On the substrate specificity of human CYP27A1: implications for bile acid and cholestanol formation

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Abstract The mitochondrial sterol 27-hydroxylase (CYP27A1) is required for degradation of the C27-sterol side chain in bile acid biosynthesis. CYP27A1 seems, however, to have roles beyond this, as illustrated by patients with a deficient sterol 27-hydroxylase due to mutations of the CYP27A1 gene [cerebrotendinous xanthomatosis (CTX)]. These subjects have symptoms ranging from accumulation of bile alcohols and cholestanol to accelerated atherosclerosis and progressive neurologic impairment. The present work describes a detailed investigation on the substrate specificity of recombinant human CYP27A1. In accordance with some previous work with rat liver mitochondria, the activity in general increased with the polarity of the substrate. An obvious example was the finding that cholesterol was 27-hydroxylated more efficiently than cholesterol oleate but less efficiently than cholesterol sulfate. The oxysterols 24S-hydroxycholesterol and 25-hydroxycholesterol were 27-hydroxylated less efficiently than cholesterol, possibly due to steric hindrance. Surprisingly, sterols with a 3-oxo-Δ4 structure were found to be hydroxylated at a much higher rate than the corresponding sterols with a 3β-hydroxy-Δ5 structure. The rates of hydroxylation of the sterols were: 7α-hydroxy-4-cholesten-3-one > 4-cholesten-3-one > 7α-hydroxycholesterol > 24S-hydroxy-4-cholesten-3-one > cholesterol > 25S-hydroxy-4-cholesten-3-one > 24S-hydroxycholesterol > 25S-hydroxycholesterol. The possibility is discussed that the findings may have implications for oxysterol-mediated regulation of gene expression. The very high activity of CYP27A1 towards the cholestanol precursor 4-cholesten-3-one may be of importance in connection with the accumulation of cholestanol in patients with CTX. Supplementary key words sterol 27-hydroxylase • cerebrotendinous xanthomatosis • cholesterol sulfate

Mitochondrial sterol 27-hydroxylase (CYP27A1; P450c27, mitochondrial vitamin D₃ 25-hydroxylase) is a multifunctional P450 enzyme that catalyzes hydroxylations in both bile acid biosynthesis and bioactivation of vitamin D₃ (1–5). The conversion of cholesterol into bile acids and the biliary excretion of cholesterol are the most important mechanisms for the ultimate removal of cholesterol from the body. In the classical or neutral pathway of bile acid biosynthesis, starting with the 7α-hydroxylation of cholesterol, CYP27A1 initiates degradation of the C27-steroid side chain. In the acidic pathway, it catalyzes the initial 27-hydroxylation of cholesterol (6). Recent data suggest that CYP27A1 plays several important roles in cholesterol homeostasis and affects atherogenesis. A novel mechanism has been described for the elimination of cholesterol from human lung macrophages and cells in arterial endothelium. This mechanism involves 27-oxygenation of cholesterol by CYP27A1 (7). Patients with the rare inherited lipid storage disease, cerebrotendinous xanthomatosis (CTX), have a sterol 27-hydroxylase deficiency due to point mutations of the CYP27A1 gene. Manifestations of this genetically determined CYP27A1 deficiency range from accelerated atherosclerosis to progressive neurologic impairment. The major symptoms in CTX are due to the generalized accumulation of cholesterol and cholestanol in almost every tissue, including the nervous system (8, 9).

CYP27A1 is expressed in many tissues, and both hepatic and extrahepatic CYP27A1 are active in the 27-hydroxylation of several C27-sterols (1, 3). Besides cholesterol and various intermediates in bile acid biosynthesis, CYP27A1 may also catalyze 27-hydroxylation of different oxysterols. These oxysterols have been reported to affect the expression of genes in cholesterol homeostasis, at least under in vitro conditions, and a 27-hydroxylation may alter their biological activity (10). Currently, it is not clear if oxysterols are metabolized by CYP27A1. Several 3β-hydroxy-Δ5 sterols are converted by 3β-hydroxy-Δ5-C27 steroid dehydrogenase(s) in liver and other tissues into the corresponding 3-oxo-Δ4 sterols. 7α-Hydroxy-4-cholesten-3-one,
an intermediate in the classical pathway for bile acid biosynthesis, is known to be 27-hydroxylated by the mitochondrial sterol 27-hydroxylase (11, 12). However, there is no information regarding whether, or to what extent, 4-cholesten-3-one or 3-oxo-Δ4 metabolites of oxysterols can be metabolized by CYP27A1.

The objectives of the current study were to examine the substrate specificity of human CYP27A1 with respect to C27-steroids and to get more information on the relationship between sterol structure and CYP27A1 activity. Considering that cholesterol, having a 3β-hydroxy Δ5 structure, is 27-hydroxylated much less efficiently by CYP27A1 than the saturated steroids 5β-cholestan-3α, 7α, 12α-triol and 5β-cholestan-3α, 7α-diol (3, 11, 12), it was hypothesized that sterols with a 3-oxo-Δ4 structure might be hydroxylated at a higher rate. In this paper, we report that CYP27A1 has a low activity toward 24-hydroxycholesterol and 25-hydroxycholesterol, and that 3-oxo-Δ4 sterols are 27-hydroxylated much more efficiently than the corresponding 3β-hydroxy-Δ5 sterols. The finding that 4-cholesten-3-one, a suggested precursor to cholestanol, is 27-hydroxylated is discussed in relation to bile acid and cholestanol formation.

MATERIALS AND METHODS

Materials

Cholesterol and cholesterol oxidase (Cellulomonas sp) were from Sigma. 7α-Hydroxycholesterol and 25-hydroxycholesterol were purchased from Steraloids Inc. (Wilton, NH). 7α-Hydroxy-4-cholesten-3-one and 24-hydroxycholesterol were synthesized as described previously (12, 13). 27-Hydroxycholesterol was a gift from Dr. L. Tökes, Syntex, Palo Alto, CA. 4-Cholesterol-3-one (cholestene-1), 24-hydroxy-4-cholesten-3-one, and 25-hydroxy-4-cholesten-3-one were prepared enzymatically by oxidation of cholesterol, 24-hydroxycholesterol, and 25-hydroxycholesterol, respectively, with cholesterol oxidase. Octyl-Sepharose CL-4B was from Pharmacia and hydroxyapatite (Macroprep Ceramic HTP) was from Bio-Rad. Tris-acetate buffer (pH 7.4) containing 20% glycerol and 0.15% sodium cholate instead of a linear gradient of potassium phosphate (10–400 mM) in 20% glycerol and 0.15% sodium cholate was used as the source of CYP27A1.

Expression and purification of recombinant human CYP27A1 in Escherichia coli

The methods used for expression of human CYP27A1 in E. coli were essentially the same as those described by Pikuleva et al. (14), with the exception that DH5α cells were used instead of TOP10 cells. Recombinant CYP27A1 was partially purified using the initial steps, including sodium cholate solubilization and octyl-Sepharose as described by Pikuleva, Björkhem, and Waterman (14). The adrenodoxin-Sepharose chromatography was omitted, and cytochrome P450 was eluted from the hydroxyapatite column with 500 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol and 0.15% sodium cholate instead of a linear gradient of potassium phosphate (10–400 mM) in 20% glycerol and 0.15% sodium cholate. Fractions with a spectrophotometric index (A418/A280) higher than 0.9 were pooled, dialyzed against 50 mM phosphate buffer containing 20% glycerol and 0.1 mM EDTA, and used as source of CYP27A1.

Incubation procedures

Incubations of recombinant human CYP27A1 (0.1–0.5 μM) with cholesterol, 4-cholesten-3-one, 7α-hydroxycholesterol, 7α-hydroxy-4-cholesten-3-one, 24-hydroxycholesterol, 24-hydroxy-4-cholesten-3-one, 25-hydroxycholesterol, or 25-hydroxy-4-cholesten-3-one (15 μM of each dissolved in acetone) were carried out for 5–30 min in the presence of adrenodoxin (2 μM or 4 μM), adrenodoxin reductase (0.2 μM or 0.4 μM), and NADPH (1 μM) in a total volume of 1 ml of 50 mM Tris-acetate buffer (pH 7.4) containing 20% glycerol and 0.1 mM EDTA. Incubations with cholesterol (13 μM) and cholesterol sulfate (13 μM) were performed under the same conditions.

Incubations with racemic 24-hydroxycholesterol or 24-hydroxy-4-cholesten-3-one (15 μM) and pig liver mitochondria (3 mg of protein) were carried out for 30 min in the presence of isocitrate (7 mM) and MgCl2 (10 mM) in a final volume of 1 ml of 50 mM Tris-acetate buffer (pH 7.4) containing 20% glycerol and 0.1 mM EDTA.

Incubations with 27-hydroxycholesterol and 27-hydroxy-4-cholesten-3-one with recombinant and purified CYP7A1 and CYP7B1, respectively, were carried out according to procedures described previously (15).

Analysis of incubation mixtures

Incubation mixtures with cholesterol, 7α-hydroxycholesterol, 24-hydroxycholesterol, and 25-hydroxycholesterol were extracted, oxidized by cholesterol oxidase, and reextracted prior to HPLC analysis as described previously (15). Mixtures from incubations with 4-cholesten-3-one, 7α-hydroxy-4-cholesten-3-one, 24-hydroxy-4-cholesten-3-one, and 25-hydroxy-4-cholesten-3-one were extracted in the same way but were not oxidized by cholesterol oxidase prior to HPLC. The sterol extracts were analyzed by normal-phase HPLC (15). The column used was LiChrosphere Si 60, 5 μm (Merck, Rahway, NJ). The flow rate was 0.7 ml/min and the monitoring absorbance was 240 nm. The detector used was a 2151 Variable Wavelength Monitor (from LKB). The following mobile phases were used: hexane-isopropanol (96:4; v/v) for products from incubations with cholesterol and 4-cholesten-3-one; hexane-isopropanol (87:13; v/v) for products from incubations with 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one; hexane-isopropanol (92:8; v/v) for products from incubations with 24-hydroxycholesterol and 24-hydroxy-4-cholesten-3-one; and hexane-isopropanol (90:10; v/v) for products from incubations with 25-hydroxycholesterol and 25-hydroxy-4-cholesten-3-one.

In a separate set of experiments, incubation mixtures with cholesterol and cholesterol sulfate were directly analyzed after acid solvolysis as described previously (16). Ether extracts of the mixtures were washed with water until neutral, converted into trimethylsilyl ether, and analyzed by gas chromatography-mass spectrometry as described below. The recovery of cholesterol in connection with solvolysis of cholestenyl sulfate by this method was shown to be more than 90%. In the calculation of the conversion of cholesterol sulfate into 27-hydroxycholesterol sulfate, it was assumed that the recovery of 27-hydroxycholesterol is the same as that of cholesterol.

Analysis by combined gas chromatography-mass spectrometry

Lipid extracts or isolated chromatographic fractions were converted into trimethylsilyl ether and analyzed by combined gas chromatography-mass spectrometry under the conditions previously described (3, 7, 16). In the cases of the incubations with cholesterol and cholesterol sulfate, the extent of conversion of the substrate into the corresponding 27-hydroxylated product was calculated from the height of the peaks corresponding to cholesterol and 27-hydroxycholesterol in the total ion current.
chromatogram. The total ion current signal was found to be similar for cholesterol and 27-hydroxycholesterol, and no correction factor was used to correct for different responses.

Other methods

An oxysterol 7α-hydroxylase (CYP7B1) from pig liver microsomes was purified as described previously (17). Recombinant human CYP27A1 was expressed in E. coli and purified as described (15). Purification of adrenodoxin and adrenodoxin reductase from bovine adrenal mitochondria and preparation of mitochondria from pig liver were carried out as described previously (11, 18).

RESULTS

Metabolism of cholesterol and 4-cholesten-3-one by recombinant human CYP27A1

Mixtures from incubations of cholesterol with recombinant human CYP27A1 in the presence of NADPH and the mitochondrial electron transporters, adrenodoxin, and adrenodoxin reductase, were analyzed by HPLC (after conversion of product into 3-oxo-Δ4 structure prior to analysis). Cholesterol was converted into one major product with the same retention time as authentic 27-hydroxy-4-cholesten-3-one (Fig. 1A). CYP27A1 was incubated with 4-cholesten-3-one to investigate the ability of the enzyme to 27-hydroxylate this sterol. The formation of 27-hydroxy-4-cholesten-3-one was found to be about 10-fold higher than with cholesterol (Fig. 1B). No products were formed when incubations were carried out in the absence of the mitochondrial electron transporting proteins (Fig. 1C).

Metabolism of cholesterol sulfate and cholesterol oleate by recombinant human CYP27A1

Incubations with cholesterol sulfate were analyzed by gas chromatography-mass spectrometry. After incubation of cholesterol sulfate with recombinant human CYP27A1 and acid solvolysis, a product peak occurred in the chromatogram corresponding to 27-hydroxycholesterol. When equivalent amounts of cholesterol and cholesterol sulfate were incubated with the same amount of CYP27A1 under identical conditions, the rate of conversion of cholesterol into 27-hydroxycholesterol was 7%, whereas the corresponding conversion of cholesterol sulfate was 19%. In another set of experiments, the rate of conversion of cholesterol

Fig. 1. Metabolism of cholesterol and 4-cholesten-3-one by recombinant human CYP27A1. A: Incubations of cholesterol with recombinant human CYP27A1 (HPLC analysis after conversion of product into 3-oxo-Δ4 structure prior to analysis). Cholesterol was converted into one major product with the same retention time as authentic 27-hydroxy-4-cholesten-3-one. B: Incubation of 4-cholesten-3-one with CYP27A1 under the same conditions as in A. The formation of 27-hydroxy-4-cholesten-3-one was about 10-fold higher than with cholesterol. C: Incubation of 4-cholesten-3-one with CYP27A1 in the absence of the mitochondrial electron transporting proteins adrenodoxin and adrenodoxin reductase (negative control). The mobile phase was hexane-isopropanol (96:4, v/v). For details of incubation procedures, see Materials and Methods.
Fig. 2. Metabolism of 24-hydroxycholesterol and 24-hydroxy-4-cholesten-3-one by recombinant human CYP27A1. A: Incubation of racemic 24-hydroxycholesterol with CYP27A1 and subsequent conversion to 3-oxo-Δ4-derivatives prior to HPLC analysis. Three products (P1, P2, and P3) were obtained. B: Incubation of racemic 24-hydroxy-4-cholesten-3-one with CYP27A1 under the same conditions as in A. The conversion into the products was about 10-fold higher. C: No products were formed when the substrate was incubated with CYP27A1 in the absence of the mitochondrial electron transporters. Std, testosterone standard. Mobile phase was hexane-isopropanol (92:8, v/v). For details of incubation procedures, see Materials and Methods.
and cholesterol sulfate was 6% and 27%, respectively. Cholesterol sulfate is thus a better substrate for CYP27A1 than cholesterol itself. When cholesterol oleate was incubated under identical conditions using the same preparation of enzyme and the same molar concentration of substrate, there was no significant conversion into the 27-hydroxylated product (<0.2% conversion).

**Metabolism of 24-hydroxycholesterol and 24-hydroxy-4-cholesten-3-one by recombinant human CYP27A1 and liver mitochondria**

Incubation of racemic 24-hydroxycholesterol with CYP27A1 and subsequent conversion to 3-oxo-$\Delta^4$-derivatives gave three product peaks on HPLC analysis (Fig. 2A). Incubation of racemic 24-hydroxy-4-cholesten-3-one with CYP27A1 under the same conditions resulted in the same three product peaks, but the conversion into the products was about 10-fold higher (Fig. 2B). All three products increased with time in parallel (Fig. 3). No products were formed when the substrate was incubated with CYP27A1 in the absence of the mitochondrial electron transporters (Fig. 2C).

In separate experiments, 24-hydroxycholesterol or 24-hydroxy-4-cholesten-3-one were incubated with pig liver mitochondria. The same pattern of products was obtained as with recombinant human CYP27A1. Also, with liver mitochondria the rate of product formation was five to ten times higher with 24-hydroxy-4-cholesten-3-one than with 24-hydroxycholesterol.
from three to five experiments. The differences in hydroxylation rates were statistically significant (P < 0.01) in all cases except for the difference between 24-hydroxycholesterol and 25-hydroxycholesterol (P > 0.1).

1 Sum of three products.

24-hydroxycholesterol. The results show that recombinant CYP27A1 expressed in E. coli has the same catalytic properties as CYP27A1 in isolated liver mitochondria.

Gas chromatography-mass spectrometry analysis of products formed by CYP27A1 with cholestenone and 24-hydroxy-4-cholestene-3-one

The product formed from cholestenone by CYP27A1 was found to have the same retention time on gas chromatography (as trimethylsilyl ether) and the same mass spectrum as authentic 27-hydroxy-4-cholesten-3-one formed by oxidation of 27-hydroxycholesterol with cholesterol oxidase. The three products from incubations with CYP27A1 and racemic 24-hydroxycholesterol and subsequent oxidation or directly from 24-hydroxy-4-cholesten-3-one (Fig. 2A, B) were also analyzed by combined gas chromatography-mass spectrometry as trimethylsilyl ethers. The materials corresponding to Peaks 2 and 3 had identical mass spectra, with prominent peaks at m/z 470 (M-90) and 380 (M-2190), as expected for the 24R- and 24S-isomers of 24,27-dihydroxy-4-cholesten-3-one. The identity of Peak 1 in Fig. 2A and B could not be established.

Table 2 summarizes the rate of product formation by recombinant human CYP27A1 with various 3β-hydroxy-Δ5 and 3-oxo-Δ4 sterols. In this table, 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one, two known substrates for CYP27A1, are also included. The rates of CYP27A1-mediated hydroxylation of the various sterols were: 7α-hydroxy-4-cholesten-3-one > 4-cholesten-3-one > 7α-hydroxycholesterol > 24-hydroxy-4-cholesten-3-one > cholesterol > 25-hydroxycholesterol > 24-hydroxycholesterolol ≥ 25-hydroxycholesterol.

As shown in the table, the rate of hydroxylation by CYP27A1 was five to ten times higher with the 3-oxo-Δ4 sterols than with the corresponding 3β-hydroxy-Δ5 compounds.

Experiments with CYP7A1, CYP7B1, and 27-hydroxy-4-cholesten-3-one

Experiments were carried out to study if 7α-hydroxylating enzymes are able to further metabolize 27-hydroxy-4-cholesten-3-one. The two enzymes, CYP7A1 and CYP7B1, are known to catalyze 7α-hydroxylation of 27-hydroxycholesterol (15, 19). As shown in Table 2, both purified pig liver CYP7B1 and recombinant human CYP7A1 converted 27-hydroxycholesterol into 7α-hydroxylated product. No 7α-hydroxylated product could be detected when CYP7B1 was incubated with 27-hydroxy-4-cholesten-3-one. CYP7A1, on the other hand, converted this 3-oxo-Δ4 sterol into the 7α-hydroxylated product at a rate of about 10% of that with 27-hydroxycholesterol (Table 2).

DISCUSSION

Previous studies with purified and recombinant CYP27A1 have shown that in general, polar substrates are hydroxylated at a higher rate than less polar substrates. Thus, 5β-cholestan-3α,7α,12α-triol is 27-hydroxylated at the highest rate of all known substrates for CYP27A, and 5β-cholestan-3α,7α-diol is hydroxylated much more efficiently than cholesterol (3, 11, 14). From results with purified hepatic CYP27A, the relative rates of 27-hydroxylation of 5β-cholestan-3α,7α,12α-triol, 5β-cholestan-3α,7α-diol, and 7α-
hydroxy-4-cholesten-3-one are approximately 8:3:1 (20). The present study provides further information on structural features affecting the rate of CYP27A1-catalyzed hydroxylation. It is apparent that sterols with a 3-oxo-Δ^4 structure are more efficiently 27-hydroxylated by CYP27A1 than the corresponding 3β-hydroxy-Δ^5 sterols. Accordingly, cholestenone was 27-hydroxylated at a rate about 10 times higher than cholesterol and 24- and 25-hydroxycholestenone were hydroxylated at a much faster rate than 24- and 25-hydroxycholesterol.

The finding that CYP27A1 is able to 27-hydroxylate 24- and 25-hydroxycholesterol may have implications for oxysterol-mediated regulation of gene expression. Oxysterols such as 24-hydroxycholesterol and 25-hydroxycholesterol have been suggested to be potential regulators of genes in cholesterol homeostasis (10, 21). Metabolism may alter their biological activity and is required for elimination of the oxysterols from the body. The 7α-hydroxylation of 24- and 25-hydroxycholesterol by CYP7A1, CYP7B1, and CYP39A1 has previously been described (15, 19, 22). Here we demonstrate that 24S- and 24R-hydroxycholesterol and 25-hydroxycholesterol are 27-hydroxylated by human CYP27A1, although at a low rate. Since CYP27A1 is not confined to the liver but is expressed in almost every tissue in the body, this metabolism of oxysterols may be important in extrahepatic cells. It is possible that the 27-hydroxylation, as well as the 7α-hydroxylation, of oxysterols alters their effects on gene expression. That CYP27A1-mediated hydroxylation is involved in the elimination of oxysterols from the body is supported by a recent report (16). Labeled 24-hydroxycholesterol was found to be converted into cholic and chenodeoxycholic acids and free and conjugated 5-cholestene-3β,24,27-triol by human primary hepatocytes. The conjugated 27-hydroxylated product was also found in human intestinal content. It should be emphasized, however, that it is possible that conjugated rather than free 24S-hydroxycholesterol may be the normal substrate for CYP27A1. In this connection, it is interesting that cholesterol sulfate was found to be 27-hydroxylated by CYP27A1 at a higher rate than cholesterol itself.

The most intriguing finding in the present work is the efficient 27-hydroxylation of cholestenone by CYP27A1 and its relation to bile acid and cholestanol biosynthesis. Cholestenone is a precursor in the two pathways described for cholestanol formation. It is generally believed that the major pathway in biosynthesis of cholestanol in healthy subjects involves an oxidation of cholesterol into cholestenone by an NAD-dependent 3β-hydroxy-Δ^5-ste-roid dehydrogenase [reviewed in ref. (8)]. Cholestenone is then converted to cholestanol by the action of a 5α-reductase and a 3β-hydroxysteroid dehydrogenase. In patients with CTX, an alternative pathway has been described involving intermediates in bile acid biosynthesis as precursors. This pathway starts with dehydration of 7α-hydroxy-4-cholesten-3-one by a specific liver enzyme to yield cholesta-4,6-dien-3-one, which is converted into 4-cholesten-3-one. The final steps are the same as in the major pathway. It has been calculated that the alternative pathway is responsible for at most 20% of the cholestanol formed in healthy humans (8).

Cholestanol is known to be degraded to 5α-bile acids, allocholic, and allochenodeoxycholic acid by liver enzymes similar to those involved in biosynthesis of 5β-bile acids (8). Interestingly, cholestanol is also a substrate for CYP27A1 and is 27-hydroxylated by this enzyme at a rate similar to that of cholesterol (23). The results of the present study

**Fig. 5.** Proposed pathways for formation of bile acids from cholestenone and metabolic consequences of CYP27A1 deficiency for cholestanol formation. In cerebrotendinous xanthomatosis, CYP27A1 is deficient. 3βHSD, 3β-hydroxy-Δ^5-C27-steroid dehydrogenase.
suggest the possible existence of a minor pathway for bile acid biosynthesis involving 27-hydroxylolation of cholesterol by CYP27A1. The 27-hydroxycholestenone formed may be 7α-hydroxylated by CYP7A1, and the resulting 7α,27-dihydroxy-4-cholesten-3-one may be further converted into bile acids (Fig. 5). It may be mentioned that we have shown that cholestenone is a substrate for human CYP7A1, although 7α-hydroxylation of this substrate occurs at a rate considerably lower than that of cholesterol and 27-hydroxycholesterol (M. Norlin et al., unpublished observations). Most probably, a pathway including this reaction is of little or no importance from a quantitative point of view.

Formation of cholestanol is dramatically increased in patients with CTX having a CYP27A1 deficiency. Currently, there are three hypotheses for the mechanisms behind the increased synthesis of cholestanol in patients with CTX: 1) the activity of the 3β-hydroxy-Δ5-steroid dehydrogenase catalyzing the first step in the major pathway may be increased in CTX patients; 2) the increased biosynthesis of cholestanol may be secondary to the increased cholesterol biosynthesis; and 3) the increased biosynthesis of cholestanol may be due to increased utilization of bile acid intermediates as precursors for cholestanol (8). In addition to the increased formation of cholestanol in CTX, the accumulation of cholestanol in this disease may in part be due to a reduced removal of this steroid from macrophages as a consequence of the lack of CYP27A1 in the macrophages (23).

The results of the present study provide an additional explanation for the increased levels of cholestanol in CTX patients. The ability of CYP27A1 to 27-hydroxylolate cholestenone indicates that cholestenone may be continuously drained from the cholestenol-forming pathways under normal conditions, probably leading to formation of a bile acid (Fig. 5). According to these results, the increased cholestanol levels in CTX patients may in part be the consequence of the abolished CYP27A1-catalyzed 27-hydroxylation of cholestenone directing cholestenone exclusively into cholestanol formation.

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REFERENCES

1. Andersson, S., D. L. Davis, H. Dahlbäck, H. Jörnvall, and D. W. Russell. 1989. Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile acid biosynthetic enzyme. J. Biol. Chem. 264:8222–8229.

2. Cali, J. J., and D. W. Russell. 1991. Characterization of human sterol 27-hydroxylase. A mitochondrial cytochrome P-450 that catalyzes multiple oxidation reaction in bile acid biosynthesis. J. Biol. Chem. 266:7774–7778.

3. Pikuleva, I. A., A. Babiker, M. R. Waterman, and I. Björkhem. 1998. Activities of recombinant human cytochrome P450c27 (CYP27) which produce intermediates of alternative bile acid biosynthetic pathways. J. Biol. Chem. 273:18153–18160.

4. Usui, E., M. Noshiro, Y. Ohyama, and K. Okuda. 1990. Unique property of liver mitochondrial P450 to catalyze the two physiologically important reactions involved in both cholesterol catabolism and vitamin D activation. FEBS Lett. 274:175–177.

5. Su, P. H., R. M. Shaw, C. R. Yamamoto, Y. M. Zheng, S. Addya, J. F. Strauss III, and N. G. Avadhani. 1990. A cDNA encoding a rat mitochondrial cytochrome P450 catalyzing both the 26-hydroxylation of cholesterol and 25-hydroxylation of vitamin D3: gonadotropic regulation of the cognate mRNA in ovaries. DNA Cell Biol. 9:657–665.

6. Vlahcevic, Z. R., R. T. Stratvis, D. M. Heuman, P. B. Hylemon, and W. M. Pandak. 1997. Quantitative estimations of the contribution of different bile acid pathways to total bile acid synthesis in the rat. Gastroenterology. 113:1949–1957.

7. Babiker, A., O. Andersson, E. Lund, R. J. Xiu, S. Deeb, A. Reshef, E. Leitersdorf, U. Diczfalusy, and I. Björkhem. 1997. Elimination of cholesterol in macrophages and endothelial cells by the sterol 27-hydroxylase mechanism. Comparison with high density lipoprotein-mediated reverse cholesterol transport. J. Biol. Chem. 272:26253–26261.

8. Björkhem, I., K. M. Boberg, and E. Leitersdorf. 2001. Inborn errors in bile acid biosynthesis and storage of steroids other than cholesterol. In The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Inc., New York. 2961–2988.

9. Cali, J. J., C. L. Huech, U. Francke, and D. W. Russell. 1991. Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. J. Biol. Chem. 266:7779–7783.

10. Russell, D. W. 2000. Oxyysterol biosynthetic enzymes. Biochim. Biophys. Acta. 1529:126–135.

11. Wikvall, K. 1984. Hydroxylations in biosynthesis of bile acids. Isolation of a cytochrome P-450 from rabbit liver mitochondria catalyzing 26-hydroxylation of C27-steroids. J. Biol. Chem. 259:3800–3804.

12. Björkhem, I., and J. Gustafsson. 1973. Omega-hydroxylation of steroid side-chain in biosynthesis of bile acids. Exer. J. Biochem. 36:201–212.

13. Lund, E., O. Breuer, and I. Björkhem. 1992. Evidence that 24- and 27-hydroxylations are not involved in the cholesterol-induced down-regulation of hydroxymethylglutaryl-CoA reductase in mouse liver. J. Biol. Chem. 267:25092–25097.

14. Pikuleva, I. A., I. Björkhem, and M. R. Waterman. 1997. Expression, purification, and enzymatic properties of recombinant human cytochrome P450c27 (CYP27). Arch. Biochem. Biophys. 343:123–130.

15. Norlin, M., U. Andersson, I. Björkhem, and K. Wikvall. 2000. Oxysterol 7α-hydroxylase activity by cholesterol 7α-hydroxylase (CYP7A1). J. Biol. Chem. 275:34046–34053.

16. Björkhem, I., U. Andersson, E. Ellis, G. Alvelius, L. Ellegård, U. Diczfalusy, J. Sjövall, and C. Einarsson. 2001. From brain to bile. Evidence that conjugation and omega-hydroxylation are important for elimination of 24S-hydroxysterol (cerebrosterol) in humans. J. Biol. Chem. 276:57004–57010.

17. Norlin, M., and K. Wikvall. 1998. Biochemical characterization of the 7α-hydroxylation activities towards 27-hydroxycholesterol and dehydroepiandrosterone in pig liver microsomes. Biochim. Biophys. Acta. 1390:269–281.

18. Norlin, M. 2002. Expression of key enzymes in bile acid biosynthesis during development: CYP7B1-mediated activities show tissue-specific differences. J. Lipid Res. 43:721–731.

19. Li-Hawkins, J., E. G. Lund, S. D. Turley, and D. W. Russell. 2000. Disruption of the oxysterol 7α-hydroxylase gene in mice. J. Biol. Chem. 275:16536–16542.

20. Wikvall, K. 1993. Sterol 26-hydroxylase. In Handbook of Experimental Pharmacology. Vol. 105. J. B. Schenkenman and H. Greim, editors. Springer-Verlag, Berlin. 705–718.

21. Björkhem, I. 2002. Do oxyysterols control cholesterol homeostasis? J. Clin. Invest. 110:725–730.

22. Li-Hawkins, J., E. G. Lund, A. D. Bronson, and D. W. Russell. 2000. Expression cloning of an oxysterol 7α-hydroxylase selective for 24-hydroxycholesterol. J. Biol. Chem. 275:16543–16549.

23. von Bahr, S., T. Movin, N. Papadogiannakis, I. Pikuleva, P. Rönnov, U. Diczfalusy, and I. Björkhem. 2002. Mechanism of accumulation of cholesterol and cholestanol in tendons and the role of sterol 27-hydroxylase (CYP27A1). Arterioscler. Thromb. Vasc. Biol. 22:1129–1135.