashion to the perfusate plasma [3]. Suramin could be detected unchanged neither in the HPLC chromatograms, nor in the effluent fraction corresponding to suramin of the pooled bile samples; while the recovery of [^{14}C]suramin from the spiked bile samples was only slightly reduced compared with that of the perfusate and liver samples.

DISCUSSION

Suramin retains importance in the treatment of onchocerciasis, although its toxicity has limited its use in mass chemotherapy. The drug has shown some promise in the therapy of Acquired Immune Deficiency Syndrome, however in two recent studies, it predictably caused a number of side effects, including some slight hepatic dysfunction [1, 2]. In the present study we have determined the hepatic component of suramin disposition, free from the influences of other organs.

At 30 min post drug administration, almost 100% of the [^{14}C] radioactivity and unchanged suramin were located in the perfusate plasma. Since during the study hepatic uptake of suramin and [^{14}C] radioactivity was relatively low, ≃95% dose remained in the perfusate plasma at 5 hr. This was reflected in the calculated volume of distribution for suramin (102.4 ± 23.7 ml) at 5 hr, which corresponded closely to the IPRL circuit volume (100 ml plus liver volume). Suramin binding to plasma proteins *in vivo* has been reported to exceed 99.7% [9]; hence in the present study the drug was probably extensively bound to perfusate albumin.

The localisation of radioactivity corresponded to that of suramin in the liver at 5 hr, although there was no difference in the distribution of suramin between the 10,000 *g* pellet and supernatant, significantly more drug was located in the lysosome-rich 105,000 *g* supernatant, in comparison with the 105,000 *g* pellet. This is in close agreement with a previous study in the whole rat, where suramin was found to be located in the mitochondrial and lysosomal fractions of the liver [10].

During the course of the study, approximately half of the [^{14}C] radioactivity taken up by the liver was eliminated in bile (Table 1). However, unchanged suramin could not be detected in biliary radioactivity, which comprised unidentified and highly polar suramin derivatives. This lack of biliary parent drug could conceivably be due to the possible biotransformation of suramin. Previous studies *in vivo*, however, have failed to detect metabolites [1, 11]. This may be due to the marked hydrophilic nature of suramin, its avid protein binding [9] and its reported accumulation in Kupffer cell lysosomes [10]. These factors, together with the minimal hepatic uptake seen in the present study, indicate suramin to be amenable only to very low level biotransformation. However, the IPRL is known to facilitate the detection and measurement of metabolites which could not be determined *in vivo* [12]. In addition, due to the high molecular weight of suramin (1429) the biliary route is most likely to be the major excretion pathway for suramin metabolites. Hence our studies indicate that although suramin hepatic handling is limited and the drug is localised in lysosome-rich liver fractions, previously undetected suramin metabolites may well account for the unidentified [^{14}C] radioactivity excreted into the bile.

Acknowledgements—This study was supported in part by the British Council and the World Bank/U.N.D.P./W.H.O. Special Programme for Research and Training in Tropical Disease. The authors wish to thank Dr James L. Maggs for his advice during this work, and Mrs Carole Clarke for typing the manuscript.

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