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Molecular Case Studies

RESEARCH REPORT

Clinical and metabolic consequences of L-serine supplementation in hereditary sensory and autonomic neuropathy type 1C

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Abstract Hereditary sensory neuropathy type 1 (HSAN1) may be the first genetic neuropathy amenable to a specific mechanism-based treatment, as L-serine supplementation can be used to lower the neurotoxic levels of 1-deoxysphingolipids (1-deoxySL) that cause the neurodegeneration. The treatment is so far untested in HSAN1C caused by variants in the serine palmitoyl transferase subunit 2 (*SPTLC2*) gene. The aim of this study was to establish whether oral L-serine lowers 1-deoxySL in a patient with HSAN1C, to perform a dose escalation to find the minimal effective dose, and to assess the safety profile and global metabolic effects of the treatment. Our patient underwent a 52-wk treatment in which the L-serine dose was titrated up to 400 mg/kg/day. She was followed up by repeated clinical examination, nerve conduction testing, and skin biopsies to document effects on small nerve fibers. Serum was assayed for 1-deoxySL and metabolomics analysis of 111 metabolites. We found a robust lowering of 1-deoxySL, which correlated in a near-linear fashion with increased serum L-serine levels. Metabolomics analysis showed a modest elevation in glycine and a marked reduction in the level of cytosine, whereas most of the other assayed metabolites did not change. There were no direct side effects from the treatment, but the patient developed a transitory toe ulceration during the course of the study. The Charcot–Marie–Tooth neuropathy score increased by 1 point. We conclude that oral supplementation of L-serine decreases 1-deoxySL in HSAN1C without major global effects on metabolism. L-serine is therefore a potential treatment for HSAN1C.

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INTRODUCTION

Hereditary neuropathies, commonly known as Charcot–Marie–Tooth disease (CMT), are a genetically diverse group of disorders with a total population frequency of approximately 1 in 2500 (Reilly et al. 2011). In two specific hereditary neuropathies a toxic disease mechanism is implicated: hereditary sensory and autonomic neuropathy (HSAN) types IA and IC are caused by variants in the genes *SPTLC1* (Dawkins et al. 2001) and *SPTLC2* (Rotthier et al. 2010), respectively. These genes encode subunits of serine palmitoyl transferase (SPT), a

pyridoxal 5′-phosphate (PLP)-dependent enzyme needed for sphingolipid biosynthesis (Hanada 2003). Disease-causing variants lead to the incorporation of L-alanine or glycine in the place of L-serine, causing the accumulation of neurotoxic deoxysphingolipids (1-deoxySL) and deoxymethylsphingolipids in the bloodstream (Penno et al. 2010; Rotthier et al. 2010).

Increasing the substrate availability of SPT by oral supplementation with L-serine lowers 1-deoxySL levels in a mouse model and humans with *SPTLC1* variants (Garofalo et al. 2011). Therefore, L-serine is being tested as a treatment for HSAN1A (clinicaltrials.gov; NCT01733407) but so far the supplementation is untested in HSAN1C. L-serine supplementation is also used in congenital disorders of L-serine synthesis (El-Hattab 2016) and has been tested as a potential neuroprotective agent in amyotrophic lateral sclerosis (ALS) (Levine et al. 2017).

Enzymatic activity of SPT requires SPTLC1 together with the PLP-binding catalytic subunit STPLC2, which is present in lower amounts and is therefore rate-limiting. In addition, there is a third subunit, SPTLC3, whose expression is highly tissue-specific (Hornemann et al. 2006). SPTLC2 and SPTLC3 are structurally similar and can be replaced by each other. Their tissue-specific expression may allow the adaptation to specific requirements for sphingolipid synthesis depending on cell type and developmental stage (Hornemann et al. 2007). Mutations in SPTLC1 and SPTLC2 probably cause 1-deoxySL elevations through similar mechanisms, as most of the known mutations of both subunits are situated near the PLP binding site in the enzyme's three-dimensional structure (Bode et al. 2016). However, given that SPTLC2 is the rate-limiting catalytic subunit, and that it may be functionally redundant in certain tissues, it is of interest to explore the effects of L-serine supplementation also in the setting of SPTLC2 mutation. Moreover, it is important to perform a dose-escalation study in order to determine the lowest L-serine dose at which 1-deoxySL levels are suppressed. The aim of this study was to test whether L-serine lowers 1-deoxySL levels in HSAN1C and to gain knowledge of the clinical and metabolic consequences of prolonged exposure to high-dose L-serine.

RESULTS

Experimental Treatment Protocol and Clinical Findings

The previously described patient 323 (Suriyanarayanan et al. 2016), is a female with sensory-predominant axonal neuropathy and small fiber neuropathy starting at age 54 caused by *SPTLC2* p.(Arg183Trp). At the start of this trial, the patient's age was 67, weight 98 kg, and height 165 cm. Her permanent medication consisted of losartan, bisoprolol, and hydrochlorothiazide for hypertension and nortriptyline for neuropathic pain.

L-serine (Sterling Supplements and the Serine Store; http://theserinestore.com/) was administered orally in the form of a powder mixed in water. The dose was titrated at 3-wk intervals at 50–100–200–300 mg/kg/day such that the target dose of 400 mg/kg/day was reached on week 13, which was the highest dose previously tested for HSAN1A patients (Garofalo et al. 2011). The study was continued for a total of 52 wk.

The patient did not develop immediate side effects from the treatment. Her subjective sensations of neuropathic symptoms were essentially stable but she did report some increased tingling and pains distally in her hands soon after the start of the study. For this, she was placed on gabapentin, which continued until the end of the study. At week 45 (i.e., when she had used 400 mg/kg/day L-serine for 32 wk), swelling and an ulceration had developed on one toe. Therefore, as a precaution the dose was lowered to 200 mg/day combined with oral cephalosporin for 14 d and careful local nurture. After this, the swelling and ulceration subsided. A peripheral circulatory disturbance was excluded

Table 1. Clinical and neurophysiologic parameters

	Prestudy	Week 22	End of study
L-serine dose	0	400 mg/kg/day	200 mg/kg/day
Serum 1-deoxySO	0.385 μM	0.192 μM	0.218 μM
Serum 1-deoxySA	0.154 μM	0.031 μM	0.061 μM
Sensory symptoms	3	3	2
Motor symptoms (legs)	1	1	1
Motor symptoms (arms)	1	1	1
Pinprick sensibility	2	2	2
Vibration	2	2	2
Strength (legs)	1	1	1
Strength (arms)	0	1	2
Ulnar CMAP	7.4 mV (0)	6.5 mV (0)	7.6 mV (0)
Radial SAP	22.8 μV (0)	20.8 μV (0)	20.8 μV (0)
TOTAL CMTNS	10	11	11

Clinical symptoms and findings were scored according to the Charcot–Marie–Tooth neuropathy score second version (CMTNS) on a scale of 0–4 for each item, as previously described (Murphy et al. 2011). For the neurophysiological parameters, the measured values are given together with the CMTNS score in parentheses.
CMAP, compound muscle action potential; SAP, sensory nerve action potential.

by a normal ankle-brachial pressure index, but MRI of the foot showed chronic skeletal deformities that may have predisposed to abrasion.

No other new symptoms developed during the trial. Her Charcot–Marie–Tooth neuropathy score (second version) increased by one point during the study, because of increased weakness in the arms, whereas sensory symptoms improved slightly. No changes were observed on nerve conduction studies (NCS) and quantitative sensory testing parameters after the study (Table 1). Her prestudy skin biopsy had been negative for intraepidermal nerve fibers (Suriyanarayanan et al. 2016). Repeated biopsies at weeks 22 and 52 remained negative, although in the latter there were slight features indicative of regenerating fibers that nevertheless did not reach the quantitative threshold.

Metabolic Effects of L-Serine Supplementation

Upon routine laboratory testing, no significant changes were observed on blood cell counts or plasma cholesterol, triglycerides, glucose, or liver transaminases.

In metabolomics analysis, the serum level of L-serine showed a strong increase as the L-serine dose was titrated upward (Fig. 1A). The serum L-serine level correlated with the supplemented dose, approximating a second-order polynomial relationship ($R^2 = 0.90$; Fig. 1B). On the 400 mg/kg/day dose, the serum L-serine level was increased on average 3.2 ± 0.38-fold (average ± standard deviation) of the pretreatment levels. 1-DeoxySL levels showed close to linear correlation with serum L-serine ($R^2 = 0.63$ for 1-deoxysphingosine [1-deoxySO] and $R^2 = 0.75$ for 1-deoxysphinganine [1-deoxySA]; Fig. 1C). The levels of deoxymethylsphinganine, which is formed upon glycine incorporation, were suppressed upon L-serine supplementation but the levels were close to the detection limit and therefore the data may not be reliable (Supplemental Fig. 1A). On 400 mg/kg/day L-serine, total 1-deoxySL (1-deoxySO + 1-deoxySA) reached the normal range, <0.3 μM, corresponding to the 95th percentile measured in healthy individuals (Fig. 1A). Of the normal C_{18} sphingoid base products of SPT, sphingosine (SO) and sphinganine (SA), there were no significant

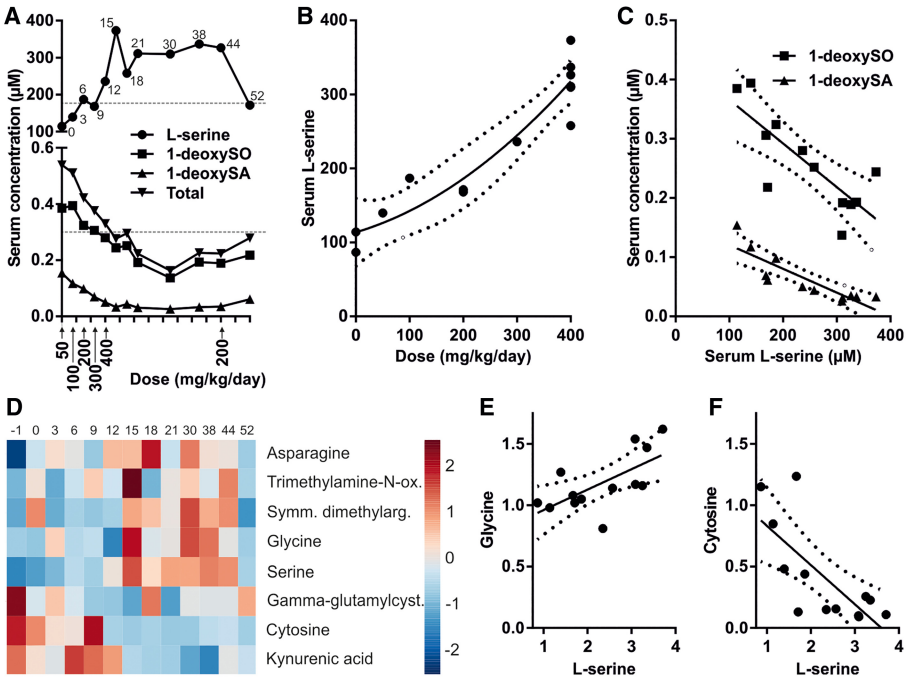

Figure 1. Serum metabolites during L-serine supplementation. (*A*) The serum L-serine level increased and 1-deoxySL level decreased over the course of the study. Shown on the horizontal axis are the time points at which a new L-serine dose was begun. Next to the L-serine trace are the week numbers at which the sample was taken. The upper limit of normal for L-serine (177 μM) is indicated by the *upper* gray dotted line and the upper limit of normal (0.3 μM) for total 1-deoxySL (1-deoxySO + 1-deoxySA) is indicated by the *lower* gray dotted line. (*B*) Serum L-serine plotted against the oral dose, the trend line is a second-order polynomial curve (R^2 = 0.90). (*C*) Plotting the serum 1-deoxySL against serum L-serine shows that 1-deoxySL levels decrease upon administration of L-serine. (*D*) Metabolomic analyses of 111 serum metabolites were performed and plotted on a heatmap. Data are shown for metabolites showing a negative correlation with serum L-serine: γ-glutamylcysteine (R^2 = 0.30), cytosine (R^2 = 0.48), kynurenic acid (R^2 = 0.37); or a positive correlation with L-serine: asparagine (R^2 = 0.36), trimethylamine-*N*-oxide (R^2 = 0.40), symmetric dimethylarginine (R^2 = 0.42), and glycine (R^2 = 0.44). The correlations were statistically significant (Benjamini–Hochberg method for 95 observations assuming 33% false discovery rate) for cytosine, glycine, symmetric dimethylarginine, and trimethyl-*N*-oxide. Data are organized according to the sampling weeks as indicated above the plot (week −1 corresponds to a sample that was taken before initiation of the trial). (*E*) The relative level of serum glycine is shown in relation to the serum level of L-serine, such that the average pretreatment levels are taken as 1. (*F*) The corresponding curve for serum cytosine; note that the trend line is shown only for the portion above the horizontal axis. Trend lines are shown with 95% confidence intervals (dotted lines).

changes, which likely reflects their regulation by metabolic feedback mechanisms (Supplemental Fig. 1B).

A heatmap was generated to analyze the levels of all other assayed metabolites. Selected metabolites are shown in Figure 1D, the whole heatmap in Supplemental Figure 2, and numeric metabolomics data in Supplemental Table 1. Although the majority of metabolites showed no correlation with serum L-serine, a positive correlation was found between the serum L-serine and glycine (R^2 = 0.44; Fig. 1E). This was expected, because glycine can be directly synthesized from L-serine. On 400 mg/kg/day L-serine, serum glycine was on average 1.35 ± 0.22-fold increased compared with pretreatment levels. The most significant negative correlation to serum L-serine was found for cytosine, which decreased on average to 0.16 ± 0.07-fold on the highest L-serine dose (Fig. 1F).

DISCUSSION

This study provides the first data on the effects of L-serine supplementation in HSAN1C. The results are important considering the potential use of L-serine in future trials for HSAN1C and for treating other disorders such as HSAN1A and ALS.

L-serine supplementation is effective at lowering 1-deoxySL in HSAN1A disease. However, there are differences between the SPTLC1 and SPTLC2 subunits—namely that SPTLC2 is the catalytic subunit that binds the pyridoxal 5′-phosphate coenzyme, whereas SPTLC1 does not (Hanada 2003), and that the expression of SPTLC2 determines the catalytic rate. Therefore, the effects of L-serine supplementation may not be the same in HSAN1A and HSAN1C. We chose a dose escalation approach in order to find the lowest dose at which 1-deoxySL levels become suppressed. Our data show that the serum 1-deoxySL has a near linear correlation to the supplemented dose, reaching normal levels at 400 mg/kg/day. This suggests that more clinical benefit may be anticipated at higher doses. The level of 1-deoxySO decreased slower than that of 1-deoxySA, which is consistent with 1-deoxySO being the downstream product of 1-deoxySA (Alecu et al. 2017). These data show that L-serine supplementation is also effective in lowering plasma 1-deoxySL in HSAN1C and is a potential treatment for both forms of HSAN1.

Based on this single case and one-year follow-up, we cannot conclude whether the achieved 1-deoxySL reduction was sufficient to stop or slow the progression of the disease. Our patient's CMTNS increased by one point because of a reduced arm strength, although we observed a slight improvement in her sensory symptoms. The NCS measurements remained stable. The rarity and slow natural progression of HSAN1C makes it difficult to set up sufficiently powered clinical trials to confirm a mild clinical outcome measure. To our knowledge, nine families with HSAN1C have so far been reported (Rotthier et al. 2010; Murphy et al. 2013; Ernst et al. 2015; Suriyanarayanan et al. 2016). It was shown recently that 1-deoxySL, although not degraded by the canonical sphingolipid catabolic pathway, is metabolized by a set of CYP4F enzymes as part of a physiological lipid detoxification mechanism (Alecu et al. 2017). A stimulation of this pathway (e.g., by fibrates) (Othman et al. 2015) should be synergistic to L-serine supplementation. Thus a more potent 1-deoxySL decrease could be reached at lower doses of L-serine.

The utility of CMTNS in clinical trials is limited by its low sensitivity (Mannil et al. 2014). Therefore, we used skin biopsy with PGP9.5 staining for small fibers as a second biomarker. The biopsy was negative for small fibers before the start of the study, and no recovery of small fibers was observed at the end of the study. This suggests that nerve fibers that have already been lost do not effectively regenerate despite the reduced 1-deoxySL levels, or that a biopsy from the distal part of the leg is not sensitive enough to document a slight regenerative change. Biopsies from a more proximal site (Fridman et al. 2015) or imaging of corneal small fibers by confocal microscopy (Tavakoli et al. 2010) could be a more sensitive method for following small fiber integrity in future studies.

L-serine supplementation was not associated with any evident side effects in our patient. The patient did develop an ulcer after being on the 400 mg/kg/day (40 g/day) dose for 32 wk that later healed when the dose was decreased to 200 mg/kg/day. However, ulceration may have rather been a consequence of absent distal sensation and skeletal deformities, but because she had not reported ulcers previously we cannot fully exclude that it was also related to the treatment. It is therefore important to pay special attention to skin changes in future trials for HSAN1C. The overall tolerance of L-serine in our patient is in line with its reported safety in the previous trial for ALS where the highest dose was 30 g/day for up to 6 mo (Levine et al. 2017), HSAN1A where doses reached 400 mg/kg/day (Garofalo et al. 2011), and a toxicity study in rats using daily doses of up to 2900 mg/kg (Tada et al.

2010). The use of nortriptyline by our patient is important to note. Tricyclic antidepressants have been shown to affect sphingolipid metabolism by inhibiting acid sphingomyelinase (ASM), which produces ceramide through hydrolysis of sphingomyelin (Beckmann et al. 2014). Altered signaling from ceramide-induced membrane domains has been implicated in depression and other disorders (Beckmann et al. 2014; Gulbins et al. 2015). Ceramide is also produced de novo via the SPT-catalyzed reaction. Thus nortriptyline and L-serine both have the potential to affect ceramide-dependent signaling, but the absence of unexpected side effects such as mood changes in our patient suggests that their combination is safe.

In addition to being incorporated into proteins, L-serine serves as a precursor for several essential metabolites including sphingolipids, the plasma membrane component phosphatidyl-serine, and the neurotransmitters glycine and D-serine. L-serine can be removed through conversion into glycine by serine hydroxymethyl-transferase or through degradation into pyruvate and ammonia by serine hydratase (El-Hattab 2016). Because of the involvement of L-serine in multiple metabolic pathways, we chose an unbiased metabolomics approach to document unexpected metabolic effects. We found a trend toward a second-order relationship between serum L-serine concentration and the supplemented dose. This suggests that the pathways catabolizing L-serine may become saturated at very high doses, which could theoretically increase the risk of toxicity. We also found a moderate increase in serum glycine, as expected because of its conversion from L-serine. As the deoxymethylsphingolipids levels were also suppressed by L-serine supplementation, the glycine elevation does not appear to limit the efficiency of the treatment.

Furthermore, we observed a significant drop in the level of the DNA/RNA base cytosine. The biochemical mechanism of this decrease is not known but may be related to the central role of L-serine in one-carbon metabolism (Meiser et al. 2016). There was no indication of defective nucleic acid metabolism such as cytopenia, so the clinical significance of the cytosine depletion is not clear. The vast majority of the assayed metabolites did not change significantly in response to L-serine. We therefore conclude that there does not seem to be significant global metabolic effects from high-dose L-serine supplementation.

In conclusion, we have shown that L-serine is effective at lowering 1-deoxySL in HSAN1C with restricted effects on global metabolite levels. Doses up to 400 mg/kg/day are likely to be safe in this patient population.

METHODS

Standard clinical testing was performed at HUSLAB laboratory, Helsinki University Hospital. Metabolomics analyses were carried out at the Metabolomics Unit, Technology Centre, Institute for Molecular Medicine Finland FIMM, University of Helsinki. A detailed description of the method for metabolomics analysis from serum for 100 metabolites has been published recently (Nikkanen et al. 2016). For 11 additional metabolites, the same extracted samples from 100 metabolites analysis were injected under different chromatography and mass spectrometry conditions for the analysis of methionine cycle metabolites together with calibration curve. Instrumental and analytical conditions were the same as for 100 metabolites analysis except that metabolites were separated in 5.6 min of run time using 10 mM ammonium formate, pH 3 in the mobile phase A and B. The detection system, a Xevo TQ-S tandem triple quadrupole mass spectrometer, was operated in only positive polarity and optimized mass-dependent parameters like declustering potential (DP) and collision energy (CE) were used for detection of the methionine cycle metabolites. Data were analyzed with MetaboAnalyst 3.0. (http://www.metaboanalyst.ca/), excluding variables with missing values due to being below the detection limit.

Total sphingoid base levels after hydrolysis (SO, SA, 1-deoxySO, and 1-deoxySA) were measured as described previously (Suriyanarayanan et al. 2016). The patient underwent serial clinical evaluation using the Charcot–Marie–Tooth neuropathy score (second version, CMTNS) (Murphy et al. 2011), NCS, quantitative sensory testing, and skin punch biopsies taken 10 cm proximal to the lateral malleolus with PGP9.5 immunostaining for intraepidermal nerve fibers. Statistical analyses were performed with GraphPad.

ADDITIONAL INFORMATION

Data Deposition and Access

Metabolomics data have been deposited to Metabolomics Workbench (www.metabolomicsworkbench.org/), study number ST000876.

Ethics Statement

The study was approved by the institutional ethics review board of Helsinki University Hospital (decision number 130/13/03/01/2015), and the patient gave written informed consent.

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Competing Interest Statement

The authors have declared no competing interest.

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Author Contributions

M.A., H.T., T.H., and E.Y. conceived and designed the experiments. J.T. performed neurophysiologic analyses. A.P. performed pathologic analyses. M.A. and E.Y. performed clinical analysis. S.S. and M.A.L. performed experiments. E.Y. drafted the manuscript. All authors critically reviewed the manuscript.

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