Hereditary Tyrosinemia Type I

Self-induced Correction of the Fumarylacetoacetase Defect

E. A. Kvittingen,* H. Rootwelt,* P. Brandtzaeg,† A. Bergan,* and R. Berger^{||}

*Institute of Clinical Biochemistry, †Institute of Pathology, and ‡Department of Surgery, University of Oslo, Rikshospitalet Oslo, Norway; and ||Department of Pediatrics, Wilhelmina Kinderziekenhuis, Utrecht, The Netherlands

Abstract

Two Norwegian patients with chronic tyrosinemia type I showed > 50% residual fumarylacetoacetase activity in liver samples obtained during liver transplantation. The enzyme characteristics of both patients were comparable with those of a normal control. Immunohistochemistry on liver sections from these patients and from three other Norwegian tyrosinemia patients revealed a mosaicism of fumarylacetoacetase immunoreactivity corresponding completely or partly to some of the regenerating nodules. This appearance of enzyme protein is presumably induced by the disease process. The mechanism involved remains unclear and could be caused by a genetic alteration, regained translation of messenger RNA, or to enhanced stability of an abnormal enzyme. (*J. Clin. Invest.* 1993. 91:1816–1821.) Key words: liver disease • amino acid metabolism • mosaicism • immunohistochemistry • mutagenesis

Introduction

Hereditary tyrosinemia type I (McKusick 27670) is an autosomal recessive disorder caused by an almost complete deficiency of fumarylacetoacetase (FAH),¹ the last enzyme in tyrosine degradation, functionally important in the liver and kidneys (1). Because of the enzyme block, the metabolites fumaryl- and maleylacetoacetate accumulate. These alkylating agents are presumably responsible for the injuries of the disorder: progressive liver damage and kidney disease. The disorder is clinically heterogeneous and is referred to as acute or chronic. Patients with the acute form die within the first year of life because of liver failure. The chronic form presents later in childhood, usually with rickets and moderate or few signs of liver disease, but the patients die of progressive liver disease in young age. Development of hepatocellular carcinoma is frequent in chronic tyrosinemia (2).

Address correspondence to E. A. Kvittingen, Institute of Clinical Biochemistry, Rikshospitalet, N-0027 Oslo, Norway.

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1. Abbreviations used in this paper: APAAP, alkaline phosphatase/anti-alkaline phosphatase; FAH, fumarylacetoacetase.

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Distinction between acute and chronic tyrosinemia has been claimed on the basis of residual FAH activity and the amount of immunoreactive FAH protein by some investigators (3), but this has not been observed by us in previous studies (4). We have found < 2% residual enzyme activity in liver tissue from both chronic and acute patients.

In a Norwegian tyrosinemia patient, Western blots revealed presence of FAH immunoreactivity in liver tissue but not in fibroblast extract. This led to the discovery of high residual FAH activity in some sections of the liver, and immunohistochemical investigations demonstrated a mosaic distribution of immunoreactive FAH material. Here, we report the findings in this patient and similar findings in four other Norwegian patients with chronic tyrosinemia.

Methods

The diagnosis of tyrosinemia in the five studied patients were established between 1 and 6 yr of age by demonstration of low FAH activity in fibroblasts and/or lymphocytes and elevated urinary excretion of succinylacetone (7–700 $\mu\text{mol}/\text{mmol}$ creatinine vs normal < 0.2 $\mu\text{mol}/\text{mmol}$). Succinylacetone was identified by capillary gas chromatography-mass spectrometry and quantified by the δ -aminolevulinic dehydratase inhibition assay (5). FAH activity was measured as previously described (4). Calculation of enzyme kinetics and inhibition effects of ZnCl_2 , CuCl_2 , and *p*-chloromercuribenzoate were investigated as previously reported (6).

Two of the patients were transplanted because of hepatocellular carcinoma at 7 yr (patient 1) and 14 yr (patient 3) of age; the others were transplanted at age 3 (patient 4), 6 (patient 5), and 8 (patient 2) yr without evidence of liver cancer.

Liver tissue was either frozen immediately in liquid nitrogen or at -70°C , or fixed in 4% neutral formaldehyde. Subcellular fractionation on fresh liver tissue (from patient 2) was performed by differential centrifugation as described by de Duve (7). Western blots were run as previously reported (4, 8).

Immunohistochemical investigation for FAH was performed in several liver specimens from the five tyrosinemia patients, in liver tissue from three normal controls, in two patients with cirrhosis caused by chronic autoimmune hepatitis, and in one patient (liver transplanted) with cirrhosis from chronic active hepatitis. An alkaline phosphatase/anti-alkaline phosphatase (APAAP) method was used (9); dewaxed 5- μm sections of formalin-fixed and paraffin-embedded tissue blocks were incubated with a rabbit antiserum (1:800) of established specificity for bovine FAH and known to crossreact with the human counterpart (4, 8). The developed sections were lightly counterstained with hematoxylin.

Results

Fig. 1 shows a Western blot of fibroblast and liver extract from patient 1; no immunoreactive FAH was seen in fibroblasts but

Table I. FAH Activity in Various Tissues from the Five Tyrosinemia Patients and Their Parents

	Liver tissue	FAH	
		Fibroblasts	Lymphocytes
		U/g protein	
Patient 1	6-32	< 0.15	< 0.2
Mother			1.8
Patient 2	26*	< 0.15	< 0.2
Mother		1.3	2.6
Father		1.7	
Patient 3	0.4-4	< 0.15	
Mother		1.5	1.1
Father		1.3	1.5
Patient 4	ND	< 0.15	
Mother		1.7	1.4
Father		1.6	1.6
Patient 5	ND	< 0.15	
Mother		1.5	
Father		1.7	
Controls	33-66 (n = 7)	2.3-3.9 (n = 26)	2.5-4.2 (n = 174)

1 U is the amount of enzyme hydrolysing 1 μ mol fumarylacetoacetate/min at 37°C. * High enzyme activity was found in all frozen liver samples. ND, not determined (frozen tissue unavailable).

significant amounts of cross-reactive material were present in liver tissue. Immunoreactivity was likewise absent in fibroblasts from the other four patients.

The FAH activity in various tissues from the patients and their parents is given in Table I. Substantial enzyme activity was present in frozen liver samples from patient 1, > 50% of the normal mean activity being seen in some samples. High FAH activity was found in all frozen liver samples from patient 2, whereas only some residual enzyme activity was measured in a single liver sample from patient 3. Liver tissue for enzyme determination was not available in patients 4 and 5. The parents had enzyme activities compatible with a heterozygous genotype.

Subcellular fractionation of fresh liver tissue from patient 2 located the FAH activity to the cytosol as normal. The enzyme of both patients 1 and 2 showed K_m and V_{max} values, pH opti-

Figure 1. Immunoblotting of fibroblast and liver extracts in tyrosinemia patient 1 (P) and control (C). Applied samples: fibroblast protein, 100 μ g; liver tissue protein, 10 and 5 μ g from patient (lanes 5 and 6, respectively) and control (lanes 3 and 4).

mum, inhibition pattern towards metal ions, and freeze/thaw stability comparable with a normal control (Table II). In patient 1, the K_m and V_{max} values altered slightly after leaving the enzyme on ice for 7 h.

Immunohistochemistry on liver tissue from patient 2 is shown in Fig. 2; a distinct mosaic pattern of FAH with immunoreactivity confined to only two regeneration nodules was seen in one section (A), whereas in another section (B), all nodules were positive. There was no apparent morphological difference between positive and negative hepatocytes (C and D) in this patient. Similar staining for FAH in patient 4 (Fig. 3) showed several immunoreactive nodules (A). Centrally in one nodule, there was a focus of positive cells (B and D) that peripherally mingled with negative ones (C). Mosaicism was observed also in the other three patients (1, 3, and 5); some sections showed mostly immunoreactive nodules while other areas were mainly negative. Liver tissue from the normal controls always showed an even distribution of FAH immunoreactivity. The three cirrhotic patients with either chronic active hepatitis (Fig. 4) or chronic autoimmune hepatitis had immunoreactive FAH protein in all regenerating nodules.

Discussion

The level of FAH activity observed in liver tissue from the tyrosinemia patients and the mosaic distribution of FAH in regenerating nodules presumably reflect a correction of the enzyme defect in clones of proliferating hepatocytes. Because the phenomenon occurs in liver tissue, this correction might be related either to the accumulated metabolites directly or to the regeneration process. The mechanism underlying the correction is not clear. Reexpression of a fetal form of the FAH protein is unlikely as prenatal diagnosis of tyrosinemia is per-

Table II. Kinetic Constants, Inhibition Effects and Freeze/Thaw Stability of Liver Fumarylacetoacetase in Tyrosinemia Patients 1 and 2 Compared with a Normal Control

	K_m	V_{max}	pH-Optimum	Percent inhibition by ZnCl ₂				Percent inhibition by CuCl ₂			Percent inhibition by p-cmb*			% retained activity by freeze/thaw $\times 10$
				0.01	0.05	0.1	1.0	0.05	0.1	1.0	0.05	0.1	0.5	
Patient 1	1.2	1.0	7-8.5	10	43	64	61	14	41	75	0	5	94	95
	3.2 [‡]	0.7 [‡]												
Patient 2	0.8	0.8	7-8.5	12	56	73	73	10	36	72	0	4	93	96
Experiment control	1.2	0.7	7-8.5	13	49	64	60	19	54	64	0	13	95	95

* p-cmb, p-chloromercuribenzoic acid; [‡] K_m and V_{max} values obtained after leaving the enzyme solution on ice for 7 h.

A

B

C

D

Figure 2. Immunohistochemical staining (APAAP) of two liver sections from patient 2. The overviews show one section (A) with fumarylacetoacetase immunoreactivity in only two regenerating nodules whereas another section (B) contains positive nodules throughout the field. Higher magnification (C) of field from same section as A shows a clear-cut delineation between a positive and a negative nodule separated by connective tissue. By H & E staining (D) of comparable field in adjacent section, no morphological difference appears between hepatocytes in the two nodules.

Figure 3. Immunohistochemical staining (APAAP) of liver section from patient 4. Several immunoreactive regenerating nodules are seen in overview (A). In the middle of one nodule there is a focus (arrow) of positive cells shown at higher magnification in (B); H & E staining of the same nodule in adjacent section is seen in D. At the periphery of this focus the immunoreactive cells mingle with negative ones (C).

Figure 4. Immunohistochemical staining (APAAP) of liver tissue from a cirrhotic patient with chronic active hepatitis (liver transplanted). Even distribution of staining is seen in all regenerating nodules.

formed by assay of succinylacetone and FAH activity from the first trimester (10), and only one gene for FAH has been indicated by in situ hybridization of FAH cDNA (11). Enhanced stability of an unstable protein may also be a less likely explanation, because the enzyme characteristics of the two patients corresponded well to those of a control (Table II), indicating a normal configuration of their enzyme. A stabilization of mRNA, with correct coding sequences, or a reversion of some transcription/translation inhibition could result in expression of a normal enzyme. The immunohistochemical result, showing an apparent clonal distribution of the enzyme protein, suggests a stable correction within a set of cells. A genetic correction would account for both a normal enzyme and a stable reappearance of the protein. There would be a strong positive selection pressure on cells containing the enzyme. If the tyrosinemia patients showing the mosaicism were compound heterozygotes, mitotic recombination between homologous chromatids could theoretically explain the observed enzyme correction. Mitotic recombination is presumed to occur in mammalian tissue in vivo (12, 13). Alternatively a somatic mutation could be introduced in a way to correct the primary defect. Somatic cell mosaicism has been postulated for dominantly inherited and x-linked disorders (14) and in a case of xeroderma pigmentosa, a recessively inherited deficiency of a DNA repair enzyme (15).

The metabolites accumulating in tyrosinemia have a number of effects in the cell; they inhibit adenosylmethionine synthase (16) and mixed function oxidases, result in reduced glutathione level (17), and affect cellular growth (18). The liver is the major production site of these metabolites, and liver cancer is more frequent in tyrosinemia than in patients with liver cirrhosis of other causes (2). The metabolites, presumably indirectly, induce rapid cell division with implemented mutagenesis and carcinogenesis. Direct mutagenic effect of the metabolites has yet to be demonstrated.

The present findings imply that diagnosis of tyrosinemia by assay of FAH activity in liver tissue from chronic patients

could be misleading. When mosaicism exists, the enzyme level found depends entirely on the condition of the actual biopsy sample. We speculate that the substantial residual FAH activity reported in chronic tyrosinemia by others (3) is a result of mosaicism. Furthermore, substantial correction of the enzyme activity could possibly contribute to the long-time survival of some tyrosinemia patients. Reappearance of FAH activity in a cell abolishes the metabolite production in this cell, but does not protect surrounding cells from further damage and malignant transformation. Elucidation of the mechanism(s) underlying the correction of the enzyme defect in tyrosinemia may give further insight into various cellular mechanisms.

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