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Cerebrotendinous xanthomatosis: An inborn error in bile acid synthesis with defined mutations but still a challenge

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ABSTRACT

Cerebrotendinous xanthomatosis [CTX] is a rare disease characterized by the accumulation of cholesterol and cholestanol in brain and tendons caused by a mutation in the sterol 27-hydroxylase gene [CYP27A1] involved in bile acid synthesis. Disruption of this gene in mice does not give rise to xanthomas. The gene defect leads to reduced bile acid synthesis with a compensatory increase in the activity of the rate-limiting enzyme in bile acid synthesis, cholesterol 7α-hydroxylase. This leads to a marked accumulation of 7α-hydroxylated bile acid precursors, in particular 7α-hydroxy-4-cholesten-3-one. The latter oxysterol passes the blood–brain barrier and is an efficient precursor to cholestanol. The activity of cholesterol 7α-hydroxylase is normalized by treatment with bile acids. Such treatment reduces the xanthomas in CTX patients in parallel with decreased cholestanol levels. The relationship between the accumulation of cholestanol and the development of cholesterol-rich xanthomas has however not been clarified and a suitable animal model is still lacking.

The first report of a patient with cerebrotendinous xanthomatosis [CTX] came from van Bogaert et al. [1]. The patient they described suffered from dementia, ataxia, cataracts and xanthomas of the tendons and the brain. Since then several hundred patients have been diagnosed, with clusters reported from Japan, Israel and The Netherlands [2]. In general CTX patients have normal levels of cholesterol in the blood but raised levels in tissues. In contrast to the xanthomas in patients with familiar hypercholesterolemia, xanthomas from CTX patients contain high levels, up to 30%, of the 5α-saturated analogue of cholesterol, cholestanol. The typical onset of the disease consists of early bilateral cataracts and diarrhoea, followed by cerebellar and pyramidal signs, mental retardation and xanthomas. The most serious consequence of the disease is the development of xanthomas in the brain and the neurological symptoms caused by this. The preferential site of the brain xanthomas is in the white matter of the cerebellum.

In 1974, Setoguchi et al. made the surprising finding that this neurological disease is linked to a defect in bile acid synthesis [3]. Thus the patients were found to excrete high amounts of 25-hydroxylated bile alcohols in urine and feces (pathway B in Fig. 1). Formation of bile acids was reduced, with an almost complete lack of chenodeoxycholic acid. This group of researchers suggested that a normal pathway from cholesterol to bile acids involves a 25-hydroxylation of the steroid side-chain followed by a 24-hydroxylation and cleavage of acetone (pathway B in Fig. 1) [4]. The 24-hydroxylase step is important in the latter pathway and based on some in vitro experiments it was suggested that patients with CTX have a reduced 24-hydroxylation [5]. The new pathway described challenged the pathway for cholesterol synthesis previously described, involving a mitochondrial 27-hydroxylation as a key step in the degradation of the steroid side-chain (pathway A in Fig. 1).

In collaboration with a Norwegian group we could however show that a liver biopsy from a patient with CTX had an almost complete lack of mitochondrial sterol 27-hydroxylase activity [6]. In addition we could demonstrate a very marked accumulation of various substrates for the sterol 27-hydroxylase in the liver of patients with CTX [7]. A 27-hydroxylated intermediate in bile acid biosynthesis was found to be rapidly converted into cholic acid in patients with CTX, with a much slower conversion of the corresponding intermediate without a 27-hydroxyl group [8]. Using 27-^{14}C-labeled cholesterol we could later demonstrate that the pathway involving cleavage of acetone from 25-hydroxylated bile alcohols contributes to normal bile acid synthesis by less than 2% [9]. We concluded that the normal pathway to bile acids is the one involving a 27-hydroxylase step (pathway A) and that the new pathway involving a 25-hydroxylase step (pathway B) is normally of minor importance. When the 27-hydroxylase pathway is blocked, however, the 25-hydroxylase pathway may become of major importance for the biosynthesis of bile acids.

Importantly, pathway B produces only cholic acid, explaining the fact that almost no chenodeoxycholic acid is present in the bile of patients with CTX.

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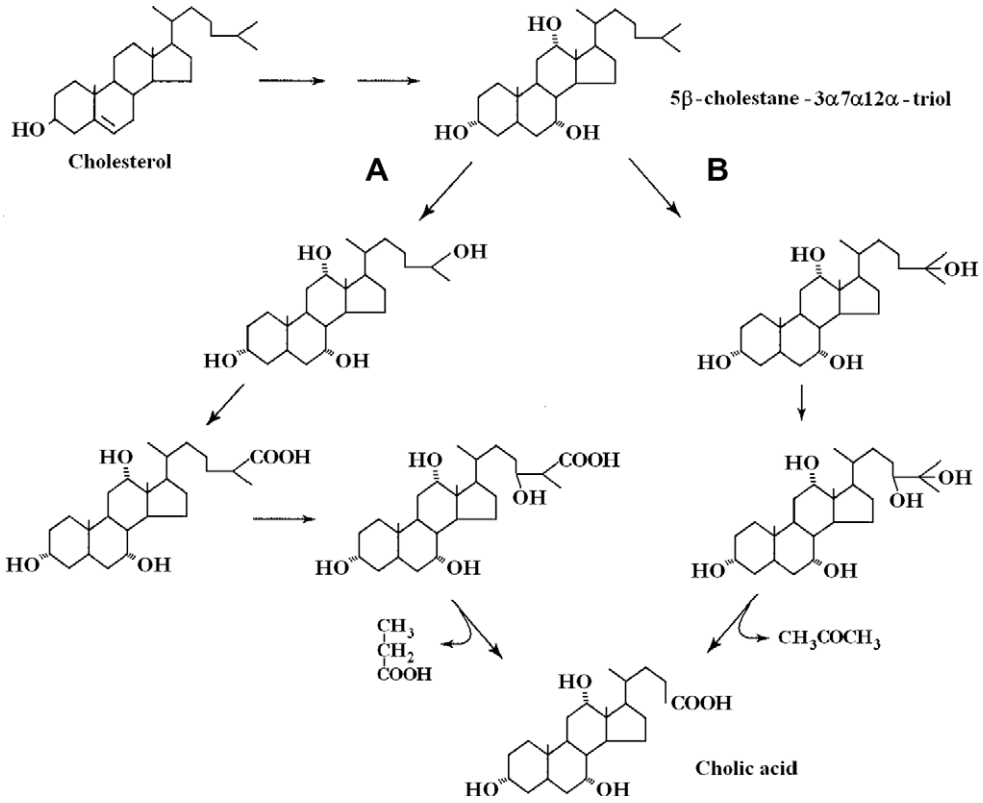

Fig. 1. Conversion of cholesterol into cholic acid by the classical pathway involving a mitochondrial 27-hydroxylation (pathway A) and the pathway involving microsomal 25- and 24-hydroxylations described by Shefer et al. [4,5] (pathway B).

The gene coding for the human sterol 27-hydroxylase [CYP27A1] was cloned and characterized in 1991 by the group of Russell [10]. The gene contains nine exons and spans 18.6 kb of DNA. The enzyme is comprised of 498 amino acids, containing putative binding sites for heme ligands and adrenodoxin. Evidence for the presence of a mutation in this gene in CTX was presented by the same group [11]. Since then more than 30 different mutations in the CYP27A1 gene have been described (missense, nonsense, frameshift and splice junction). The mutations are located in the mapping region to the adrenodoxin binding region or the heme ligand binding site (for a general review – see Ref. [12]).

Sterol 27-hydroxylase, located on the inner membranes of the mitochondria, is involved in the normal oxidation of the steroid side-chain in connection with biosynthesis of bile acids in the liver [13]. The most important pathway from cholesterol into bile acids starts with an hydroxylation in the 7α-position, and the preferred substrates for CYP27A1 are thus 7α-hydroxylated intermediates in bile acid synthesis. The enzyme is however also active on cholesterol. Because of the fact that the enzyme is present and active in almost all cells in the body, and all cells contain cholesterol, there is some production of 27-hydroxycholesterol in all cells. Since 27-hydroxycholesterol is able to pass cell membranes, there is a continuous concentration-dependent flux of 27-hydroxycholesterol to the two organs with the most efficient metabolizing systems: the liver and the brain. In the liver 27-hydroxycholesterol and its peripheral metabolites are further oxidized into bile acids [13]. In the brain, 27-hydroxycholesterol is oxidized to a steroidal acid, 7α-hydroxy-3-oxo-4-cholestenoic acid [14]. The latter acid is able to pass the blood–brain barrier into the circulation. It is then taken up in the liver and further oxidized into bile acids. The flux of 27-hydroxycholesterol and its metabolites from extrahepatic tissues to the liver can be regarded as an alternative mechanism to the classical HDL-dependent reversed cholesterol transport and is antiatherogenic [15]. In accordance with this the levels of sterol 27-hydroxylase are particularly high in macrophages and endothelial cells.

The activity of CYP27A1 is dependent upon NADPH and the availability of two electron transporters, adrenodoxin and adrenodoxin reductase. In addition to this it seems likely that specific transporters are required for the transport of cholesterol to the location of the cytochrome on the inner mitochondrial membranes. At the present point in time no mutation causing CTX has been defined in any other gene than CYP27A1. We have however described a CTX patient with a heterozygous mutation in CYP27A1 who is likely to have a mutation in some additional gene, possibly encoding for a protein responsible for transport of cholesterol into the mitochondria [16]. This subject has very low levels of 27-hydroxycholesterol in the circulation. Normally, subjects with a heterozygous mutation in the CYP27A1 gene have levels of 27-hydroxycholesterol about half of that found in the normal population and have no symptoms.

Most probably it is the generation of cholestanol that is the "driving force" in the development of xanthomas in this disease, and accumulation of cholesterol is likely to be secondary to this. Intravenous administration of a mixture of 4-^{14}C- and 7α-^{3}H-labeled cholesterol in patients with CTX resulted in a production of cholestanol that had lost most of the ^{3}H label [17]. Based on these experiments we concluded that most of the cholestanol accumulating in patients with CTX is derived from 7α-hydroxylated metabolites of cholesterol. Among these intermediates 7α-hydroxy-4-cholesten-3-one is probably the most important. We could show that this oxysterol is an efficient precursor to cholestanol in various tissues by a pathway involving cholesta-4,6-dien-3-one as an intermediate (Fig. 2) [2,18]. 7α-Hydroxy-4-cholesten-3-one is present in levels 100-fold higher than normal in the circulation of patients with CTX [19]. The reason for this accumulation is the upregulation of the rate-limiting enzyme in bile acid synthesis,

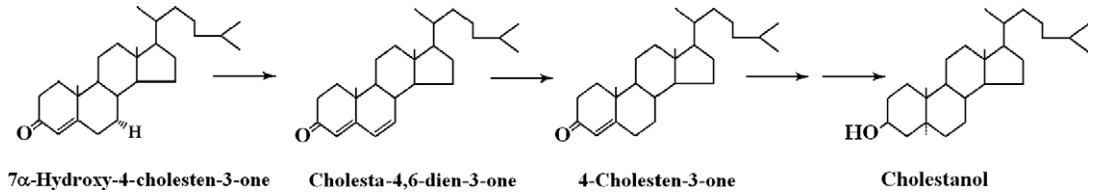

Fig. 2. Conversion of the bile acid intermediate 7α-hydroxy-4-cholesten-3-one into cholestanol [2,18,19,22].

cholesterol 7α-hydroxylase [CYP7A1]. It has been shown that this enzyme is upregulated by a factor of about 20 in the liver of CTX patients [20]. The reason for this upregulation is the markedly reduced formation of chenodeoxycholic acid in CTX. The latter bile acid is the most effective suppressor of the rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1) in man [21].

We recently showed that 7α-hydroxy-4-cholesten-3-one is able to pass an in vitro model of the blood–brain barrier more efficiently than other oxysterols [22]. Furthermore there was an efficient conversion of this oxysterol into cholestanol in cultured astrocytes, glial cells and cells of neuronal origin [22]. In preliminary experiments we have shown that intravenous administration of 7α-hydroxy-4-cholesten-3-one in a mouse with a disruption of the cyp27a1 gene results in an increased level of cholestanol in the brain [unpublished]. Thus it seems likely that there is a continuous flux of 7α-hydroxy-4-cholesten-3-one across the blood–brain barrier in patients with CTX with a subsequent formation of cholestanol.

If the production of cholestanol from 7α-hydroxylated bile acid intermediates is of key importance for the development of xanthomas in CTX, a suppression of CYP7A1 would be the most optimal treatment. In accordance with this, treatment with chenodeoxycholic acid reverses all the biochemical changes including levels of 7α-hydroxy-4-cholesten-3-one and cholestanol in the circulation. Not only the xanthomas in the tendons but also those in the brain may be reduced or disappear as a consequence of such treatment [2,23]. Treatment with chenodeoxycholic acid is thus a very effective treatment. In view of this it is important to get an early diagnosis.

The reason for the accumulation of cholesterol in parallel with cholestanol is not clear. Cholestanol is however less efficient in suppressing cholesterol synthesis [24] and a dilution of a cholesterol pool with cholestanol may cause a compensatory increased synthesis of cholesterol. In some experiments with experimental animals dietary treatment with cholestanol has in fact been shown to increase cholesterol synthesis [25,26].

In addition to formation of cholestanol, the accumulation of 7α-hydroxylated intermediates in bile acid synthesis leads to formation of 7α- and 25-hydroxylated alcohols, e.g. 5β-cholestane-3α,7α,12α,25-tetrol. These alcohols may be secreted in bile and urine in gram amounts and detection of these compounds is generally used in the diagnosis of the disease [2]. Fig. 3 summarizes the metabolic relations between 7α-hydroxy-4-cholesten-3-one, cholestanol and the 25-hydroxylated bile alcohols in patients with CTX. It should be emphasized that only a small shunting of the 7α-hydroxylated intermediates into the cholestanol pathway is required to explain the accumulation of cholestanol in CTX. A minor part of the 25-hydroxylated intermediates are further converted into cholic acid by the pathway B described in Fig. 1.

It is important to note that a disruption of the gene coding for the sterol 27-hydroxylase in mice does not lead to formation of xanthomas in tendons or brain [27]. There is some accumulation of cholestanol in these tissues, in particular in female mice, but this accumulation is less marked and is not accompanied by accumula-

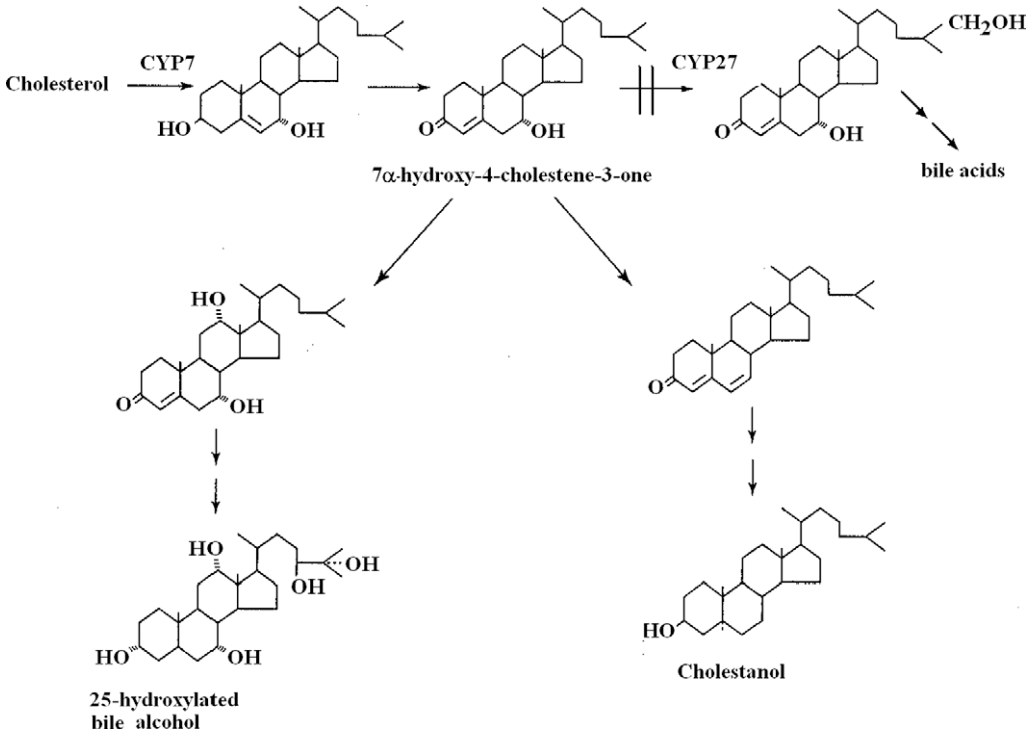

Fig. 3. Metabolic relations between 7α-hydroxy-4-cholesten-3-one and the synthesis of bile alcohols and cholestanol [2].

tion of cholesterol. The reason for the difference between human CTX and the corresponding mouse model is not known with certainty. Part of the explanation may be that the degree of upregulation of cholesterol 7α-hydroxylase as a consequence of the lack of sterol 27-hydroxylase is less marked in the mouse model than in patients with CTX.

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