

A case of cerebrotendinous xanthomatosis II: the sterol content of a cataractous lens

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Abstract

The cholestanol content of a cataractous lens nucleus from a patient with cerebrotendinous xanthomatosis (CTX) was quantified by gas chromatography-mass spectrometry and found to be 0.27 μg per mg freeze-dried lens tissue. The cholestanol-cholesterol ratio of 1.7% in the lens nucleus was similar to that in the serum of the CTX patient. The cholestanol content and cholestanol-cholesterol ratio in the CTX lens were approximately four-fold and six-fold greater respectively than the mean levels found in three senile cataractous lens nuclei analysed simultaneously for comparative purposes.

Cerebrotendinous xanthomatosis (CTX) is caused by a rare inborn error of bile acid synthesis.¹ Its main clinical features include cataracts, tendon xanthomata, and neurological abnormalities, which usually first become apparent during the second and third decades of life.² The biochemical defect lies in hydroxylation of the steroid side-chain of cholesterol (5-cholesten-3 β -ol), prior to oxidative cleavage, which occurs during hepatic conversion to cholic and chenodeoxycholic acids (Fig 1).¹ The consequent reduced level of the latter bile acid (cholic acid can be synthesised by an alternative minor pathway) decreases the negative feedback inhibition on the rate-limiting enzymes for cholesterol and bile acid synthesis, namely, 3-hydroxy-3-methylglutaryl CoA reductase and cholesterol 7 α -hydroxylase respectively.^{3,4} This results in increased hepatic synthesis of cholesterol and bile acid precursors.⁵ Otherwise minor side-products of the bile acid pathway such as cholestanol (5 α -cholestan-3 β -ol) and 'bile alcohols' (cholesterol derivatives hydroxylated on the steroid nucleus and side-chain without side chain cleavage) are also synthesised in increased amounts.¹⁵ There is increased biliary secretion of

cholestanol and 'bile alcohol' glucuronides, raised serum levels of cholestanol, but not serum cholesterol, and excessive cholestanol and cholesterol deposition in tissues.^{1,6,7}

Sterol deposition is widespread, with xanthomata in the cerebellum and forebrain as well as in tendons; raised cholestanol levels have been found in virtually every tissue examined.^{2,7,8} An earlier report of the cholestanol content of a cataractous lens from a CTX patient describes the cholestanol content as 'significantly elevated'.⁹ Others, however, have reported that cholesterol is the only sterol found in human cadaver lens and senile cataract.¹⁰

The aim of this study was to quantify the cholestanol content in a cataractous lens nucleus of a CTX patient. A case report giving full details has been published.¹¹ The clinical features were typical of CTX, and although bilateral cataracts were not recorded until the age of 40 years, the patient was first prescribed spectacles at 21, when his best corrected visual acuity was 6/18 in each eye. One year after his second cataract extraction with intraocular lens insertion his visual acuities were 6/6 part right, 6/6 left compared with preoperative acuities of 2/60 and 4/60 respectively.

Material and methods

Left lens extracapsular cataract extraction was performed on the 42-year-old male CTX patient. Three senile cataractous lens nuclei from male patients in the eighth decade (mean age 77 years) were also obtained following extracapsular extraction. The nuclei were stored at -70°C under nitrogen until analysed simultaneously. Immediately prior to analysis the nuclei were freeze-dried to obtain a representative weight. The total lipid content of each nucleus was extracted by the Folch procedure.¹² The subsequent steps were performed in duplicate. The free sterols were obtained by saponification of a known amount (approximately 20%) of the lipid extract. Cholestane, added prior to saponification, was used as an internal standard. The free sterols were analysed by gas chromatography-mass spectrophotometry (GC-MS) in the selective ion quantitative mode,¹³ monitoring peaks with mass-charge ratio of 388, 386, and 372 (molecular weights of cholestanol, cholesterol, and cholestane are 388, 386, and 372 respectively).

Standards were prepared from known amounts of cholestanol and a fixed amount of cholesterol and cholestane subject to the same saponification procedure. A standard curve was plotted relating the cholestanol/cholestane content of the standard samples to the cholestanol/cholesterol area ratio. The amount of cholestanol in the lens nucleus samples was estimated from this standard curve by means of the calculated cholestanol/cholesterol ratio of the unknown lens sample. The free sterol peaks, but not internal standard, overlapped in the gas chromatography trace. Because of this the cholestanol component was estimated from the GC-MS peak with mass-charge ratio of 388 after compensation for the cholesterol component. The cholesterol component of the peak at 388 was calculated from the peak at 386 by means of the ratio

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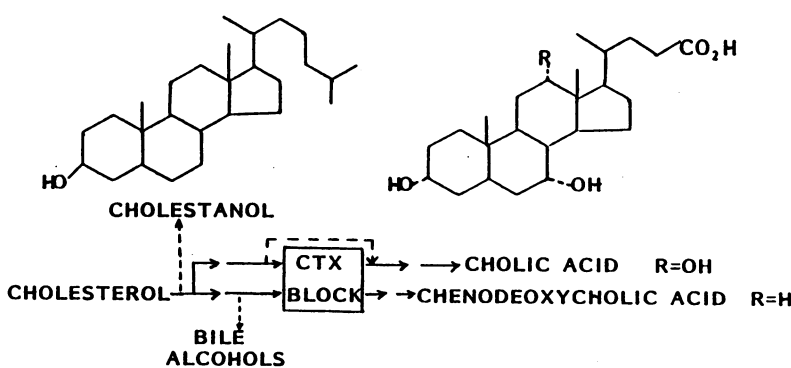


Figure 1 Bile acid pathway in CTX.

Table 1 The sterol content of cataractous lens nuclei

	CTX	Senile
Total cholestanol content per lens	13.1 µg	2.8 µg
Cholestanol content per mg freeze-dried mass of lens	0.27 µg	0.07 µg
Cholestanol/cholesterol ratio	1.7%	0.3%

Table 2 Serum sterol values from the CTX patient

Cholestanol	93 µmol/l (<10)
Cholestanol/cholesterol ratio	1.5% (<0.2%)

Reference values for normal serum in parentheses.

obtained from cholestanol-free standards. The cholestanol-cholesterol mass ratio was calculated from the peak ratio after calibration with standard samples of known cholestanol and cholesterol mass.

Results

The sterol content of the CTX lens nucleus is shown in Table 1 together with the mean values of three senile lens nuclei for comparative purposes (in the absence of reference values). Corresponding serum values are given in Table 2.

The lens nucleus from the CTX patient had approximately a four-fold greater cholestanol content (corrected for freeze-dried lens nucleus mass) compared with the senile lens nuclei in which cholestanol was also detected. The cholestanol/cholesterol ratio in the CTX lens nucleus was similar to that in the serum of the CTX patient, but approximately six-fold greater than in the senile lens nuclei. The amount of cortex attached to the lens nuclei might differ between patients owing to the nature of the extracapsular extraction procedure; it is unlikely to influence the results significantly.

Discussion

It is likely that the cholestanol found in the lens nuclei in the senile cataracts and in the cataract secondary to CTX has an exogenous origin. The source of the cholestanol in both the CTX and senile cataracts is probably hepatic; the bile acid pathway is not known to occur in extrahepatic sites. Although cholestanol is present in the human diet, it is found only at very low levels (estimated at 0.1% of cholesterol) and is not well absorbed into the body.² Serum lipoproteins poorly penetrate aqueous humour,¹⁴ but the similarity of the lens and serum cholestanol content suggest that cholestanol is deposited in the lens by equilibration from serum. The low levels of serum high density lipoprotein typically found in CTX patients may also inhibit sterol removal from peripheral tissues,^{2,15} but this is of unknown significance in relatively isolated tissues such as the lens.

Whether cholestanol has a role in the mechanism of cataractogenesis is unclear. Other cholesterol derivatives, such as 20,25-diazacholesterol and 3-β (2-diethylaminoethoxy)-androst-5-en-17-one hydrochloride, which also are inhibitors of cholesterol synthesis, have been shown to be cataractogenic in experimental animals.^{16,17} Triparanol, a non-steroidal inhibitor

of cholesterol synthesis, also causes cataract formation in man.¹⁸ One mechanism of cataractogenesis by such agents has been postulated to be mediated via alterations in intracellular electrolyte concentrations in the lens and subsequent effects on lens proteins, especially γ-crystallins.¹⁷ Although cholestanol is not an inhibitor of cholesterol synthesis, incorporation of cholestanol in the lens cellular membranes, partially replacing cholesterol, might cause a functional defect equivalent to cholesterol deficiency. Thus in lens tissue, which is critically isolated from systemic cholesterol metabolism, such a functional deficit might activate the same mechanisms leading to cataractogenesis as those previously postulated for direct inhibition of cholesterol synthesis.¹⁷

It can therefore be concluded that there is excess deposition of cholestanol in the nucleus of the CTX lens in association with premature cataract formation. In the light of cataractogenesis by other agents this suggests, but does not prove, a causative role for cholestanol. If cholestanol has an aetiological role, a general mechanism for cataractogenesis could be postulated based on interference with cholesterol function, possibly an alteration in cell membrane permeability rather than cholesterol deficiency itself.

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