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# Experimental animal model for mucopolysaccharidosis: Suramin-induced glycosaminoglycan and sphingolipid accumulation in the rat

(neuronal inclusions/lysosomal enzymes/noncompetitive inhibition/ganglioside storage)

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ABSTRACT **Intracerebral injection of the trypanocidal drug suramin in rats caused the formation of membranous neuronal and neuroglial inclusions. Here we show that intravenous administration of suramin, 500 mg/kg, to 2-month-old rats causes a 5- to 8-fold increase of glycosaminoglycan concentration in the liver within 10 days and a 6-fold increase in urinary glycosaminoglycan excretion. The excess glycosaminoglycans consist of heparan sulfate and dermatan sulfate. Intracerebral injection of 250 μg of suramin results in a small increase of glycosaminoglycan and larger increase of ganglioside $G_{M2}$, $G_{M3}$, and $G_{D3}$ concentrations in the treated region of the brain. The activities of the lysosomal enzymes iduronate sulfatase, β-glucuronidase, and hyaluronidase in the liver of the suramin-treated mature rats were consistently decreased, whereas those of α-L-iduronidase, heparan *N*-sulfatase, arylsulfatase B, and others were considerably increased. The activity of iduronate sulfatase was completely inhibited *in vitro* by suramin at concentrations of 50 μM or higher. The activity of β-glucuronidase was also strongly inhibited by low concentrations of suramin, but this inhibition was partially decreased at higher concentrations of the drug. The inhibition of both enzymes by suramin was noncompetitive. The suramin-treated rat may be a useful experimental animal model of mucopolysaccharidosis.**

Mucopolysaccharidoses (MPS) are diseases caused by the heritable deficiency of lysosomal enzymes necessary for the degradation of glycosaminoglycans (GAG) (1, 2). The genetic defect results in tissue storage and excessive urinary excretion of various partially degraded GAG. In addition, most of these disorders are characterized by sphingolipid abnormalities, particularly of the brain (3). Recently, one of us found that intracerebral injection of the trypanocidal drug suramin resulted in the formation of membranous neuronal and neuroglial inclusions (4). It was suggested that these inclusion bodies may consist of glycolipids and GAG and that the experiment indicated a possible experimental model for storage diseases. The present study was conducted to examine biochemical alterations in the liver and in the brain of rats after intravenous and intracerebral injection of suramin. The pathologic changes found are similar to those detected in the genetic MPS (5) and suggest that the administration of suramin to rats may provide a useful model for the study of the pathogenesis of these diseases. A preliminary report of this work has been published (6).

## MATERIALS AND METHODS

**Materials.** Suramin was kindly supplied by J. Lloyd, Biochemistry Department, Keele University, Keele, England, and Fairfield Hospital, Melbourne, Victoria, Australia. The enzymes papain (EC 3.4.22.2), trypsin (EC 3.4.21.4), and hyaluronidase (EC 3.2.1.35) were purchased from Worthington, and Pronase (*Streptomyces griseus* protease, B grade) from Calbiochem. GAG standards were supplied by Martin B. Mathews, University of Chicago. *p*-Nitrophenyl glycosides were purchased from Sigma and Research Products International (Elk Grove Village, IL). Phenyl-α-L-iduronide was a gift from B. Weissmann of the University of Illinois College of Medicine, and *O*-(α-L-idopyranosyluronic acid 2-sulfate)-2,5-anhydro-D-[$^3$H]mannitol 6-sulfate was prepared from heparin (7). [*N-sulfonate*-$^{35}$S]Heparin (substrate for heparan *N*-sulfatase) with specific activity of 33.5 mCi/g (1 Ci = $3.7 \times 10^{10}$ becquerels) was purchased from Amersham. Cellulose polyacetate electrophoresis strips (Sepraphore III) were from Gelman (Ann Arbor, MI).

**Administration of Suramin.** Experiments were performed on hooded rats. Intravenous injections of suramin in 0.5 ml of saline were administered via the left jugular vein to five groups of rats as shown in Table 1. Three groups of control rats received 0.5 ml of saline. Multiple intracerebral injections of suramin, totaling 40–250 μg in 10 μl of saline (i.e., 4–25 μg/μl) were given to 10-day-old and to 2-month-old rats. Control rats were given 10 μl of saline. The rats were sacrificed between 7 and 10 days after injection. The livers were removed, weighed, and frozen in liquid nitrogen. Rats receiving intracerebral injections were sacrificed after 2–5 days. The regions of brain about 4 mm in diameter surrounding the sites of injection were removed, weighed, and frozen.

**Extraction of Lipids and Isolation of GAG.** Frozen tissues were thawed and portions were used for measurement of water content. Lipids were extracted from the minced tissues with 10 vol of $CHCl_3$/MeOH, 2:1 (vol/vol). An equal volume of $CHCl_3$/MeOH 1:2 was added and the contents of the tubes were mixed and centrifuged at $10,000 \times g$ for 10 min. The residue was extracted once more with 20 ml of $CHCl_3$/MeOH 1:2 containing 5% $H_2O$ followed by 10 ml of $CHCl_3$/MeOH 1:1 and 10 ml of ethyl ether. The combined lipid extracts were used for lipid analysis. GAG were isolated from the lipid-free dry residue after papain digestion as described (8) or by the procedure of Breen *et al* (9). The GAG were further purified by chromatography on small ECTEOLA-cellulose ($Cl^-$) columns (3 cm × 0.7 cm inside diameter). Impurities were removed by washing with 50 ml of 0.9% NaCl, and GAG were eluted with five 1.0-ml portions of 3.0 M NaCl and precipitated with 2.5 vol of 95% (vol/vol) alcohol. The chromatographic method was used for the isolation of rat urine GAG. Urine was collected from rats of groups M and N (Table 1) on days 7, 8, 9, and 10 after suramin (500 mg/kg) was administered intravenously to the rats of group M. The urine was diluted with distilled water 1:10 prior to application on the ECTEOLA ($Cl^-$)

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Abbreviations: MPS, mucopolysaccharidoses; GAG, glycosaminoglycan(s); ganglioside nomenclature is according to Svennerholm (29).

column. GAG concentration was estimated from the uronic acid content, measured by the method of Bitter and Muir (10). Molecular weight distribution was determined by gel filtration (11). The isolated GAG were separated by electrophoresis on Sepraphore III cellulose polyacetate strips, using the following two solvent systems: (*i*) copper acetate (20 g), glacial acetic acid (100 ml), and distilled water (900 ml), pH 3.6, at a constant current of 0.5 mA/cm for 2 hr (12) or (*ii*) 0.1 M barium acetate, pH 8.0, constant current of 1 mA/cm for 4.5 hr (13). The procedures used for partition and analysis of lipids have been described in detail (5).

**Enzyme Assays.** Tissues were homogenized with an all-glass homogenizer in 3 vol of ice-cold deionized water. The homogenate was frozen and thawed once and then was centrifuged at 1000 × *g* for 10 min. The supernatant was used for the enzyme assays. The pellet was treated with 0.1% Triton X-100 and was again centrifuged at 1000 × *g* for 10 min. About 10% of the total protein was recovered after the Triton treatment of the pellet. α-*N*-Acetylglucosaminidase was assayed by the method of O'Brien (14). Lysosomal hyaluronidase activity was measured by a modification of the method of Hutterer (15). The activities of all the other enzymes were measured under the conditions described by Hall *et al.* (16) for fibroblasts, with a few modifications. In all enzyme assays the incubation mixtures were scaled down to 100–200 μl (from 500 μl), and in several the assay mixture, after completion of the incubation, was cleared by precipitation of the protein with 20% trichloroacetic acid (to 5% final concentration). The effect of suramin *in vitro* was studied by measuring the activity of the enzymes in the presence of the drug, which was added to the incubation mixtures as an aqueous solution to a final concentration of 0.01–10 mM.

## RESULTS

Table 1 shows the outline of the experiments and some analytical data on the rat livers. The weight of the suramin-treated mature rats decreased by about 25% during the 10 days after administration of the drug. In contrast, the weight of the liver of all the treated animals increased by about 25–30% in the 2-month-old rats and 50% in the pups. There was also a concomitant increase in water content, total lipids, and total GAG concentration in the livers of the treated rats. Total GAG concentration in the liver increased about 3 times in the pups and 5–8 times in the mature rats. The relative proportion of heparan sulfate and dermatan sulfate (the two components of the mature rat liver GAG) changed (Fig. 1). The concentration of heparan sulfate increased about 12 times, while the concentration of dermatan sulfate increased about 6 times.

Two to five days after intracerebral injection of 80–250 μg of suramin, there was a 30–80% increase in the total GAG concentration of the treated region of the brain. This increase in GAG content could be accounted for by a 7-fold increase in heparan sulfate, a 50% increase in the dermatan sulfate–hyaluronic acid band (that could represent a large increase in dermatan sulfate, which is a minor GAG component of the normal rat brain), and possibly a small increase in chondroitin 4-sulfate.

Patients with MPS excrete large amounts of partially degraded GAG in their urine. We therefore examined the urine of suramin-treated and control rats to see if the abnormal metabolism of GAG found in the liver and brain is also reflected in their urine. Urine from control rats contained 18 μg of GAG per mg of creatinine, whereas the urine of the group M rats contained 101 μg of GAG per mg of creatinine. The urinary GAG of the control rats consist mainly of heparan sulfate (probably undersulfated) and a small amount of dermatan sulfate. Urinary GAG in the suramin-treated rats consisted of approximately equal amounts of heparan sulfate and dermatan sulfate, indicating that the abnormal metabolism involves both of these GAG. A 7-fold increase was also found in urinary GAG degradation products. These are low molecular weight, hexuronic acid-containing anionic compounds which are not retained by the ECTEOLA column but are retained by a Dowex 1 column.

There was no difference in the molecular weight distribution of brain GAG in the control and suramin-treated rats. However, GAG from liver of suramin-treated animals showed a shift towards the smaller molecules, indicating abnormal degradation.

Intracerebral injection of 80–150 μg of suramin to 7-day-old rats caused, in addition to the increase in the GAG content of the treated region of the brain, a slight accumulation of the gangliosides $G_{M2}$ and $G_{M3}$ and a larger increase of $G_{D3}$. The increase of all these gangliosides was most marked in the brains of 2-month-old rats 3 days after intracerebral injection of 250 μg of suramin (Fig. 2). The concentration of ceramide monohexosides and sulfatides was reduced in the brain of the suramin-treated rats but it was increased markedly in their livers.

The results demonstrated that heparan sulfate and dermatan sulfate accumulate in the liver and brain and that their excretion in the urine is increased in the suramin-treated rats and suggested that enzymes of GAG degradation may be inhibited. The activities of iduronate sulfatase, β-glucuronidase, and hyaluronidase were consistently decreased in the liver of the mature rats treated with suramin (Table 2). α-*N*-Acetylglucosaminidase, heparan *N*-sulfatase, sulfatase B, and α-L-iduronidase were increased in activity. In addition, the activities

Table 1. Experimental design and analytical data on rat liver

| Animal group | No. of animals | Age at injection | Average weight,* g | Suramin dose, mg/kg | Duration of experiment, days | Liver | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Average weight, g | Water content, % | Total lipids, % of dry weight | GAG, % of lipid-free dry weight |
| A | 3 | 2 months | 142 | 500 | 10 | 10.6 | 74.5 | 30.0 | 0.32 |
| B | 3 | 2 months | 144 | 250 | 10 | 10.4 | 78.0 | 29.5 | 0.18 |
| C | 4 | 2 months | 190 | None | 10 | 8.5 | 69.3 | 23.6 | 0.04 |
| L-1 | 4 | 10 days | 45 | 500 | 7 | 2.0 | 73.7 | 28.5 | 0.20 |
| L-2 | 2 | 10 days | 45 | 250 | 7 | 2.0 | 72.7 | 28.4 | 0.17 |
| L-C | 9 | 10 days | 45 | None | 7 | 1.3 | 72.2 | 21.6 | 0.07 |
| M | 3 | 2 months | 137 | 500 | 10 | 8.3 | 75.2 | 35.0 | 0.39 |
| N | 4 | 2 months | 175 | None | 10 | 6.8 | 71.3 | 24.3 | 0.08 |

* At conclusion of experiment.

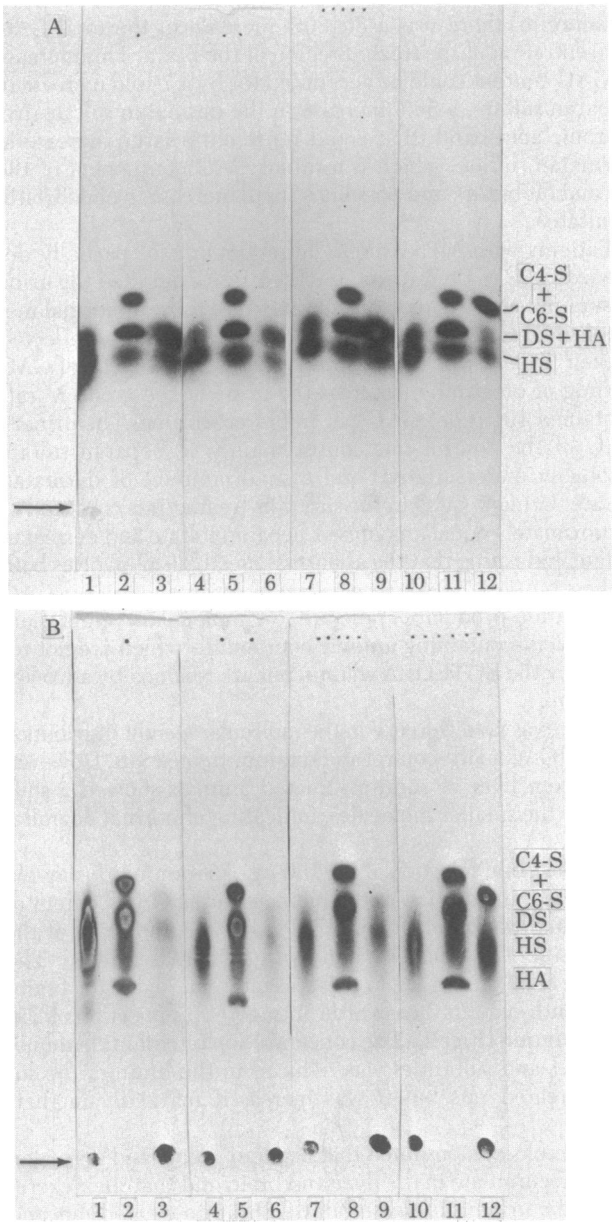


FIG. 1. Cellulose acetate electrophoresis of liver GAG from 2-month-old suramin-treated and control rats. Spots represent GAG from: lanes 2, 5, 8, and 11, standard mixture; lane 1, suramin-treated, 2.5 mg of wet tissue; lane 3, control, 50 mg of tissue; lane 4, suramin-treated, 5 mg of tissue; lane 6, control, 25 mg of tissue; lane 7, suramin-treated, 5 mg of tissue; lane 9, control, 50 mg of tissue; lane 10, suramin-treated, 5 mg of tissue; lane 12, control, 25 mg of tissue + standard chondroitin 4-sulfate. C4-S and C6-S, chondroitin 4-sulfate and 6-sulfate; DS, dermatan sulfate; HS, heparan sulfate; HA, hyaluronic acid. (*A*) Electrophoretic separation in 0.1 M barium acetate (pH 8.0); constant current 1 mA/cm for 4.5 hr, staining with alcian blue. (*B*) Separation in copper acetate/glacial acetic acid (pH 3.6); constant current 0.5 mA/cm for 2 hr.

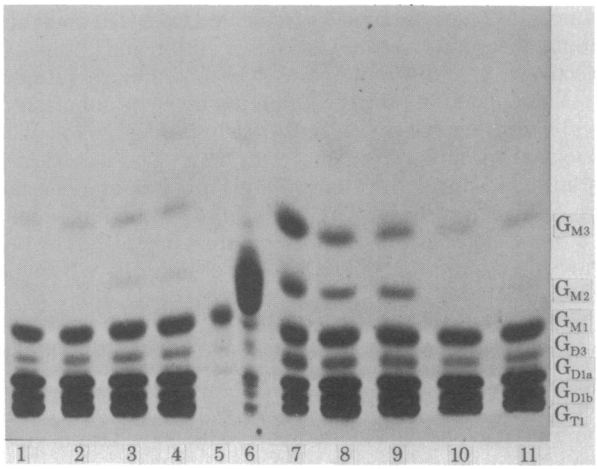


FIG. 2. Thin-layer chromatogram of gangliosides from brains of 2-month-old suramin-treated and control rats. Spots represent: lanes 1, 2, 10, and 11, control rats; lanes 3 and 4, rats with 40 $\mu$g of suramin; lane 5, $G_{M1}$ standard; lane 6, Tay–Sachs brain ($G_{M2}$); lane 7, MPS III A brain; lanes 8 and 9, rats with 250 $\mu$g of suramin. Gangliosides equivalent to 40 mg of tissue (wet weight) were applied per spot on silica gel 60 plates previously activated at 110°C for 1 hr. Solvent system: $CHCl_3$/MeOH/0.75% $CaCl_2$ (60:35:8, vol/vol). Chromatography for 8 hr at room temperature; resorcinol spray.

of the enzymes $\alpha$-galactosidase, $\beta$-galactosidase, and $\beta$-glucosaminidase were increased, whereas the activity of acid phosphatase was decreased in the liver of the suramin-treated rats. The assay of hyaluronidase by using *N*-acetylglucosamine end-group determination revealed increased amounts of such groups in the liver of the suramin-treated rats. Mixing experiments did not demonstrate the presence of an inhibitor of $\beta$-glucuronidase in the liver tissue of the suramin-treated rats. However, there was a small amount of inhibition of hyaluronidase in these experiments. Because of the presence of non-dialyzable inhibitor(s) (other than suramin) of iduronate sulfatase in the liver homogenates of both control and suramin-treated rats, mixing experiments with this enzyme were not meaningful.

In the brain of the 10-day-old rats that were treated with 80–150 $\mu$g of suramin, the activities of these enzymes were less changed in either direction. Thus, the activities of $\alpha$-L-iduronidase, heparan *N*-sulfatase, arylsulfatase B, $\alpha$-*N*-acetylglucosaminidase, and arylsulfatase A increased by 90%, 3%, 45%, 0%, and 24%, respectively, and the activity of $\beta$-glucuronidase decreased by 12%. However, the activity of iduronate sulfatase increased by 120%. Also, the increase in ganglioside content was negligible. Similarly, in the livers of the 10-day-old rats the changes in GAG content (Table 1) and enzymic activities were smaller.

The effect of suramin, *in vitro*, on the activity of 12 lysosomal enzymes is shown in Table 3. The activity of iduronate sulfatase was inhibited about 20% by 0.01 mM suramin and 100% by 0.05 mM suramin, and the inhibition was sustained at higher concentrations of the drug. $\beta$-Glucuronidase was inhibited about 15% by 0.01 mM suramin and 82% (maximum) by 0.5 mM suramin, but the inhibition was partially decreased at higher concentrations. The activity of hyaluronidase was not affected at low concentrations of suramin but was markedly inhibited at 0.5 mM, and the inhibition was sustained at higher concentrations.

Lineweaver–Burk plots for liver $\beta$-glucuronidase without inhibitor gave $K_m$ and $V_{max}$ values of 390 $\mu$M and 2940 nmol per hr per mg of protein and with 0.5 mM suramin 370 $\mu$M and 667 nmol per hr per mg of protein, respectively. Similarly for brain iduronate sulfatase, without inhibitor $K_m$ and $V_{max}$ values were 28 $\mu$M and 1538 pmol per hr per mg of protein and with 25 $\mu$M suramin, 25 $\mu$M and 250 pmol per hr per mg of protein. Thus in both cases the inhibitor had no effect on the $K_m$ but simply reduced the velocity. Therefore the inhibition of iduronate sulfatase and $\beta$-glucuronidase by suramin is noncompetitive.

The effect of suramin on the activity of several other enzymes may be represented by a hyperbola. Increasing concentration of suramin resulted in gradual decrease of the enzymatic ac-

Table 2. Effect of suramin on the activity of lysosomal enzymes of mature rat liver *in vivo*

| Enzyme | Disease caused by deficiency of enzyme | % change in enzyme activity* |
|---|---|---|
| α-L-Iduronidase (EC 3.2.1.76) | MPS IH (Hurler), MPS IS (Sheie) | +329 (205 to 420) |
| L-Iduronate sulfatase | MPS II (Hunter) | −26 (−10 to −41.5) |
| Heparan *N*-sulfatase | MPS IIIA (Sanfilippo A) | +85 (25 to 220) |
| α-*N*-Acetylglucosaminidase (EC 3.2.1.50) | MPS IIIB (Sanfilippo B) | +10 (2 to 22) |
| Arylsulfatase B (EC 3.1.6.1) | MPS VI (Maroteaux–Lamy) | +161 (32 to 290) |
| β-Glucuronidase (EC 3.2.1.31) | MPS VII | −37.5 (−16 to −66) |
| β-*N*-Acetylglucosaminidase (EC 3.2.1.30) | | +49 (25 to 87) |
| α-Galactosidase (EC 3.2.1.22) | | +107 (55 to 152) |
| β-Galactosidase (EC 3.2.1.23) | | +60 (16 to 160) |
| Arylsulfatase A (EC 3.1.6.1) | | +39 (20 to 62) |
| Acid phosphatase (EC 3.1.3.2) | | −34.6 (−20 to −48) |
| Hyaluronidase (EC 3.2.1.35) | | −31 (−20 to −39) |

* Change is suramin-treated compared to control. Numbers in parentheses are range of the change.

tivity, which reached a plateau at about 1 mM, then increased again at higher concentrations of the drug.

## DISCUSSION

The results presented here demonstrate that intravenous administration of the antitrypanosomal drug suramin to rats causes marked accumulation of GAG in the liver and excessive excretion in the urine. The stored GAG were identified as heparan sulfate and dermatan sulfate. Three enzymes required for the degradation of GAG—namely, iduronate sulfatase, β-glucuronidase, and hyaluronidase—were partially deficient in the liver of the suramin-treated rats, whereas the activity of four other such enzymes—α-L-iduronidase, α-*N*-acetylglucosaminidase, heparan *N*-sulfatase, and arylsulfatase B—was invariably increased. Also, intracerebral injection of 250 μg of suramin resulted in an 80% increase of the total GAG content of the brain and an increase of the gangliosides $G_{M2}$, $G_{M3}$, and $G_{D3}$. The findings were similar to those found in the genetic MPS (5) and are more remarkable because the changes occur within 10 days after administration of the drug.

The enzyme iduronate sulfatase is required for the removal of the sulfate group located on position 2 of the iduronic acid residues of dermatan sulfate, heparan sulfate, and heparin (17). This enzyme is deficient in MPS II (Hunter disease). Both dermatan sulfate and heparan sulfate are stored in this disease. β-Glucuronidase is required for the cleavage of β-glucuronosyl linkages found in chondroitin 4- and 6-sulfates, in hyaluronic acid, and also in dermatan sulfate and heparan sulfate. This enzyme is deficient in MPS VII. Different patterns of GAG storage have been reported in each of the few identified patients with MPS VII, with modified amounts of chondroitin 4- and 6-sulfates, dermatan sulfate, heparan sulfate, or dermatan and heparan sulfate (18). It has been reported (15) that lysosomal hyaluronidase cleaves, in addition to hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, and heparan sulfate, though at much slower rate. Dermatan sulfate is partially degraded by testicular hyaluronidase (19). No mucopolysaccharide storage may be expected from the deficiency of hyaluronidase, because it is believed that its activity can be replaced by the combined action of β-glucuronidase and β-hexosaminidase. *In vitro*, the activity of iduronate sulfatase was inhibited about 100% by 0.05 mM suramin, and the inhibition was sustained at higher concentrations of the drug. The inhibition was noncompetitive. The activity of β-glucuronidase was also strongly inhibited by 0.025–0.5 mM suramin, but the inhibition was partially decreased at higher concentrations. The inhibition of β-glucuronidase was also noncompetitive.

Smeesters and Jacques (20) reported that 2 days after intravenous injection into rats of suramin (250 mg/kg), 14% of the administered dose was found in the liver. They also presented some evidence that the drug was bound in the lysosomes. In addition, they found that the activities of the enzymes β-glycerophosphatase, β-*N*-acetylglucosaminidase, and β-glucuronidase were decreased by 66%, 32%, and 20%, respectively, *in*

Table 3. Effect of suramin on the activity of rat liver lysosomal enzymes *in vitro*

| Enzyme | Enzyme activity in the presence of suramin,* % of control | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.01 mM | 0.025 mM | 0.05 mM | 0.10 mM | 0.50 mM | 1.0 mM | 5.0 mM | 10.0 mM |
| α-L-Iduronidase | 100.0 | — | — | 100.0 | — | 56.0 | 0.0 | 0.0 |
| L-Iduronate sulfatase | 80.0 | 67.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Heparan *N*-sulfatase | 121.0 | — | — | 104.0 | — | 45.0 | 0.0 | — |
| α-*N*-Acetylglucosaminidase | 100.0 | — | — | 99.0 | — | 102.0 | 115.0 | 110.0 |
| Arylsulfatase B | 100.0 | — | — | 100.0 | 91.0 | 79.5 | 63.5 | — |
| β-Glucuronidase | 85.0 | 49.0 | 34.0 | 25.5 | 22.0 | 30.0 | 45.0 | 47.5 |
| β-*N*-Acetylglucosaminidase | 80.0 | — | — | 39.0 | — | 48.0 | 56.0 | — |
| α-Galactosidase | 93.0 | — | 62.0 | 55.0 | 61.0 | 71.0 | 90.0 | 115.0 |
| β-Galactosidase | 82.0 | — | — | 28.3 | 33.0 | 33.0 | 38.0 | 38.0 |
| Arylsulfatase A | 91.0 | — | — | 85.0 | — | 54.0 | 42.0 | — |
| Acid phosphatase | 84.0 | — | 72.0 | 48.0 | 29.0 | 25.0 | 13.5 | — |
| Hyaluronidase | 97.0 | — | — | 118.0 | 27.0 | 9.0 | 0.0 | 0.0 |

Tissue homogenates and incubation times for the assay of the enzymes were: 20–50 μg of protein and 15–30 min for β-*N*-acetylglucosaminidase and acid phosphatase; 100 μg and 4 hr for L-iduronate sulfatase; 100 μg and 1 hr for arylsulfatase B, β-glucuronidase, α-galactosidase, and arylsulfatase A; 200–500 μg and 1 hr for β-galactosidase; 200–500 μg and 18 hr for α-L-iduronidase; 500 μg and 24 hr for heparan *N*-sulfatase; and 0.5–2 mg and 4 hr for α-*N*-acetylglucosaminidase.

* Mean values of multiple assays.

*vivo*. However, in their experiments, suramin *in vitro* failed to show any effect on the activity of those three enzymes or β-galactosidase, acid maltase, or cathepsin D. Suramin also inhibits a host of other enzymes, including L-α-glycerophosphate oxidase and dihydrofolate reductase (21), and is known to be teratogenic (22, 23). Our results suggest that suramin binds to various lysosomal enzymes, including those involved in GAG degradation. One may speculate that its presence causes an increase in the synthesis of many (if not all) lysosomal enzymes, but the activity of some of them is inhibited by certain concentrations of the drug, resulting in the accumulation of their substrates. The mechanism of disposition of suramin is not known, but there is evidence that it is excreted in the urine. Thus removal of suramin should permit the recovery of various enzyme activities. This could explain the increased activity of iduronate sulfatase found in the brain and liver of the young rats. Increased activity of lysosomal enzymes other than the deficient one has been seen in the tissues of patients with MPS (24), generalized gangliosidosis (25), and many other lysosomal diseases, and in rat liver and spleen of animals injected with phenylhydrazine and human erythrocyte stroma (26). This presumably occurs via the redundant stimulation by the accumulated substrate of new enzyme synthesis in a nonspecific way (27).

The accumulation of sphingolipids may be a consequence of the accumulation of the GAG or a direct effect of the suramin. Further work is necessary to answer this and other questions. It is possible that the teratogenic effect of suramin is mediated by its interference with lysosomal enzymes and the metabolism of GAG. It has been proposed that the latter may play a role in cellular differentiation and thus in the pathogenesis of the MPS (28).

This animal model may provide a convenient and useful tool for the study of mucopolysaccharidosis. The accumulation of GAG simulates the natural disease, and the attendant ganglioside changes are likewise similar. The importance of iduronate sulfatase, β-glucuronidase, and hyaluronidase in the production of these changes require further experimentation to determine the mechanism.

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