Gyrate atrophy with hyperornithinaemia: different types of responsiveness to vitamin B₆

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SUMMARY Three cases of Japanese patients with gyrate atrophy of the choroid and retina with hyperornithinaemia were studied clinically and biochemically. The types of disease differed in responsiveness to vitamin B₆. In-vivo responsiveness to vitamin B₆ was correlated with in-vitro data. It is suggested that the in-vitro examination of the influence of pyridoxal phosphate on ornithine ketoacid transaminase activity in cultured fibroblasts may be useful in ascertaining the efficacy of vitamin B₆ treatment in gyrate atrophy. In addition the early development of the fundus lesions was observed in one case (case 1), and the ciliary body abnormality and chorioretinal atrophy were noted in another (case 3).

Large doses of vitamin B₆ have been tried in the treatment of gyrate atrophy of choroid and retina since Simell and Takki¹ found hyperornithinaemia in these cases and several investigators²⁻⁵ confirmed deficient activity of ornithine ketoacid aminotransferase in cultured fibroblasts or lymphocytes from the affected patients. Genetic heterogeneity of the disease in responsiveness to vitamin B₆ was suggested recently.^{3,6,7} But a study of both in-vivo and in-vitro responsiveness to vitamin B₆ has been lacking. We therefore investigated whether in-vivo responsiveness to vitamin B₆ is correlated with in-vitro data.

Case reports

Three patients with gyrate atrophy with hyperornithinaemia, their parents and a sister, and normal controls were examined in this study.

Case 1. A 5-year-old boy was first seen at the Departments of Paediatrics and Ophthalmology of Tohoku University Hospital at the age of 2 years, because his mother noticed he had thick speech and brown hair. Delivery and development were normal. The boy was the second son of a consanguineous marriage. On examination he appeared normal, except for hyperornithinaemia (8.48 mg/dl (84.8 mg/l) serum, Table 1) and hyperornithinuria. Thick speech and brown hair were not observed. Ophthalmological

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examination showed no abnormal lesion in the ocular fundi at the ages of 2 and 3 years. Yellow-white spots at the peripheral fundus (Fig. 1) and an abnormal reflex in the macular area were first noted at the age of 4 years. The visual acuity of both eyes was 0.3 with correction of myopia (-2 D). The optical media were clear. He complained of night blindness at the age of 5 years. The electroretinogram was subnormal. The ciliary processes were normal by cycloscopy. The clinical diagnosis of early gyrate atrophy was made.

Case 2. An 8-year-old boy first noted decreased

Table 1 Amino acid concentration in serum (mg/dl)

	Case 1	Case 2	Case 3	Normal	
				Mean	Range (2 SD)
Ornithine	8.48	12.95	6.92	0.96	0.36-1.56
Lysine	1.06	1.49	2.15	2.23	1.49-2.97
Histidine	1.61	1.74	1.73	1.38	1.04-1.72
Arginine	1.03	2.06	1.26	1.25	0.49-1.72
Threonine	0.96	1.48	2.20	1.30	0.78-1.82
Serine	1.94	1.76	2.48	1.66	1.14-2.18
Glu. + glutamin	6.51	8.25	9.22		
Glycine	1.71	2.01	2.67	2.03	1.23-2.83
Alanine	3.16	3.27	2.93	3.28	1.70-4.86
Valine	2.27	2.71	3.19	2.62	1.50-3.74
Cystine	0.88	1.26	0.49		
Methionine	0.54	0.41	0.44	0.34	0.20-0.48
Isoleucine	0.88	0.83	0.98	0.86	0.32-1.44
Leucine	1.76	1.78	2.22	1.64	0.66-2.60
Tyrosine	1.12	1.38	1.10	1.16	0.62-1.70
Phenylalanine	1.12	1.04	1.38	1.27	0.69-1.85
Proline	1.30	0.95	2.87	1.66	0.82-2.50
Citrulline			0.30	0.37	0.09-0.65

SI conversion: g/l=mg/ml×1.

Fig. 1 Fundus photograph of case 1. Yellow-white spots at the peripheral fundus at the age of 4 years.

vision at the age of 5 years, and glasses were prescribed for myopia. At the age of 8 he was referred to the retina service of our department suffering from night blindness. Ophthalmological examination showed best corrected visual acuity was RE 0.4 with correction of myopia (−8 D), LE 0.4 with correction of myopia (−10 D). The lens in each eye was clear. Typical chorioretinal atrophy with scallop margins at

the peripheral fundus and an abnormal reflex in the macular area were ophthalmoscopically noted (Fig. 2). The Goldmann visual fields showed a concentric constriction. The dark adaptation curve was monophasic. The electroretinogram was subnormal, and the electro-oculogram was flat. The ciliary processes were normal by cycloscopy.

There was no history of any other family members with ocular disease. Consanguinity was denied. No physical or mental abnormalities were found. Hyperornithinaemia (12.95 mg/dl (129.5 mg/l)) was noted (Table 1). The clinical diagnosis of typical gyrate atrophy was made.

Case 3. An 18-year-old man was first seen at the Department of Ophthalmology at Kagoshima University at the age of 17 because his vision had deteriorated with age. The clinical diagnosis of gyrate atrophy was made there, and he was referred to our department.

The parents were first cousins. There was no family history of night blindness. Two weeks after birth he had suffered from erysipelas. At that time heterotaxis was noted. Ophthalmological examination at the age of 17 showed best corrected visual acuity was RE 0.03 with correction of myopia (−13 D), LE 0.6 with correction of myopia (−14 D). A posterior subcapsular cataract was observed in both eyes, more severe in the right eye.

Ophthalmoscopically chorioretinal atrophy with the characteristic scalloped border approached the posterior pole (Fig. 3). Cycloscopically short and scanty ciliary processes were noted (Fig. 4). The

Fig. 2 Fundus photograph of case 2. Irregular and sharply defined atrophy at the periphery.

Fig. 3 Fundus photograph of case 3. Chorioretinal atrophy with scalloped border approached to the posterior pole.

Goldmann visual fields showed a concentric constriction. The electroretinogram was subnormal. No physical or mental abnormalities were found. Hyperornithinaemia (6.92 mg/dl (69.2 mg/l)) was noted (Table 1). The clinical diagnosis of typical gyrate atrophy was made.

Parents and a sister. The parents and a sister of these patients had good visual acuity and no abnormal atrophic lesions in the ocular fundi. Serum amino acids were within normal limits.

Materials and methods

Serum amino acids were measured on a Hitachi amino acid analyser (type 835).

Fig. 4 Cyclogram of case 3. Short and scanty ciliary processes.

Skin biopsy material was cut into small fragments and cultured in Eagle's medium (MEM) supplemented with 10% fetal calf serum and nonessential amino acids. When the monolayers had grown to confluency, they were trypsinised and the fibroblasts were harvested for enzyme analysis. Cell lysates were prepared by twice freezing and thawing and further sonication at 60 kilocycles for 10 s of the washed cells in saline. For one enzyme assay a cell lysate obtained from 1 to 2×10^6 cells was required.

Cell extracts of phytohaemagglutinin-transformed lymphocytes were prepared by the method of Berger.⁸ Briefly, the peripheral blood lymphocytes prepared from heparinised blood were cultured for 72 hours in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal calf serum and phytohaemagglutinin (5 μ g per ml). Cells harvested were sonicated at 60 kilocycles for 10 s for enzyme assay.

Ornithine ketoacid aminotransferase activity was determined by spectrophotometric and radioisotopic methods. Spectrophotometric assay is a modification of the method of Katsunuma and coworkers.⁹ Briefly, the reaction mixture usually contained 4 μ mol ornithine, 1 μ mol α -ketoglutarate, 40 nmol pyridoxal phosphate, and enzyme source (cell lysate) at pH 8.0 in a total volume of 0.4 ml. Incubation was carried out at 37°C for 30 min. The amount of pyrroline-5-carboxylate formed was determined spectrophotometrically.

Radioisotopic assay as described by Phang *et al.*^{10,11} was employed. Briefly, the reaction mixture usually contained ornithine-1-¹⁴C (1 μ Ci), α -ketoglutarate

Fig. 5 Serum ornithine level after vitamin B₆ administration in case 3.

(0.7 mM), pyridoxal phosphate (4 µg/ml), 0.1 M phosphate buffer (pH 8.0), and cell lysate (100 µl) in a final volume of 0.25 ml. Incubation was carried out at 37°C for 1 h. The reaction was terminated with addition of 50 µl of 6N HCl. The radioactivity of ¹⁴C-Δ-pyrroline-5-carboxylate formed was determined after separation from ¹⁴C-ornithine by an ion-exchange column.

Protein content was determined by the method of Lowry *et al.*¹²

All values are expressed as mean of 3 different experiments.

Table 2 Ornithine ketoacid aminotransferase activity *in vitro* in PHA-transformed lymphocytes and cultured skin fibroblasts

	Specific enzyme activity	
	PHA-transformed lymphocytes*	Cultured skin fibroblasts†
Family 1		
Patient (case 1)	0.045	Not detectable
Father	1.075	
Mother	1.538	
Family 2		
Patient (case 2)	0.011	Not detectable
Father	0.535	
Mother	0.945	
Sister	1.028	
Family 3		
Patient (case 3)	Not detectable	Not detectable
Father		
Normal controls		
Mean ± SD (n=5)	2.02 ± 0.29	148 ± 26

*n Moles/mg protein/h. Radioisotopically assayed at 10⁻⁴ M pyridoxal phosphate.

†n Moles/mg protein/30 min. Spectrophotometrically assayed at 10⁻⁴ M pyridoxal phosphate.

Results

As shown in Table 1, these 3 patients showed a marked increase in serum ornithine and almost normal values of the other amino acids.

IN-VIVO RESPONSE TO VITAMIN B₆

After 2 weeks of high dosage of vitamin B₆ (300 mg per day) the serum ornithine level and ERG did not change in cases 1 and 2. The serum ornithine level following vitamin B₆ (300–600 mg per day) in case 3 is shown in Fig. 5. The serum ornithine level in case 3 decreased with a maximum of 60% reduction, responding to concentration of vitamin B₆. After stopping the administration of vitamin B₆ the ornithine level returned to the initial level. High-dose vitamin B₆ treatment showed no significant improvement in the electroretinogram, visual acuity, or visual field in case 3.

IN-VITRO RESPONSE TO PYRIDOXAL PHOSPHATE (VITAMIN B₆-AL-PHOSPHATE)

Ornithine ketoacid aminotransferase activity in the phytohaemagglutinin-transformed lymphocytes and cultured skin fibroblasts is shown in Table 2. When assayed at 10⁻⁴ M pyridoxal phosphate, little or no enzyme activity was detected in all 3 affected patients. The activities in carrier parents and sister showed approximately 50% of the mean control values, indicating heterozygotes. Fibroblasts from case 3 showed an increase in enzyme activity when concentrations of pyridoxal phosphate were increased in the assay medium (Fig. 6). In the presence of 2 × 10⁻³ M pyridoxal phosphate, ornithine ketoacid aminotransferase activity in the fibroblasts in this patient

increased up to 25% of normal levels. Fibroblasts from cases 1 and 2 did not respond to pyridoxal phosphate in the ornithine ketoacid aminotransferase activity.

Discussion

The present study points to several conclusions. Firstly, there are types of gyrate atrophy with hyper-ornithinaemia which differ in their responsiveness to vitamin B₆, as shown by the responsive case 3 and the nonresponsive cases 1 and 2. Secondly, in-vivo responsiveness to vitamin B₆ (Fig. 5) is correlated with in-vitro data (Fig. 6).

It seems probable that the vitamin B₆ responsive type (case 3) has the enzyme of high K_m (Michaelis constant) value for pyridoxal phosphate. Our previous data¹³ show that the K_m value for pyridoxal phosphate of the enzyme in the bovine ciliary body and iris is 1.0×10^{-5} M. Fibroblasts from the vitamin B₆ responsive type (case 3) required a high concentration (over 1×10^{-3} M) of pyridoxal phosphate for in-vitro enzyme activity. The data suggest the presence of enzyme with high K_m for pyridoxal phosphate in the vitamin B₆ responsive type and enzyme deficiency in the vitamin B₆ nonresponsive type of the disease.

Although heterogeneity of gyrate atrophy in vitamin B₆ response has been previously reported,^{3,6,7} the relationship between in-vitro and in-vivo responsiveness has not been noted. Shih and coworkers³ reported only the in-vitro data of the effect of pyridoxal phosphate, while Kaiser-Kupfer and coworkers⁷ showed only the in-vivo reduction of plasma ornithine level in response to oral vitamin B₆. The value of the enzyme activity was not described in the preliminary report of Welber *et al.*⁶ The present study confirms the correlation of in-vitro and in-vitro responsiveness of gyrate atrophy to vitamin B₆ and the presence of different types. It also strongly suggests that the in-vitro examination of the influence of pyridoxal phosphate on enzyme activity in the cultured fibroblasts may help us to determine the efficacy of vitamin B₆ treatment in gyrate atrophy.

Case 1 had a normal appearance of the ocular fundi at the ages of 2 and 3 years. The yellowish white atrophic patches began at the age of 4 years. This is in agreement with the observation^{14,15} that ocular lesions begin at around the age of 4 years.

Cycloscopic observation is also interesting. We previously¹⁶ reported the high enzyme activity of ornithine ketoacid transaminase in the bovine retinal pigment epithelium, ciliary body, and iris. A cyclogram of case 3 (Fig. 4) shows the short and scanty ciliary processes. Cycloscopically, the ciliary processes in the affected children (cases 1 and 2) were normal, while those in the young adult patient (case

Fig. 6 Ornithine ketoacid aminotransferase in vitro in the cultured skin fibroblasts and pyridoxal phosphate concentration.

3) were short and scanty. Another young adult (21 years old) with gyrate atrophy showed similar lesions of the ciliary body (unpublished observation). The data suggested involvement of the ciliary body with age as well as retinochoroidal atrophy in the gyrate atrophy.

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References

- 1 Simell O, Takki K. Raised plasma-ornithine and gyrate atrophy of the choroid and retina. *Lancet* 1973; **i**: 1031-3.
- 2 Senger RCA, Trijbels JMF, Brussaard JH, Deutman AF. Gyrate atrophy of the choroid and retina and ornithine-ketoacid aminotransferase deficiency. *Pediatr Res* 1976; **10**: 894.
- 3 Shih VE, Berson EL, Mandell R, Schmidt SY. Ornithine ketoacid transaminase deficiency gyrate atrophy of the choroid and retina. *Am J Hum Genet* 1978; **30**: 174-9.
- 4 Kaiser-Kupfer MI, Valle D, Del Valle LA. A specific enzyme defect in gyrate atrophy. *Am J Ophthalmol* 1978; **85**: 200-4.
- 5 O'Donnell JJ, Sandman RP, Martin SR. Gyrate atrophy of the retina. Inborn error of L-ornithine: 2-oxoacid aminotransferase. *Science* 1978; **200**: 200-1.
- 6 Welber RG, Kennaway NG, Buist NR. Vitamin B₆ in management of gyrate atrophy of choroid and retina. *Lancet* 1978; **ii**: 1213.

- 7 Kaiser-Kupfer MI, Valle D, Bron AJ. Clinical and biochemical heterogeneity in gyrate atrophy. *Am J Ophthalmol* 1980; **89**: 219–22.
- 8 Berger SL. Lymphocytes as resting cells. In: Jakoby WB, Pastan IH, eds. *Methods in Enzymology*. New York: Academic Press. 1979; **58**: 486–94.
- 9 Katsunuma N, Matsuda Y, Tomino I. Studies on ornithine-ketoacid transaminase. I. Purification and properties. *J Biochem* 1964; **56**: 499–503.
- 10 Phang JM, Downing SJ, Valle D. A radioisotopic assay for ornithine-transaminase. *Anal Biochem* 1973; **55**: 272–7.
- 11 Valle D, Blaese RM, Phang JM. Increased sensitivity of lymphocyte pyrroline-5-carboxylate reductase to inhibition by proline with transformation. *Nature* 1975; **253**: 214.
- 12 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. *J Biol Chem* 1951; **193**: 265–75.
- 13 Shiono T, Hayasaka S, Mizuno K. Partial purification and certain properties of ornithine ketoacid aminotransferase in bovine ciliary body and iris. *Exp Eye Res* in press.
- 14 Yatziv S, Statter M, Merin S. Metabolic studies in two families with hyperornithinemia and gyrate atrophy of choroid and retina. *J Lab Clin Med* 1979; **93**: 749–57.
- 15 Rinaldi E, Stoppoloni GP, Savastano S, Russo S, Cotticelli L. Gyrate atrophy of choroid associated with hyperornithinemia: report of the first case in Italy. *J Pediatr Ophthalmol* 1979; **16**: 133–5.
- 16 Hayasaka S, Shiono T, Takaku Y, Mizuno K. Ornithine ketoacid aminotransferase in the bovine eye. *Invest Ophthalmol Visual Sci* 1980; **19**: 1457–60.