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Clinical Report

Simvastatin Treatment in the SLO Syndrome: A Safe Approach?

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Soon after the discovery of reduced cholesterol synthesis in the Smith-Lemli-Opitz syndrome (SLOS), several trials with dietary supplementation were initiated with the aim of increasing cholesterol and reducing the de novo synthesis and accumulation of 7- and 8-dehydrocholesterol (DHC). Dietary cholesterol raises cholesterol levels in the circulation with only marginal effects on levels of DHC. Photosensitivity and polyneuropathy have been reported to be improved by the treatment, but other effects have been difficult to evaluate. In order to see whether inhibition of hydroxymethylglutaryl CoA reductase is of benefit, two of our patients have been treated with simvastatin in addition to the long-term treatment with cholesterol and bile acids. Absolute as well as relative levels of DHC were reduced. In one patient, creatine kinase increased moderately after 2 months of treatment. In the other patient, the treatment had to be interrupted because of hepatotoxic side effects with a marked increase in alanine aminotransferase and aggravation of the hypocholesterolemia and photosensitivity. We conclude that even if the levels of accumulated intermediates can be reduced, treatment with a statin may be harmful in some patients with SLOS.

KEY WORDS: Smith-Lemli-Opitz syndrome; cholesterol; dehydrocholesterols; dietary choles-terol; simvastatin; statin hepatotoxicity

INTRODUCTION

In the metabolic malformation syndrome Smith-Lemli-Opitz (SLOS), a reduced activity of 7-dehydrocholesterol (7-DHC) reductase in the final step of the synthesis leads to hypocholesterolemia and accumulation of 7-DHC and its epimere 8-dehydrocholesterol (8-DHC) [Tint et al., 1994]. The defect is caused by mutations in the gene for 7-DHC reductase (*DHCR7*). The gene coding for this enzyme has been cloned [Moebius et al., 1998; Wassif et al., 1998], and 80 different mutations have so far been described [Nowaczyk et al., 2001]. Biochemical diagnostic methods have been developed that can also be used prenatally [Irons and Tint, 1998] and on Guthrie cards [Starck and Lövgren, 2000].The incidence of the SLOS varies among different ethnic groups but seems in some to be at least 1/60,000 [Ryan et al., 1998]. An incidence of 1/29,000 in whites of European ancestry in Canada has recently been reported [Nowaczyk, 2001]. The true incidence of the syndrome is difficult to estimate without screening because of the wide variability of the phenotypical spectrum, with microcephaly, dysmorphic features, genital abnormalities, minor abnormalities of the limbs, and mental retardation. Internal malformations, severe feeding problems, photosensitivity and increased susceptibility to infections are common.

The discovery of the metabolic basis for the syndrome raised hopes of therapeutic intervention with the goal of increasing cholesterol in plasma and tissues. Such an increase would be expected to down-regulate the synthesis with decrease of the accumulated and possibly toxic precursors.

Dietary supplementation with cholesterol with or without bile acids has shown that some specific features like photosensitivity [Azurdia et al., 2001] and polyneuropathy [Starck et al., 1999] are treatable, but the improvement has not been correlated to biochemical changes. There has been no dramatic improvement in developmental ability, and it has been difficult to

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evaluate the effect of different treatment protocols because of small numbers and the clinical and biochemical variability. An increase of plasma cholesterol has been seen in most treatment trials, but the decrease of the dehydrocholesterols has been variable and marginal.

The pathogenetic mechanisms behind the consequences of the deficiency of cholesterol and the accumulation of DHCs are not fully understood, and it is not known which of the two is most harmful. The DHCs seem to be a less effective substrate for bile acids [Natowics et al., 1999], for myelin [Fumagalli et al., 1969], and for adrenal steroids [Andersson et al., 1999]. Accumulation of 7-DHC has been found in a cataract from an SLO patient [Elias et al., 1997].

We have treated two girls of the same age but with different severity of the syndrome with simvastatin, an inhibitor of the rate-limiting enzyme of the cholesterol synthesis hydroxymethylglutaryl (HMG)-CoenzymeA reductase, in addition to dietary supplementation with cholesterol and bile acids. To evaluate the effect on the synthesis, we have used lathosterol plasma levels, besides analyses of ordinary sterols.

The aim was to find out if statins are safe to use in the SLOS, if this approach is of clinical benefit, and if more knowledge about pathophysiological mechanisms can be obtained.

MATERIALS AND METHODS

The present study was approved by the Ethics Board at Huddinge University Hospital.

Patient A

Patient A has typical stigmata: microcephaly, cleft hard palate, short stature, but no internal malformations. She can walk with a frame, has no speech, and is fed orally. She had her menarche at the age of 13. She has no cataract, no increased susceptibility to infections, and no polyneuropathy. The aminotransferases were moderately increased during the first 4 years but have gradually normalized. Her main symptom is severe photosensitivity, which was the main reason for starting dietary supplementation with cholesterol and bile acids 3 years before the present study. Her light tolerance improved, which was also verified by ultraviolet A (UVA)-light tests.

At the age of 15 years, she was treated for 2 months with 0.5–0.7 mg/kg simvastatin in one evening dose in addition to 100 mg cholesterol and 8 mg taurocholate per kilogram. Sterols including lathosterol, aminotransferases, alkaline phosphatase, cortisol, and creatine kinase (CK) were checked weekly. Medical check-ups were done monthly, and observation of her general condition and behavior daily by the staff of the nursing home where she lives. A UVA-light test was done before and after the treatment.

Patient B

Patient B was also 15 years old, but more severely affected than patient A. She has typical stigmata and no internal malformations, apart from a patent ductus arteriosus that was closed operatively at the age of 3 weeks. She has no independent ambulation, no speech, and is still fed by means of a feeding tube. She is photosensitive, susceptible to infections, and she has severe contractures. At the age of 7 she was deteriorating, with increasing polyneuropathy, precocious puberty, more infections, and weight stagnation. Dietary supplementation with cholesterol and bile acids was started about 2 years later. The progressive course was interrupted and the polyneuropathy improved, which has been reported earlier [Starck et al., 1999].

At about the age of 14, white, coarse hairs started to grow on her chin. Endocrinological investigations including 17-α-hydroxy progesterone, estradiol, testosterone, dihydroepiandrosteronesulphatase and ultrasound examination of her ovaries and adrenals were normal. Her liver enzymes were rising, her polyneuropathy was advanced according to measurement of nerve conduction velocities, and a peripheral cataract not formerly observed was diagnosed.

Treatment with 0.5 mg simvastatin per kilogram was started in addition to 90 mg cholesterol and 9 mg sodium taurocholate per kilogram. The dose was decreased during the treatment, which continued for a month and, after an interval of 2 months, for another 3 months with a dose of 0.3 to 0.4 mg per kilogram. The same weekly laboratory analyses, observations, and UVA-light tests were performed as in patient A.

The malformation score according to Kelley and Hennekam, where 10 embryologically separate organ systems are scored as either "1" or "2" and the sum is normalized to 100 [Kelley and Hennekam, 2000], is 17 for patient A and 11 for patient B.

The mutations in the *DHCR7* gene in patient B are N287K;IVS8−1G→C [Yu et al., 2000]. Molecular data from patient A are lacking.

RESULTS

The dose of simvastatin was gradually increased in patient A. The effects on sterols including lathosterol are shown in Figure 1a. The ratio of DHC to sterols decreased from 0.26 to 0.15. Creatine kinase increased from 1.7 to 4.0 μcat/L the last week (upper limit of normal 2.5 μcat/L). Liver enzymes and cortisol were not affected. She gained 2 kg of weight during the treatment, which she later lost. There was no other change clinically or in her response to UVA light. A year after the trial, still on dietary supplementation, her cholesterol level was 2.55 mmol/L (lower limit of normal 2.5 mmol/L), and the ratio of DHC to sterols was the same as with simvastatin.

Patient B was started on the same dose as patient A, but it was reduced after a week because of rising hepatic enzymes. The treatment was interrupted after 5 weeks because of markedly elevated aminotransferases. Clinically she was more tired. After an interval of 2 months, simvastatin was given again in reduced doses. After another 3 months, alanine aminotransferase (ALAT) again rose to the same level and the treatment was stopped.

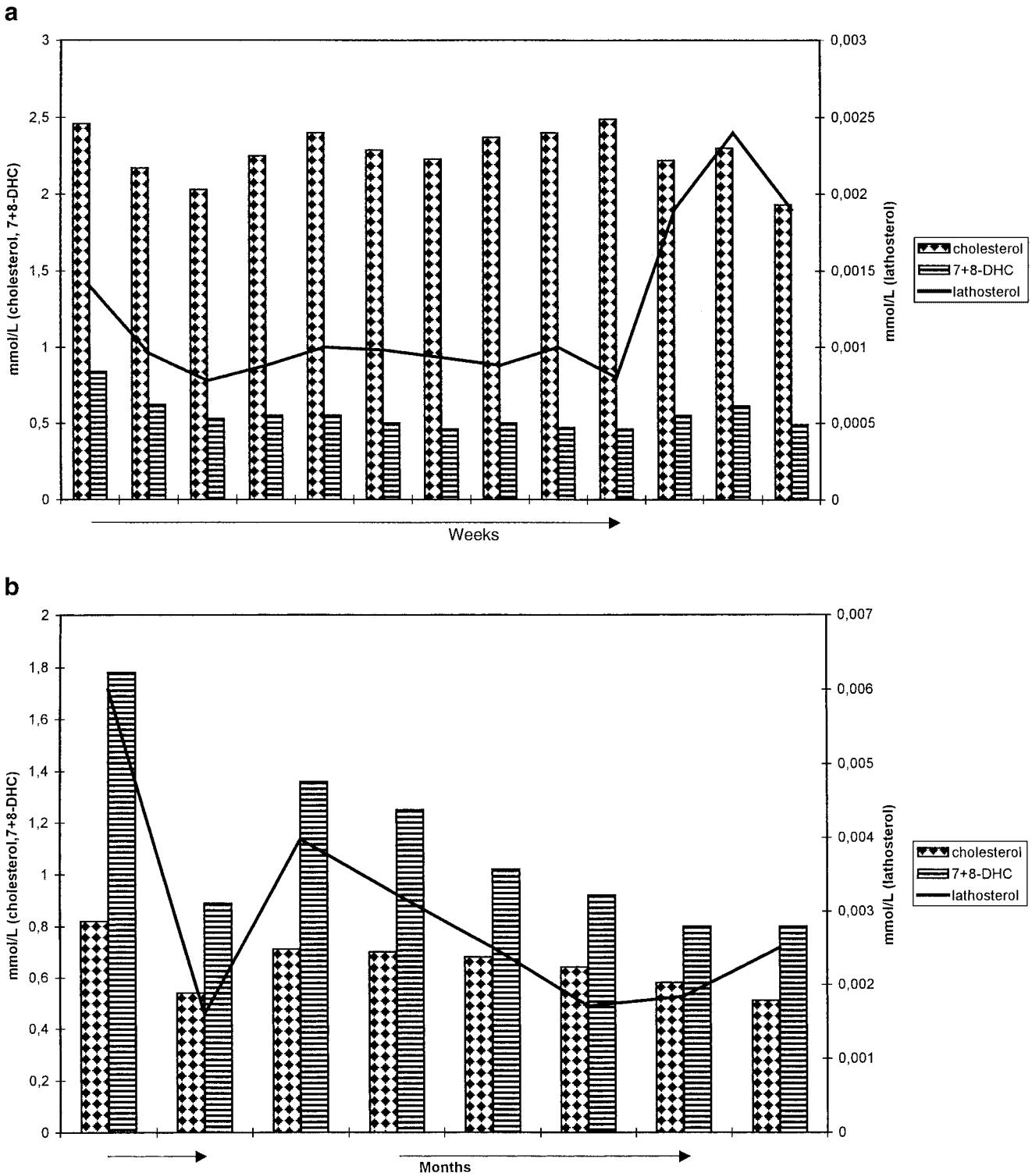

Fig. 1. **a:** Effects of simvastatin on sterols and rate of synthesis in patient A. Arrow shows period with simvastatin. **b:** Effects of simvastatin on sterols and rate of synthesis in patient B. Arrows show periods with simvastatin.

The effect of simvastatin during the 6 months of the trial on sterols including lathosterol and on DHCs and alanine transferase are shown in Figure 1b and Figure 2, respectively. The ratio of DHC to sterols decreased from 0.68 to 0.58. Figure 3 shows ALAT during 9 years and DHCs 6 years before simvastatin was given.

No other medication was given during the first month of treatment. The week before the second hepatic

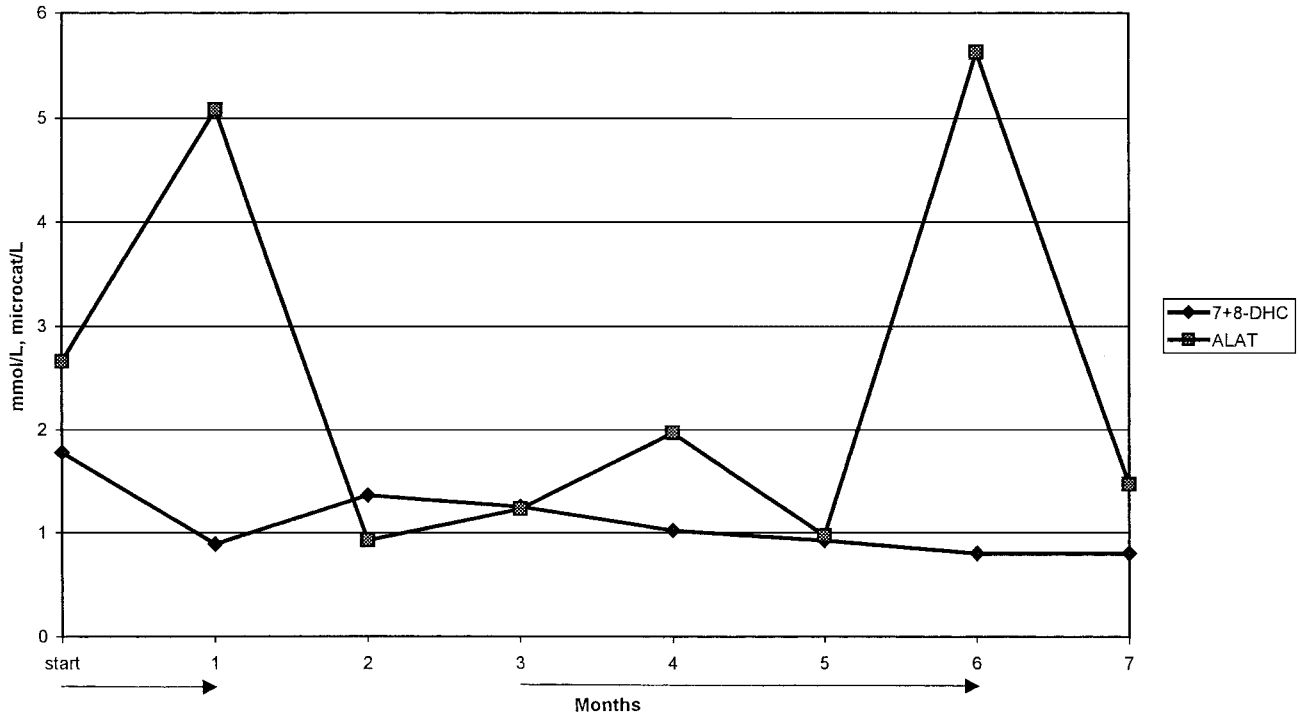

Fig. 2. Effects of simvastatin on dehydrocholesterol (DHC) and alanine aminotransferase (ALAT) in patient B. Arrows show periods with simvastatin.

reaction, she was treated with antibiotics (amoxicillin–clavulanic acid) because of pneumonia on a vacation trip. She also received five doses of ibuprofen and two doses of paracetamol.

She gained 2 kg during the treatment. The photosensitivity was aggravated according to the UVA-light tests. The cataract did not increase. Plasma cortisol and creatine kinase were normal during both periods. The changes of sterols on dietary supplementation with and without statins in the two patients are displayed in Figure 4.

DISCUSSION

It was possible to treat patient A with an HMG-CoA reductase inhibitor in addition to extra cholesterol

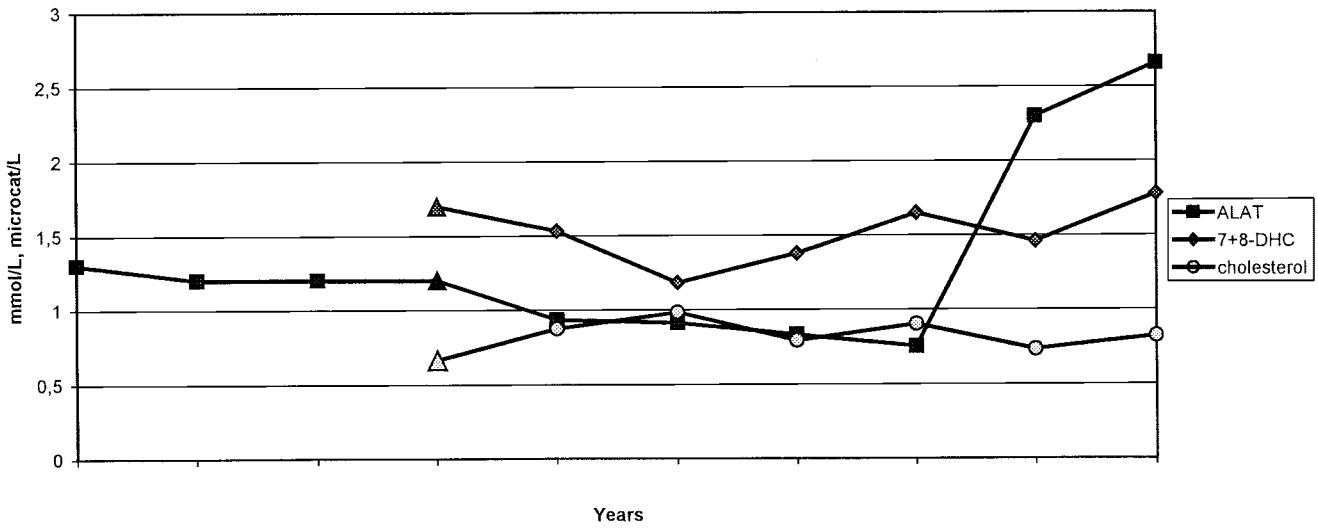

Fig. 3. Sterols and alanine aminotransferase (ALAT) in patient B before simvastatin. Triangles show start of dietary supplementation. DHC, dehydrocholesterol.

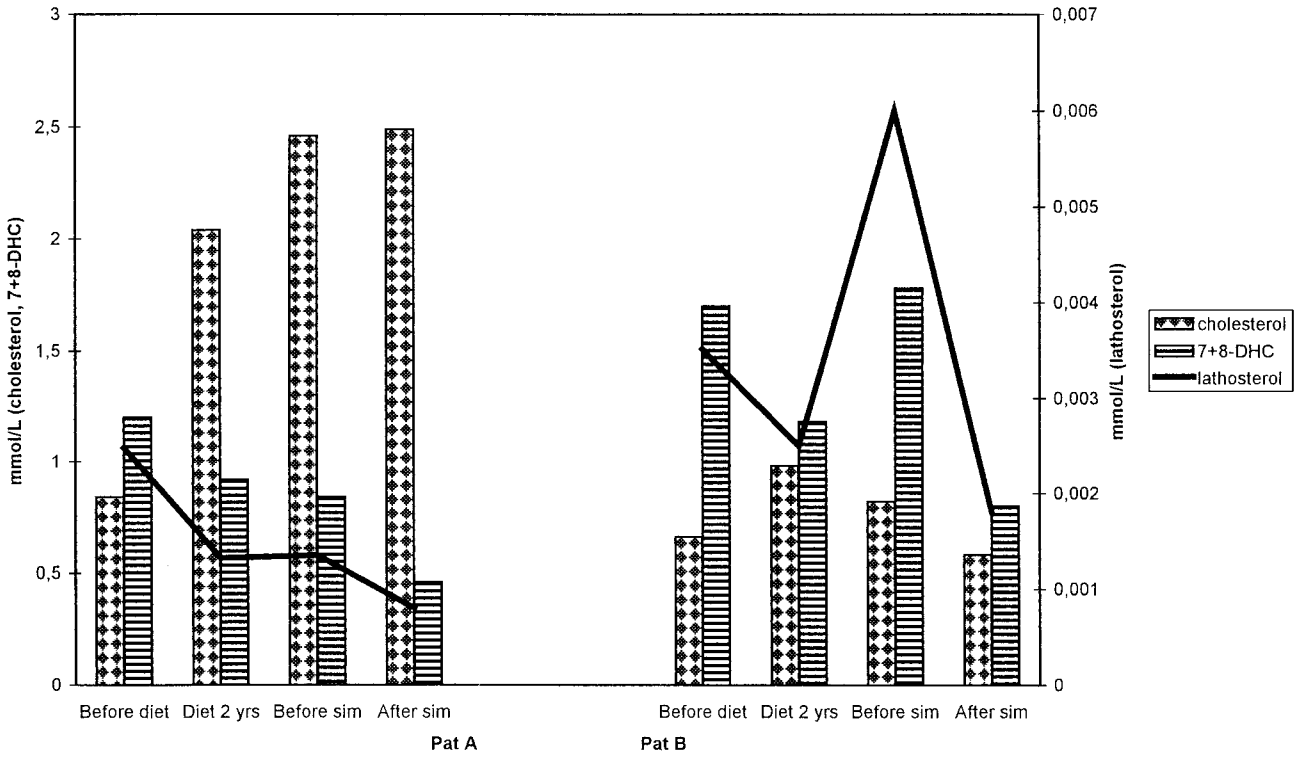

Fig. 4. Effects of diet only and addition of simvastatin on sterols in two patients. DHC, dehydrocholesterol.

and bile acids without serious side effects. The rate of synthesis, according to measurements of lathosterol, and the DHCs were reduced with only a minor and transient decrease of plasma cholesterol levels. Apart from some weight gain, no clinical change was observed. The moderate rise of CK was the only laboratory deviation, but we do not know if it would have been normalized if we had continued the treatment.

Patient B has a more severe disease, with DHCs as the major circulating sterols. She had received dietary supplementation for 6 years with some beneficial effects that, however, seemed to be temporary. Because the beard-like growth of hair, the rising hepatic enzymes, progression of the polyneuropathy, and the newly diagnosed peripheral cataract could all theoretically be connected with the accumulation of the DHCs, treatment with simvastatin seemed indicated.

The rate of synthesis and the DHCs were reduced and plasma cortisol was not influenced. However, the cholesterol levels also decreased. One of her mutations, IVS8−1G→C, is a splice site mutation resulting in a functional null allele [Fitzky et al., 1998]. The second mutation, N287K, is a missense mutation in exon 8 with a tentative location close to the putative transmembrane domain. The loss of enzymatic activity for mutations in this area probably results from protein instability [Witsch-Baumgartner et al., 2000]. We have not found literature data for the enzyme activity of this mutation. However, in an earlier study of the defective *DHCR7* activity in cultured fibroblasts, this patient had less than 5% of the enzyme activity found in fibroblasts from healthy controls [Lund et al., 1996]. It seems that the further decrease of her small endogenous source of cholesterol caused by the statin treatment could not be compensated for by exogenous supply. The aggravated reaction to UVA light at the end of the trial seems to support the hypothesis that hypocholesterolemia is pathogenetically related to the photosensitivity in the SLOS [Anstey et al., 1999]. The pneumonia that she developed during the treatment was more severe than any of her previous infections during recent years. The possibility must be considered that cholesterol deficiency in cellular membranes may disturb the immunological defense system.

The rise of ALAT to more than nine times the upper limit for normal range caused interruption of the treatment twice. The hepatic injury can probably be ascribed to simvastatin, although other medication may on the second occasion have increased the hepatic load by the cytochrome P 450 (CYP) system, which is involved in the metabolism of statins [Bottorff and Hansten, 2000]. Ibuprofen, however, is metabolized by P2CP and paracetamol by 2E1, and not by the isoenzyme P3A4 like simvastatin. The rise of ALAT was of the same magnitude and the was evolution temporary, with decreasing levels on withdrawal.

The hepatic effect of the treatment was double-edged. Two months after the start of the simvastatin medication, hepatic transferases were almost normalized. During the second period, this was repeated after the

same interval, this time while she was still receiving simvastatin. The outcome is consistent with a hepatotoxic effect of the rising DHC the year before the treatment, which was reduced by falling DHC. Dietary supplementation initially decreased DHCs and the slightly elevated liver enzymes.

Statins have been used in children with hypercholesterolemia and have generally been considered safe, with few adverse events. Transient elevations of CK and hepatic transferases have been observed [Duplaga, 1999], but not to such a level that the medication had to be interrupted. In adults, hepatic enzyme levels are increased in 1–3% of the patients, most often during the first 3 months [Farmer and Torre-Aminione, 2000]. More serious hepatic adverse events have been reported [Hartleb et al., 1999], as well as myopathy including rhabdomyolyses. The latter has most often occurred in combination with other medication, for example cyclosporin, erythromycin, or certain antifungal agents. The risk in monotherapy has been estimated to 0.01% [Farmer and Torre-Aminione, 2000].

Hoffmann et al. [1993] described alarming adverse effects with rhabdomyolyses in two children when trying to reduce the accumulated precursor with lovastatin in mevalonic aciduria, an inborn error of cholesterol and nonsterol isoprene metabolism.

Jira et al. [1997] first described the use of statins in the SLOS in one patient who was treated with repeated peritoneal dialysis and in whom no side effects were seen. In August 2000, after the initiation of treatment in one of our patients, the same group reported the results of prolonged simvastatin treatment in two young patients for 23 and 14 months, respectively, with no serious side effects Jira et al. [2000]. Both patients had a less severe form of the syndrome, comparable to patient A in the present study, and both were treated with initial dialysis. The children were fed on standard formula and one of them was given extra cholesterol from the age of 11 months. The ratio of dehydrocholesterols to sterols was substantially reduced in both and plasma cholesterol levels were normalized.

The efficacy of dietary cholesterol in the SLOS has been limited, and generally accepted strategies for optimal therapy are still lacking. In spite of the reported impressive biochemical effects of statins, their place in the treatment remains to be established. In the present study of two patients treated with simvastatin, one had no obvious clinical benefit and laboratory signs of muscular involvement of unclear significance. Besides, her improvement with extra dietary cholesterol and bile acids has been satisfactory and continuous. In the other one, a possible benefit of decreasing levels of precursors could not compensate for negative effects of hypocholesterolemia and a severe hepatotoxic reaction. The malformation scoring system used here is valuable in order to compare prenatal effects of different mutations in the *DHCR7* gene. As judged from the present study, it seems less useful for evaluation of postnatal severity and expected treatment response.

In conclusion, treatment with HMG-CoA reductase inhibitors in SLOS does not seem uncomplicated and safe, especially not for severely affected patients. This could affect the possibility of performing long-term studies of larger groups of patients covering the broad spectra of biochemical and phenotypical variations in this syndrome.

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