L-Threonine in the Treatment of Spasticity

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Summary: Preclinical data indicate that the administration of the amino acid l-threonine increases glycine levels in rat spinal cord. In order to investigate glycergic mechanisms in spasticity, and other signs of the upper motor syndrome, we gave 4.5 and 6.0 g/day of l-threonine to 18 patients with familial spastic paraparesis (FSP) according to a double-blind, crossover protocol. The response to treatment at the end of each 2-week period was based upon three measures: the physician's global impressions; the patients' global impressions; and semiquantitative ratings of strength, muscle tone, DTRs, walking, hopping, and running. Blood and CSF were collected during each treatment period for amino acid analyses. Based upon the severity rating scales, there was a statistically significant (p < 0.02) decrease in motor impairment and spasticity during l-threonine administration compared to placebo treatment; significant treatment effects were not found on the physician's and patients' global impressions. Plasma and CSF levels of threonine increased significantly during l-threonine treatment but glycine levels did not change. These data indicate that l-threonine significantly suppressed the signs of spasticity even though the benefits were not clinically valuable. Key Words: L-Threonine—Glycine—Amino acid—Spasticity.

Familial spastic paraparesis (FSP) is a slowly progressive neurological disorder that generally begins in the third or fourth decade of life and leads to major disability over a course of 10 to 15 years (1–5). Clinically, the initial and dominant symptom of FSP is difficulty walking due to spasticity in the legs; additional symptoms that occur later in the course of the illness include urinary urgency and incontinence. Deep tendon reflexes are increased in the lower extremities, and clonus and bilateral Babinski signs are usually present. Although spasticity is present, the chief source of disability in FSP patients is uncertain; strength is generally preserved. Neuropathologically, the principal lesion in FSP is symmetric degeneration of the descending corticospinal tracts. Aside from variable mild involvement of the posterior columns, other descending and ascending long tracts

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in the white matter are spared, as are the interneurons and anterior horn cells of
the spinal cord gray matter (1,2,4–6). The disease is probably genetically hetero-
genous: Autosomal-dominant genetic transmission accounts for the majority of
cases, but an indistinguishable disorder apparently occurs in a few families as a
result of autosomal-recessive inheritance (3,6).

Drugs used in the treatment of spasticity (7–9) have aimed either at enhancing
presynaptic inhibition (benzodiazepines such as diazepam; baclofen) or at dimin-
ishing muscle contraction (dantrolene). These treatments may have intolerable
side effects and are often ineffective in suppressing symptoms of spasticity in
patients with FSP. This study describes an alternate therapeutic approach, with a
drug thought to increase the inhibitory glycinergic tone of spinal cord interneu-
rions. This rational neuropharmacological strategy was impossible to implement
prior to the discovery that exogenously administered threonine increases glycine
levels in rat spinal cord (10).

METHODS

Subjects

Eighteen men and women with FSP participated in this research project ac-
cording to the provisions of a protocol approved by the Massachusetts General
Hospital (MGH) Subcommittee on Human Subject Research (Table I). The diag-
osis of FSP was based upon clinical criteria (1,3–5). Subjects included 4 women
and 14 men whose ages ranged between 23 and 70 years old; the duration of illness

<table>
<thead>
<tr>
<th>Pt. #</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Duration of illness (years)</th>
<th>Chief symptoms</th>
<th>Global severity</th>
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<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>M</td>
<td>20</td>
<td>3 0 1</td>
<td>Moderate</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
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<td>3</td>
<td>49</td>
<td>M</td>
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<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>M</td>
<td>14</td>
<td>4 0 2</td>
<td>Moderate</td>
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<tr>
<td>5</td>
<td>62</td>
<td>M</td>
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<td>6</td>
<td>50</td>
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<td>22</td>
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<tr>
<td>7</td>
<td>58</td>
<td>F</td>
<td>23</td>
<td>4 2 0</td>
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<tr>
<td>8</td>
<td>64</td>
<td>M</td>
<td>35</td>
<td>4 2 2</td>
<td>Severe</td>
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<tr>
<td>9</td>
<td>31</td>
<td>M</td>
<td>8</td>
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<td>10</td>
<td>27</td>
<td>F</td>
<td>10</td>
<td>0 1 1</td>
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<td>11</td>
<td>34</td>
<td>F</td>
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<td>1 0 2</td>
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<tr>
<td>12</td>
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</tr>
<tr>
<td>18</td>
<td>63</td>
<td>M</td>
<td>3</td>
<td>2 1 2</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Gait: 0 = normal; 1 = stiff legs; 2 = stiff legs + circumduction; 3 = stiff legs + circumduction and
effortful walk; 4 = needs cane or other support.
Bladder: 0 = normal; 1 = frequency and urgency; 2 = incontinence.
Flexor spasms: 0 = none; 1 = rare; 2 = 1–4 times/week; 2 = every night.
was 2 to 35 years. Five patients had severe disease; they could not run, hop, or walk unsupported. Their ages ranged between 50 and 70 years with a mean age of 59.0 years; their mean duration of illness was 20.4 years (range of 8 to 35 years). Eight patients had moderate disease; they could run and walk unsupported but had pronounced leg stiffness and circumduction. Their ages ranged between 31 and 66 years with a mean of 50.1 years; their duration of illness ranged from 3 to 20 years with a mean of 12.6 years. Five patients had mild disease; they could run, walk unsupported, and hop with good spring. Their ages ranged between 23 and 41 years with a mean of 30.4 years; their duration of illness was between 2 and 10 years with a mean of 6.0 years. None of the patients received treatments for spasticity in the 2 weeks prior to joining the study.

Drugs

L-threonine was supplied as a purified amino acid by Ajinomoto Inc. (Tokyo, Japan) and incorporated into 500 mg capsules by the research pharmacy at the MGH. Seven patients took 1.5 g t.i.d. (4.5 g/day total dose) of L-threonine, and nine patients took 2.0 g t.i.d. (6.0 g/day) of L-threonine; two patients (#3 and #4) took both doses (completed the double-blind study twice) and their scores for the 6 g/day dose only were used in clinical data analyses. These doses were selected based upon a preliminary open-label, dose-finding study (11). The placebo capsules contained lactose NF and were prepared by the pharmacy in a manner similar to the procedure followed for the preparation of the L-threonine capsules. L-threonine and placebo capsules had an identical appearance, texture, and taste when intact.

Assessment Measures

Prior to entering the research protocol, subjects related a complete medical history, underwent physical and neurological examinations, and had a chest radiograph, electrocardiogram (ECG), complete blood count (CBC), urinalysis, and a complete routine blood chemical analysis. All laboratory blood tests were repeated at the end of each drug treatment period (e.g., after l-threonine and after placebo) and 2 weeks after completion of the entire drug study. Only one chest radiograph and one ECG were obtained.

A detailed neurological examination was performed on each visit that included semiquantitative estimates of the following measures of motor dysfunction: strength, muscle tone, deep tendon reflexes (DTRs), clonus, plantar responses, gait, ability to hop unsupported on either leg, and ability to run. For each sign, a score of 0 represented normal function (including no evidence of spasticity), and higher scores represented increasing severity of dysfunction. Leg strength was tested and scored for the iliopsoas, quadriceps, hamstrings, anterior tibials, gastrocnemii, extensor hallucis, hip adductors, and hip abductors bilaterally, using the scale of 0 = normal, 1 = slightly weak, 2 = hold against gravity, 3 = inability to sustain posture against gravity, and 4 = flicker of movement. Muscle tone was estimated according to a scale in which 0 = normal, 1 = increased only with remote activation, 2 = definite increase, 3 = moderate increase, and 4 = stiff as a board. Knee, ankle, and adductor reflexes were scored as 0 = normal,
3 = hyperactive, and 4 = hyperactive with clonus. Clonus was rated independent of DTRs as 0 = no clonus and 4 = present. The scores for gait and running were based upon 0 = normal, 1 = slightly stiff, 2 = stiff with circumduction, 3 = stiff, circumduction, and effortful, and 4 = needs cane or other support. Scores for hopping unsupported on right and left foot were 0 = normal, 1 = slightly stiff, 2 = stiff with no spring, 3 = cannot hop, and 4 = cannot stand unsupported on one foot. In order to avoid problems in data analysis caused by variations in interrater reliability, all examinations were conducted and scored by the same neurologist (J.H.G.).

In addition to the semiquantitative rating scale, there were two assessments of global change. At the end of each treatment period, the examining physician (J.H.G.) assessed the patient's signs and symptoms as improved, deteriorated, or unchanged. The parameters assessed were strength, muscle tone, DTRs, frequency of flexor spasms, walking, running, bladder function, and overall global impression of change during treatment. In a separate global rating scale, each patient evaluated treatment by answering a questionnaire at the end of each 2-week treatment period. The questionnaire asked whether he or she had noticed any change in walking, strength, stiffness, frequency of flexor spasms, bladder control, energy, sleeping, mood, and emotions.

Protocols

Patients were enrolled from September 1987 to April 1989. The study was conducted according to a double-blind, crossover design, in which there were two treatment periods, each lasting 2 weeks, interrupted by a 2-week washout period during which no experimental drug was prescribed. Treatment group assignment was made by the dispensing research pharmacist according to a randomized, computer-generated order of administration that was counterbalanced across patients. Thus, within each block of 10 patients, 5 received L-threonine and 5 received placebo during the first treatment period. Neither the clinical investigators nor the patients knew the order in which the drugs were dispensed.

Ten of the 18 patients were admitted to the General Clinical Research Center (CRC) at the MGH during the last 2 days of each treatment period for spinal fluid examinations. Of the remaining eight patients, seven refused lumbar punctures (LPS), and one had an initial LP and refused it the second time. Cerebrospinal fluid (CSF) was obtained by conventional technique (lateral decubitus position; L3-4 interspace; #22 gauge spinal needle) at 10 a.m. Patients remained at bedrest for 8 h prior to the LP in order to avoid potential effects of mixing amino acid pools due to physical activity or time of day. The first 2 ml of CSF were always sent for routine studies (cell count, protein, and glucose), and the next 2 ml aliquot was frozen and stored at \(-70^\circ C\) for amino acid analysis. At the time of the LP, blood was collected from a forearm vein: 5 cc of plasma was frozen at \(-70^\circ C\) and stored for amino acid analysis.

Method of Amino Acid Analyses

Plasma and CSF samples were prepared for amino acid analysis by adding equal volumes of 5% sulfosalicylic acid and precipitating the protein by centrifugation at

3.000 rpm for 10 min at 4°C. Aliquots (100 µl) of the supernatant fluid were then analyzed for amino acid content using a Beckman Automatic Amino Acid Analyzer. This method utilized a 30 cm heated ion-exchange column, lithium citrate buffers, and postcolumn ninhydrin derivatization. Detection of the derivatized amino acids was accomplished by colorimetric analysis at 440 and 570 nm. Typical retention times with quantitatively acceptable peak resolution for threonine, serine, and glycine were 47, 49, and 88 min, respectively. The area under each amino acid’s peak was automatically integrated and compared to reference standards; concentrations of each amino acid were expressed as nmol/ml of CSF or plasma.

Statistical Analysis

We summed the semiquantitative rating scale scores for strength in individual leg muscle groups, right and left leg muscle tone, leg DTRs, clonus, gait, hopping, and running in each patient and divided by the number of measurements in order to derive a motor disability score. We then calculated the difference between each patient’s posttreatment scores and the baseline score before beginning L-threonine or placebo. The difference in score for the baseline placebo to placebo treatment condition was then compared to the difference in score for the baseline L-threonine to L-threonine treatment (12). This method of analysis tests for an effect of treatment in the presence of a possible period effect. The same method was used to test for treatment effects on threonine and glycine levels in plasma and CSF, and for differences in symptom scores. Tests comparing changes in severity score or amino acid levels between treatments were one sided; all other tests were two sided.

Comparison of treatment effects based upon the physician’s and patients’ assessments were made using McNemar’s test (13).

RESULTS

Treatment Response by Disability Score

Based upon the disability scores, there was a statistically significant ($p < 0.02$) improvement during L-threonine administration compared to placebo treatment. The mean spasticity score went from $1.56 \pm 0.16$ to $1.54 \pm 0.18$ on placebo and from $1.64 \pm 0.17$ to $1.40 \pm 0.16$ on L-threonine. The differences were $0.2 \pm 0.05$ and $-0.24 \pm 0.08$, respectively. No carryover effect was detected ($p = 0.15$). When the first period of the trial was analyzed separately (i.e., ignoring the second period of treatment and therefore examining the study from the standpoint of a parallel design), the difference favoring scores during L-threonine treatment over placebo remained significant ($p < 0.02$).

When the scores for each clinical observation were tested separately, benefits always favored the L-threonine treatment condition; decreased spasticity (lessened DTRs and reduced clonus) was the most important change (Table 2). Leg strength was normal in almost every patient before treatment and remained strong throughout the study. Muscle tone was increased before treatment in all patients.
TABLE 2. Changes in scores rating spasticity variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg strength</td>
<td>-0.01 ± 0.03</td>
<td>0.61</td>
</tr>
<tr>
<td>Leg tone</td>
<td>-0.06 ± 0.18</td>
<td>0.77</td>
</tr>
<tr>
<td>Leg DTR</td>
<td>-0.32 ± 0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Clonus</td>
<td>-2.46 ± 0.85</td>
<td>0.01</td>
</tr>
<tr>
<td>Walk</td>
<td>-0.15 ± 0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Hop on left foot</td>
<td>-0.15 ± 0.11</td>
<td>0.19</td>
</tr>
<tr>
<td>Hop on right foot</td>
<td>-0.20 ± 0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>Run</td>
<td>0.00 ± 0.10</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean summed severity score</td>
<td>-0.27 ± 0.10</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are expressed as the spasticity score during placebo minus the score during L-threonine treatment. Positive scores favor placebo, and negative scores favor L-threonine.

and insignificantly decreased during threonine administration. Estimates of walking and hopping ability tended to improve during threonine treatment.

Global Clinical Assessment

Based upon the physician’s overall clinical assessments and upon the patients’ self-evaluations of response, there were no significant differences between threonine and placebo treatment conditions. Among separate features of spasticity, four patients reported decreases in leg flexor spasms during L-threonine treatment, and none reported benefit during placebo treatment. Three patients reported improved bladder function with L-threonine and one improved during placebo administration. Less clonus was detected in four patients and decreased hyperactivity of DTRs in three other patients during L-threonine administration; no changes in these measures were detected during placebo. None of these subjective differences was statistically significant. There were no noticeable clinical side effects of L-threonine administration; the results of hematologic, hepatic, and renal function tests did not change during treatment.

Biochemical Results

There were no significant differences in either blood or CSF levels of threonine or glycine between patients receiving 4.5 g/day and those receiving 6.0 g/day of L-threonine (Fig. 1). Plasma threonine levels in FSP patients during placebo treatment ranged between 109 and 439.7 nmol/ml, with a mean ± SEM of 192.3 ± 20 nmol/ml; CSF threonine levels ranged between 32.8 and 120.2 nmol/ml with a mean of 51 ± 6.4 nmol/ml. During the same condition, glycine levels in the plasma ranged between 140 and 422.9 nmol/ml with a mean of 248 ± 24.1 nmol/ml and in the CSF between 5.1 and 15.7 nmol/ml with a mean of 8.4 ± 0.7 nmol/ml. Administration of L-threonine in doses of 4.5 and 6.0 g/day increased the mean blood threonine level to 415 ± 31.9 nmol/ml (p < 0.05) and mean CSF threonine to 101 ± 9.7 nmol/ml (p < 0.05). Despite a significant increase in threonine levels during L-threonine administration, the calculated plasma:CSF threonine ratio remained constant. Mean glycine levels in plasma of 279.8 ± 21.0 nmol/ml and in CSF of 7.6...
FIG. 1. L-threonine in doses of 4.5 and 6.0 g/day produced comparable significant increases in threonine levels in plasma.

0.8 nmol/ml were not significantly different from levels noted during placebo administration (Table 3).

DISCUSSION

The results of this study indicate that L-threonine in oral doses of 4.5 and 6.0 g/day did not produce clinically valuable improvement in motor function in FSP. Nonetheless, the finding that mean spasticity rating scores improved significantly during L-threonine administration suggests that L-threonine has promise in the treatment of symptoms due to upper motor neuron lesions including spasticity.

Drugs currently used in the treatment of spasticity (7-9) aim at either enhancing presynaptic inhibition (benzodiazepines, baclofen) or at diminishing muscular contraction (dantrolene). Benzodiazepines increase presynaptic inhibition by increasing the affinity of γ-aminobutyric acid (GABA) receptor sites for the endogenous ligand. Dantrolene’s therapeutic effect occurs directly on contractile mech-

TABLE 3. Changes in threonine and glycine levels during placebo and L-threonine administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Threonine (nmol/ml)</th>
<th>Glycine (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>CSF</td>
</tr>
<tr>
<td>Placebo</td>
<td>192.3 ± 20.0</td>
<td>51.0 ± 6.4</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>415.0 ± 31.9*</td>
<td>101.0 ± 9.7*</td>
</tr>
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</table>

* p < 0.05 compared to placebo.
anisms within muscle, without any specific action on reflex pathways. These current modalities for treating spasticity often have significant side effects and, at effective doses, may cause or exacerbate weakness.

In the present study, we used a novel approach to the treatment of spasticity, and sought to increase the inhibitory glycinergic tone of spinal cord interneurons. This strategy is based upon the knowledge that glycine is a major postsynaptic inhibitory neurotransmitter in the spinal cord (14–16), and upon the premise that threonine administration increases glycine levels in the spinal cords of laboratory animals (10). Prior attempts to increase brain levels of glycine by administering the amino acid glycine itself were generally unsuccessful due to very limited passage of glycine across the blood–brain barrier (17). Serine is a natural precursor of glycine that also crosses the blood–brain barrier only poorly; its administration did not increase brain glycine levels (18,19), perhaps because the catalytic enzyme serine transhydroxymethylase (STHM) is almost fully saturated with serine under physiological conditions. L-threonine is another substrate for STHM; moreover, the enzyme is highly unsaturated with this substrate. This observation led to Maher and Wurtman’s seminal discovery that threonine administration to rats caused dose-dependent increases in spinal cord threonine and glycine levels (10). Shortly after that report was published, Barbeau et al. (20) reported that 500 mg/day of L-threonine suppressed spasticity in six of six patients with spinocerebellar degeneration; all showed improvement with lessening of tendon reflexes and muscle spasms. Despite this encouraging preliminary report, there apparently have been no other clinical studies in which L-threonine has been tested. For our study, we selected patients with FSP because they have relatively pure involvement of the corticospinal (pyramidal) tracts uncontaminated by other central nervous system lesions; importantly, interneurons whose inhibitory actions are mediated by glycine are intact (21–23). Proof that conversion of L-threonine to glycine occurs in humans, and that the resulting increases in cord glycine levels can lessen the symptoms of spasticity, is inferential. The most direct evidence for a glycine mechanism would be glycine measurements in the spinal cord under different treatment conditions during life. CSF is considerably more accessible than spinal cord tissue, but this body fluid provides an indirect, and possibly misleading, estimate of actual glycine concentrations in spinal cord synapses.

L-threonine in the doses administered did not clinically benefit patients with FSP. Although several patients reported specific improvements in spasticity symptoms during L-threonine administration, as a group there were no significant clinical effects during either L-threonine or placebo treatment. Among several possible explanations (e.g., threonine failed to increase spinal cord glycine levels, and glycinergic transmission is not involved in spasticity), the possibility that we used an insufficient dose of L-threonine to obtain the required increase in glycine release seems most reasonable. Had more L-threonine been given, the subclinical improvement detected on analyses of the semiquantitative assessments might have been more evident. Even with the prescribed doses, however, several variables on the motor disability rating scale did improve during threonine administration, including features of spasticity (clonus and abnormal DTRs). These observations imply that spasticity, strictly defined as the exaggerated velocity-

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dependent stretch reflex (24), does not contribute to the motor disabilities of patients with FSP.

In contrast to conventional antispasticity drugs, L-threonine did not produce weakness. Preserved strength may reflect, in part, the unique pathology of FSP: of suprasegmental descending pathways, only the corticospinal tract is affected (25). Lessened spasticity but preserved strength may also be specific to increased glycnergic transmission: segmental connections between interneurons, anterior horn cells, and muscle are anatomically intact and therefore responsive to physiologically induced increases in inhibitory tone. According to this formulation, strength is unaffected because activity in the motor unit is maintained; spasticity is lessened because firing patterns in anterior horn cells are modulated by glycine.

Acknowledgment: This work was supported by a grant (FD-R-00-270) from the Federal Food and Drug Administration. The MGH General Clinical Research Center is supported by CLR 5-M01-RR-01066. We would like to thank Drs. Raymond Adams, Edwin Kolodny, and Rose-Mary Boustany for referring patients to the research study, and Mrs. Rowena Wilder (MGH Research Pharmacist) for preparing L-threonine and placebo capsules. Dr. T. J. Maher provided assistance with amino acid analyses.

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