Oxysterols in the circulation of patients with the Smith-Lemli-Opitz syndrome: abnormal levels of 24S- and 27-hydroxycholesterol

Ingemar Björkhem,† Lena Starck,* Ulla Andersson,* Dieter Lütjohann,* Sara von Bahr,* Irina Pikuleva,** Amir Babiker,* and Ulf Diczfalusy*

Division of Clinical Chemistry,* Huddinge University Hospital, SE-141 86 Huddinge, Sweden, and Sachs’ Children’s Hospital,† Karolinska Institutet, SE 11669 Stockholm, Sweden, and Department of Clinical Pharmacology,*§ University of Bonn, DE 53105 Bonn, Germany, Department of Pharmacology and Toxicology,** and University of Texas Medical Branch, Galveston, TX 77555-1031

Abstract Infants with the cholesterol synthesis defect Smith-Lemli-Opitz syndrome (SLO) have reduced activity of the enzyme 7-dehydrocholesterol-7-reductase and accumulate 7-dehydrocholesterol, with the highest concentration in the brain. As a result of the generally reduced content of cholesterol, plasma levels of oxysterols would be expected to be reduced. 24S-hydroxycholesterol is almost exclusively formed in the brain, whereas 27-hydroxycholesterol is mainly formed from extrahepatic and extracerebral cholesterol. In accordance with the expectations, sterol-correlated plasma levels of 24S-hydroxycholesterol were reduced in infants with SLO (by about 50%). In contrast, the sterol-correlated levels of 27-hydroxycholesterol in the circulation were markedly increased. No side-chain oxidized metabolites of 7-dehydrocholesterol were detected in the circulation. Recombinant human CYP27 had markedly lower 27-hydroxylase activity toward 7-dehydrocholesterol than toward cholesterol. HEK293 cells expressing 24S-hydroxylase active toward cholesterol had no significant activity toward 7-dehydrocholesterol. The plasma levels of 3β,7α-dihydroxy-5-cholestenoic acid in the patients were reduced, suggesting a generally reduced metabolism of 27-oxygenated steroids. It is concluded that the accumulation of 7-dehydrocholesterol in the brains of patients with SLO reduces formation of 24S-hydroxycholesterol. The condition is associated with markedly increased circulating levels of 27-hydroxycholesterol, most probably due to reduced metabolism of this oxysterol. We discuss the possibility that the circulating levels of 24S-hydroxycholesterol may be used as a marker for the severity of the disease.

The Smith-Lemli-Opitz syndrome (SLO) is an autosomal-recessive, severely debilitating disorder caused by a block in the last enzymatic step in cholesterol biosynthesis (1–3). The mutations behind the disease have recently been defined (4, 5).

The affected infants have markedly reduced levels of cholesterol in tissues and plasma, combined with accumulation of 7- and 8-dehydrocholesterol. Because there is little or no transfer of cholesterol over the blood-brain barrier, and most of the cholesterol in the brain is formed by local synthesis, the accumulation of 7- and 8-dehydrocholesterol is higher in the brain than elsewhere in the SLO patients (3). Although it is possible to increase the ratio of cholesterol to dehydro-cholesterol in the circulation of SLO patients by dietary means (6, 7), it is unlikely that the diet can change the sterol composition in the brain.

24S-hydroxycholesterol is an oxysterol formed in the brain by a specific cytochrome P-450 species, CYP46 (8), and is able to pass the blood-brain barrier much more efficiently than cholesterol (9–11). We recently showed that most or all of the 24S-hydroxycholesterol present in human circulation originates from the brain (10). If 7- and 8-dehydrocholesterol are substrates for the cerebral cholesterol 24S-hydroxylase, the pattern of 24-hydroxylated products in the circulation of SLO infants would give direct information about the sterol composition in the brain. If CYP46 is unable to hydroxylate the dehydrocholesterols in the brain, and if these steroids dominate greatly over cholesterol, a reduced flux of 24S-hydroxycholesterol from the brain would be expected.

The circulating levels of 24S-hydroxycholesterol are affected not only by the flux of the steroid over the blood-brain barrier but also by the metabolic capacity of the liver. As the ratio of cerebral production to hepatic meta-
bolic capacity decreases during the first two decades of life, plasma concentrations of 24S-hydroxycholesterol decrease by a factor of about five during this period (12). Owing to the fact that 24S-hydroxycholesterol is carried in the circulation by the same lipoproteins as cholesterol, cholesterol and lipoprotein levels also affect the absolute levels of oxysterol in the circulation.

27-Hydroxycholesterol is another major oxysterol in human circulation. This steroid is formed by the action of sterol 27-hydroxylase, CYP27. This enzyme is involved in the formation of bile acids in the liver, but has a broad distribution in most tissues and organs. We have shown that there is a net flux of 27-hydroxycholesterol from extrahepatic sources to the liver (13), and most of the 27-hydroxycholesterol in the circulation seems to originate from extrahepatic sources (14). Whether or not 7- and 8-dehydrocholesterol are substrates for the sterol 27-hydroxylase in humans is not known. It has been reported, however, that the accumulation of 7- and 8-dehydrocholesterol in the livers of SLO patients may have an inhibitory effect on both cholesterol 7α-hydroxylase and sterol 27-hydroxylase activity, with possible consequences for bile acid biosynthesis (15).

The aim of the present work was to characterize the pattern of side-chain oxidized oxysterols in the circulation of SLO patients. In particular, we were interested to know whether or not 24- and/or 27-hydroxylated metabolites of 7- and 8-dehydrocholesterol are present and whether a quantitative analysis of the 24-hydroxylated steroids can provide information about the sterol composition in the brain.

MATERIALS AND METHODS

Subjects

The study included six Swedish patients with SLO, with ages varying from 2 to 12 years. All the patients had the typical biochemical findings, with low cholesterol levels (0.3–1.4 mmol/l) and similar levels of 7-dehydro cholesterol and 8-dehydrocholesterol (0.2–1.0 mmol/l) (Table 1). All patients were treated with sodium taurocholate and cholesterol (7) during at least part of the study period (Fig. 1). The treatment led to beneficial effects, some not correlated to changes in plasma sterols (7). One of the patients (Patient 1) has been reported on previously (7).

The study also included 59 control infants 1–15 years old (Fig. 1). Most were treated in the hospital for medical or surgical illnesses. In all cases the plasma had been collected for diagnostic purposes, and ethical permission was obtained to use excess plasma only for the present study. (Ethical permission to use excess plasma from the control subjects and the patients was obtained from the local ethical committee at Huddinge University Hospital.) Samples from a few apparently healthy, well-defined infants, who were hetero- or homozygotes with respect to sitosterolemia, were also included (n = 3 and 2, respectively), as well as nine infants with short-bowel syndrome, atroin-jejunum defect, chronic diarrhea, or Mb Crohn. The levels of 24S-hydroxycholesterol from the latter infants were similar to those of age-matched infants with an undefined medical or surgical illness. In addition, plasma was obtained from a number of infants (n = 16) with clinical findings similar to those of SLO patients but with normal sterol patterns in the circulation. Also in these cases, the levels of 24S- and 27-hydroxycholesterol were similar to those of the apparently healthy infants.

Analyses and analytical methods

The sterol composition in the SLO patients was measured by combined gas chromatography-mass spectrometry as described previously (7). Serum concentrations of cholesterol were measured by isotope dilution-mass spectrometry with the use of deuterium-labeled cholesterol as the internal standard, as described previously (16). Levels of 24S-hydroxycholesterol and 27-hydroxycholesterol were also assayed by isotope dilution-mass spectrometry after extraction and solid phase adsorption using racemic 23,24,24-dihydroxy-5-cholestenoic acid, re-

TABLE 1. Sterol patterns in SLO patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Cholesterol</th>
<th>7-Dehydrocholesterol</th>
<th>8-Dehydrocholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/ml</td>
<td>mg/ml</td>
<td>mg/ml</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>394</td>
<td>285</td>
<td>342</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>259</td>
<td>164</td>
<td>238</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>121</td>
<td>142</td>
<td>145</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>197</td>
<td>144</td>
<td>166</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>674</td>
<td>158</td>
<td>206</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>627</td>
<td>129</td>
<td>132</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>604</td>
<td>346</td>
<td>465</td>
</tr>
</tbody>
</table>

* All the subjects were treated at the time of collection of plasma.
sterol (m/z 456), 27-hydroxy-7-dehydrocholesterol (m/z 454), or 7α,27-dihydroxycholesterol (m/z 544). The general conditions for the chromatography were as described in detail previously (9–11). There was separation of the deuterium-labeled and unlabeled steroids on the column, allowing the determination of conversion of both unlabeled and deuterium-labeled cholesterol into 24S-hydroxycholesterol. The conversion of one steroid into another was calculated from the height of the peak in the ion chromatograms corresponding to the two steroids, using a factor correcting for the difference in the intensity of the two ions.

Incubations with recombinant human CYP27

Human recombinant CYP27 was incubated with adrenodoxin, adrenodoxin reductase, NADPH, and a sterol extract under the conditions previously described for studies on conversion of cholesterol into 27-hydroxycholesterol (19). The sterol extract was obtained from the plasma of an SLO patient and contained cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol in about equal concentrations. In addition, the system was incubated with pure cholesterol, pure 7-dehydrocholesterol, and a mixture of these compounds under the same conditions. An extract of each incubation was analyzed by combined gas chromatography-mass spectrometry as described above.

Expression of CYP46 in HEK293 cells and measurement of CYP46 enzymatic activity toward endogenous cholesterol and 7-dehydrocholesterol

Human Embryonic Kidney 293 cells (ATCC CRL 1573) were cultured in Minimum Essential Medium (MEM) Eagle (Sigma, St. Louis, MO), containing 10% horse serum (GIBCO, Täby, Sweden), 0.1 nm MEM nonessential amino acids (Sigma), sodium pyruvate (1 mM), and penicillin-streptomycin (GIBCO) (20). The cells were transfected with the cDNA of CYP46 (8) in pcDNA 3.1 vector using Tfx™-20 (Promega, Madison, WI) at approximately 60% confluence. The stably transfected cells were selected with 400 μg/ml Geneticin (Sigma). The cells were cultured in 100-mm tissue culture dishes (Falcon, Franklin Lakes, NJ) until approximately 70% confluent. The cells were then treated with 20 mg/ml 2-hydroxypropyl-β-cyclodextrin (Sigma) for 1 h to reduce the cholesterol content of the membranes (8). Serum in the medium was exchanged for charcoal-stripped deproteinized cell serum (Sigma) in this treatment. Approximately 5 x 10⁶ cholesterol-depleted cells were then incubated with 50 or 100 μg deuterium-labeled cholesterol, 50 μg cholesterol + 50 μg of 7-dehydrocholesterol, or 50 μg 7-dehydrocholesterol for 48 h at 37°C in an atmosphere of 5% CO₂ in 10 ml of the same medium as above. The added steroids were dissolved in 20 μl of ethanol. An extract of the incubation medium was purified by C-18 chromatography (7) and analyzed as described above.

Measurements of oxysterol 7α-hydroxylase activity in COS-7 cells

COS-7 cells were cultured as described previously (20) but without gentamicin. At 70% confluence the cells (about 5 x 10⁶ in each incubation) were incubated with 20 μg 27-hydroxycholesterol in 20 μl ethanol with or without addition of 50 μg deuterium-labeled cholesterol, 100 μg deuterium-labeled cholesterol, 50 μg 7-dehydrocholesterol, or 50 μg deuterium-labeled cholesterol + 50 μg 7-dehydrocholesterol. Incubations were performed at 37°C for 4 h in 10 ml of the medium (20) in an atmosphere of 5% CO₂. Medium was extracted as above, and the steroids were isolated by silicic acid chromatography as described (20). The steroid fraction was analyzed by combined gas chromatography-mass spectrometry as described above.

RESULTS

Absence of 24S- and 27-hydroxylated metabolites of 7-dehydrocholesterol and 8-dehydrocholesterol in the circulation

In view of the fact that the levels of 7-dehydrocholesterol and 8-dehydrocholesterol are similar to those of cholesterol in the SLO patients (Table 1), the possibility must be considered that the two dehydrocholesterols may be 24S- and/or 27-hydroxylated. However, no compounds with chromatographic and mass spectrometric properties, as expected for 24S- and 27-hydroxy derivatives of 7- and 8-dehydrocholesterol, could be found in the circulation of the SLO patients.

7-dehydrocholesterol is not a substrate for human cholesterol 24S-hydroxylase (CYP46) and is a considerably less efficient substrate than cholesterol for human sterol 27-hydroxylase (CYP27)

HEK293 cells transfected with CYP46 cDNA and depleted of cholesterol were found to have a relatively high 24S-hydroxylase activity toward added deuterium-labeled cholesterol (about 10% product recovered in medium) (8). When cholesterol was replaced with 7-dehydrocholesterol, there was no significant conversion (less than 0.5%).

A preparation of human CYP27 was incubated with adrenodoxin, adrenodoxin reductase, NADPH, and a sterol extract from the serum of a patient with SLO containing a mixture of cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol (Table 1). About 8.5% of the cholesterol was converted into the corresponding 27-hydroxylated product, whereas the conversion of the two dehydrocholesteryl terols was less than 0.5%. The same human CYP27 system was incubated with pure cholesterol (10 μg) or pure 7-dehydrocholesterol (10 μg). There was a clear formation of the 27-hydroxylated product of cholesterol (about 9.5%), and formation of a small amount of the 27-hydroxylated product of 7-dehydrocholesterol (about 1.8%). In another experiment, the CYP27 system was incubated with a mixture of cholesterol (10 μg) and 7-dehydrocholesterol (10 μg). In this case, too, the rate of 27-hydroxylation of cholesterol was about 5-fold higher than the hydroxylation of 7-dehydrocholesterol.

Low levels of 24S-hydroxycholesterol and high levels of 27-hydroxycholesterol in the circulation of SLO patients

Table 1 summarizes the sterol patterns in the circulation of SLO patients. All the patients had low levels of cholesterol, and had levels of 7-dehydrocholesterol and 8-dehydrocholesterol similar to the cholesterol levels.

Figure 1 and Table 2 summarize the results of the measurements of 24S-hydroxycholesterol in the SLO patients and in the controls. The levels are presented in relation to the levels of sterols in plasma in Fig. 1. The levels of 24S-hydroxycholesterol in plasma are highly correlated to the levels of cholesterol (and presumably other sterols as well) in plasma, most probably due to the fact that the oxysterols are distributed and transported in the lipoproteins in the same way as cholesterol is (12).
As in previous work (12), the sterol-correlated levels of 24S-hydroxycholesterol decreased with age. This decrease is probably due to the decreasing ratio of cerebral production to hepatic metabolism of the oxysterol. As in previous results (12), there was no sex-related difference in the sterol-correlated levels of 24S-hydroxycholesterol.

The sterol-correlated levels of 24S-hydroxycholesterol in the circulation of SLO patients were lower than those in the controls by about half.

Figure 2 summarizes the results of measurements of sterol-correlated levels of 27-hydroxycholesterol in the controls and in the SLO patients (absolute levels are given in Table 2). As in a previous investigation (9), there were few or no age-dependent variations in the controls or in the patients.

The sterol-correlated levels of 27-hydroxycholesterol were, however, markedly higher in the SLO patients than in the controls (2- to 11-fold).

Low levels of 3β,7α-dihydroxy-5-cholestenolic acid in SLO patients

The very high levels of 27-hydroxycholesterol in the plasma of the SLO patients may be due to reduced metabolism. The oxysterol 7α-hydroxylase (CYP7B) is known to be most important for the metabolism of 27-hydroxycholesterol as well as for cholestenolic acid (21, 22). The predominant metabolite formed by the action of this cytochrome P-450 is 3β,7α-dihydroxy-5-cholestenolic acid (23).

Adult control subjects (n = 17) were found to have a plasma concentration of this acid of 25 ± 6 ng/ml (mean ± SE), whereas the level was 22 ± 2 ng/ml in four control infants of the same age as the SLO infants. The level was found to be 9 ± 1 ng/ml in five SLO infants. The ratio of 3β,7α-dihydroxy-5-cholestenolic acid to cholestenolic acid was 0.40 ± 0.10 in the control subjects and 0.15 ± 0.03 in the infants with SLO.

Dilution of cholesterol with 7-dehydrocholesterol reduces formation of 24S-hydroxycholesterol

As reported above, HEK293 cells transfected with CYP46 cDNA were found to have a high 24S-hydroxylase activity toward added deuterium-labeled cholesterol (15–30% conversion of 50 μg of added 4H6-cholesterol). Addition of 50 μg 7-dehydrocholesterol reduced the above conversion by 20–40% (Table 3). Further addition of 50 μg cholesterol reduced the same conversion by 30–50%. It is evident that the slight inhibitory effect obtained by 7-dehydrocholesterol can be explained by the dilution of the substrate. However, no significant hydroxylation of 7-dehydrocholesterol was employed under any of the conditions.

7-dehydrocholesterol has no direct inhibitory effect on the enzymatic activity of oxysterol 7α-hydroxylase (CYP7B)

COS cells have a high endogenous oxysterol 7α-hydroxylase activity toward 27-hydroxycholesterol. Addition of 7-dehydrocholesterol to such cells had no significant ef-

![Fig. 2. Ratio 27-hydroxycholesterol/cholesterol in the circulation of 59 control infants (shown as means ± SEM) and seven SLO infants of varying ages. Measurements were made on one to three different occasions in the latter case.](image-url)
fect on this activity. In a typical experiment, the degree of conversion of 20 μg 27-hydroxycholesterol added to the COS cells was about 11% after incubation for 4 h. Addition of 50 μg 7-dehydrocholesterol did not significantly reduce the conversion (conversion was 10%). Addition of deuterium-labeled cholesterol, 50 and 100 μg, also had no effect on the oxygenated 7α-hydroxylase activity (conversion was about 10% in both cases).

DISCUSSION

What is the explanation for the high plasma levels of 27-hydroxycholesterol in SLO?

The most significant abnormality in the pattern of oxygenated steroids in the circulation of the SLO infants was the marked increase in the level of 27-hydroxycholesterol. In view of the high concentrations of 7- and 8-dehydrocholesterol in all tissues of patients with SLO, a reduced formation of 27-hydroxycholesterol would be expected, regardless of whether or not the dehydrocholesterols are substrates for the enzyme. It was clearly shown that 7- and 8-dehydrocholestrols are 27-hydroxylated by the human sterol 27-hydroxylase at a rate considerably lower than that with cholesterol as substrate. The high levels of 27-hydroxycholesterol may be explained by an increased synthesis or a decreased metabolism.

An increased upregulation of the sterol 27-hydroxylase cannot be completely excluded, but it seems unlikely. However, experiments supported the hypothesis of a reduced metabolism of 27-oxygenated steroids in SLO. Oxygenated 7α-hydroxylase (CYP7B) has a key role in the metabolism of both 27-hydroxycholesterol and cholestenolic acid, and both these compounds were found to accumulate in an infant with a defective oxygenated 7α-hydroxylase (22). It was recently reported that mice with a disruption of the oxygenated 7α-hydroxylase gene had markedly elevated levels of 27-hydroxycholesterol in the circulation (24). CYP7B catalyzes conversion of 27-hydroxycholesterol into 7α,27-dihydroxycholesterol, as well as cholestenolic acid into 3β,7α-dihydroxy-5-cholestenolic acid. 7α,27-dihydroxycholesterol is not normally detectable in the circulation, whereas 3α,7β-dihydroxy-5-cholestenolic acid is present in human circulation in relatively high concentrations (18, 23). In view of this we believe that the ratio of 3β,7α-dihydroxy-5-cholestenolic acid to cholestenolic acid may function as a marker for the in vivo activity of CYP7B in humans. This ratio was markedly decreased in the SLO infants.

It should be mentioned that we have previously observed one adult with markedly elevated levels of 27-hydroxycholesterol combined with a very low ratio of 3β,7α-dihydroxy-5-cholestenolic acid to cholestenolic acid. Analysis of a new plasma sample from this subject some years later showed normal levels of 27-hydroxycholesterol and a normal ratio of 3β,7α-dihydroxy-5-cholestenolic acid to cholestenolic acid (I. Björkhem and A. Babiker, unpublished observation). A likely explanation is that there was a transient decrease in the activity of the CYP7B in this case, resulting in accumulation of 27-hydroxycholesterol in the circulation. The reason for the decrease in activity of CYP7B could not be defined, but the observation supports the contention that there may be a link between low levels of 3β,7α-dihydroxy-5-cholestenolic acid and 27-hydroxycholesterol in humans.

The possibility was tested here that direct exposure of the CYP7B system to 7-dehydrocholesterol could reduce the activity toward 27-hydroxycholesterol. Addition of 7-dehydrocholesterol to HEK293 cells expressing CYP7B did not significantly reduce 7α-hydroxylase activity toward added 27-hydroxycholesterol. The experimental conditions, however, were different from those occurring in vivo with endogenous dehydrocholesterol species integrated in the endoplasmic reticulum. Thus it is difficult to draw firm conclusions from this experiment.

It may be mentioned here that the cholesterol 7α-hydroxylase (CYP7A) involved in bile acid biosynthesis has been shown to be competitively inhibited by 7- and 8-dehydrocholesterol (15).

Factors affecting plasma levels of 24S-hydroxycholesterol in SLO

Tint et al. have reported that the sterol fractions of the brains of a 1-day-old deceased infant and a 20-week fetus, both with clinical features of the most severe form of SLO, contained less than 5% cholesterol (3). In contrast, the sterol fraction from their non-brain tissues contained about 20% cholesterol. The SLO infants studied here, both treated and untreated, had cholesterol content in their plasma sterols varying between 30% and 60%. In view of the fact that there is little or no transport of cholesterol over the blood-brain barrier, the cholesterol content of the sterols in the brain should be considerably lower than that of plasma in our SLO infants.

In view of this, it is surprising that the circulating levels of 24S-hydroxycholesterol were reduced by only about 50%. Our experiments with CYP46 showed that addition of 7-dehydrocholesterol had a slight inhibitory effect on 24S-hydroxylation of cholesterol, an effect that could be explained by the dilution of the substrate. The CYP46 enzyme had no activity toward 7-dehydrocholesterol, and we could not find any 24S-hydroxylated 7-dehydrocholesterol in the circulation of the SLO infants. It is evident that the cholesterol 24S-hydroxylase has a relatively high substrate specificity.

The relatively moderate reduction in circulating levels of 24S-hydroxycholesterol in the SLO infants may be due to an interference with the metabolism. We have shown that CYP7A has at least some enzymatic activity toward 24S-hydroxycholesterol (26). Because the activity of CYP7A is likely to be reduced in SLO (15), this may, in part, explain the relatively modest decrease in the levels of 24S-hydroxycholesterol. Very recently, a new species of cytochrome P-450 was detected that had a high specificity toward 24S-hydroxycholesterol (27). This cytochrome P-450, CYP39A, has a similarity to CYP7A of about 83%. The possibility that this enzyme may also be affected in SLO must be considered.
Is it possible to use reduction in the levels of 24S-hydroxycholesterol as a marker for the severity of the disease?

Cholesterol 24S-hydroxylase seems to be localized almost exclusively in neurons in the normal brain (8), whereas the product, 24S-hydroxycholesterol, has a distribution similar to that of cholesterol. We recently showed that patients with severe neurological diseases associated with reduction in the number of neurons have reduced levels of 24S-hydroxycholesterol in the circulation (28). During the phase characterized by destruction of the neurons and demyelination, however, a transient slight increase in the level may occur (29). In patients with SLO, the circulating sterol-correlated levels of 24S-hydroxycholesterol may reflect both reduced availability of substrate cholesterol in the brain and a reduced metabolism. The former factor would tend to reduce the circulating levels and the latter factor to increase them. In preliminary attempts to relate the level of 24S-hydroxycholesterol to the clinical severity of the disease, we have not seen any clear correlations. Thus we conclude that the plasma level of 24S-hydroxycholesterol alone is probably not suitable as a marker for the severity of the disease.

The skillful technical assistance of Mrs. Anita Lövgren Sandblom is gratefully acknowledged. This work was supported by The Swedish Medical Research Council, The Swedish Heart-Lung Foundation, The Strategic Foundation, and The European Community (PL 963191). We are grateful to Professor D. W. Russell and Dr. E. G. Lund for a generous gift of CYP46 cDNA.

References