Distribution of Suramin, an Antitrypanosomal Drug, across the Blood-Brain and Blood-Cerebrospinal Fluid Interfaces in Wild-Type and P-Glycoprotein Transporter-Deficient Mice

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Received 20 March 2007/Returned for modification 22 May 2007/Accepted 10 June 2007

Although 60 million people are exposed to human African trypanosomiasis, drug companies have not been interested in developing new drugs due to the lack of financial reward. No new drugs will be available for several years. A clearer understanding of the distribution of existing drugs into the brains of sleeping sickness patients is needed if we are to use the treatments that are available more safely and effectively. This proposal addresses this issue by using established animal models. Using in situ brain perfusion and isolated incubated choroid plexus techniques, we investigated the distribution of [3H]suramin into the central nervous systems (CNS) of male BALB/c, FVB (wild-type), and P-glycoprotein-deficient (Mdr1a/Mdr1b-targeted mutation) mice. There was no difference in the [3H]suramin distributions between the three strains of mice. [3H]suramin had a distribution similar to that of the vascular marker, [14C]sucrose, into the regions of the brain parenchyma that have a blood-brain barrier. However, the association of [3H]suramin with the circumventricular organ samples, including the choroid plexus, was higher than that of [14C]sucrose. The association of [3H]suramin with the choroid plexus was also sensitive to phenylarsine oxide, an inhibitor of endocytosis. The distribution of [3H]suramin to the brain was not affected by the presence of other antitrypanosomal drugs or the P-glycoprotein efflux transporter. Overall, the results confirm that [3H]suramin would be unlikely to treat the second or CNS stage of sleeping sickness.

Suramin, a polysulfonated naphthylurea, is used to treat the first stage of infection, when the parasites are largely found in the blood, but it is considered ineffective once the parasite has invaded the CNS. This is thought to be due to the inability of suramin to cross the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF) barrier in sufficient quantities to reach active concentrations in the target tissues. Nonetheless, there is experimental evidence that suramin can reach specific parts of the CNS, as suramin successfully cleared Trypanosoma brucei rhodesiense from the cerebral cortex, but not the choroid plexus or ventricular wall, of infected mice (34). Interestingly, suramin is taken up by human dermal microvascular endothelial (HMEC-1) cells by an active process involving the caveola system (14). In addition, suramin is thought to slowly enter the parasite by receptor-mediated endocytosis (31), which is possibly linked to host low-density lipoprotein (LDL) endocytosis (40). Furthermore, LDL receptors are expressed at the BBB (29) and are believed to be involved in the transcytosis of LDL from the blood to the brain (11). However, no study has investigated if suramin uses any form of receptor-mediated endocytosis to enter the CNS. If suramin does use this type of pathway to cross the brain barriers, different regional expression of the receptors may explain the ability of suramin to reach select areas of the CNS. Furthermore, coadministration of suramin with drugs that are active against the second, CNS stage, namely, melarsoprol, nifurtimox, and eflornithine, has been shown to improve cure rates (2, 7, 9, 19, 34). Inhibition by suramin of the P-glycoprotein (P-gp) transporter at the BBB, thus preventing the removal of the second-stage drug from the brain, has been put forward as a possible explanation for this observation (12, 13), but this hypothesis remains untested.
A clear understanding of how these drugs enter and leave the CNS is essential if we are to optimize their use in terms of improving efficacy and reducing toxicity and in suggesting the use of new combinations of existing drugs. Human studies on the CNS are limited to CSF analysis and postmortem examinations. Hence, experimental models in animals are necessary if we are to further our understanding of the potential efficacies of the drugs. This study uses brain/choroid plexus perfusion and isolated incubated choroid plexus techniques with wild-type and P-gp transporter-deficient mice to examine the distribution of suramin into the CNS in detail.

**Materials and Methods**

**Materials.** [3H]suramin sodium (5.1 to 6.2 Ci/mmol) and [14C]sucrose (498 mCi/mmol) were purchased from Morris Biochemicals, Inc., Brea, CA. [1,2,4,6,7]-H]dexamethasone (90.0 Ci/mmol) was purchased from GE Healthcare UK Ltd., Buckinghamshire, United Kingdom. [3H]mannitol (14.2 Ci/mmol) was purchased from Perkin-Elmer Life and Analytical Sciences, Boston, MA. [3H]butanol (50 to 60 mCi/mmol) was purchased from American Radiolabeled Chemicals, St. Louis, MO. Unlabeled suramin, sodium salt (molecular weight, 1,429.2; purity, >98%), was purchased from Calbiochem, Merck Biosciences Ltd., Nottingham, United Kingdom.

**Animals.** Adult male BALB/c mice (weight, approximately 25 g) were purchased from Harlan UK Ltd., Oxon, United Kingdom. BALB/c mice are a very highly controlled inbred strain that originated from albino mice in 1932. Adult FVB Mdr1a/Mdr1b−/− mice, which carry disruptions of the multidrug resistance genes Mdr1a and Mdr1b, and the animal was decapitated. The brain was removed and samples were taken for 10-min perfusion with the artificial plasma that also contained [3H]suramin (0.2 μM) and the vascular marker [14C]sucrose (1.0 μM) in both the wild-type and P-gp transporter-deficient mice. In order to examine the ability of suramin to inhibit the P-gp transporter, a further experiment involved an isotope-free perfusion, followed by a 2.5-min perfusion with artificial plasma containing [3H]suramin and [14C]sucrose. As the plasma was obtained from the patients after treatment with standard dosage regimens. The following drugs and concentrations were used: 250 μM etornithine (30), 10 μM pentamidine (41), 6 μM nifurtimox (17), and 30 μM melsaropil. This was then followed by a further 10-min perfusion with the artificial plasma containing [3H]suramin and [14C]sucrose. The effects of additional antitypansomal drugs on [3H]suramin uptake into the CNS were also examined. Male BALB/c mice were perfused for 10 min with a range of antitypansomal drugs at concentrations equivalent to those found in the plasma of patients after treatment with standard dosage regimens. The perfusions in BALB/c mice were also performed with 150 μM phenylarsine oxide (a trivalent mono-substituted organoarsenic compound which is an inhibitor of P-gp) and [3H]suramin and [14C]sucrose. Hence, experimental models in animals are necessary if we are to optimize their use in terms of improving efficacy and reducing toxicity and in suggesting the use of new combinations of existing drugs. Human studies on the CNS are limited to CSF analysis and postmortem examinations. Hence, experimental models in animals are necessary if we are to further our understanding of the potential efficacies of the drugs. This study uses brain/choroid plexus perfusion and isolated incubated choroid plexus techniques with wild-type and P-gp transporter-deficient mice to examine the distribution of suramin into the CNS in detail.
incubation in which $[^{14}C]$butanol (0.25 to 0.3 $\mu$M) and $[^{3}H]$sucrose (0.76 $\mu$M) were also present. The tissue was then removed and weighed. The choroid plexus was solubilized in 0.5 ml of solvable (Perkin-Elmer Life and Analytical Sciences) over 24 h and taken with samples of the incubation medium (artificial CSF) for liquid scintillation counting. The levels of radioactivity in the choroid plexus (dpm/g) were measured as a ratio of the concentration in the CSF (dpm/ml). The association of $[^{14}C]$butanol with the choroid plexus was corrected for the extra-cellular space component by subtracting the $[^{14}C]$sucrose ratio. The choroid plexuses taken from BALB/c mice were also incubated in the presence of 150 $\mu$M phenylarsine oxide. The phenylarsine oxide was dissolved in DMSO and diluted with artificial CSF to achieve a final concentration of 150 $\mu$M phenylarsine oxide in 0.05% DMSO. A set of control experiments also contained 0.05% DMSO.

Octanol-saline partition coefficient and protein binding. The octanol-saline partition coefficients of $[^{14}C]$butanol and $[^{3}H]$sucrose were determined in triplicate, as described previously (1). The percentages of binding to proteins in the artificial plasma, mouse plasma, and human plasma were determined by ultrafiltration centrifugal dialysis (16). Mouse plasma was obtained by exsanguination via the heart of male FVB mice. The blood was collected into heparinized syringes and centrifuged at 5,400 $\times$ g for 10 min, and the plasma was removed. Lyophilized human plasma was purchased from Sigma Chemical Company and was reconstituted in 1 ml deionized water. The plasma had been prepared from whole blood that had been mixed with the anticoagulant 3.8% trisodium citrate [9:1] and then centrifuged. The resulting plasma was then filtered through 0.45-$\mu$m-pore-size filters and lyophilized.) Ultrafiltration of $[^{14}C]$butanol (0.14 $\mu$C) dissolved in 0.7 ml of a warmed sample was carried out with the use of a Centrifree micropartition device (Amicon, Beverly, MA). In addition, to confirm that the majority of the protein was retained by the partition filter, the protein concentration was determined by the Lowry method with bovine serum albumin as the standard. The saline and artificial plasma samples produced no detectable protein in their ultrafiltrates, and only 1.5% and 0.5% of the total protein present in the mouse plasma and the human plasma samples, respectively, were detected in their ultrafiltrates.

Data analysis. The data from all the experiments are presented as means $\pm$ standard errors of the means (SEMs). The relationship between $[^{14}C]$butanol clearance into the CNS and artificial plasma flow rate was examined for linearity and the correlation coefficient ($r$), the $F$-test statistic, and the level of significance that the flow rate could be used to predict the clearance of $[^{14}C]$butanol were reported. Statistical analysis was carried out with Sigma Stat software (SPSS Science Software UK Ltd., Birmingham, United Kingdom), and significance was taken as a $P$ value of $<0.05$.

HPLC analysis. To ensure the integrity of the radiolabeled suramin during perfusion through the cerebral circulation, samples of the arterial inflow (i.e., $[^{14}C]$butanol in artificial plasma) and the venous outflow, taken after a 10-min perfusion with $[^{14}C]$butanol, were examined. These were compared to the venous outflow taken at 7 min during a perfusion with artificial plasma only (data not shown). Both arterial inflow and venous outflow samples were analyzed by high-pressure liquid chromatography (HPLC) after the following extraction procedure of Kassack and Nickel (23). Briefly, 250 $\mu$L of artificial plasma was vortexed with an equal volume of 1 M tetrabutylammonium bromide, and then the supernatant removed. The supernatants and unextracted $[^{3}H]$suramin standards in water were then injected, separately, onto a Hypersil MOS1 5-$\mu$m RP8 (100 by 2.1 mm) column (23). Separation was carried out by gradient elution on a Jasco HPLC system (Jasco Great Dunmow, Essex, United Kingdom). The eluant was a mixture of solvents A and B, where solvent A was 25% methanol in 0.02 M phosphate buffer (pH 6.5) containing 6.25 mM tetrabutylammonium hydrogen sulfate and solvent B was 100% methanol. The sample was eluted at a flow rate of 0.6 ml/min by using a gradient of 20 to 53.6% solvent B over 8 to 10 min, followed by a 4-min linear gradient to 100% solvent B (8 to 12 min). Conditions were held at 100% solvent B for a further 2 min (12 to 14 min) and were then returned to the initial conditions of 20% solvent B over 3 min (14 to 17 min), and the column was allowed to equilibrate (17 to 20 min). The UV absorbance was monitored at 238 nm, and then the column eluant was passed into a radioactive detector (Packard, Pangbourne, United Kingdom), where it was mixed 1:3 with scintillation fluid (Ultima Flo M; Packard) and passed through a 0.5-ml flow cell for real-time radioactive analysis.

FIG. 1. Initial (20-s) uptake clearance of $[^{14}C]$butanol into the different brain regions, including the choroid plexus, as a function of the artificial plasma perfusion rate through the heart. The solid line represents the linear regression of the data. Values are means $\pm$ SEMs.

RESULTS

$[^{14}C]$butanol clearance. The clearance of $[^{14}C]$butanol into different brain regions after heart perfusion at fluid flow rates ranging from 1.8 to 12 ml/min is illustrated in Fig. 1 and can be seen to increase with increasing flow. There was a linear relationship between $[^{14}C]$butanol clearance and the heart perfusion fluid flow rate in the frontal cortex, caudate putamen, occipital cortex, hippocampus, cerebellum, pons, hypothala-
mus, thalamus, pituitary gland, and choroid plexus. There was a significant difference between the median clearance values obtained for $^{14}$C]butanol in the different regions (Kruskal-Wallis one-way analysis of variance [ANOVA] on ranks). At a flow rate of 5 ml/min, the initial uptake clearance for $^{14}$C]butanol into the occipital cortex was $11.4 \pm 2.8$ ml min$^{-1}$ 100 g$^{-1}$. This rose to $45.4 \pm 9.9$ ml min$^{-1}$ 100 g$^{-1}$ at a flow rate of 12 ml/min. The vascular or $[^{3}$H]mannitol space measured in the different brain regions at 20 s with a perfusion fluid flow rate of 5 ml/min varied from $0.95\% \pm 0.14\%$ ($n = 6$) in the hippocampus to $2.63\% \pm 0.21\%$ ($n = 6$) in the pons.

**Brain/choroid plexus perfusion in BALB/c mice.** The distribution of $[^{3}$H]suramin and $[^{1}$C]sucrose into the perfused (5 ml/min) mouse brain is illustrated in Fig. 2. In the regions...
consisting of brain parenchyma (frontal cortex, occipital cortex, caudate putamen, hippocampus, hypothalamus, thalamus, pons, and cerebellum), the percentage of \([^{14}C]\)sucrose detected relative to that found in the plasma varied slightly, depending on the brain region itself and the time of perfusion \((P < 0.001 \text{ in both cases; two-way ANOVA})\). The vascular space in the pons and cerebellum was consistently larger than that measured in the other brain regions sampled at all time points. However, the \(R_{\text{Tissue}}\) values for \([^{3}H]\)suramin were not greater than those achieved for the vascular space marker, \([^{14}C]\)sucrose, in any of these brain regions or in the CSF. In contrast, the levels \((R_{\text{Tissue}}\%)\) of \([^{3}H]\)suramin were significantly higher than those of \([^{14}C]\)sucrose in the IVth ventricle choroid plexus \((P < 0.001)\), pineal gland \((P < 0.001)\), and pituitary gland \((P < 0.001; \text{two-way ANOVA})\) (Fig. 2). The \([^{3}H]\)suramin levels \((R_{\text{corrTissue}})\) reached 163% ± 32% in the choroid plexus, 388% ± 105% in the pineal gland, and 39% ± 13% in the pituitary gland after a 30-min perfusion. Capillary depletion analysis of the remaining brain tissue produced an endothelial cell-enriched pellet, as well as a brain parenchyma-containing supernatant; and the results achieved for \([^{3}H]\)suramin and \([^{14}C]\)sucrose following a 10-min perfusion are shown in Fig. 3. In the supernatant, the percentage of \([^{3}H]\)suramin was lower than that of \([^{14}C]\)sucrose \((R_{\text{Tissue}}\%)\), but the difference observed was not statistically significant at all time points \((P = 0.062; \text{two-way ANOVA})\). In contrast, the association of \([^{3}H]\)suramin with the pellet sample \((R_{\text{Tissue}}\%)\) was significantly greater than that achieved for \([^{14}C]\)sucrose at each time point \((P < 0.001; \text{two-way ANOVA})\) and reached a maximum of 1.56% ± 0.15% at 10 min when the association was corrected for the \([^{14}C]\)sucrose space (i.e., \(R_{\text{corrTissue}}\)).

For further transport studies, a 10-min perfusion with unlabeled inhibitor/drug was carried out prior to a further 10-min perfusion with \([^{3}H]\)suramin and the vascular space marker, \([^{14}C]\)sucrose. This preperfusion did not affect the integrity of the barrier, as measured by \([^{14}C]\)sucrose, in any of the regions tested \((P > 0.05; \text{Student's } t \text{ test})\). The CNS associations of both \([^{3}H]\)suramin and \([^{14}C]\)sucrose were not affected by the presence of unlabeled suramin, pentamidine, melarsoprol, ef-lornithine, or nifurtimox \((P > 0.05 \text{ for all of the regions tested, including the capillary depletion samples; one-way ANOVA})\), as illustrated in Fig. 4. DMSO was used to dissolve phenylarsine oxide, and control experiments revealed that DMSO did not affect the integrity of the barriers to \([^{14}C]\)sucrose \((P > 0.05; \text{Student's } t \text{ test})\). Interestingly, although DMSO did not change the percentage of \([^{3}H]\)suramin measured in the majority of CNS regions (Student's \(t\) test), DMSO significantly increased the distribution of \([^{3}H]\)suramin into the choroid plexus from 70.3% ± 7.4% to 99.4% ± 3.3% \((P < 0.009; \text{Student's } t \text{ test}; \text{values were corrected for the vascular space})\). There was no affect of phenylarsine oxide on the integrity of the barriers in any region except the thalamus, where a significant increase in the \([^{14}C]\)sucrose \(R_{\text{Tissue}}\) from 1.5% ± 0.1% to 2.2% ± 0.1% was observed \((P = 0.004; \text{Student's } t \text{ test})\). However, no such increase in the percentage of \([^{3}H]\)suramin was observed in the thalamus or any other brain region. Interestingly, phenylarsine oxide reduced the association of \([^{3}H]\)suramin from 120.9% ±
4.4% to 75.9% ± 14.7% (P < 0.02; Student’s t test) in the choroid plexus.

**Brain/choroid plexus perfusion in wild-type and P-gp transporter-deficient mice.** The profile of [3H]suramin and [14C]sucrose distribution in the brains of both FVB Mdr1a/Mdr1b+/+ and FVB Mdr1a/Mdr1b−/− mice was similar to that in BALB/c mice (Fig. 2 and 5). No significant differences in the [14C]sucrose or [3H]suramin values (R_Tissue %) were observed between the three mouse strains for any of the regions tested, including the supernatants and pellets obtained by capillary depletion of the remaining brain tissue sample (P > 0.05 for each region; two-way ANOVA). The percentage of [3H]suramin in the frontal cortex, occipital cortex, caudate putamen, hypothalamus, thalamus, pons, cerebellum and CSF was not greater than that of [14C]sucrose at any time point for either the FVB Mdr1a/Mdr1b+/+ or the FVB Mdr1a/Mdr1b−/− strain. However, as with the BALB/c mice, for both wild-type and knockout mice the percentage of [3H]suramin was significantly greater than that of [14C]sucrose in the choroid plexus (P < 0.001 and P < 0.001 for the wild-type and knockout mice, respectively; two-way ANOVA), pituitary gland (P = 0.027 and P = 0.006, respectively; two-way ANOVA), and pineal gland (P = 0.002 and P < 0.001, respectively; two-way ANOVA). After a 30-min perfusion in the FVB Mdr1a/Mdr1b+/+ or FVB Mdr1a/Mdr1b−/− strains, the [3H]suramin R_Tissue values reached 100% ± 48% and 97% ± 26%, respectively, in the choroid plexus; 263% ± 60% and 437% ± 145%, respectively, in the pineal gland; and 46% ± 4% and 57% ± 32%, respectively, in the pituitary gland (all R_Tissue values are corrected for the vascular space). Brain tissue samples that had been processed for capillary depletion analysis to produce an endothelial cell-depleted supernatant and an endothelial cell-enriched
pellet were then examined. The distribution of \([3H]\)suramin was significantly lower than that of \([14C]\)sucrose in the supernatant for wild-type and P-gp transporter-deficient mice (\(P = 0.010\) and \(P < 0.001\), respectively; two-way ANOVA). In contrast, the association of \([3H]\)suramin with the pellet for both the FVB \(Mdr1a/Mdr1b^{+/+}\) and the FVB \(Mdr1a/Mdr1b^{-/-}\) strains was significantly greater than that achieved for \([14C]\)sucrose at each time point (\(P < 0.001\); two-way ANOVA). This association was not time dependent, and the \([14C]\)sucrose \(R_{\text{corrTissue}}\) values were \(2.4\% \pm 0.7\%\) and \(1.4\% \pm 0.03\%\) at 10 min for the wild-type and knockout mice, respectively.

The distributions of \([3H]\)dexamethasone and \([14C]\)sucrose were examined in FVB \(Mdr1a/Mdr1b^{+/+}\) and FVB \(Mdr1a/Mdr1b^{-/-}\) mice after a 2.5-min perfusion time. The \([14C]\)sucrose \(R_{\text{corrTissue}}\) values were not significantly different between wild-type and P-gp transporter-deficient mice in any CNS region measured (\(P > 0.05\) for all regions; Student’s \(t\) test). The \([3H]\)dexamethasone \(R_{\text{corrTissue}}\%\) values were greater than those for the vascular space marker, \([14C]\)sucrose, for all of the regions tested except the CSF in both FVB \(Mdr1a/Mdr1b^{+/+}\) and FVB \(Mdr1a/Mdr1b^{-/-}\) mice. In the wild-type mice, the \(R_{\text{corrTissue}}\) values for \([3H]\)dexamethasone ranged from \(1.1\% \pm 0.2\%\) for the caudate putamen to \(2.2\% \pm 0.6\%\) for the hypothalamus, while in the circumventricular organs (CVOs) they were higher, at \(83.2\% \pm 21.2\%\) for the IVth ventricle choroid plexus, \(54.4\% \pm 10.6\%\) for the pineal gland, and \(80.4\% \pm 28.1\%\) for the pituitary gland. In contrast, a significant increase in the distribution of \([3H]\)dexamethasone (corrected for the vascular space) was observed in FVB \(Mdr1a/Mdr1b^{-/-}\) mice for the frontal cortex (\(2.5\% \pm 0.3\%\)), caudate putamen (\(2.5\% \pm 0.4\%\)), hypothalamus (\(5.8\% \pm 0.7\%\)), thalamus (\(3.4\% \pm 0.2\%\)), and cerebellum (\(2.5\% \pm 0.4\%\)) compared with that in FVB \(Mdr1a/Mdr1b^{+/+}\) mice, thus confirming the efflux of \([3H]\)dexamethasone via the P-gp transporter in these regions (Fig. 6). Capillary depletion analysis of the remaining brain tissue sample showed that the distribution of \([3H]\)dexamethasone was also significantly higher in the homogenate and the supernatant from the P-gp transporter-knockout mice than from the wild-type mice. Although the association of tritiated dexamethasone was higher in the pellet obtained from the knockout mice than that in the pellet obtained from the wild-type mice, this difference did not attain statistical significance. A significant change in the distribution of \([3H]\)dexamethasone in P-gp transporter-deficient mice compared to that in wild-type mice could not, however, be detected in the occipital cortex, hippocampus, pons, or the CVOs (pineal gland, choroid plexus, or pituitary gland) by use of a perfusion time of 2.5 min. Figure 6 also shows the results of a further experiment.
carried out to determine whether the addition of 150 μM unlabeled suramin to the perfusion medium could inhibit the removal of [3H]dexamethasone in regions where the transport of the P-gp transporter was shown to be significant. No increase in the distribution of [3H]dexamethasone was observed in any of these brain regions upon exposure to suramin; on the contrary, there appeared to be a reduction in the distribution for all brain tissue samples, but this was significant only in the frontal cortex and caudate putamen (Fig. 6). As observed in BALB/c mice, a 20-min exposure to unlabeled suramin did not affect the integrity of the barrier, as measured by the level of [14C]sucrose, in any of the regions tested for FVB Mdr1a/Mdr1b mice (P < 0.05; Student’s t test).

**Incubated choroid plexus studies.** The accumulation of [3H]suramin relative to that of the extracellular space marker, [14C]sucrose, was measured by using incubated choroid plexus tissue isolated from BALB/c, FVB Mdr1a/Mdr1b/++, and FVB Mdr1a/Mdr1b−/− mice. The results are illustrated in Table 1. No difference was observed in the values achieved for [3H]suramin or [14C]sucrose between the three murine strains. Other observations were that [3H]suramin was accumulated by the tissue over time and that 0.05% DMSO did not significantly affect either [3H]suramin or [14C]sucrose levels. Interestingly, phenylarsine oxide increased the association of [3H]suramin and [14C]sucrose in the choroid plexus, but this difference did not attain statistical significance.

**HPLC analysis.** The HPLC chromatograms shown in Fig. 7 confirm that the integrity of the [3H]suramin was maintained over a 10-min perfusion period. The [3H]suramin in the arterial inflow and venous outflow samples was eluted from the column and detected by the radiodetector at 9.3 ± 0.02 min (n = 6 samples). This matched the results from the radiolabeled suramin standard. Suramin was not present in any sample at sufficient concentrations to be detected by UV absorption. An additional radioactive peak was also eluted with the solvent front from the arterial inflow and venous outflow samples. This peak area was similar in both the arterial inflow and venous outflow samples (P = 0.611; Student’s t test; n = 3 mice) but was not present in the [3H]suramin standard; hence, it would appear to be the result of the extraction procedure. The area of the [3H]suramin peak for the venous outflow samples was not significantly different from that for the arterial inflow samples (P = 0.333; Student’s t test; n = 3). No additional radioactive or UV peaks were observed in either the venous outflow samples or the arterial inflow samples.

### TABLE 1. Levels of [3H]suramin and [14C]sucrose in isolated incubated choroid plexus over time and in the presence of phenylarsine oxide

<table>
<thead>
<tr>
<th>Mouse strain or condition</th>
<th>[3H]suramin (ratio %)</th>
<th>[14C]sucrose (ratio %)</th>
<th>Suramin accumulation (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB Mdr1a/Mdr1b+/++ (2.5 min)</td>
<td>9.5 ± 3.6</td>
<td>0.9 ± 0.2</td>
<td>8.6 ± 3.4</td>
<td>0.545</td>
</tr>
<tr>
<td>FVB Mdr1a/Mdr1b−/− (2.5 min)</td>
<td>12.5 ± 1.6</td>
<td>1.5 ± 0.6</td>
<td>10.9 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>FVB Mdr1a/Mdr1b−/− (30 min)</td>
<td>27.2 ± 3.0</td>
<td>2.1 ± 0.2</td>
<td>25.3 ± 2.8</td>
<td>0.826</td>
</tr>
<tr>
<td>FVB Mdr1a/Mdr1b−/− (30 min)</td>
<td>29.3 ± 5.1</td>
<td>2.6 ± 0.4</td>
<td>26.6 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>BALB/c (2.5 min, 0.05% DMSO)</td>
<td>7.4 ± 1.9</td>
<td>0.7 ± 0.1</td>
<td>6.7 ± 1.9</td>
<td>0.177</td>
</tr>
<tr>
<td>With 150 μM phenylarsine oxide</td>
<td>13.0 ± 2.4</td>
<td>1.6 ± 0.2</td>
<td>11.4 ± 2.2</td>
<td></td>
</tr>
</tbody>
</table>

*The accumulation of [3H]suramin was corrected for the extracellular space, as measured by [14C]sucrose. Values are means ± SEMs, and each point represents three to five mice. A Student’s t test was used to compare wild-type and P-gp transporter-deficient mice, as well as the presence versus the absence of phenylarsine oxide in BALB/c mice.*

### Octanol-saline partition coefficients and protein binding.

The octanol-saline partition coefficients for [3H]suramin and [14C]sucrose were 0.00023 ± 0.00006 and 0.0007 ± 0.0002, respectively. Ultrafiltration centrifugal dialysis suggested that...
95.3% ± 1.8% of the \[^{3}H\]suramin was protein bound in the artificial plasma, which contains 0.1% bovine serum albumin. Similar values were observed for mouse plasma (97.0% ± 0.2%) and human plasma (99.4% ± 0.03%). However, it is worth noting that 36.2% ± 4.3% of the \[^{3}H\]suramin was retained by the ultrafiltration device when no protein was present, as measured with saline.

**DISCUSSION**

The brain/choroid plexus perfusion method for examination of the distribution of molecules into the brain and CSF is an established technique in the rat (24) and guinea pig (16, 38). Murine brain perfusion via a carotid artery with flow rates of 1 to 2.5 ml/min is also an established method (10) and allows examination of molecule movement into the perfused cerebrum. To examine the distribution of a drug into the murine brain and CSF compartments, a heart perfusion method that perfuses both carotid arteries and, therefore, both cerebral hemispheres can be used (4, 15). Banks et al. (4) used a flow rate of 2 ml/min through the heart, which filled the vascular space without disrupting the BBB. In this study we explored the effect of using higher flow rates on barrier integrity and \[^{14}C\]butanol clearance (5, 10). A linear relationship between \[^{14}C\]butanol clearance and flow rate was observed in all brain regions. The \[^{14}C\]butanol clearance values achieved after perfusion through the heart were lower than those achieved after perfusion through the carotid artery (10). Interestingly, at a fluid flow rate of 12 ml/min through the heart, the clearance of \[^{14}C\]butanol into the occipital cortex was 45.4 ± 9.9 ml min\(^{-1}\) 100 g\(^{-1}\). This is not as high as the 255 ml min\(^{-1}\) 100 g\(^{-1}\) measured for \[^{3}H\]diazepam at a flow rate of 2.5 ml/min through the carotid artery (10). In the present study no barrier disruption was observed at any flow rate measured (up to 12 ml/min). At a flow rate of 5 ml/min through the heart, the \[^{14}C\]sucrose space in the occipital cortex reached 1.5 ± 0.2 ml/100 g, which is similar to the 1.3 ± 0.2 ml/100 g achieved by perfusion of the carotid artery of the mouse (10). The differences between the brain regions, such as higher \[^{14}C\]sucrose values in the cerebellum, were also in agreement with the findings presented in previous reports (10). The cardiac output in a mouse has been measured by various groups and ranges from 5.2 to 32.5 ml/min for a 25-g mouse (18). Based on all these findings and the flow rates used in earlier studies, we used 5 ml/min for the following heart perfusion experiments, as this was a practical working flow rate that ensured that the cerebral vascular space was filled and that the integrity of the barriers was maintained.

The distribution of suramin into the CNS has not previously been studied. \[^{14}C\]sucrose, a nonmetabolizable carbohydrate, is excluded from the brain, as it does not cross the BBB (25), and thus, \[^{14}C\]sucrose is used as a cerebral vascular space marker. Our results indicate that \[^{3}H\]suramin does not rapidly cross the BBB in the frontal cortex, caudate putamen, occipital cortex, hippocampus, hypothalamus, thalamus, cerebellum, and pons. However, in the CVOs the \[^{3}H\]suramin \(R_{Tissue}\%\) value was higher than the \[^{14}C\]sucrose \(R_{Tissue}\%\). In contrast to the majority of brain capillaries, the capillaries of the CVOs have discontinuous interendothelial tight junctions, fenestrated walls, and more pinocytic vesicles and are permeable to proteins and polar molecules like \[^{14}C\]sucrose. Overall, the results indicate that suramin is restricted from crossing the majority of cerebral capillaries due to the presence of continuous bands of tight junctions between the endothelial cells and the lack of vesicles. Interestingly, \[^{3}H\]suramin was found to be associated with the endothelial cell pellet at higher levels than \[^{14}C\]sucrose. Suramin is a symmetrical polysulfonated naphthylamine derivative of urea. Because of the low \(pK_a\) value (<−1) of the arylsulfonate groups, suramin remains negatively charged under physiological conditions. It is speculated that suramin adsorbs to cell membranes by the sulfonate groups interacting with positively charged membrane moieties, such as phosphatidylcholine, and the organic, benzene rich-ring internaphthalene bridge hydrophobically interacting with the lipid (36). This could explain the higher level of association of \[^{3}H\]suramin in the endothelium compared with that of \[^{14}C\]sucrose. In addition, its octanol-saline partition coefficient indicates that suramin is hydrophilic; thus, suramin is unlikely to passively diffuse across cell membranes to any great extent. It is therefore likely that suramin is internalized by a combination of adsorptive endocytosis and pinocytosis (36), and this may explain why \[^{3}H\]suramin reaches higher concentrations than \[^{14}C\]sucrose in the CVOs. Interestingly, suramin endocytosis into the parasite occurs (31), and this is possibly indirectly linked to LDL (40). In addition, the caveola system is important in \[^{3}H\]suramin uptake by the human dermal microvascular endothelium (14). Phenylarsine oxide inhibits the endocytosis of molecules at the BBB (37) and of LDL in human umbilical vein endothelial cells (8). Although the mechanism for this effect is unknown, it is thought that phenylarsine oxide covalently modifies cellular sulfhydryl groups, resulting in the inhibition of endocytosis (42). In this study, phenylarsine oxide did not affect the distribution of \[^{3}H\]suramin in the brain. However, the distribution of \[^{3}H\]suramin to the choroid plexus was reduced, which suggests that \[^{3}H\]suramin accumulates by an endocytotic mechanism in this tissue. Examining the choroid plexus in more detail by the isolated incubation method, phenylarsine oxide did not significantly affect the accumulation of \[^{3}H\]suramin. However, this in vitro method focuses on molecule movement across the apical/CSF side of the choroid plexus, in contrast to the luminal/basolateral side of the choroid plexus that is examined by the brain perfusion technique. Interestingly, excess unlabeled suramin did not affect the distribution of \[^{3}H\]suramin into any CNS region, as measured by the in situ method, and this suggests that high-affinity (i.e., \(K_{on} < 150 \mu M\)) saturable mechanisms are not responsible for the higher level of accumulation of \[^{3}H\]suramin compared with that of \[^{14}C\]sucrose into the CVOs or the capillary endothelial cell pellet.

In the in situ study, \[^{3}H\]suramin was detected in the CSF, although it was not present at levels above those of \[^{14}C\]sucrose. Taken together with the choroid plexus values, this indicates that although suramin is accumulated by the choroid plexus, it does not cross the cells to reach the CSF. It is likely that the high \[^{3}H\]suramin levels compared with the \[^{14}C\]sucrose levels in the other CVOs also reflects suramin binding to the capillaries and not necessarily vesicular transcytosis across the endothelium. The accumulation of suramin by the CVOs would explain the ability of suramin to cure the early CNS stage of trypanosomiasis (20).
The P-gp efflux transporter has a broad substrate recognition and affects the pharmacokinetics of drugs. The hypothesis that [3H]suramin does not reach the brain due to the expression of the P-gp transporter on the luminal and possibly abluminal surfaces of the capillaries (6) was tested. In mice, the P-gp transporter is encoded by Mdr1a and Mdr1b, which have 90% sequence homology to each other and 80% sequence homology to human MDR1. Interestingly, the locations of the two isoforms in mice vary, but there is evidence to suggest that both Mdr1a and Mdr1b are expressed at the BBB (22, 39). Thus, we used mice lacking Mdr1a and Mdr1b to ensure that P-gp transporter activity at the BBB was completely eliminated. Initial results with [3H]dexamethasone, a P-gp transporter substrate (28), revealed that molecule efflux via the P-gp transporter could be confirmed by using heart perfusions in Mdr1a/ Mdr1b+/ mouse and Mdr1a/Mdr1b−/− mice. The distribution of [3H]dexamethasone in the frontal cortex, hypothalamus, thalamus, cerebellum, and caudate putamen was higher in mice that did not express the P-gp transporter. Interestingly, the P-gp transporter did not influence the distribution of [3H]dexamethasone to the CVOs. This agrees with the findings of a study that examined the distribution of [3H]dexamethasone to the pituitary (28). Furthermore, the P-gp transporter has a subapical localization in the choroid plexus epithelium (33), and its functional role in molecule movement at the choroid plexus is unclear (1, 3). Our studies with Mdr1a/Mdr1b+/+ and Mdr1a/Mdr1b−/− mice revealed that [3H]suramin was not prevented from reaching the CNS because of P-gp. It is possible that suramin inhibits the CNS removal of P-gp transporter substrates (12, 13, 26), and this may explain the synergistic effect observed when patients are treated with suramin in combination with melarsoprol, nifurtimox, and efornithine. However, we found no evidence that unlabeled suramin inhibited the distribution of [3H]dexamethasone. Further studies indicated that pentamide, melarsoprol, nifurtimox, and efornithine did not significantly affect the distribution of [3H]suramin into the CNS.

The considerable ability of suramin to bind to plasma proteins is expected, as the six negatively charged sulfonate groups allow this compound to bind to proteins, presumably because of a weak ionic interaction between these residues and basic amino acids. Clinical pharmacokinetic studies have shown that 99.7% of suramin is bound to plasma proteins, mainly albumin, and that suramin has a plasma half-life of 44 to 55 days. As such, [3H]suramin would be expected to be poorly metabolized (14, 36) and, as confirmed by HPLC analysis, to remain intact and retain its integrity to the radiolabel during the perfusion periods.

In summary, suramin does not significantly cross the BBB or the blood-CSF barrier. We found no evidence for the involvement of a transporter, including the P-gp transporter, in the movement of this molecule into or out of the CNS. However, there was evidence of some form of interaction between suramin and plasma membranes, which is possibly linked to endocytosis. Furthermore, the enhanced clinical efficacy of suramin in combination with other antitrypanosomal drugs is not correlated to a change in the CNS distribution of suramin or the use of the P-gp efflux transporter.

ACKNOWLEDGMENT

This work was supported by The Wellcome Trust (grant codes 073542, 080268, and V506).

REFERENCES


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