Cerebrotendinous xanthomatosis: a family study of sterol 27-hydroxylase mutations and pharmacotherapy

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Summary

We examined the phenotypic characteristics, molecular genetics and optimal pharmacological treatment of cerebrotendinous xanthomatosis (CTX) in an English family with combined hyperlipidaemia. The proband presented in adulthood with classical clinical characteristics of CTX, a greater than ten-fold elevation in plasma cholestanol and combined hyperlipidaemia. His brother also had typical features of CTX without the presence of dyslipidaemia. Genotyping revealed that the two brothers were compound heterozygotes for a novel missense mutation in exon 2 (R94Q) and for a recently described nonsense mutation in exon 5, of the sterol 27-hydroxylase gene (CYP27). Analysis of all available family members revealed that hyperlipidaemia did not co-segregate with the presence of a CYP27 mutant allele. Trial of therapy showed that the lowest plasma sterol and triglyceride concentrations and cholestanol:cholesterol ratio were achieved with the combination of chenodeoxycholic acid (CDCA) 750 mg/day, a primary bile acid, and simvastatin 40 mg/day, an inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase. CDCA alone and simvastatin alone significantly lowered plasma cholestanol concentration, but the decrease was greater with the former. After 1 year there was significant improvement in both cognitive and motor function with regression of tendon xanthomata on computerized tomography. We conclude that CTX in this English pedigree is probably due to compound mutant alleles in CYP27, that combined hyperlipidaemia in this family is unrelated to CTX, and that this complicated condition responds optimally to the combination of CDCA and simvastatin.

Introduction

Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive disorder which results in disabling neurological disease, tendon xanthomata, cataracts and premature atherosclerosis.¹ The metabolic defect in CTX is due to underactivity of 27-hydroxylase (EC 1.14.13.15), a mitochondrial enzyme member of the cytochrome P450s that catalyses the oxidation of the side-chain of sterol intermediates in the pathway for converting cholesterol to bile acids.² There is reduced hepatic synthesis of bile acids and increased production of cholestanol,³ the 5α-dihydro derivative of cholesterol, which accumulates in plasma and in all tissues,⁴ notably the central nervous system and tendon sheaths. Affected patients almost invariably have normal plasma lipid levels.⁵ None of the cases so far reported have been from an English pedigree.

Treatment of CTX relies on pharmacological inhibition of the formation of cholestanol to decrease its concentration in plasma and ultimately regress or

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stabilize organ damage. The most effective agent is reportedly chenodeoxycholic acid (CDCA), which expands the bile acid pool and reciprocally decreases cholestanol synthesis. There may also be benefit from inhibiting 3-hydroxy-3-methyl-glutaryl coenzyme A (HMGCoA) reductase with a statin, but studies have produced conflicting results. The therapeutic role of simvastatin, a highly effective HMGCoA reductase inhibitor, has not been established.

In CTX the presence of hyperlipidaemia, however, may justify using statins in combination therapy. Since untreated CTX is a fatal disorder, early detection is critical, and molecular genetics offers the definitive method for screening at-risk patients. Several mutant alleles of the sterol 27-hydroxylase gene (CYP27) thought to be responsible for CTX have been characterized, mostly in patients of Jewish origin. The condition is probably genetically heterogeneous and the full range of mutations has yet to be identified.

In the present study, we describe the first cases of CTX in an English pedigree that also appeared to exhibit combined hyperlipidaemia. We aimed to examine the optimal therapy, with specific reference to the use of simvastatin, and to characterize the molecular defect of the disorder.

Methods

Case report

A 40-year-old man (Study number #240-9; Pedigree number II-5) presented to a neurologist with difficulty in walking, and swelling of the ankles and wrists. His mother reported that over the last 5 years he had been progressively unsteady on his feet, and that memory, writing and reading abilities had deteriorated significantly. He had done poorly at school and had undergone removal of juvenile cataracts. Examination revealed large Achilles and extensor tendon xanthomata, truncal ataxia, hyperreflexia, bilateral Babinski responses and vibration loss at the ankles. Cognition and mental function were impaired, with grossly abnormal verbal and visual recall, and an IQ of 65 on psychometry. Body mass index was 25 kg/m² with normal blood pressure, measured by the CHOD-PAP enzymic colorimetric method (Boehringer); interassay CV < 3.5%. Plasma triglyceride was also measured enzymically (Wako Chemicals); interassay CV < 3%. High-density lipoprotein (HDL) cholesterol was assayed after precipitation of apolipoprotein B with heparin manganese/dextran sulphate. Low-density lipoprotein (LDL) cholesterol was derived from the Friedewald formula. After hydrolysis and extraction, plasma cholestanol was quantified as the ketone derivative by gas or xanthomata. Achilles tendon xanthomata were examined by computerized tomography (CT) scan.

Pedigree study

Of fifteen close family members, thirteen consented to be examined. A 42-year-old brother (Study number #240-13; Pedigree number II-6) had abnormal clinical findings, and these are compared with the proband in Table 1. The remainder of the family members did not show the phenotypic characteristics of CTX. The maternal grandparents were first cousins, but there was no other evidence of consanguinity. There was a family history of coronary artery disease, three maternal relations having died prematurely from myocardial infarction as well as the paternal grandfather at 62 years.

Therapeutic regimens

The proband and his affected brother were both treated for three consecutive 4-month periods with simvastatin (40 mg/day) alone, simvastatin (40 mg/day) plus CDCA (750 mg/day), and CDCA (750 mg/day) alone. Both were prescribed an isocaloric, fat-modified diet throughout the treatment periods, with monthly checks on compliance by packet count and on drug safety by clinical enquiry and measurement of liver and muscle enzymes. Since simvastatin + CDCA was subsequently shown to achieve the lowest cholestanol levels, both patients were treated with this drug regimen for a further 12 months, at the end of which neurological assessment, psychometry, EEG, EMG, brain MRI and CT scan of both Achilles tendons were repeated.

Chemical analysis

Venous blood was collected after a 12 h fast into tubes containing Na₂EDTA (final concentration 1 mg/ml) for lipid, lipoprotein and cholestanol analyses. A biopsy of the left Achilles tendon was also obtained from the proband for sterol analysis. Because following therapy plasma cholestanol takes approximately 12 weeks to approach a nadir, blood was only sampled on three separate occasions during the fourth month of the three periods of treatment. The plasma concentration of cholesterol was measured by the CHOD-PAP enzymic colorimetric method (Boehringer); interassay CV < 3.5%. Plasma triglyceride was also measured enzymically (Wako Chemicals); interassay CV < 3%. High-density lipoprotein (HDL) cholesterol was assayed after precipitation of apolipoprotein B with heparin manganese/dextran sulphate. Low-density lipoprotein (LDL) cholesterol was derived from the Friedewald formula. After hydrolysis and extraction, plasma cholestanol was quantified as the ketone derivative by gas
Cerebrotendinous xanthomatosis

Chromatography (GC) using a Hewlett Packard 5890 Series II Gas Chromatograph fitted with flame ionization detector. Coprostanol (Steraloids Ltd) was used as the internal standard. The concentration of plasma cholestanol was determined by comparing peak height ratios of cholestanol to coprostanol in the plasma sample with standards. The ketones were separated using an HP17 series 530 column (15 m long), oven temperature 240 °C, detector temperature 280 °C and helium as the carrier gas. The cholestanol method had a between assay CV of 7.4%. The concentrations (μg/mg wet weight) of cholestanol and cholesterol in the biopsy of the Achilles tendon were measured (in patient #240-II-6) by the same method as described for plasma. To exclude phytosterolaemia, plasma beta-sitosterol and campesterol concentrations were measured as the underivatised sterols by the same method as cholestanol, excluding the ketone derivatization stage.

**Results**

Table 1 confirms that the proband (#240-II-5) and his brother (#240-II-6) had classical clinical features of CTX with extreme elevation in plasma cholestanol concentration. In the proband, the cholesterol and cholestanol contents of the Achilles tendon biopsy were 49.7 and 3.5 μg/mg wet weight, respectively, consistent with other cases of CTX. Patient #240-II-5 had combined hyperlipidaemia according to newly published definitions, but #240-II-6 had normal plasma lipid and lipoprotein concentrations. In neither patient were the plasma concentrations of phytosterols elevated.

Table 2 shows the plasma sterol, triglyceride and HDL cholesterol concentrations and the cholestanol:cholesterol ratio in patients #240-II-5 and #240-II-6 in response to treatment with simvastatin alone, simvastatin + CDCA and CDCA alone; normal values for plasma cholestanol and cholestanol:cholesterol ratio in 10 control subjects were 0.33(± SEM 0.07) mg/dl and 0.17(± 0.05)% respectively. In patient #240-II-5, simvastatin achieved significant reductions in mean plasma cholesterol (−37%) and...
The mean cholestanol:cholesterol ratio also fell by 14%, but this failed to reach significance. The addition of CDCA to simvastatin resulted in further decreases in plasma cholestanol (−82%), cholestanol:cholesterol ratio (−74%) and triglyceride (−53%), but the mean fall in cholesterol was less than with simvastatin alone. With CDCA alone, there was a significant fall in cholestanol (−60%), triglyceride (−46%) and the cholestanol:cholesterol ratio (−58%), but these were all less than with combination therapy and there was no significant change in cholesterol. HDL cholesterol concentrations did not change significantly with any of the regimens. Only CDCA + simvastatin dropped the cholestanol concentration and ratio to a level that was not significantly different from normal values. In patient #240-II-6, significant decreases in plasma lipids were seen with all agents. The mean reductions in plasma cholestanol and cholestanol:cholesterol ratio, with simvastatin, simvastatin + CDCA and CDCA alone, were (cholestanol) −62%, −84% and −66%, and (ratio) −25%, −76% and −64%, respectively. The pattern of fall in plasma sterol levels in this patient was similar to #240-II-5. Compliance with the drug regimens was >95% and no untoward side-effects were noted in either patient.

After 1 year of treatment with simvastatin + CDCA, patient #240-II-5 showed dramatic improvement in mental and physical abilities. Psychometry revealed an increase in IQ to 75 with marked improvement in visuospatial ability. Paretic and cerebellar abnormalities resolved significantly. These changes were paralleled by improvements in EEG and EMG abnormalities. MRI of the brain showed some resolution of the low attenuation areas in the dentate nuclei, although the change was barely discernible. Repeat CT scan of the Achilles tendons did, however, show evidence of regression of xanthomata (Figure 1). His brother (#240-II-6) showed less dramatic improvement alone, there was a significant fall in cholestanol (−25%); the mean cholestanol:cholesterol ratio fell by 14%, but this failed to reach significance. The addition of CDCA to simvastatin resulted in further decreases in plasma cholestanol (−82%), cholestanol:cholesterol ratio (−74%) and triglyceride (−53%), but the mean fall in cholesterol was less than with simvastatin alone. With CDCA alone, there was a significant fall in cholestanol (−60%), triglyceride (−46%) and the cholestanol:cholesterol ratio (−58%), but these were all less than with combination therapy and there was no significant change in cholesterol. HDL cholesterol concentrations did not change significantly with any of the regimens. Only CDCA + simvastatin dropped the cholestanol concentration and ratio to a level that was not significantly different from normal values. In patient #240-II-6, significant decreases in plasma lipids were seen with all agents. The mean reductions in plasma cholestanol and cholestanol:cholesterol ratio, with simvastatin, simvastatin + CDCA and CDCA alone, were (cholestanol) −62%, −84% and −66%, and (ratio) −25%, −76% and −64%, respectively. The pattern of fall in plasma sterol levels in this patient was similar to #240-II-5. Compliance with the drug regimens was >95% and no untoward side-effects were noted in either patient.

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Figure 1. Effect of treatment of CTX with simvastatin (40 mg/day) and chenodeoxycholic acid (750 mg/day) for a period of 12–24 months in patient #240-II-5 as assessed by serial computerized tomography (CT) of both Achilles tendons. Cross-sectional CT images of tendon xanthomata (white arrow) before (a) and after (b) treatment. Note regression of cross-sectional area of xanthomatosus tissue.

Figure 2. Detection and characterization of CYP27 mutant alleles in all members of family #240 except for individuals #240-I-1 and #240-II-4. a PCR-SSCP analysis of exon 2 and its flanking splice junction sequences. Unaffected control DNA (lane C), individuals II-2, II-1, I-2, I-3, III-1, II-7, III-3, I-4, II-6, II-3, II-8, III-4, II-5, III-2, correspond to lanes 1–14, respectively. b PCR-PIRA analysis of exon 5, following RsaI digestion. DNA size marker (Phi-X digested by HaeIII, lane M), unaffected control DNA (lane UC, undigested PCR product; lane C, RsaI-digested DNA), individuals II-2, II-1, I-2, I-3, III-1, II-7, III-3, I-4, II-6, II-3, II-8, III-4, II-5, III-2 (lanes 1–14), respectively. c Pedigree trace showing mutant alleles in exon 2 (oblique lines in pedigree symbols) and in exon 5 (horizontal lines in pedigree symbols) of CYP27.
Discussion

We have described the first cases of CTX in an English family, and the optimal mode of therapy and genetic mutations responsible for this disorder. Both affected individuals were compound heterozygotes for mutations in exons 2 (R94Q) and 5 (stop 251) of CYP27. We confirm that sustained reduction in plasma cholestanol may in some cases dramatically improve clinical outcome and regress tendon xanthomata. Consistent with the recent report of Kuriyama et al., we also propose that the best treatment for this condition is the combination of a statin and CDCA. This is particularly relevant in the proband who, exceptionally for a case of CTX, exhibited combined hyperlipidaemia.

There is no doubt that patients #240-II-5 and #240-II-6 had classical CTX. The diagnostic features were the typical neurological findings, tendon xanthomata, premature cataracts and markedly elevated plasma and tissue cholestanol concentrations. Dementia and grossly abnormal neurological signs have been described previously even in the absence of ostensible brain lesions on MRI or CT scan. The clinical features of the two cases confirm that CTX is phenotypically heterogenous, but the basis for this remains undefined. Hyperlipidaemia is not a consistent finding in CTX. The combined hyperlipidaemia in the proband may have been due to familial combined hyperlipidaemia, given the lipid results in the remainder of the pedigree and the family history of premature atherosclerosis. The two important differential diagnoses at presentation were familial hypercholesterolaemia and phytosterolaemia, but these were excluded by the neurological involvement, absence of significant hypercholesterolaemia or premature coronary artery disease in the pedigree, and normal plasma plant sterol levels.

The metabolic lesion in CTX is a block in the conversion of cholesterol to bile acids, now attributed to underactivity of the mitochondrial 27-hydroxylase. Since the synthesis of bile acids is regulated by the quantity that returns to the liver in the enterohepatic circulation, the reduced formation of bile acids in CTX results in increased conversion of cholesterol to bile-acid precursors and simultaneous production of cholestanol. Although we did not measure biliary or plasma bile alcohol concentrations in the present study, we would expect these to be markedly increased in both the affected patients. The precise mechanism for overproduction of cholestanol in CTX has been disputed. Cholestanol is derived from cholesterol via 4-cholesten-3-one. This precursor of cholestanol may be synthesized directly from cholesterol or indirectly by 7α-dehydroxylation of 7α-hydroxy-4-cholesten-3-one, an early precursor of bile acids. The response of our patients to the drug regimens suggest that both the direct and indirect pathways of cholestanol biosynthesis were operational: simvastatin would have specifically decreased cholesterol synthesis by inhibiting HMGCoA reductase, and CDCA would have predominantly decreased the production of 7α-hydroxy-4-cholesten-3-one by inhibiting cholesterol 7α-hydroxylase. The clinical manifestations of CTX are due to the spatial effects of xanthomatous deposits, comprising cholestanol and cholesterol, and to demyelination of neuronal tissue resulting from replacement of cholesterol by cholestanol in nerve myelin. Central nervous system involvement has also been shown to result from increased permeability of the blood–brain barrier to plasma lipoproteins. Previous analysis of plasma lipids and lipoproteins in CTX families of Druze origin revealed the absence of any measurable effect of the presence of a CYP27 mutant allele on these parameters.

In the family analysed here, we have excluded the possibility of co-segregation of hypercholesterolaemia or hypertriglyceridaemia with the presence of a mutant CYP27 allele. We may therefore conclude that the proband’s combined hyperlipidaemia was unrelated to the metabolic defect in CTX. These results demonstrate the inherent advantage of family studies as compared to that of unrelated individuals having CTX. In addition, they further support our conclusion that the presence of a CYP27 mutant allele does not affect the plasma concentration of routinely measured lipids and lipoproteins.

Since CTX is an autosomal recessive disorder, it is most prevalent in highly inbred populations. Significant advances in the understanding of the molecular basis for CTX followed the histochemical identification of deficiency in the sterol 27-hydroxylase, cloning of its cDNA, characterization of the structure of CYP27, and identification of several novel mutations. In North African Jews, five different genotypes including compound heterozygotes have been characterized. In Afrikaners of English family, and the optimal mode of therapy and genetic mutations responsible for this disorder. Both affected individuals were compound heterozygotes for mutations in exons 2 (R94Q) and 5 (stop 251) of CYP27. We confirm that sustained reduction in plasma cholestanol may in some cases dramatically improve clinical outcome and regress tendon xanthomata. Consistent with the recent report of Kuriyama et al., we also propose that the best treatment for this condition is the combination of a statin and CDCA. This is particularly relevant in the proband who, exceptionally for a case of CTX, exhibited combined hyperlipidaemia.

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Table 3  Plasma lipids, lipoproteins and cholestanol concentrations, cholestanol:cholesterol ratios and allelic distribution of mutations of the sterol 27-hydroxylase gene in family #240

<table>
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<th>Study number</th>
<th>Pedigree number</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL-cholesterol (mg/dl)</th>
<th>LDL-cholesterol (mg/dl)</th>
<th>Cholestanol (mg/dl)</th>
<th>Cholestanol: cholesterol (%)</th>
<th>G→A transversion in exon 2</th>
<th>A→T transversion in exon 5</th>
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<td>−/−−</td>
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<td>−/−−</td>
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G, guanosine; A, adenosine; T, thymidine.
Dutch origin, an adenosine to thymidine transversion in exon 5 of the gene was previously shown to result in a premature termination codon, a mutation also demonstrated in this English pedigree. The additional missense mutation in exon 2 (R94Q) was identified as a band shift in SSCP, but did not apparently create or eliminate a restriction site. In vitro expression studies should show whether this new mutation impairs enzyme activity. Whether the various mutant alleles determine the phenotypic expressions and response to therapy also warrants examination. It is also interesting to speculate whether the presence of stop 251 in the present pedigree and in Afrikaners reflects a gene flow between England, the Netherlands and South Africa. Elucidation of the molecular basis of CTX in the Netherlands, where a relatively large number of CTX cases were reported, is essential and may shed more light on this matter.

The treatment of CTX is based on pharmacological inhibition of the hepatic production of cholesterol, in order to decrease its plasma concentration to a level below which there is net and sustained eflux of cholesterol from tissues. The most widely recommended approach is to restore the bile-acid pool with CDCA, thereby inhibiting 7α-hydroxylase, the rate-limiting enzyme for bile-acid synthesis. Another approach is to decrease selectively the input of cholesterol into the bile-alcohol pool by competitively inhibiting HMGCoA reductase, the rate-limiting enzyme for cholesterol synthesis, with a statin. CDCA alone has been shown to significantly reduce plasma cholesterol and to dramatically improve neurological symptoms and signs, but this was not paralleled by regression of tendon xanthomata. The dose of CDCA employed in the present study was similar to that of Salen et al. but higher than used by Nakamura et al., which may account for differences in the efficacy of this agent in reducing plasma cholesterol. By contrast to Kuriyama et al., we did not find that CDCA alone induced an ‘atherogenic’ lipid profile in either patient, and this may be due to racial or genetic variations. The results in CTX with statins have been contradictory, which may be due to class differences in inhibitory potency on HMGCoA reductase and 7α-hydroxylase. Genetic variation in enzyme activities may also account for disparate therapeutic responses. Simvastatin was shown to be more efficacious in reducing plasma cholesterol than CDCA, but in that study, patients were intolerant of the latter agent. The excellent clinical response to combination therapy in our proband testifies that the process of accumulation of sterols in brain and Achilles tendons is potentially reversible, but why the response was less dramatic in his affected brother is unclear. Simvastatin has not been previously shown to improve clinical symptoms in CTX, but the duration of treatment was insufficiently long to maximally mobilize the large tissue pools of cholesterol.

Treatment of hypercholesterolaemia per se may reduce tissue deposition of cholesterol, but the degree of change recorded in the proband is unlikely to have accounted for the regression of tendon xanthomata. The improvement in combined hyperlipidaemia in the proband with simvastatin is likely to have been due to the effect of a reduced intrahepatic cholesterol pool to increase LDL receptors as well as to a decrease in the secretion of apolipoprotein B. Turnover studies will be required to dissect the relative importance of each of the two treatment modalities on plasma lipids and lipoproteins in the two affected brothers reported here, who may differ in respect to the presence of combined hyperlipidaemia.

Our study does have important practical implications. The diagnosis of CTX must be considered in all patients with neurological dysfunction, tendon xanthomata and normal or mild elevation of plasma lipids. The most efficacious treatment to reverse or stabilize end-organ damage is simvastatin plus CDCA, especially in the presence of concomitant combined hyperlipidaemia, since the risk of premature atherosclerosis is increased in both these conditions. In patients without concomitant hyperlipidaemia, treatment should start with CDCA, with the addition of a statin if normal plasma levels of cholesterol are not achieved by 6 months or if an atherogenic lipid profile develops. In all patients with CTX and hyperlipidaemia, we would recommend combination treatment with CDCA and a statin. Molecular characterization of mutations in CYP27 is useful in making a definitive diagnosis of CTX, this being particularly relevant for presymptomatic screening and for offering genetic counselling to highly inbred populations.

Acknowledgments

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