Antiepileptic Drugs Increase Plasma Levels of 4β-Hydroxycholesterol in Humans

EVIDENCE FOR INVOLVEMENT OF CYTOCHROME P450 3A4*

Received for publication, June 5, 2001, and in revised form, August 17, 2001
Published, JBC Papers in Press, August 20, 2001 DOI 10.1074/jbc.M105127200

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The major cholesterol oxidation products in the human circulation are 27-hydroxycholesterol, 24-hydroxycholesterol, and 7α-hydroxycholesterol. These oxysterols are formed from cholesterol by specific cytochrome P450 enzymes, CYP27, CYP46, and CYP7A, respectively. An additional oxysterol present in concentrations comparable with 7α- and 24-hydroxycholesterol is 4β-hydroxycholesterol. We now report that patients treated with the antiepileptic drugs phenobarbital, carbamazepine, or phenytoin have elevated plasma levels of 4β-hydroxycholesterol. When patients with uncomplicated cholesterol gallstone disease were treated with ursodeoxycholic acid, plasma 4β-hydroxycholesterol increased by 45%. Ursodeoxycholic acid, as well as all the antiepileptic drugs, are known to induce cytochrome P450 3A. Recombinant CYP3A4 was shown to convert cholesterol to 4β-hydroxycholesterol, whereas no conversion was observed with CYP1A2, CYP2C9, or CYP2B6. The concentration of 4β-hydroxycholesterol in plasma was lower than the concentration of 4α-hydroxycholesterol and not affected by treatment with the antiepileptic drugs or ursodeoxycholic acid. Together, these data suggest that 4β-hydroxycholesterol in human circulation is formed by a cytochrome P450 enzyme.

Cholesterol oxidation products (oxysterols) have recently attracted great interest because of their numerous biological actions. They have been implicated in bile acid biosynthesis, cholesterol transport, and gene regulation (1). In addition, many oxysterols are toxic to cells and induce apoptosis (2–4). These compounds can be formed either by cholesterol auto-oxidation or by the action of cholesterol-metabolizing enzymes. Several oxysterols can be formed by both mechanisms, i.e. 7α-hydroxycholesterol. This oxysterol is a predominant cholesterol auto-oxidation product but is also formed by the hepatic enzyme cholesterol 7α-hydroxylase. Major oxysterols in the human circulation include 27-hydroxycholesterol, 24-hydroxycholesterol, and 7α-hydroxycholesterol (5). One additional oxysterol present in human plasma at a relatively high concentration is 4β-hydroxycholesterol (6). Very little is known about its formation or metabolism. We have shown earlier that small amounts of this oxysterol are formed, together with 4α-hydroxycholesterol, during in vitro oxidation of low density lipoprotein, and low levels of the two oxysterols were also found in human atherosclerotic plaques (7). The ratio between 4α- and 4β-hydroxycholesterol was close to one both in oxidized LDL and in plaques, and the amount formed in oxidized LDL was only a small percent of the dominating oxysterol, 7α-cholesterol. These data suggested that very little 4β-hydroxycholesterol is formed by cholesterol auto-oxidation. Because relatively high levels were reported in human plasma we hypothesized that this compound is formed in vivo by an enzymatic reaction. 4α- and 4β-hydroxycholesterol were determined in plasma from volunteers and patients, and it was found that patients treated with certain antiepileptic drugs, known to influence cytochrome P450 enzymes, had 10–20-fold higher plasma levels of 4β-hydroxycholesterol than untreated control subjects. Attempts to identify the cytochrome P450 responsible for the conversion of cholesterol into 4β-hydroxycholesterol were made using recombinant human cytochrome P450 enzymes expressed in insect cells.

EXPERIMENTAL PROCEDURES

Chemicals

Chloroform, ethyl acetate, hexane, and toluene, analytical grade, and methanol (high pressure liquid chromatography grade) were obtained from Merck. 2-Propanol (high pressure liquid chromatography grade) was from Labscan Ltd. (Dublin, Ireland). Butylated hydroxytoluene was obtained from Sigma. EDTA disodium salt and potassium bromide were obtained from Merck. Solid-phase extraction cartridges (100 mg of Isolute silica) were obtained from Sorbent (Mid Glamorgan, United Kingdom). tert-Butyldimethylsilylimidazole-dimethylformamide was obtained from Supelco Inc. (Bellefonte, PA).

Testosterone, dichlofenac, and phenacetin were obtained from Sigma. Microsomes (SUPERSOMES) of recombinant human P450 2C18 (CYP1A2, CYP2B6, CYP2C9 (with Arg144), and CYP3A4) were obtained from insect cells (BTI-TN-5B1–4), together with NADPH-P450 reductase and cytochrome b5 (except CYP1A2), were purchased from Gentest (Woburn, MA). All other reagents and chemicals were high purity standard commercial products.

Synthesis of Cholesterol-5-ene-3β,4β-diol

Cholesterol-5-ene-3β,4β-diol (4β-hydroxycholesterol) and [26,26,27,27,27-3H]4β-hydroxycholesterol were synthesized as described previously (6).

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* This work was supported by grants from Gunvor och Josef Aneré stipendium, Swedish Heart Lung Foundation, Stiftelsen Serafimerlasaret, and Swedish Medical Research Council Project 3143. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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† The abbreviations used are: LDL, low density lipoprotein; GC-MS, gas chromatography-mass spectrometry; LXRα, liver X receptor α.
Plasma samples were stored at 20 °C. The plasma of each volunteer was fractionated separately. Incubations were also carried out with different amounts of protein (0, 50, 100, 200, and 400 μg) for 2 h with 25 pmol of enzyme. Incubations were terminated after 2 h by adding 50 μl of methanol. After a rapid cooling the samples were subjected to GC-MS analysis as described for plasma samples but without alkaline hydrolysis. Incubations were performed according to the manufacturers' recommendations.

The formation of 4β-hydroxycholesterol by CYP3A4 was characterized in more detail by incubating cholesterol at five different concentrations (0, 50, 100, 200, and 400 μM) for 2 h with 25 pmol of enzyme. Incubations were also carried out with different amounts of protein (0, 12.5, 25, 50, and 100 μM), at 200 μM cholesterol, and for different times (0, 0.5, 1, 2, and 3 h) at 100 μM cholesterol.

Because no conversion of cholesterol to 4β-hydroxycholesterol was observed with CYP1A2, CYP2C9, or CYP2B6, control incubations were carried out without phenacetin, diclofenac, and testosterone as substrates, respectively. All three enzymes converted the control substrates to the expected products, acetaminophen, 4-hydroxycyclofenac, and 16β-hydroxytestosterone, respectively.

### Determination of Cholesterol

Cholesterol in plasma and lipoprotein fractions was determined using a commercial enzymatic method (Roche Diagnostics/Hitachi 917 system). Total cholesterol in tissue homogenate was determined as described earlier (10) by using isotope dilution GC-MS with [2H6]cholesterol as internal standard.

#### Determination of 4α- and 4β-Hydroxycholesterol

**Alkaline Hydrolysis and Extraction**—The procedure for alkaline hydrolysis and extraction of 4β-hydroxycholesterol in tissue homogenate and plasma has been described previously (6). Briefly, to 1 ml of plasma 10 μg of butylated hydroxytoluene and 100 ng of [1H3]4β-hydroxycholesterol dissolved in 40 μl of toluene was added. Aragon was flushed through the vial for 20 min to remove air. Freshly prepared 0.35 M potassium hydroxide in ethanol (10 ml) was added. The alkaline hydrolysis was allowed to proceed for 2 h at room temperature with continuous magnetic stirring. The reaction mixture was transferred to a separatory funnel, and the pH value was adjusted to 7 with phosphoric acid. 18 ml of chloroform and 6 ml of 0.15 M NaCl were added. Thereafter the funnel was vigorously shaken. The organic phase was transferred to a round-bottom flask, and the solvent was evaporated using a rotary evaporator. The residue was dried with ethanol and finally dissolved in 1 ml of toluene. A 100-μg silica solid-phase extraction column (International Sorbent Technology, Mid Glanorgan, UK) was used to separate 4-hydroxycholesterol from cholesterol. The column was conditioned with 2 ml of hexane. The sample (dissolved in toluene) was applied to the column followed by 1 ml of hexane. Cholesterol was eluted with 8 ml of 0.5% 2-propanol in hexane and by adding 5 ml of 30% 2-propanol, 4α- and 4β-hydroxycholesterol were eluted. The solvent was evaporated under a gentle stream of argon, and the residue was derivatized.

**Derivatization**—4β-Hydroxycholesterol was converted into a tert-butyldimethylsilyl ether by treatment with 100 μl of tert-butyldimethylsilyltrimethylchlorosilane (Supelco Inc., Bellefonte, PA) at 50 °C overnight, followed by addition of 1 ml of water and extraction twice with 1 ml of ethyl acetate. After derivatization and removal of solvent under a stream of argon, the samples were dissolved in 100 μl of hexane (6).

**Analysis by Gas Chromatography-Mass Spectrometry**—Gas chromatography-mass spectrometry was performed on a Hewlett Packard 5890 Series II Plus gas chromatograph equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25-μm phase thickness), connected to an HP 5972 mass selective detector and an HP 7673A automatic sample injector. The oven temperature program was as follows: 180 °C for 1 min, 35 °C/min to 270 °C, and then 20 °C/min to 310 °C where the temperature was kept for 17.0 min. Helium was used as a carrier gas with a flow rate of 0.8 ml/min. Samples were splitless injected (1 μl), and the detector temperature was 270 °C. The detector transfer line temperature was set to 280 °C. The mass spectrometer was used in the selected ion monitoring mode, and the following ions (m/z) were monitored (retention times in brackets): 575,367 (4α-hydroxycholesterol [16.1 min], 4α-hydroxycholesterol [17.0 min]) and 579,373 (4β-hydroxycholesterol [16.0 min]). The electron ionization energy was 70 eV.

### Ethical Aspects

All studies were approved by the Ethics Committee of Karolinska Institutet at Huddinge University Hospital (Huddinge, Sweden).

#### RESULTS

**Determination of Plasma 4α- and 4β-Hydroxycholesterol in Healthy Volunteers**—Plasma concentrations of 4α- and 4β-hydroxycholesterol were determined in 125 healthy volunteers (Table I), and the mean concentrations were found to be 6.6 and 29 ng/ml, respectively. The distribution of plasma 4β-hydroxycholesterol concentrations in the volunteers is shown in Fig. 1.

**Table I**

| Plasma concentrations of 4α- and 4β-hydroxycholesterol in healthy volunteers |
|-----------------------------|-----------------------------|
| 4α-Hydroxycholesterol | 4β-Hydroxycholesterol | Cholesterol |
| ng/ml | ng/ml | mmol/l |
|---|---|---|
| All volunteers (n = 125) | 6.6 ± 2.8 | 29 ± 10 | 4.5 ± 0.8 |
| Males (n = 49) | 6.8 ± 3.6 | 26 ± 11 | 4.3 ± 0.8 |
| Females (n = 76) | 6.5 ± 2.1 | 30 ± 10 | 4.7 ± 0.7 |
| *Mean ± S.D.*
$4\beta$-Hydroxycholesterol was present both in its free form and esterified to long-chain fatty acids. The degree of esterification was determined in plasma from 10 healthy volunteers. $4\beta$-Hydroxycholesterol was present in esterified form to 83% in plasma whereas $4\alpha$-hydroxycholesterol was esterified to 70%.

Distribution of $4\beta$-Hydroxycholesterol in Lipoprotein Fractions—Total cholesterol and $4\alpha$- and $4\beta$-hydroxycholesterol were determined in plasma collected from four healthy volunteers. The plasma was fractionated into very low density lipoprotein, LDL, and high density lipoprotein fractions, and cholesterol and $4\alpha$- and $4\beta$-hydroxycholesterol were determined in the fractions. As shown in Table II, the distribution of $4\alpha$- and $4\beta$-hydroxycholesterol paralleled completely the distribution of cholesterol in the lipoprotein fractions with the major part residing in the LDL fraction.

Determination of $4\beta$-Hydroxycholesterol in Human Tissues—The ratio of $4\beta$-hydroxycholesterol to cholesterol was determined in several human tissues from autopsy material. As shown in Table III this ratio did not differ much in the tissues examined except for the brain, which contained 5–6 times higher relative amounts compared with most other tissues. We can not judge how representative these results are, because autopsy material from each tissue was only obtained from one subject.

Increased Levels of $4\beta$-Hydroxycholesterol in Patients Treated with Certain Antiepileptic Drugs—Plasma concentrations of $4\alpha$- and $4\beta$-hydroxycholesterol were determined in patients on monotherapy with different antiepileptic drugs. As shown in Table IV, patients treated with valproate had plasma levels of the two oxysterols very similar to those in healthy subjects (Table I). Patients treated with carbamazepine, phenytoin, and phenobarbital all had significantly ($p < 0.0001$) increased plasma concentrations of $4\beta$-hydroxycholesterol, 7–8-fold higher than those in healthy subjects. Some patients had a 20-fold increase in $4\beta$-hydroxycholesterol. None of the antiepileptic drugs influenced the plasma level of $4\alpha$-hydroxycholesterol. Plasma from one patient treated with carbamazepine, with a $4\beta$-hydroxycholesterol concentration of 600 ng/ml, was analyzed for other oxysterols (5). Normal values were found for all oxysterols analyzed, i.e. $7\alpha$- and $7\beta$-hydroxycholesterol, cholestan-3$\beta,5\alpha,6\beta$-triol, 7-oxocholesterol, and 24-, 25-, and 27-hydroxycholesterol (data not shown). The identity of $4\beta$-hydroxycholesterol was ascertained by full scan GC-MS, and the mass spectrum was found to be identical to what was published previously (7).

Treatment of Patients with Ursodeoxycholic Acid Leads to Increased Plasma Levels of $4\beta$-Hydroxycholesterol—Four patients, three females and one male, were treated with ursodeoxycholic acid for 3 weeks. Plasma samples were taken before and immediately after treatment. The plasma concentration of $4\beta$-hydroxycholesterol was on average 45% higher after treatment ($p < 0.006$) whereas $4\alpha$-hydroxycholesterol was not influenced by the treatment as shown in Table V.

Formation of $4\beta$-Hydroxycholesterol by Recombinant Cytochrome P450 3A4—Insect cell microsomes (SUPERSOMES®) containing recombinant human cytochrome P450 enzymes were incubated with cholesterol. Four different microsomal preparations containing CYP3A4, CYP1A2, CYP2B6, and CYP2C9 were used. Microsomes containing CYP3A4 converted cholesterol into $4\beta$-hydroxycholesterol with a $K_m$ of $\sim 50$ $\mu M$, whereas no conversion could be detected with CYP1A2 and CYP2C9 or CYP2B6. The $4\beta$-hydroxylation of cholesterol by recombinant CYP3A4 was characterized in some detail. Incubations with different amounts of enzyme showed an almost linear response between 25 and 100 pmol CYP3A4/ml (Fig. 2). Substrate saturation was obtained above 100 $\mu M$ (Fig. 2). Control microsomes without expressed human cytochrome P450 enzymes did not convert cholesterol into $4\beta$-hydroxycholesterol.

**DISCUSSION**

The major circulating oxysterols in man are $7\alpha$-, 24- and 27-hydroxycholesterol. They are all products of enzymatic reactions (11–13). An additional oxysterol present in relatively high concentrations in human plasma is $4\beta$-hydroxycholesterol (6). It has not been known, however, how this oxysterol is formed. In vitro oxidation of low density lipoprotein resulted in the formation of small amounts of $4\alpha$- and $4\beta$-hydroxycholesterol in a ratio close to one (7) suggesting that the oxysterols were formed by auto-oxidation. The high levels in the human
induce cytochrome P450 enzymes, had normal plasma levels of cholesterol compared with patients treated with valproate (Table 2). Three weeks of treatment with ursodeoxycholic acid for three weeks showed higher levels of 4β-hydroxycholesterol with a mean ratio of 4.7. One subject (not included in the group of healthy volunteers), treated with the antiepileptic drug carbamazepine, had a plasma 4β-hydroxycholesterol concentration over 600 ng/ml compared with 30 ng/ml in the average healthy volunteer (Table I). This led us to investigate the effect of different antiepileptic drugs on the plasma levels of 4α- and 4β-hydroxycholesterol. Patients treated with carbamazepine, phenytoin, or phenobarbital, three drugs known to induce cytochrome P450 enzymes (14, 15), had significantly elevated plasma levels of 4β-hydroxycholesterol compared with patients treated with valproate (Table IV) whereas 4α-hydroxycholesterol levels were similar. Patients on monotherapy with valproate, a drug that does not induce cytochrome P450 enzymes, had normal plasma levels of 4β-hydroxycholesterol.

Because three antiepileptic drugs known to induce cytochrome P450 enzymes did not result in elevated plasma levels of 4β-hydroxycholesterol. This showed that epilepsy per se does not result in plasma levels of 4β-hydroxycholesterol. Because three antiepileptic drugs known to induce cytochrome P450 enzymes elevated plasma 4β-hydroxycholesterol, a number of recombinant human cytochrome P450 enzymes were tested for the ability to 4β-hydroxylate cholesterol. The major human liver P450 enzymes involved in drug metabolism are CYP3A4, CYP1A2, CYP2D6, and the CYP2C subfamily (15). The CYP3A4 enzyme metabolizes the widest range of drugs and endogenous compounds of the different cytochromes (15). Phenytoin, phenobarbital, and carbamazepine induce CYP1A2, CYP2B6, CYP2C9, and CYP3A4 (14–16). Therefore, these cytochromes were tested for cholesterol 4β-hydroxylase activity. The substrates for CYP2D6 are all bases, and their binding to the active site depends on ion-pair interactions (17, 18). Consequently, cholesterol is not expected to be a substrate for CYP2D6.

Although CYP1A2, CYP2C9, and CYP2B6 did not metabolize cholesterol, CYP3A4 converted cholesterol into a polar compound co-chromatographing with 4β-hydroxycholesterol on GC-MS with selected ion monitoring, using an isotope-dilution technique. The identity of the product was verified by full scan GC-MS. Ursodeoxycholic acid has been shown to induce murine CYP3A (19). Plasma concentrations of 4β-hydroxycholesterol in patients before and after treatment with ursodeoxycholic acid was therefore determined. Three weeks of treatment with ur-
sodeoxycholic acid increased plasma 4β-hydroxycholesterol by 45%. This finding is in accordance with our assumption that CYP3A4 is the enzyme responsible for the conversion of cholesterol into 4β-hydroxycholesterol.

Although recombinant CYP3A4 was shown to convert cholesterol into 4β-hydroxycholesterol in vitro, it can not be excluded that another cytochrome is responsible for the normal production of this sterol. Many drugs that induce cytochrome P450 enzymes are relatively nonspecific and may induce several enzymes. Because CYP3A4 is known to be induced by antiepileptics, is abundant in the liver, and was shown to convert cholesterol into 4β-hydroxycholesterol in vitro it is a strong candidate enzyme, but it is still possible that another enzyme is responsible for this conversion in vivo.

Very little is known about the biological effects of 4β-hydroxycholesterol. It has been reported that 4β-hydroxycholesterol is almost as good an activator for the nuclear receptor liver X receptor α (LXRα) as 24S-hydroxycholesterol, 20S-hydroxycholesterol, and 22R-hydroxycholesterol (20). The most effective activator of LXRs, 24(S),25-epoxycholesterol, binds to the receptor with a $K_d$ of ~200 nM (21). In healthy volunteers the plasma concentration of 4β-hydroxycholesterol is 75 nM, but patients treated with carbamazepine may have 4β-hydroxycholesterol concentrations up to 1500 nM. LXRs are an important transcription factor involved in gene regulation of genes important for cholesterol homeostasis. It is possible that the highly elevated levels of 4β-hydroxycholesterol in patients treated with some antiepileptic drugs may effect transcription of genes responsive to LXRs. One such gene is sterol regulatory element-binding protein-1c, which controls transcription of lipogenic genes (22). A known side effect of treatment with certain antiepileptic drugs is weight gain. Further research will show whether drug-induced formation of 4β-hydroxycholesterol may be related to weight increase seen in patients treated with antiepileptics. 4β-Hydroxylated bile acids have been identified in fetal gallbladder bile where they constituted 5–15% of total biliary bile acids (23, 24).

In summary, we have found evidence for an enzymatic formation of 4β-hydroxycholesterol in man. Patients treated with antiepileptics known to induce CYP3A4 had highly elevated plasma levels of 4β-hydroxycholesterol. Ursodeoxycholic acid, which has also been reported to induce CYP3A, caused an increase in plasma 4β-hydroxycholesterol when given to gallstone patients. Plasma 4α-hydroxycholesterol concentrations were not influenced by treatments that elevated 4β-hydroxycholesterol, indicating that 4α-hydroxycholesterol is formed by cholesterol auto-oxidation or an enzyme not affected by carbamazepine, phenytoin, phenobarbital, or ursodeoxycholic acid.

REFERENCES
1. Russell, D. W. (2000) Biochim. Biophys. Acta 1529, 126–135
2. Schreepfer, G. J., Jr. (2000) Physiol. Rev. 80, 361–554
3. Yin, J., Chauffour, X., McAllister, C., McGuire, M., White, G., King, N., and Hamblin, B. (2000) Atherosclerosis 148, 365–374
4. Nishio, E., and Watanabe, Y. (1996) Biochem. Biophys. Res. Commun. 226, 928–934
5. Dzeletovic, S., Breuer, O., Lund, E., and Diczfalussy, U. (1995) Anal. Biochem. 225, 73–80
6. Breuer, O. (1995) J. Lipid Res. 36, 2275–2281
7. Breuer, O., Dzeletovic, S., Lund, E., and Diczfalussy, U. (1996) Biochim. Biophys. Acta 1302, 145–152
8. Havel, R. J., Eder, H. A., and Braden, J. H. (1955) J. Clin. Invest. 34, 1345–1353
9. Martin, K. O., Budai, K., and Javitt, N. B. (1993) J. Lipid Res. 34, 581–588
10. Björkhem, I., Blomstrand, R., and Svenssen, L. (1974) Clin. Chem. Acta 54, 185–193
11. Cohen, J. C., Cali, J. J., Jelenik, D. F., Mehrabian, M., Sparkes, R. S., Lasis, A. J., Russell, D. W., and Hobbs, H. H. (1992) Genomics 14, 153–161
12. Lund, E. G., Guileymard, J. M., and Russell, D. W. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 728–7243
13. Cali, J. J., and Russell, D. W. (1991) J. Biol. Chem. 266, 7774–7778
14. Tanaka, E. (1999) J. Clin. Pharmacol. Ther. 24, 87–92
15. Michaelson, E. L. (1998) Pharmacotherapy 18, 84–112
16. Pascucci, J.-M., Gerbal-Chaloin, S., Fahren, J.-M., Maurel, P., and Vila, M.-J. (2000) Mol. Pharmacol. 58, 1441–1450
17. Bertilsson, L. (1995) Clin. Pharmacokinet. 29, 192–209
18. Anzenbacher, P., and Anzenbacherová, E. (2001) Cell. Mol. Life Sci. 58, 757–747
19. Paolini, M., Puzzetti, L., Piazza, F., Cantelli-Forti, G., and Roda, A. (1999) Hepatology 30, 730–739
20. Janowsky, B. A., Willy, P. J., Devi, T. R., Falck, J. R., and Mangelsdorf, D. J. (1996) Nature 383, 728–731
21. Janowsky, B. A., Gregan, M. J., Jones, S. A., Wisely, G. B., Kliever, S. A., Coreyc, E. J., and Mangelsdorf, D. J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 266–271
22. Schultz, J. R., Tu, H., Luk, A., Repa, J. J., Medina, J. C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D. J., Lustig, K. D., and Shab, B. (2000) Genes Dev. 14, 2831–2838
23. Dumaswala, R., Setchell, K. D. R., Zimmer-Nechenas, L., Iida, T., Goto, J., and Nambara, T. (1989) J. Lipid Res. 30, 847–856
24. Setchell, K. D. R., Dumaswala, R., Colombo, C., and Ronchi, M. (1988) J. Biol. Chem. 263, 16637–16644