3-Methylglutaconic Acidemia in Smith-Lemli-Opitz Syndrome

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ABSTRACT

The branched-chain organic acid, 3-methylglutaconic acid, is an intermediate (as the CoA thioester) in the leucine degradative pathway as well as the mevalonate shunt, a pathway that links isoprenoid metabolism with mitochondrial acetyl-CoA metabolism. Because the majority of patients with abnormal 3-methylglutaconic aciduria or acidemia appear to have normal leucine metabolism, we have speculated that some patients with 3-methylglutaconic aciduria may have defects of polyisoprenoid or sterol biosynthesis leading to overflow of isoprenoid precursors to 3-methylglutaconate via the mevalonate shunt. We therefore measured plasma levels of 3-methylglutaconic acid in patients with a known defect of sterol biosynthesis, Smith-Lemli-Opitz syndrome, and found that the patients with the lowest cholesterol levels had abnormally increased plasma levels of 3-methylglutaconic acid, similar in magnitude to those of patients with idiopathic 3-methylglutaconic aciduria. This finding suggests that some patients with unexplained 3-methylglutaconic aciduria may have defects of isoprenoid or sterol biosynthesis underlying their abnormal organic aciduria. (*Pediatr Res* **37: 671–674, 1995**)

Abbreviations
3MGC, 3-methylglutaconic acid
SLOS, Smith-Lemli-Opitz syndrome
7DHC, 7-Dehydrocholesterol
HMG, 3-hydroxy-3-methylglutarate

Although 3MGC is best known as a CoA intermediate in the catabolism of L-leucine, the urinary excretion of 3MGC appears to be independent of the metabolism of leucine in the majority of patients with abnormal 3MGC-aciduria (1, 2). Except in several patients with mild neurologic abnormalities caused by a genetic deficiency of 3-methylglutaconyl-CoA hydratase (3, 4), provocative testing including fasting, oral loading with L-leucine, and high protein diets have had little effect on the excretion of 3MGC, even in patients with 50- to 100-fold increased excretion of 3MGC. And whereas a few patients with excessive 3MGC aciduria have been found to have a primary defect of mitochondrial energy metabolism (5, 6), most searches for a specific metabolic lesion in patients with 3MGC aciduria prove fruitless.

Because patients with the syndrome of X-linked cardiomyopathy and neutropenia (Barth syndrome) have both increased 3MGC aciduria and hypocholesterolemia (1), we have speculated that some idiopathic syndromes associated with 3MGC aciduria may be caused by defects of sterol or isoprenoid

metabolism, causing overflow of mevalonate carbon through the mevalonate shunt pathway to 3MGC (Fig. 1). In the mevalonate shunt first proposed in 1974 by Edmond and Popjak (7), dimethylallyl pyrophosphate is dephosphorylated to the free alcohol, oxidized in two steps to 3-methylcrotonic acid, and then activated with CoA to form 3-methylcrotonyl-CoA, the precursor of 3MGC-CoA in the normal leucine catabolic pathway. Other plausible links between the two pathways at the level of higher order isoprenoids, such as geraniol and farnesol, have been described by Schroepfer (8). Also possible is formation of 3MGC-CoA by dehydration of HMG-CoA, either spontaneously or by reverse activity of 3MGC-CoA hydratase. Physiologic evidence that the mevalonate shunt or a related shunt pathway is significant in humans and other mammals has been provided by several laboratories (9–12), but whether the 3MGC in some patients with abnormal 3MGC-aciduria derives from isoprenoid precursors is unknown.

The well-known congenital malformation syndrome, SLOS (13), has recently been found to have a block in cholesterol biosynthesis at the level of 3β-hydroxysteroid-Δ^7-reductase, the terminal enzyme of the Kandutsch-Russell (14) pathway for synthesis of cholesterol (15). The unusually high plasma levels of 7DHC found in patients with SLOS suggested to us that if 3MGC were a product of a mevalonate shunt pathway, then the high levels of cholesterol precursors likely to occur in
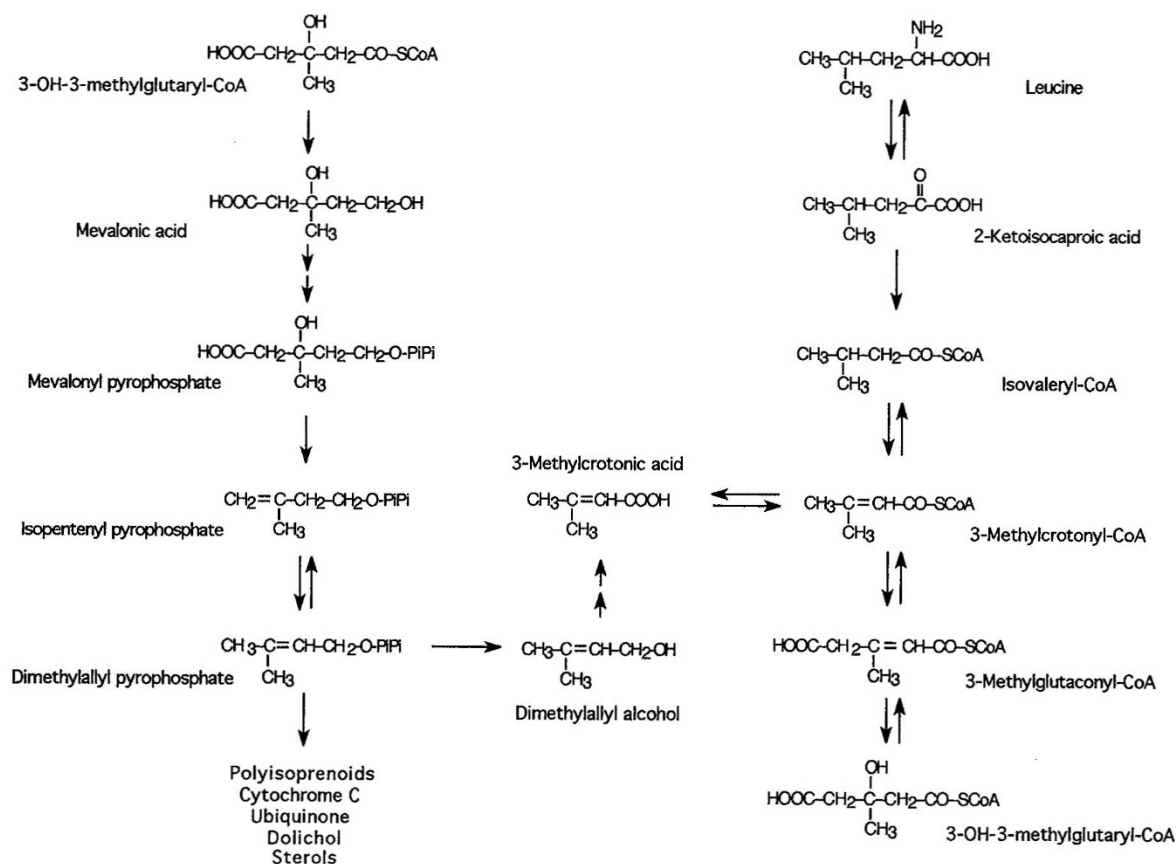
Figure 1. Pathways for isoprenoid biosynthesis and leucine catabolism showing the proposed mevalonate shunt linking the two pathways at the level of 3-methylcrotonyl-CoA. The diagram indicates two separate pools of 3-hydroxy-3-methylglutaryl-CoA, one for the microsomal/peroxisomal isoprenoid pathways (left) and one for the mitochondrial leucine catabolic pathway (right).

SLOS might lead to increased amounts of 3MGC by "overflow" of isoprenoids through the mevalonate shunt. Accordingly, we decided to measure the level of 3MGC in the plasma of a large number of patients with SLOS and 7-dehydrocholesterolemia of varying severity.

METHODS

Heparinized plasma samples originally submitted for biochemical diagnosis of SLOS were recovered from freezer ($-20°C$) storage. All 42 patients with SLOS in the study had abnormally increased levels of 7DHC, which varied from 20 to 1000 times normal (16). Patients with both clinically defined extremes of severity of SLOS—classic type I (13) and severe type II (17)—as well as patients of intermediate severity were represented in the study sample. All except two teenage patients with SLOS were less than 10 y old. Reference plasma specimens were archived samples from either sibling controls of patients with disorders other than SLOS or patients ultimately determined not to have a recognizable metabolic disease.

The levels of plasma sterols in patients with SLOS were measured by a standard ion-ratio gas chromatography/mass spectrometry technique, with minor modification, using 20-μL plasma samples and epicoprostanol as the internal standard (18, 19). The level of 3MGC in plasma was measured by stable isotope-dilution gas chromatography/mass spectrometry in 400-μL samples using 3-$[2,4,6-^{13}C_3]$methylglutaconic acid as the diluting standard, as described previously (20). For seven patients with SLOS, insufficient plasma for quantification of 3MGC remained after completing the initial diagnostic sterol analysis.

RESULTS

Figure 2A shows the relationship between the plasma levels of 7DHC and cholesterol for children with SLOS. The level of 7DHC ranged from 2 to 470 μg/mL (normal $\pm$ SD = 0.10 $\pm$ 0.05 μg/mL; n = 52). In general, children with SLOS who were on diets containing moderate to large amounts of cholesterol had normal or low normal plasma levels of cholesterol and lower levels of 7DHC than children fed proprietary formulas, most of which contain very little cholesterol. The inverse relationship between the level of 7DHC and that of cholesterol suggests increased endogenous synthesis of 7DHC when plasma levels of cholesterol are low. When the same plasma specimens were assayed for 3MGC, a similar inverse relationship between the plasma levels of 3MGC and cholesterol was found (Fig. 2B). As the plasma level of cholesterol decreased, the level of 3MGC increased, reaching abnormally high levels at cholesterol levels below 200 μg/mL. The highest plasma levels of 3MGC in the SLOS patients were similar in magnitude to those of patients with Barth syndrome (1) or other idiopathic forms of 3MGC aciduria, whose levels of
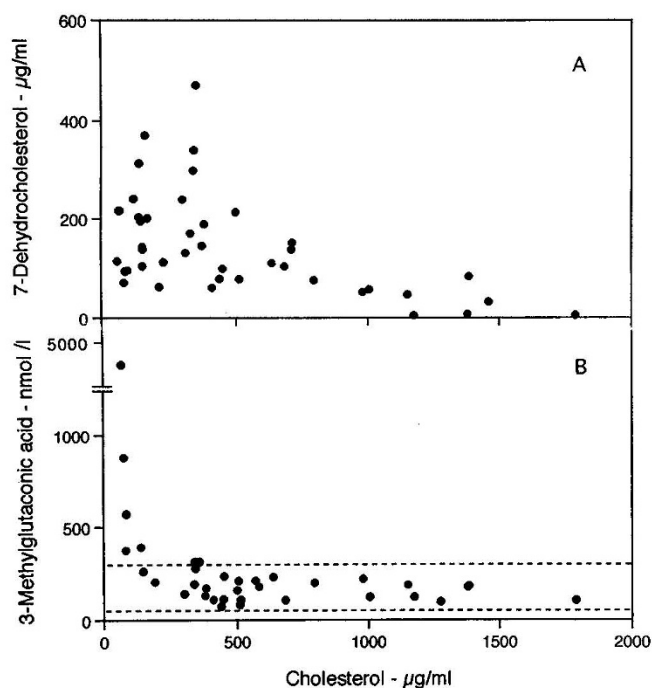
Figure 2. *A*, Relationship of plasma levels of cholesterol *vs* 7DHC in 42 patients with SLOS. *B*, Relationship of plasma levels of cholesterol *vs* 3MGC in 35 patients with SLOS. The horizontal broken lines demarcate normal mean ± 2 SD for levels of 3MGC in plasma, age 0–10 y (20).

3MGC in plasma typically range from 500 to 2000 nmol/L during infancy and early childhood (20). Although the younger, more severely affected SLOS patients with lower cholesterol levels had the highest levels of 3MGC, there was no correlation between age and the level of 3MGC, either in the SLOS patients or normal controls (20). Urine specimens from several SLOS patients with high plasma levels of 3MGC were also found to contain abnormally high levels of 3MGC, but not increased levels of 3-hydroxyisovaleric acid, which are associated with forms of 3MGC-aciduria caused by abnormal leucine catabolism (3).

DISCUSSION

The results presented here are consistent with the hypothesis that some patients with idiopathic 3MGC aciduria suffer from inborn errors of sterol or polyisoprenoid biosynthesis leading to overflow of precursor metabolites through the mevalonate shunt or other linking pathway to 3MGC (Fig. 1). Theoretically, because HMG-CoA synthase can be induced by low cholesterol levels (21), the increased levels of 3MGC in patients with SLOS and hypocholesterolemia could also be caused by increased levels of HMG-CoA which is then dehydrated to 3MGC-CoA by reversal through 3-methylglutaconyl-CoA hydratase. However, in the absence of evidence for a cytoplasmic form of 3MGC-CoA hydratase, this mechanism would require exchange between the cytoplasmic and mitochondrial pools of HMG-CoA. Although we have not found abnormal proportions of cholesterol and its neutral sterol precursors in any of our patients with idiopathic 3MGC-aciduria (Kelley R, unpublished observations), there remain numerous other possible defects of polyisoprenoid, dolichol, ubiquinone,

or bile acid biosynthesis that could lead to increased levels of precursors in the pathway and, thereby, increased synthesis of 3MGC.

There is little question that patients with idiopathic 3MGC-aciduria suffer from diverse genetic defects (2). Lactic acidosis or other evidence of mitochondrial dysfunction occur in some patients with idiopathic 3MGC-aciduria (1, 2). Similarly, several patients with well-defined primary mitochondrial disorders, such as ATP-synthase deficiency and Pearson syndrome, have been found to excrete excessive amounts of 3MGC (5, 6), suggesting that mitochondrial dysfunction may be primary in some patients with idiopathic 3MGC aciduria. In such patients, one mechanism for increased 3MGC-aciduria could be failure of mitochondrial uptake of peroxisomally or cytoplasmically synthesized 3MGC. On the other hand, because ubiquinone is, in part, a polyisoprenoid product, and because cholesterol and other polyisoprenoid derivatives are involved in the formation of mitochondrial membranes, mitochondrial abnormalities found in some patients with 3MGC-aciduria could also be secondary to a primary defect of sterol or isoprenoid metabolism. Clearly, the overall economy of 3MGC metabolism and its compartmentalization within cells and among tissues must be better understood before we can identify all of the causes of abnormal 3MGC metabolism. Nevertheless, our results indicate that a search for primary defects of polyisoprenoid and sterol metabolism among patients with idiopathic 3MGC-aciduria may prove fruitful. Although cultured skin fibroblasts from several patients with idiopathic 3MGC-aciduria have had grossly normal incorporation of acetate into cholesterol and other isoprenoids (22), so too have cells from patients with a profound deficiency of mevalonate kinase (23). Thus, there is a need for more comprehensive quantitative analysis of metabolites of isoprenoid and sterol biosynthesis before we can test adequately the hypothesis that genetic defects of isoprenoid and sterol biosynthesis other than SLOS are a cause of 3MGC aciduria.

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