CURRENT TOPIC

Disorders of cholesterol biosynthesis

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Functions of cholesterol

Sterols are important constituents of the cell membranes of most eukaryotic cells. The cell membranes of terrestrial vertebrates, including man, contain a single major sterol species—cholesterol. Cholesterol is found particularly in external cellular membranes (plasma membranes) and in the layers that make up the myelin sheaths in the central and peripheral nervous systems. In plasma membranes, the cholesterol molecules are intercalated between the phospholipid molecules of each monolayer and reduce the movement of their acyl chains (reduced “membrane fluidity”). Sterols also exert a direct effect on proteins in the membrane. For example, the function of the human red cell hexose transporter is profoundly affected by the content of cholesterol in the membrane and this cannot be related to changes in fluidity.¹ It has been discovered recently that cholesterol has important interactions with proteins which control embryonic development—the hedgehog proteins.² During biosynthesis, these proteins catalyse their own cleavage and the subsequent attachment of cholesterol to the amino terminal domain. This has a profound effect on the range of action of these signalling molecules.^{3,4} In the mouse, the absence of a functional gene for one of the hedgehog proteins (sonic hedgehog) results in multiple malformations including holoprosencephaly.² Holoprosencephaly in mice can also be caused by giving them an inhibitor of cholesterol synthesis, AY9944. In addition to its functions in cell membranes and in development, cholesterol acts as the precursor for the steroid hormones and bile acids. The bile acids, in turn, are necessary for efficient absorption of dietary lipids, including cholesterol and the fat soluble vitamins.

Given these functions of cholesterol, it is not surprising that disorders of cholesterol biosynthesis can lead to syndromes featuring major malformations, low maternal oestriol concentrations, failure of development of male external genitalia due to testosterone deficiency, and mental retardation associated with hypomyelination of the central nervous system.

Sources of cholesterol

The cholesterol present in a particular tissue has either been synthesised *de novo* in the cells of that tissue, or was derived from circulating lipoprotein cholesterol. For the organism as a whole the two possible sources are synthesis and diet (or placental transfer in the fetus).

Studies with labelled cholesterol indicate that, although as much as 20% of fetal cholesterol may be derived from the mother at the end of the first trimester, very little maternal cholesterol enters the fetal brain.^{5,6} If the mother is given labelled glucose, the label does appear in fetal brain cholesterol and glucose is thought to be the most important fuel for *de novo* cholesterol synthesis in the fetal brain. In postnatal life, a high cholesterol diet will lead to reduced synthesis of cholesterol in the liver. This occurs principally as a result of inhibition of the rate limiting step, 3-hydroxy-3-methyl-CoA (HMG-CoA) reductase, by cholesterol. In extrahepatic tissues, the uptake of lipoprotein cholesterol switches off cholesterol synthesis in the same way. Experiments in rodents with labelled cholesterol have suggested that very little lipoprotein cholesterol enters the brain,⁵ but there are at least some brain cells (microvascular endothelial cells, glial cells) which, in culture, respond to lipoprotein in the medium with a reduction in *de novo* cholesterol synthesis.^{7,8}

Biosynthesis of cholesterol

Cholesterol is synthesised from acetyl-CoA. In the brain this is derived largely from glucose, but in other tissues fatty acids and other fuels contribute. Figures 1 and 2 show a simplified version of the pathway. Until recently, the prevailing view has been that the reactions take place in the cytoplasm or endoplasmic reticulum, but there is now accumulating evidence to suggest that the early steps in the pathway at least occur in the peroxisomes.⁹ The early steps in the pathway are also required for the synthesis of non-sterol isoprenes—*isopentenyl-tRNAs*, dolichol, ubiquinone, and haem A. The *isopentenyl* groups in *tRNAs* are thought to be important in stabilising codon-anticodon interaction, thus preventing misreading of the genetic code during protein synthesis. Dolichol is required for the synthesis of glycoproteins, and ubiquinone and haem A are important components of the mitochondrial respiratory chain.

The conversion of lanosterol to cholesterol in the later stages of the pathway probably occurs via two routes: in one, the Kandutsch-Russell pathway, the immediate precursor of cholesterol is 7-dehydrocholesterol; in the second pathway, the immediate precursor of cholesterol is desmosterol. It is thought that these pathways may be independently regulated but that they share many enzymes and a

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Figure 1 Pathway for the synthesis of cholesterol and non-sterol isoprenes. HMG-CoA reductase is the rate limiting step and the major site of feedback inhibition. Mevalonic aciduria is caused by a defect in mevalonate kinase.

deficiency of one of these enzymes will seriously disrupt cholesterol synthesis. A number of the reactions require the participation of a sterol carrier protein.

Disorders affecting the synthesis of lanosterol from HMG-CoA

MEVALONIC ACIDURIA

Mevalonic aciduria (mevalonate kinase deficiency) was the first disorder of cholesterol synthesis to be recognised, and approximately 15 patients have been reported.¹⁰⁻¹² The disorder is characterised by developmental delay, failure to thrive, hypotonia, ataxia, hepatosplenomegaly, cataracts, lymphadenopathy, anaemia, myopathy, and enteropathy with fat malabsorption. In addition, many patients have recurrent febrile crises accompanied by vomiting, diarrhoea, an increased white cell count and erythrocyte sedimentation rate, and, in some cases, arthralgia, oedema, and a morbilliform rash. The dysmorphic features which have been described include microcephaly, dolicocephaly, a large fontanelle, a triangular facies with down slanted eyes and long eyelashes and low set, posteriorly rotated ears. Mild cases exist in which the only abnormal features include microcephaly, developmental delay, and recurrent fever with diarrhoea and vomiting.¹³ Routine investigations may reveal anaemia, increased transaminases, and periodic increases in creatine kinase. Serum cholesterol is normal or slightly reduced. Neuroimaging often shows progressive cerebellar atrophy. Electroencephalography may be normal or show general slowing. Biopsy specimens from the liver, muscle, and duode-

num are unremarkable. The diagnosis is made by analysing organic acids in urine by gas chromatography-mass spectrometry. This can be followed up by measuring the activity of mevalonate kinase in cultured skin fibroblasts. Prenatal diagnosis has been achieved by measurement of mevalonic acid in amniotic fluid and by measurement of mevalonate kinase activity in amniocytes and chorionic villus samples.

The pathogenesis of mevalonic aciduria is not well understood. Accumulation of mevalonic acid may have some important effects, but it is likely that deficiency of cholesterol and other products of the biosynthetic pathway play a part. Cholesterol supplementation has been attempted but led to worsening diarrhoea and general malaise. A cocktail of cholesterol, ursodeoxycholic acid, ubiquinone, and vitamin E also failed to lead to detectable clinical improvement. Suppression of mevalonic acid production by the use of the HMG-CoA reductase inhibitor, lovastatin, has also been tried, but the trials had to be aborted because of the development of severe clinical crises with fever, acute myopathy, worsening ataxia, diarrhoea, and vomiting. Steroids have been used during acute crises. Prednisone (2 mg/kg/day) led to dramatic improvement in symptoms, and there was a suggestion that growth and psychomotor development may also be improved by intermittent steroid treatment.

PEROXISOMAL DISORDERS

Patients with disorders of peroxisome biogenesis, for example Zellweger's syndrome and infantile Refsum's disease, often have low plasma cholesterol concentration. There are probably multiple reasons for this. The peroxisomal contribution to cholesterol synthesis may be impaired by the absence of the organelle and by low concentrations of sterol-carrier protein 2. There is probably also malabsorption of cholesterol due to defective bile acid secretion. These patients often have cirrhosis and this too may be associated with low plasma cholesterol.

Disorders affecting the conversion of lanosterol to cholesterol

SMITH-LEMLI-OPITZ SYNDROME

Smith-Lemli-Opitz (SLO) syndrome was first described in 1964 as a syndrome of mental retardation and multiple malformations. The biochemical basis became apparent when Tint and coworkers discovered that plasma and tissue samples from these patients contained reduced amounts of cholesterol and vastly increased amounts of 7-dehydrocholesterol together with two other unusual sterols subsequently identified as 8-dehydrocholesterol and 19-nor-5,7,9(10)-cholestatrien-3 β -ol.¹⁴⁻¹⁶ The logical explanation for this was that children with SLO syndrome had a defect in the conversion of 7-dehydrocholesterol to cholesterol, which is catalysed by the enzyme 7-dehydrocholesterol reductase (3 β -hydroxysteroid- Δ^7 -reductase). This was subsequently shown in liver microsomes and in cultured skin

Figure 2 First and last steps in the major pathways for the conversion of lanosterol to cholesterol. The two pathways probably share many enzymes. Hence inborn errors of metabolism such as desmosterolosis and SLO syndrome disrupt both routes of cholesterol synthesis.

fibroblasts.^{17, 18} The discovery of the biochemical defect in SLO syndrome has allowed clinicians to prove this diagnosis in individual patients and to build up an accurate picture of the range of clinical features that can result from 7-dehydrocholesterol reductase deficiency. In a survey of 49 biochemically proved cases from the UK, Ryan *et al* found that the most common dysmorphic features were genital abnormalities in males ranging from hypospadias to ambiguous genitalia (91%), bilateral 2/3 syndactyly of the toes (81%), microcephaly (80%), palatal abnormalities (75% including 37% with a complete or posterior cleft palate), anteverted nares (69%), micrognathia (67%), blond hair (65%), polydactyly (53%), and low set ears (47%) (Ryan AK, Bartlett K, Clayton P, *et al*; unpublished observations). All but three patients had severe feeding difficulties in infancy requiring nasogastric or gastrostomy feeds. Other gastrointestinal problems included pyloric stenosis in four of the 49 patients, Hirschsprung's disease in four, and constipation in 11. Congenital heart disease was present in 37% of cases (the most common lesions being atrioventricular septal defect and patent ductus) and renal abnormalities were present in 29% ([hypoplastic], cystic kidneys

being the most common). Cutaneous photosensitivity was also common. All patients had some degree of mental retardation, the majority having severe learning difficulties. Similar figures have been obtained in a large series of patients in the USA, who are biochemically confirmed to have SLO.^{19, 20} In the 80 patients reported by Cunniff *et al*, the best biochemical predictor of the clinical severity was the plasma cholesterol concentration at diagnosis, which was lowest in the most severely affected children.²⁰

In an infant with clinical features indicative of the severe form of 7-dehydrocholesterol reductase deficiency (SLO syndrome), the plasma/serum cholesterol measured by the standard cholesterol oxidase method is usually low and this can be an important pointer to the diagnosis. It is important to remember, however, that this method actually measures cholesterol plus 7-dehydrocholesterol plus 8-dehydrocholesterol and therefore definitive diagnosis requires quantitation of the individual sterols in plasma by gas chromatography-mass spectrometry.

When a diagnosis of SLO syndrome has been confirmed by finding greatly increased concentrations of 7-dehydrocholesterol in the plasma, tissues, or fibroblasts of a child (or of a stillborn infant or fetus), prenatal diagnosis in subsequent pregnancies can be performed quickly and reliably by analysis of the 7-dehydrocholesterol/cholesterol ratio in a chorionic villus biopsy specimen or in a sample of amniotic fluid.^{21, 22} This investigation should also be considered if maternal oestriol concentrations are very low, or if antenatal ultrasound shows features such as increased nuchal translucency, ambiguous genitalia, polydactyly, or other features consistent with SLO syndrome.²³

The gene responsible for SLO syndrome is probably the gene coding for the enzyme 3 β -hydroxysteroid- Δ^7 -reductase rather than a gene coding for an activating protein such as sterol carrier protein 2.²⁴ The gene has not yet been identified but it has been mapped to 7q32.1.²⁵

DESMOSTEROLYSIS

The elucidation of the biochemical basis of SLO syndrome has led to a search for accumulation of precursors of cholesterol in tissues from children with similar malformation syndromes. As a result, a female infant who died shortly after birth at 34 weeks' gestation was found to have marked accumulation of desmosterol in the brain, liver, and kidneys: a finding consistent with a defect in sterol- Δ^7 -reductase.²⁶ This infant had macrocephaly with frontal bossing, a hypoplastic nasal bridge, thickened alveolar ridges, gingival nodules, a cleft palate, short limbs, ambiguous genitalia, hypoplastic lungs, total anomalous pulmonary venous drainage, splenomegaly, a shortened and unrotated gut, and bilateral renal hypoplasia. Radiology showed generalised osteosclerosis.^{26, 27}

OTHERS

We have performed tissue sterol analyses on a stillborn infant with polydactyly, bilateral renal agenesis, and testicular tissue associated with female external genitalia (Clayton PT, Mills KA, Barrow M, *et al*; unpublished observations). Gas chromatography-mass spectrometry showed increased amounts of 8-dehydrocholesterol without any increase in 7-dehydrocholesterol. The biochemical basis of these findings has not yet been elucidated.

Drugs which inhibit the conversion of lanosterol to cholesterol; animal models for human disease

The conversion of lanosterol to cholesterol can be blocked by a number of drugs; these agents were soon found to be powerful teratogens. One of the first to be studied was AY9944, an inhibitor of the enzyme which is defective in SLO syndrome—7-dehydrocholesterol reductase. When given to pregnant rats on the second, third, and fourth days of gestation it leads to malformations, the most frequent of which is pituitary agenesis, which may be associated with holoprosencephaly, reduced brain weight, and reduced myelination.²⁸ Other features of the drug induced syndrome include nephrotic renal disorders, cryptorchidism, clubfoot, cleft lip, maxillary and mandibular hypoplasia, and reduced head size.¹⁴ If the pregnant rats are given a cholesterol supplement with the AY9944 on day four, and if the cholesterol is continued until day 15, the malformations can be almost entirely prevented.²⁸

The enzyme, 7-dehydrocholesterol reductase, is also inhibited by BM 15.766, a synthetic hydrophobic pyridine derivative. This drug has been used to study the effects of cholesterol supplementation in adult rats.²⁹ Cholesterol alone led to a 3.7-fold increase in plasma cholesterol, a reduction in plasma 7-dehydrocholesterol, and a fall in HMG-CoA reductase activity and mRNA. Cholic acid increased plasma cholesterol concentrations without reducing plasma 7-dehydrocholesterol. The combination of cholic acid and cholesterol produced a 9.5 fold increase in cholesterol without reducing 7-dehydrocholesterol. Recently the pathogenesis of the teratogenic effect of BM15.766 in the rat has been studied by Dehart *et al*.³⁰ On the 11th day of gestation, scanning electron microscopy reveals abnormal cell populations at the rim of the developing forebrain and in the lower alar plate of the lower midbrain and hindbrain. The affected cells appear to have lost their normal cell contacts. Dehart *et al* suggest that reduced membrane cholesterol leads to increased membrane fluidity in affected cells and also to reduced cell to cell adhesion, and that these effects may be responsible for failure of normal organogenesis.

An animal model of desmosterolosis can be created using triparanol which inhibits the sterol Δ^{24} -reductase. This drug is teratogenic in rats producing facional dysplasia, renal anomalies, anophthalmia, and neural tube defects.

Treatment of SLO syndrome with cholesterol

Before the biochemical basis of SLO syndrome was understood, it was well recognised that a significant proportion of infants with SLO syndrome had a major feeding problem associated with marked failure to thrive and irritability. Some of these infants showed an improvement in weight gain when the calorie density of their feeds was increased; others showed remarkable catch up weight gain after nasogastric or gastrostomy feeds were begun. When fed adequately, these infants were less irritable. The fact that such improvements can be achieved by correcting malnutrition must be borne in mind when assessing the results obtained with other dietary manipulations.

In 1994, Irons *et al* described the effects of supplementation with cholesterol (20–40 mg/kg/day), ursodeoxycholic acid (15 mg/kg/day), and chenodeoxycholic acid (7 mg/kg/day) in a 14 month old girl with SLO syndrome.³¹ There was a clear increase in the plasma cholesterol, but no fall in the plasma concentration of 7-dehydrocholesterol. Ullrich *et al* described two patients, both of whom received 30–70 mg/kg/day of cholesterol, and one of whom also received 15 mg/kg/day of cholic acid, chenodeoxycholic acid, and ursodeoxycholic acid.³² Normalisation of serum cholesterol was achieved within six months, but there was no discernible effect on growth, motor function, and developmental progress. It is now well established that in most children with SLO syndrome it is possible to increase the plasma cholesterol by increasing the cholesterol content of the diet, although this may require a cholesterol supplement of up to 100–125 mg/kg/day.^{33–35} Ursodeoxycholic acid does not appear to boost the effect of supplemental cholesterol and may indeed inhibit it. Chenodeoxycholic acid appears to be helpful in increasing the plasma cholesterol, but it may cause rises in transaminases. What is still unclear is whether cholesterol supplementation has a beneficial effect on the course of the disease. The numbers that have been treated are insufficient to allow statistical evaluation of the results, but in individual cases, a range of beneficial effects have been described. These can be classified as (1) anthropometric: improved weight gain, improved linear growth, improved growth of head circumference, increase in genital size, and induction of puberty in teenage males; (2) alleviation of physical symptoms: improvement in constipation or diarrhoea, diminution in the severity of photosensitivity and eczema, diminution in the frequency and severity of infections; (3) behavioural: less self injury, increased attention span, increased sociability, less irritability; and (4) developmental: two children (over the age of 10 years) who learned to walk, others who showed improved language or signing skills.

Not all children show a positive response to cholesterol supplementation; some show no change, and we have observed one infant whose parents requested that we stop the treatment because he was more irritable (Collins JE, Clayton PT; unpublished observations). It

is clear that carefully constructed trials are required to delineate what benefits can really be expected when children of different ages and with different severity scores are treated with oral cholesterol.

It is quite likely that lack of cholesterol and/or accumulation of 7-dehydrocholesterol in the brain in utero and in early infancy produce irreversible effects on brain growth and development; most children with SLO syndrome are microcephalic at birth. At the 1997 SLO syndrome conference in Boston, Mira Irons and coworkers presented data on an infant treated with cholesterol in utero. The infant was given lipoprotein cholesterol via the umbilical vein and intraperitoneally, and this led to a rise in plasma cholesterol. Unfortunately, the child still showed the features of a severe form of SLO syndrome at birth. Jira *et al* have treated a 3 month old infant with a combination of exchange transfusion and an HMG-CoA reductase inhibitor. This led to a marked reduction in the 7-dehydrocholesterol/cholesterol ratio in plasma and red cell membranes. There appeared to be an improvement in developmental progress.³⁶ It may be that, at least in young infants with SLO syndrome, significant amounts of lipoprotein cholesterol can enter the brain. In this context, an observation by Ness *et al* is of interest.³⁷ They showed that, in the brain of an infant who died of SLO syndrome at 2 months, immunocytochemical analysis revealed a remarkable increase in the expression of low density lipoprotein receptors. These results indicate an induction of these receptors in the brain, presumably as a result of reduced endogenous cholesterol synthesis.

Much remains to be learned about the role of cholesterol in cell membranes, organogenesis, and brain function. The study of inborn errors of cholesterol biosynthesis, however, has already reinforced a message that emerged with the study of peroxisomal disorders, that is that biochemical analyses have an important part to play in the investigation of infants who display dysmorphic features, malformations of internal organs, and developmental delay. It is no longer sufficient to look for chromosomal abnormalities and for evidence of intrauterine infection in such infants.

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