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THIOSULFATE-MEDIATED INCREASE OF ARYLSULFATASE ACTIVITIES IN MULTIPLE SULFATASE DEFICIENCY DISORDER FIBROBLASTS

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SUMMARY: Addition of up to 20 mM sodium thiosulfate to the culture medium of multiple sulfatase deficiency disorder fibroblasts led to a time- and dose-dependent increase of sulfatase activities. The effect was most pronounced with respect to arylsulfatase A though this enzyme too did not reach normal activity. Thiosulfate-treated fibroblasts had a higher capacity to catabolize exogenously supplied sulfatide.

Multiple sulfatase deficiency disorder (MSDD,mucosulfatidosis) is a recessively transmitted disorder which is characterized biochemically by the inactivity of all of the known specific sulfatases (1-4), i.e. of the lysosomal enzymes arylsulfatase A (galactoside-3-sulfate sulfatase), iduronide-2-sulfate sulfatase, 2-deoxyglucoside-2-sulfamate sulfatase, N-acetylgalactosamine-6-sulfate sulfatase, N-acetylglucosamine-6-sulfate sulfatase(s), arylsulfatase B (N-acetylhexosamine-6-sulfate sulfatase), and of the microsomal arylsulfatase C (steroid sulfate sulfatase). The genes for two of the sulfatases are located on the X-chromosome, while that for others are on autosomes.

The molecular basis of the multiple sulfatase deficiency still remains obscure. There is no evidence of a common structural feature between different sulfatases (5,6). Genetic complementation in heterokaryons of MSDD fibroblasts and cells of either Hunter or Sanfilippo A disease implied that the genetic defect in MSDD differs from that causing single sulfatase deficiencies (7). The finding of normal molecular activities of arylsulfatases A and B in MSDD suggested a defect in the coordinated expression of

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sulfatase proteins (8). The presence of an intact genome for arylsulfatase A was concluded from the observation that the level of this enzyme depends on pH and composition of the medium in which the deficienct fibroblasts were grown (9, 10).

Under the hypothesis that an abnormal sulfur metabolism could be responsible for depressed sulfatase activities we tested sulfur containing compounds for their effect on these enzymes.

MATERIALS AND METHODS

Cell culture: Cell line 1 from a patient with multiple sulfatase deficiency disorder was grown from a skin biopsy kindly provided by Dr. J. Couchot (Reims, France), cell line 2 (GM2407) was obtained from the Human Genetic Mutant Cell Repository, Camden,N.J.. These and several control cell lines were maintained in culture with Eagle's Minimum Essential Medium, that was modified and supplemented with non-essential amino acids, antibiotics and fetal calf serum as described previously (11) except that the originally recommended concentration of $NaHCO_3$ (2.2 g/l) was used. Cell hybridization was induced with 50 % (w/w)polyethylene glycol (mol.wt. 1400-1600) as described (12).

Determination of enzyme activities: Cultures grown to confluency in a 75 cm^2 plastic flask (Greiner, Nürtingen, Germany) were harvested by trypsinization, suspended in 1.0 ml 0.03 M Tris/HCl buffer, pH 7.4, containing 0.2 % Triton X-100 and homogenized by ultrasonication. An aliquot was taken for protein determination (13). The remaining material was divided into two portions and dialyzed overnight against 0.01 M Tris/HCl buffer, pH 7.4 with or without 0.15 M NaCl, respectively.

Assays of arylsulfatases A (14), B (14) and C (15), of iduronide-2-sulfate sulfatase (4), 2-deoxyglucoside-2-sulfamate sulfatase (16), N-acetylgalactosamine-6-sulfate sulfatase (17), N-acetylglucosamine-6-sulfate sulfatase (6) and of β-N-acetylhexosaminidase (18) were performed according to published procedures. In case of N-acetylgalactosamine-6-sulfate sulfatase, however, the buffer concentration was changed to 0.02 M sodium acetate, pH 4.0, and bovine serum albumin (17 mg/l) was included in the incubation mixture.

Uptake and degradation of sulfatide: [^{3}H]Sulfatide was kindly provided by Dr. H. Christomanou, Munich, Germany. It was brought to a specific radioactivity of 3.6×10^6 cpm/mg by addition of sulfatide from bovine brain (Serva, Heidelberg, Germany). Cultures grown to confluency in 25 cm^2 plastic flasks were incubated with 3 ml medium containing 12 µg sulfatide for 3 or 5 days. Cells were then processed as described (19). The lipid extract was applied to silica gel G plates and chromatographed in chloroform-methanol-water (14:6:1) (20). Radioactivity was quantitated by scraping 1-cm strips from the plates and counting in a scintillation spectrometer.

RESULTS

$Na_2S_2O_3$ was added to the culture medium of MSDD and norma fibroblasts. The medium was changed twice a week. As in untreated cultures the pH of the medium was 7.15 before medium replacement. Inclusion of $S_2O_3^{2-}$ into the growth medium of MSDD fibroblasts led to a time- and dose-dependent increase of arylsulfatase A activity (Fig. 1). Reversal occured after withdrawal of the drug. Arylsulfatase A activity of normal fibroblasts did not respond to thiosulfate treatment. Similarly, arylsulfatase A activity of fibroblasts from a patient with metachromatic leukodystrophy could not be influenced by $S_2O_3^{2-}$ (result not shown).

Treatment of MSDD fibroblasts for 2 weeks with up to 2 mM $S_2O_3^{2-}$ produced minor, if any effects on the activity of other sulfatases than arylsulfatase A. For example, a twofold increase of N-acetylgalactosamine-6-sulfate sulfarase activity was only observed at 10 mM $S_2O_3^{2-}$. This concentration, however, led to a decreased activity of the sulfafase of normal cells.

When MSDD fibroblasts were propagated for 1-2 months in the presence of 2 mM $S_2O_3^{2-}$ all sulfatases with the possible exception of sulfamate sulfatase were present at higher intracellular levels than in cultures treated with 2 mM SO_4^{2-} (Tab. 1). Effects on normal cells were not observed. After treatment for 3 months no further increase of sulfatase activities occured. It should be noted, however, that in no case thiosulfate treatment resulted in sulfatase activities within normal limits.

A thiosulfate-mediated effect occured only in living cells. Inclusion of $S_2O_3^{2-}$ (0.5-4 mM) into the assay mixture or dialysis of cell homogenates against $S_2O_3^{2-}$ did not influence the sulfatase activities.

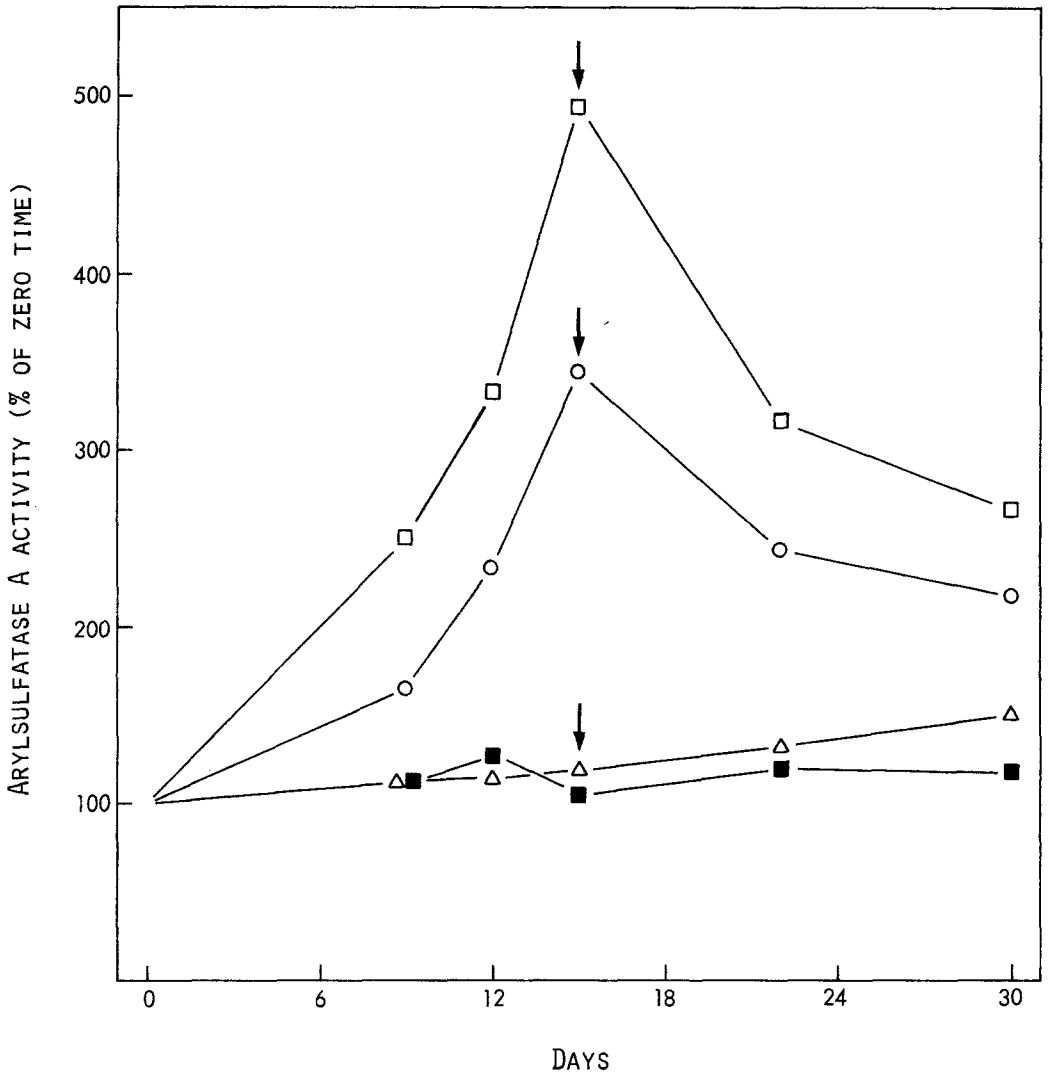

Fig. 1: Effect of thiosulfate on arylsulfatase A activity of MSDD fibroblasts.
MSDD cell line 1 (open symbols) and normal fibroblasts (closed symbols) were maintained in culture without further additions (△), or in the presence of 1 mM (○) and 2 mM (□,■) sodium thiosulfate, respectively. At zero time arylsulfatase A activity of MSDD fibroblasts was 5 nmol/hr and mg cell protein, and that of normal cells 980 nmol/hr and mg cell protein. At the time indicated by the arrows thiosulfate-containing media were changes to thiosulfate-free medium. The arylsulfatase A level of normal fibroblasts that were either grown without additions or exposed to 1 mM thiosulfate was between 95 % and 125 % of that of zero time.

Table 1

Sulfatase and glycosidase activities in MSDD fibroblasts grown in culture medium containing 2 mM $Na_2S_2O_3$ and 2 mM Na_2SO_4, respectively

	MSDD				Controls	
	Treatment for 1 month		Treatment for 2 months			
Enzyme[a]	$S_2O_3^{2-}$	SO_4^{2-}	$S_2O_3^{2-}$	SO_4^{2-}		
Arylsulfatase A	76	3.5	101	8.4	1030	(540-2500)
Arylsulfatase B	45	14	55	27	560	(400-920)
Arylsulfatase C	5	3	9	5	21	(14-32)
Iduronide-2-sulfate sulfatase	0.060	0.048	0.380	0.090	1.6	(0.5-5.5)
Sulfamate sulfatase	0.21	0.11	0.23	0.18	1.3	(0.7-2.5)
N-Acetylgalactos-amine-6-sulfate sulfatase	0.69	0.32	0.82	0.37	5.5	(3.0-8.1)
N-Acetylglucos-amine-6-sulfate sulfatase	0.023	0.007	0.023	0.013	0.33	(0.15-0.55)
β-N-Acetylhexos-aminidase	54200	51600	40600	37200	42800	(35100-59000)

[a] Activities are expressed as nmol substrate splitted at 37°C/hr and mg cell protein.

Medium was changed twice a week. MSDD fibroblasts (cell line 2) were transplanted, splitting ratio 1:2, one week before harvesting the cells. Values given for controls represent mean and range of 10-20 independent assays of normal cells and do not discriminate between cultures exposed to thiosulfate or sulfate and those grown without additions.

Thiosulfate treatment led to an improved metabolic situation of MSDD fibroblasts. Cells that had been maintained in the presence of the reagent for 2 months were challenged with [^{3}H]sulfatide. Tab. 2 shows that thiosulfate treatment did not influence the rate of sulfatide uptake but augmented the capacity for its breakdown.

Complementation analyses using cell fusion were carried out with MSDD fibroblasts and fibroblasts from a patient with meta-

Table 2

Uptake and degradation of exogenously supplied [^{3}H]sulfatide

Cell line	MSDD		MSDD		Control	
Pretreatment	2mM	SO_4^{2-}	2mM	$S_2O_3^{2-}$	2mM	SO_4^{2-}
Uptake period (days)	3	5	3	5	3	5
Uptake (µg sulfatide/ mg cell protein)	7.6	7.8	7.3	8.4	5.0	7.6
Degradation (% of internalized amount)	6.3	7.8	11.5	29.1	25.5	36.7

chromatic leukodystrophy. 3, 7 and 14 days after fusion arylsulfatase A activity was 45, 96 and 182 nmol/hr and mg cell protein, respectively, whereas in cocultivated cells the activity remained between 18 and 20 nmol/hr and mg cell protein. From the yield of multinucleate cells (32-38 %) and the normal activity (Tab. 1) one can calculate that 2 weeks were necessary to achieve full complementation.

DISCUSSION

The results presented in this paper demonstrate that in MSDD fibroblasts the levels of sulfatase activities can be influenced within a certain range by the environment in which the cells are grown. In that respect they resemble the observations on the influence of pH on arylsulfatase A activity (9, 10). As others (8), however, we found the inclusion for 2 weeks of 10 mM HEPES into the culture medium to be without effect.

The mechanism by which thiosulfate mediates an increase of sulfatase activities remains obscure. The agent does not simply activate the enzymes since it has no influence on cell homogenates, and a long period of time is required to achieve the maximal effect. It seems unlikely too that it acts by removing Ag^+ or

other metal ions as thiosulfate complexes. Previous experiments gave no indication for the presence of excessive amounts of diffusible inhibitors (4, 7). Complementation studies with MSDD and MLD fibroblasts suggest that approximately 2 weeks are required until after fusion deficient cells gain the normal amount of functional arylsulfatase A. Considering the time-course of the thiosulfate-mediated increase of arylsulfatase A activity it is conceivable that the agent or a derivative of it could protect a proportion of newly synthesized enzyme from inactivation. An effect on the pre- or posttranslational expression of sulfatases, however, cannot be excluded.

In spite of the limited effect of thiosulfate on the increase of sulfatase activities of MSDD fibroblasts the agent increased the capability of treated cells to degrade exogenously supplied sulfatide. Clinically, the major features of MSDD resemble those of metachromatic leukodystrophy. It seems thus conceivable that thiosulfate could be beneficial for MSDD patients, especially since its toxicity might be as low as that of inorganic sulfate (21).

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REFERENCES

1. Austin, J., Armstrong, D., and Shearer, L., (1965) Arch. Neurol. 13, 593-614.
2. Murphy, J.V., Wolfe, H.J., Balazs, E.A., and Moser, H.W., (1971) A patient with deficiency of arylsulfatase A, B, C and steroid sulfatase, associated with storage of sulfatide, cholesterol sulfate and glycosaminoglycans. in Lipid Storage Diseases: Enzymatic Defects and Clinical Implications (Bernsohn, J., and Grossman, H.J., eds.) pp. 67-110, Academic Press, New York.
3. Eto, Y., Wiesmann, U.N., Carson, J.H., and Herschkowitz, N.N., (1974) Arch. Neurol. 30, 153-156.
4. Basner, R., von Figura, K., Glössl, J., Klein, U., Kresse, H., and Mlekusch, W., (1979) Pediat. Res. 13, 1316-1318.

5. DeLuca, C., Brown, J.A., and Shows, T.B., (1979) Proc. Natl. Acad. Sci. USA 76, 1957-1961.
6. Basner, R., Kresse, H., and von Figura, K., (1979) J. Biol. Chem. 254, 1151-1158.
7. Horwitz, A.L., (1979) Proc. Natl. Acad. Sci. USA 76, 6496-6499.
8. Fiddler, M.B., Vine, D., Shapira, E., and Nadler, H.L., (1979) Nature 282, 98-100.
9. Fluharty, A.L., Stevens, R.L., Davis, L.L., Shapiro, L.J. and Kihara, H., (1978) Am. J. Hum. Genet. 30, 249-255.
10. Fluharty, A.L., Stevens, R.L., de la Flor, S.D., Shapiro, L.J., and Kihara, H., (1979) Am. J. Hum. Genet. 31, 574-580.
11. Cantz, M., Kresse, H., Barton, R.W., and Neufeld, E.F., (1972) Methods Enzymol. 28, 884-897.
12. Davidson, R.L., and Gerald, P.S., (1976) Somat. Cell Genet. 2, 165-176.
13. Kaltwasser, F., Wolter, P., and Pieper, J., (1967) Clin. Chim. Acta 15, 347-351.
14. Baum. H., Dodgson, K.S., and Spencer, B., (1959) Clin. Chim. Acta 4, 453-455.
15. Shapiro, L.J., Cousins, L., Fluharty, A.L., Stevens, R.L., and Kihara, H., (1977) Pediat. Res. 11, 894-897.
16. Kresse, H., (1973) Biochem. Biophys. Res. Commun. 54, 1111-1118.
17. Glössl, J., and Kresse, H., (1978) Clin. Chim. Acta 88, 111-119.
18. von Figura, K., (1977) Eur. J. Biochem. 80, 525-533.
19. Porter, M.T., Fluharty, A.L., Harris, S.E., and Kihara, H., (1970) Arch. Biochem. Biophys. 138, 646-652.
20. Harzer, K., Wässle, W., Sandhoff, K., and Jatzkewitz, H., (1968) Z. Anal. Chem. 243, 527-536.
21. Eichler, D., (1950) Die Pharmakologie anorganischer Anionen. Die Hofmeistersche Reihe. Handbuch der experimentellen Pharmakologie, Vol. 10, p. 367, Springer-Verlag, Berlin, Göttingen, Heidelberg.