Marked accumulation of 27-hydroxycholesterol in SPG5 patients with hereditary spastic paresis

Rebecca Schüle,* Teepu Siddique,† Han-Xiang Deng,† Yi Yang,† Sandra Donkervoort,† Magnus Hansson,§ Ricardo E. Madrid, Nailah Siddique,† Ludger Schöls,* and Ingemar Björkhem[1,§]**

Hertie Institute for Clinical Brain Research and Center of Neurology,* University of Tubingen, Tubingen, Germany; Davee Department of Neurology and Clinical Neurosciences,† Northwestern University Feinberg School of Medicine, Chicago, IL; Division of Clinical Chemistry,§ Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden; and Jervis Clinic Institute for Basic Research,** Staten Island, NY

Abstract Patients with a recessively inherited "pure" hereditary spastic paresis (SPG5) have mutations in the gene coding for the oxysterol 7 α hydroxylase (CYP7B1). One of the expected metabolic consequences of such mutations is accumulation of oxysterol substrates due to decreased enzyme activity. In accordance with this, we demonstrate here that four patients with the SPG5 disease have 6- to 9-fold increased plasma levels of 27-hydroxycholesterol. A much higher increase, 30- to 50-fold, was found in cerebrospinal fluid. The plasma levels of 25-hydroxycholesterol were increased about 100-fold. There were no measurable levels of this oxysterol in cerebrospinal fluid. The pattern of bile acids in serum was normal, suggesting a normal bile acid synthesis. The findings are discussed in relation to two transgenic mouse models with increased levels of 27-hydroxycholesterol in the circulation but without neurological symptoms: the cyp27a1 transgenic mouse and the cyp7b1 knockout mouse. The absolute plasma levels of 27-hydroxycholesterol in the latter models are, however, only about 20% of those in the SPG5 patients. If the accumulation of 27-hydroxycholesterol is an important pathogenetic factor, a reduction of its levels may reduce or prevent the neurological symptoms. A possible strategy to achieve this is discussed.—Schüle, R., T. Siddique, H-X. Deng, Y. Yang, S. Donkervoort, M. Hansson, R. E. Madrid, N. Siddique, L. Schöls, and I. Björkhem. **Marked accumulation of 27-hydroxycholesterol in SPG5 patients with hereditary spastic paresis. *J. Lipid Res.* 2010.** 51: **819–823.**

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"Pure" hereditary spastic paraplegias (HSP) are a clinically and genetically heterogenous group of rare neurodegenerative diseases. Patients with syndromic HSP may also have mental retardation, cerebellar ataxia, and optic and peripheral neuropathy. The spasticity occurs due to axonal degeneration of corticospinal motor neurons forming the corticospinal tracts. In addition, there is a degeneration of dorsal columns with relative preservation of the dorsal root ganglia neuron.

About 40 loci have been mapped in this heterogenous disease and causative mutations in several genes have been found in the different subgroups of the disease. These genes have diverse functions including axonal transport, mitochondrial functions, and myelin sheet formation.

A specific subgroup of HSP inherited as an autosomal recessive trait has been defined, called SPG5 (1). Very recently patients in this subgroup were shown to have mutations in the gene coding for the steroid-metabolizing enzyme, cytochrome P-4507B1 (2–4). Clinically SPG5 is characterized by a progressive spastic paraplegia with variable age at onset, which is pure in most cases, but can be complicated by mild cerebellar ataxia and optic atrophy (1–4).

Cytochrome P-4507B1 (CYP7B1) is responsible for a specific step in bile acid biosynthesis, 7α-hydroxylation of the oxysterol 27-hydroxycholesterol. This is an important reaction in the alternative pathway of bile acid synthesis from cholesterol in the liver (for a review, see ref. 5).

A knockout of this gene in mice causes accumulation of the oxysterols 27-hydroxycholesterol and 25-hydroxycholesterol, but otherwise the mice have no obvious phenotype (6). In contrast to the situation in humans, mice with a knockout of cyp7b1 thus do not develop spastic paraplegia in spite of elevated level of 27-hydroxycholesterol. A transgenic mouse model overexpressing the gene for human CYP27A1 has been developed and characterized (7). Also these mice have levels of 27-hydroxycholesterol about 6-fold higher than normal without any obvious phenotype.

[1]To whom correspondence should be addressed.
e-mail: ingemar.bjorkhem@karolinska.se

It may be concluded from the characterization of cyp7b1 deficient and CYP27A1 overexpressing mice that increasing the levels of 27-hydroxycholesterol by a factor of 6–7 is not sufficient to cause obvious neurological deficits in mice. The situation may be different in humans, because normal levels of 27-hydroxycholesterol are considerably higher in humans than in mouse, possibly as a consequence of a less efficient metabolism. Another factor that may be of relevance is the small corticospinal tract and its redundancy in rodents. Lack of Alsin resulting from loss of function mutations of the ALS2 gene results in severe disorder of human corticospinal and corticobulbar tract degeneration with primary lateral sclerosis. However, in mouse models, Alsin deletion has minimal effect on ambulation and muscle tone (8, 9).

A fatal case with mutations in the CYP7B1 gene has been reported: an infant who died in the neonatal stage with liver failure (10, 11). Plasma levels of 27-hydroxycholesterol were increased more than 1,000 times those of normal controls. This 10-wk-old boy presented with severe cholestasis, cirrhosis, and liver failure, which may have affected the 27-hydroxycholesterol levels. Another metabolite, 24-hydroxycholesterol, was also increased in this infant by a similar magnitude. This oxysterol is metabolized by CYP39A1 (5) and is not a substrate for CYP7B1 (12). More recently, another fatal neonatal case with a mutation in the CYP7B1 gene and development of cholestasis was described (13). The oxysterol levels in this patients were not reported. Whether or not the mutation was causative or a contributing factor in the death of the above two infants is not known with certainty. The possibility has been discussed that an infection and a concomitant elevation of 25-hydroxycholesterol may be a precipitating condition of liver failure in infants with CYP7B1 deficiency (11).

In the present work, we measured levels of 27-hydroxycholesterol and other side-chain oxidized oxysterols as well as bile acids in the circulation of four adult patients with the SPG5 disease and defined mutations in the CYP7B1 gene.

EXPERIMENTAL PROCEDURES

Patients

Case 1 (2) is a 26-year-old woman of Italian origin, the child of first cousins, affected with gait difficulties since childhood. The family denies anyone else being similarly affected. She exhibits a spastic gait with left greater than right weakness of the iliopsoas and glutei, which requires the use of a motorized wheelchair, flexor spasm in the legs to noxious stimuli, transient bilateral convergent spasm of extraocular muscles, and urinary retention, as well as extensive decreased superficial and proprioceptive responses. Imaging studies reveal moderate cerebral atrophy and mild cerebellar and cervical cord atrophy. She has a homozygous C1162T mutation.

Case 2 (3) is a 44-year-old female of German origin with a 26-year history of complicated HSP. In addition to spastic paraparesis, severe dorsal column affection and optic atrophy are present. She suffers from intermittent diarrhea that started a few years ago. She carries a homozygous nonsense mutation: c.825T>A, p.Y275 ×.

Cases 3 and 4 are nonconsanguineous siblings of Italian origin (3). Case 3 is a 42–year-old male who has suffered from pure HSP since the age of 11. His 45-year-old sister (case 4) developed pure HSP at the age of 12. Both siblings are compound heterozygous for a missense and a frameshift mutation: c.(1181C>A) + c.(308_309insA; 971G>A); p.(A394D) + p.(N105KfsX3) (3).

Ethical aspects

All patients gave their informed consent to this study. All the investigations of the patients and the analyses of their serum and cerebrospinal fluid were approved by ethic committees of the respective institutions.

Analyses of oxysterols and bile acids

Side-chain oxidized oxysterols, cholestenoic acid (3β-hydroxy-5-cholestenoic acid), as well as bile acids were assayed by isotope dilution mass spectrometry with use of deuterium labeled internal standards as described previously (14–16).

Analyses of tau protein and phospho-tau protein

Tau-protein (total tau) was determined using a sandwich ELISA constructed to measure both normal tau and phospho-tau (17). Phospho-tau was determined using a sandwich ELISA, with monoclonal antibody recognizing all forms of tau used as capturing body and biotinylated monoconal antibody (specific to P-Thr181) used as a detection antibody (18).

RESULTS

The absence of CYP7B1 activity will lead to a metabolic block of the alternative pathway for formation of bile acids. Because the major product of the alternative pathway in bile acid synthesis is chenodeoxycholic acid (5), a relative decrease in the level of this bile acid would be expected. As shown in **Table 1**, there was a clear tendency to decreased levels of chenodeoxycholic acid and increased levels of deoxycholic acid. The pattern of bile acids in serum with cholic acid, chenodeoxycholic acid, deoxycholic acid, and ursodeoxycholic acid as major bile acids was normal, and no abnormal bile acids could be detected.

TABLE 1. Serum levels of bile acids in SPG5 patients

	Cholic acid ng/ml	Chenodeoxycholic acid ng/ml	Deoxycholic acid ng/ml	Ursodeoxycholic acid ng/ml
Patient 1	113	150	461	16
Patient 2	124	230	522	31
Patient 3	100	277	322	48
Patient 4	100	149	558	10
Controls* (mean ± SD)	120 ± 70	320 ± 120	300 ± 150	70

* The control levels were obtained from (13) and (35).

TABLE 2. Serum levels of cholesterol and oxysterols in the SPG5 patients

	24S- Hydroxycholesterol ng/ml	25-Hydroxycholesterol ng/ml	27-Hydroxycholesterol ng/ml	Cholestenoic Acid ng/ml	Cholesterol mmol/l
Patient 1	50	176	847	51	4.6
Patient 2	52	255	1213	n.m.##	4.3
Patient 3	62	273	1316	143	4.5
Patient 4	66	162	1046	66	4.1
Heterozygous father to patient 1	47	6	249	n.m.##	4.2
Heterozygous mother to patient 2	55	17	196	n.m.##	6.4
Controls*	30-127** Mean: 64	0-11 Mean: 2	89-243** Mean: 154	59±16#	

*The control levels of the oxysterols were obtained from ref. 12 and the control levels of cholestenoic acid from (14). ** range, # mean ± SD, ## not measured.

As shown in **Table 2**, the serum levels of 27-hydroxycholesterol were 6- to 9-fold higher than controls in the four patients. The levels of 25-hydroxycholesterol, which is also a substrate for CYP7B1, were increased about 100-fold. The levels of 24S-hydroxycholesterol, which is not a substrate for the enzyme, were normal. Other oxysterols [7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-oxocholesterol) were present at normal levels (not shown)]. Heterozygotes would be expected to have lower serum levels of 27-hydroxycholesterol than the patients but higher than those of the controls. In accordance with this, the heterozygous father of patient 1 had a serum level of 27-hydroxycholesterol, slightly above the upper level of the controls and about 30% of those of the patients.

Cholestenoic acid is an important metabolite of 27–hydroxycholesterol present in the circulation (16). The level of this acid was normal or only marginally increased, however (Table 1).

27-Hydroxycholesterol was also increased in cerebrospinal fluid with levels 30- to 50-fold higher than in controls. No significant levels of 25-hydroxycholesterol were found in this compartment. The levels of 24S-hydroxycholesterol, which are known to be increased in some neurodegenerative conditions (19), were not increased. (**Table 3**).

In view of a possible neurodegenerative effect of 27--hydroxycholesterol, the levels of tau protein and phospho-tau protein in cerebrospinal fluid, biochemical markers of neurodegeneration, were also measured. The levels of these markers were normal (Table 3).

DISCUSSION

The activity of the alternative pathway in bile acid synthesis is dependent upon CYP7B1 (5) and as a consequence, a reduced formation of chenodeoxycholic acid would be expected.

As shown in Table 1 chenodeoxycholic acid made up about 25% of the total bile acids in serum (18–37%) as compared with about 40% in the controls. The difference is likely to reflect the relative importance of the alternative pathway but because of the small number of patients and the interindividual variations no firm conclusions can be drawn. Another observation was that deoxycholic acid made up about 58% of the total bile acids in serum of the SPG5 patients as compared with less than 40% of the controls. A reduced intestinal motility as a consequence of a neurological defect would be expected to give such an effect. Again, no firm conclusions can be drawn because of the small number of patients.

As expected, the plasma levels of the substrates for the CYP7B1 enzyme, 27-hydroxycholesterol and 25-hydroxycholesterol, were increased in the four SPG5 patients. The magnitude of this increase, 6- to 9-fold in case of 27-hydroxycholesterol and about 100-fold in the case of 25-hydroxycholesterol, is similar to the corresponding increase in mice with a knockout of the cyp7b1 gene (6) and slightly higher than the increase of 27-hydroxycholesterol obtained in mice with an overexpression of CYP27A1 (7).

There is an important difference, however. The basal plasma levels of the side-chain oxidized oxysterols are considerably lower in mice than in humans. The absolute plasma levels of the two side-chain oxidized oxysterols were thus about 1.3 ug/ml (corresponding to 3 uM) in the patients in this study but only about 0.25 ug/ml in the cyp7b1$^{-/-}$ mice (6) and 0.20 ug/ml in the CYP27A1 trangenic mice (7). Thus, it is likely that the critical neuronal cells of the patients are exposed to considerably higher levels of the two side-chain oxidized oxysterols

TABLE 3. Levels of oxysterols, tau protein and phospho tau protein in cerebrospinal fluid from three of the SPG5 patients

	24S-Hydroxycholesterol ng/ml	27-Hydroxycholesterol ng/ml	Tau-Protein pg/ml	Phospho-Tau pg/ml
Patient 2	<1	14	97	6
Patient 3	<1	14	127	n.m.
Patient 4	<1	24	n.m.	n.m.
Controls*	0.8-2.4 Mean: 1.4	0.5-0.8 Mean 0.5	< 400	<60

* The oxysterol control levels were obtained from (17). The cutoff levels for the levels of tau and phospho-tau were those of the producer of the diagnostic kit (15, 16).

than the corresponding cells in the two mouse models. On the other hand, the circulatory levels of the side-chain oxidized oxysterols were considerably lower than those reported in the first reported case with CYP7B1 deficiency. Most probably, the very high levels of oxysterols in that subject were secondary to the cholestasis and the liver failure. The liver failure may have been the result of other factors than the CYP7B1 mutation, although this mutation could have been an important contributing factor. To our knowledge, there are no reports of cholestasis during the neonatal period of adult patients with the SPG5 disease.

27-Hydroxycholesterol may be metabolized by further oxidation into a carboxylic acid, cholestenoic acid, and possibly also by glucuronidation or sulfatation. From a quantitative point of view, cholestanoic acid is the most important metabolite (16). In the present study, we only measured the level of cholestenoic acid in the circulation of the SPG5 patients and this level was found to be normal or only slightly increased (Table 2).

In adults, the synthesis of cholesterol in the spinal cord is 5-fold higher than in cerebrum or cerebellum (20). In spite of increased synthesis, the concentration of cholesterol in the spinal cord is only 2-fold higher than in these two brain regions. This finding indicates that the spinal cord may have a relatively high capacity for excretion of cholesterol. In the brain, the most important mechansm for this excretion is conversion of cholesterol into 24S-hydroxycholesterol, which is able to pass the blood-brain barrier (21, 22). The levels of the enzyme cholesterol 24S-hydroxylase are very low in the spinal cord in relation to the corresponding levels in the brain, indicating that there must be another mechanism for removal of the excess cholesterol from the spinal cord. There is a mechanism for removal of 27-hydroxycholesterol from the brain involving its conversion into the steroid acid 7α-hydroxy-3-oxo-4-cholestenoic acid (23). CYP7B1 is essential for this elimination pathway. It may be speculated that cholesterol is removed from the spinal cord by a primary conversion into 27-hydroxycholesterol followed by a 7α-hydroxylation by CYP7B1 and subsequent oxidation to give the above steroid acid. If this specific mechanism is of critical importance for elimination of cholesterol from the spinal cord in humans and there is a lack of CYP7B1, the accumulation of 27-hydroxycholesterol in the spinal cord may be higher than in most other compartments.The corticospinal tract and dorsal lateral columns and the spinal cord are the site of pathology in HSPs.

We have previously shown that there is a significant uptake of 27-hydroxycholesterol by the human brain from the circulation (24) and that most of the 27-hydroxycholesterol present in cerebrospinal fluid originates from the circulation (25). There is a close correlation between levels of cholesterol and 27-hydroxycholesterol in the circulation. In contrast to 27-hydroxycholesterol, cholesterol does not pass the blood-brain barrier. On account of this observation and the effect of 27-hydroxycholesterol on enzymes involved in generation of amyloid in cultured neuroblastoma cells (26), we have speculated that the influx of 27-hydroxycholesterol from the circulation into the brain may be the link between hypercholesterolemia and neurodegeneration (27, 28). The present investigation does not support the contention that increased levels of 27-hydroxycholesterol in the brain cause a general neurodegeneration that can be detected by the cerebrospinal fluid markers. There may be considerable interindividual variations and in view of the small number of the patients studied here, it is difficult to draw firm conclusions.

The long axons of the corticospinal motor neurons and the dorsal root ganglion neurons that form the corticospinal and dorsal traits in the spinal cord may be more sensitive to high levels of 27-hydroxycholesterol than most other neuronal cells.

Side-chain oxysterols, including 25- and 27-hydroxycholesterol, are known to have cytotoxic effects on cultured cells (29, 30). The relevance of such experiments for the situation in vivo is, however, difficult to evaluate. Under in vivo conditions, the oxysterols are always present together with a very high excess of cholesterol, and this cholesterol may decrease or prevent the activity of the oxysterol (31). It has been reported that 25-hydroxycholesterol induces production of interleukin 1 β and interleukin 8 from human macrophages (32, 33). It cannot be excluded that a local interleukin release as a consequence of the high levels of 25-hydroxycholesterol may be of pathogenetic importance in the SPG5 patients. The absolute levels of 25-hydroxycholesterol were, however, considerably lower than those of 27-hydroxycholesterol, and we failed to demonstrate presence of 25-hydroxycholesterol in cerebrospinal fluid.

If a clear relation between clinical symptoms and levels of 27-hydroxycholesterol in the circulation can be established in patients with SPG5, it would be beneficial to reduce the levels of the oxysterol. No specific inhibitors of 27-hydroxycholesterol are known, but because it has been shown that substrate availability is a limiting factor for CYP27A1 activity (34), a possible therapeutic strategy could be to treat the patients with statins. We have shown that the levels of 27-hydroxycholesterol and cholesterol decrease in parallel during statin therapy (unpublished observation). It would be realistic to reduce the levels of 27-hydroxycholesterol by about 50% which may well have an effect on the progression of corticospinal tract degeneration, if 27-hydroxycholesterol is a major factor in the degeneration of those tracts.

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