

suppressed after only one month's treatment.¹² We, however, found that the inhibition of bone resorption is temporary and that control of the disease needs repeated courses of treatment.

The precise mechanism causing the mineralisation defect is not known but was unrelated to changes in 1,25(OH)₂ vitamin D in this study. Possible side effects of sodium etidronate include interference with alkaline phosphatase activity, reduced synthesis of proteoglycans, and a toxic effect on bone cells.¹⁴

In conclusion, sodium etidronate 20 mg/kg/day results in a biochemical improvement in Paget's disease when given for as short a period as two to four weeks, but it produces a mineralisation defect and the effect on bone resorption is not permanent. Although the defect in mineralisation spontaneously corrects when treatment is stopped we doubt whether any manipulation of the dose or duration of treatment can dissociate the drug's beneficial effects on resorption from its adverse effects on mineralisation. It is not, therefore, ideal for long term management of patients with Paget's disease.

References

1 Smith R, Russell RGG, Bishop MC, Woods CG, Bishop M. Paget's disease of bone. *Q J Med* 1973;42:235-56.

- 2 Russell RGG, Smith R, Preston C, Walton RJ, Woods CG. Diphosphonates in Paget's disease. *Lancet* 1974;i:894-8.
- 3 Khairi MRA, Meunier P, Edouard C, et al. Quantitative bone histology in Paget's disease of bone: influence of sodium etidronate (EHDP) therapy. *Calcified Tissue Research* 1977;22(suppl):355-8.
- 4 Khairi MRA, Altman MD, DeRosa GP, Zimmerman J, Schenk RK, Johnston CC. Sodium etidronate in the treatment of Paget's disease of bone. *Ann Intern Med* 1977;87:656-63.
- 5 Boyce BF, Smith L, Fogelman I, Johnston E, Ralston S, Boyce IT. Focal osteomalacia due to low-dose diphosphonate therapy in Paget's disease. *Lancet* 1984;i:821-4.
- 6 Kanis JA, Evanson JM, Russell RGG. Paget's disease of bone: diagnosis and management. *Metab Bone Dis Relat Res* 1981;3:219-30.
- 7 Hodgkinson A, Thompson T. Measurement of the fasting urinary hydroxyproline:creatinine ratio in normal adults and its variation with age and sex. *J Clin Pathol* 1982;35:807-11.
- 8 Taylor GA, Peacock M, Pelc B, Brown W, Holmes A. Purification of plasma vitamin D metabolites for radioimmunoassay. *Clinica Chimica Acta* 1980;108:239-46.
- 9 Aaron JE, Makins NB, Francis RM, Peacock M. Staining of the calcification front in human bone, using contrasting fluorochromes in vitro. *J Histochem Cytochem* 1984;32:1251-61.
- 10 Aaron J. Histological methods. In: Nordin BEC, ed. *Calcium phosphate and magnesium metabolism*. Edinburgh: Churchill Livingstone, 1976:564-8.
- 11 Walton RJ, Russell RGG, Smith R. Changes in renal and extrarenal handling of phosphate induced by sodium etidronate (EHDP) in man. *Clinical Science and Molecular Medicine* 1975;49:45-56.
- 12 Preston CJ, Yates AJP, Beneton MNC, et al. Effective short term treatment of Paget's disease with oral etidronate. *Br Med J* 1986;292:79-80.
- 13 Siris ES, Canfield RE, Jacobs TP, Stoddard KE, Spector PJ. Clinical and biochemical effects of EHDP in Paget's disease of bone: patterns of response to initial treatment and to long-term therapy. *Metab Bone Dis Relat Res* 1981;3:301-8.
- 14 Plasmans CMT, Jap PHK, Kuigars W, Stoof TJJH. Influence of a diphosphonate on the cellular aspect of young bone tissue. *Calcif Tissue Int* 1980;32:247-56.

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Deficiency of thiosulphate sulphurtransferase (rhodanese) in Leber's hereditary optic neuropathy

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Abstract

Leber's hereditary optic neuropathy is a rare cause of progressive visual failure. Its cause is unknown, but one hypothesis is that patients have a defect in the detoxication of cyanide. One of the enzymes used in this detoxication is thiosulphate sulphurtransferase (rhodanese). The activity of this enzyme was measured in the rectal mucosa of a group of subjects with Leber's hereditary optic neuropathy, and it was found to be considerably reduced compared with that in a group of controls (p<0.001). This finding supports the hypothesis of an inborn error of cyanide detoxication in this condition.

Introduction

In 1965 Wilson showed that plasma and urinary thiocyanate concentrations were lower in a group of smokers with Leber's hereditary optic neuropathy than in a control group without this neuropathy.¹ This and the finding of raised plasma cyanocobalamin concentrations in these patients² led to the hypothesis that there is an inborn error in cyanide detoxication in patients with Leber's hereditary optic neuropathy.³ Another hypothesis is that Leber's

hereditary optic neuropathy is a hereditary vascular neuroretinopathy.⁴

The main detoxication route for cyanide is the enzymic transfer of sulphur from thiosulphate (or mercaptopyruvate) to cyanide to form thiocyanate. Wilson found no difference in the activity of thiosulphate sulphurtransferase (rhodanese; EC 2.8.1.1) in liver samples taken at necropsy of two patients who had had Leber's hereditary optic neuropathy compared with samples from controls.¹ In 1981, however, Cagianut *et al* reported reduced activity of liver rhodanese in two patients with Leber's hereditary optic neuropathy compared with living and dead controls.⁵

Rhodanese is an intramitochondrial enzyme and therefore may be found in most tissues; it occurs in large quantities in the liver and kidneys. For ethical and practical purposes rectal mucosa was chosen as the source of specimens for our enzyme assays. We compared the activity of rectal mucosa rhodanese in subjects with Leber's hereditary optic neuropathy with that in control subjects. In addition, rhodanese activity was measured in liver and rectal mucosa obtained at necropsy from controls.

Patients and methods

Ten white men with Leber's hereditary optic neuropathy from nine different families living in southern England were studied. All had optic atrophy that was inherited matroclinically and fulfilled the criteria of the diagnosis of Leber's hereditary optic neuropathy described by Lundsgaard⁶—namely, a predominantly male affection with acute or subacute bilateral visual loss with central scotomas. At least one subject on the maternal side of each pedigree had been similarly affected. Control specimens were obtained from 12 white patients (seven men, five women) undergoing investigations for various bowel disorders such as carcinoma, colitis, and idiopathic diarrhoea ("diseased" controls) and from 20 white patients (17 men, three women) undergoing routine colonoscopy for colonic polyps ("healthy" controls). Three doctor volunteers also acted as "healthy" controls. Rectal

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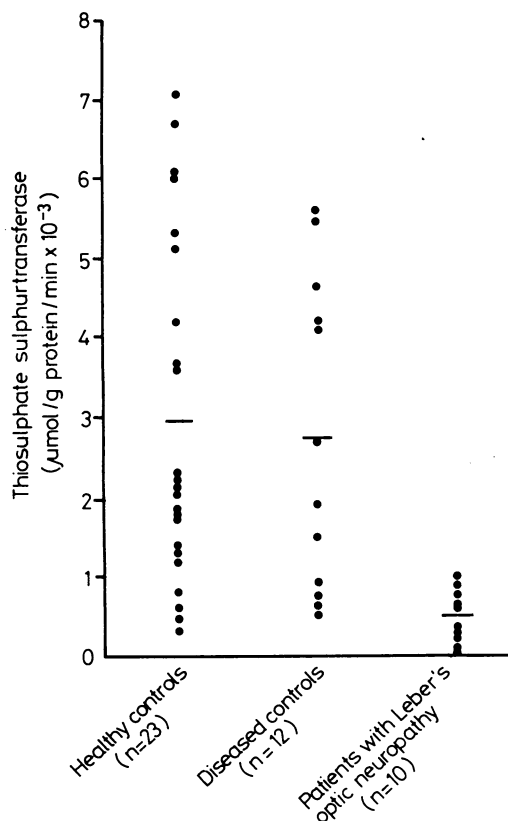
mucosa was obtained by the usual technique of sigmoidoscopy or colonoscopy with biopsy forceps. Postmortem rectal mucosa and liver samples were taken from cadavers with non-neuro-ophthalmic disease at St Thomas's Hospital, London.

Specimens were stored at -20°C for up to one month until assayed. Known weights of tissue were homogenised in ice cold glycine (0.1 mol/l) using a microtissue grinder and then centrifuged at 3000 rpm for 30 minutes at 4°C . A 5-30 mg sample of wet tissue was used to make a final homogenate volume of 400-1000 μl . After centrifugation 50 μl of the clear supernatant was used to measure rhodanese activity by the method of Sorbo.⁷ Volumes of reagents were scaled down by a factor of 10 to provide a micromethod, thereby using less of the potentially hazardous cyanide solution and also accommodating the smaller sample volume. Samples were incubated at 37°C for 20 minutes. A blank tube was set up for each test sample in which all reagents were present. These were incubated for the same time, but the tissue extract was added after the addition of the formaldehyde, which was used to stop the enzyme reaction. A further 50 μl of the supernatant was used to measure protein concentration by the Ponceau S-trichloroacetic acid micromethod.⁸ Enzyme activity was expressed as $\mu\text{mol thiocyanate/g protein/min}$.

A two tailed Mann-Whitney test was used to compare subjects with Leber's hereditary optic neuropathy with healthy and diseased controls.

Results

Mean age of the subjects with Leber's hereditary optic neuropathy was 39 (range 22-58) years. The duration of optic atrophy varied from six months to 24 years. We considered this group to be representative of patients with Leber's hereditary optic neuropathy. Mean ages of the healthy and diseased controls were 61 (30-89) years and 54 (33-79) years, respectively.



Thiolsulphate sulphurtransferase (rhodanese) activity in subjects with Leber's hereditary optic neuropathy and diseased and healthy controls.

The figure shows the results of rhodanese assay in rectal mucosa. There was a significant difference between the mean (SEM) enzyme activity for healthy controls ($2928 (447)\ \mu\text{mol thiocyanate/g protein/min}$) and for subjects with Leber's hereditary optic neuropathy ($480 (108)\ \mu\text{mol thiocyanate/g protein/min}$) ($p < 0.001$) and also between diseased controls ($2738 (563)\ \mu\text{mol thiocyanate/g protein/min}$) and subjects with Leber's hereditary optic neuropathy ($p < 0.001$). In both control groups there was no relation

between sex, age, weight of biopsy specimen, duration of storage, and enzyme activity. The intra-assay precision varied by less than 5%. Supernatant from the mucosal extract of a subject with Leber's hereditary optic neuropathy was added to that of a control sample but had no effect on the enzyme activity.

The mean (SEM) activity of rhodanese in rectal mucosa and liver obtained at necropsy was $198 (41.9)\ \mu\text{mol thiocyanate/g protein/min}$ ($n=11$) and $4712 (205.6)\ \mu\text{mol thiocyanate/g protein/min}$ ($n=8$), respectively.

Discussion

The finding of reduced rhodanese activity in the rectal mucosa of subjects with Leber's hereditary optic neuropathy substantiates the report of Cagianut *et al* of such a reduction in the livers of two patients with this neuropathy.⁵ This supports earlier evidence suggesting an inborn error of cyanide detoxication in this condition.

The sixfold reduction in the activity of rhodanese in rectal mucosa found in this study is also similar in magnitude to the reduction in hepatic tissue reported by Cagianut *et al*. The recent report of normal rhodanese activity in the anterior tibial muscle of subjects with Leber's hereditary optic neuropathy⁹ does not detract from our findings as there may be tissue specific isoenzymes of rhodanese. Defective enzyme activity may be expressed only in the rectal mucosa, hepatic tissue, and optic nerve. It is therefore important to measure the activity of rhodanese in optic nerves as well as performing family studies of rhodanese activity.

In a matroclinally inherited disease such as Leber's hereditary optic neuropathy all the offspring of a carrier woman are at risk of developing the disease. When advice is given the hazards of smoking cigarettes and ingesting dietary cyanogenetic substances, such as almonds, beans, peas, and cassava, need to be explained to patients and their relatives.

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References

- Wilson J. Leber's hereditary optic atrophy: a possible defect of cyanide metabolism. *Clin Sci* 1965;29:505-15.
- Wilson J, Linnell JC, Mathews DM. Plasma cobalamins in neuro-ophthalmological diseases. *Lancet* 1971;ii:259-61.
- Wilson J. Cyanide in human disease: a review of clinical and laboratory evidence. *Fundamental Applied Toxicology* 1983;3:397-9.
- Nikoskelainen E, Hoyt WF, Nummelin K. The ophthalmoscopic findings in Leber's hereditary optic neuropathy: II. The fundus findings in the affected family members. *Arch Ophthalmol* 1983;101:1059-68.
- Cagianut B, Rhyner K, Furrer W, Schnebli HP. Thiolsulphate-sulphur transferase (rhodanese) deficiency in Leber's hereditary optic atrophy. *Lancet* 1981;iii:981-2.
- Lundsgaard R. Leber's disease; geneologic, genetic and clinical study of 101 cases of retrobulbar optic neuritis in 20 Danish families. *Acta Ophthalmol* 1944;suppl 21:3-306.
- Sorbo BH. Crystalline rhodanese I. Purification and physicochemical examination. *Acta Chem Scand* 1955;7:1129-36.
- Pesce MA, Strande CS. A new micro method for determination of protein in CSF and urine. *Clin Chem* 1973;19:1265-7.
- Nikoskelainen E, Hassinen IE, Paljarvi L, Lang H, Kalimo H. Leber's hereditary optic neuroretinopathy, a mitochondrial disease? *Lancet* 1984;ii:1474.

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100 YEARS AGO

The Russian lady doctors find warm advocates in some of the Moscow journals. The *Russkiya Viedomosti*, for example, states that they have been most valuable in towns, in the country, and in war. They are very suited for medical duties in girls' schools, and other institutions consisting of female inmates. As in the case of the Zenana mission and similar British institutions, Russian lady-doctors have made themselves very popular amongst the Mahomedans. The writer endeavours to impress upon the St. Petersburg municipal council the importance of subsidising the female medical courses, though other help is also necessary, both from the State and from private munificence. (*British Medical Journal* 1886;ii:408.)