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Brief Report

Genistin-Rich Soy Isoflavone Extract in Substrate Reduction Therapy for Sanfilippo Syndrome: An Open-Label, Pilot Study in 10 Pediatric Patients

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ABSTRACT

BACKGROUND: Mucopolysaccharidoses (MPSs) are a group of severe metabolic disorders caused by deficiencies in enzymes involved in the degradation of glycosaminoglycans (GAGs)—long chains of sugar carbohydrates in cells that help build bone, cartilage, tendons, corneas, skin, and connective tissue. Although enzyme replacement therapy has become available for the treatment of some types of MPS, effective treatment of neurodegenerative forms of MPS has yet to be determined. Recently, genistein (4′,5,7-trihydroxyisoflavone), a specific inhibitor of protein tyrosine kinase, has been found to inhibit GAG synthesis and to reduce GAG concentrations in cultures of fibroblasts of MPS patients. Therefore, a potential substrate reduction therapy has been proposed.

OBJECTIVE: The aim of this study was to examine urinary GAG concentration, hair morphology, and cognitive function in patients receiving genistin treatment for Sanfilippo syndrome (MPS type III).

METHODS: Patients aged 3 to 14 years with a biochemically confirmed diagnosis of MPS IIIA or MPS IIIB were eligible to enroll in this open-label, pilot study. Genistin-rich soy isoflavone extract 5 mg/kg/d was administered PO for 12 months. Urinary GAG concentration, hair morphology, and cognitive function (measured using

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a modified version of the Brief Assessment Examination [BAE] and parent observations) were measured at baseline and after 12 months of treatment.

RESULTS: Ten patients (6 girls, 4 boys; mean age, 8 years [range, 3–14 years]; mean weight, 28 kg [range, 17–43 kg]) were included in the study. All patients had Sanfilippo syndrome; 5 patients had MPS IIIA and 5 had MPS IIIB. After 1 year, statistically significant improvement was found in urinary GAG concentration, hair morphology, and cognitive function. Urinary GAG concentration decreased significantly in all 5 patients with MPS IIIA and in 2 patients with MPS IIIB ($P = 0.028$). Hair morphology improved significantly in all 5 MPS IIIA patients and in 3 MPS IIIB patients ($P = 0.012$). A significant increase in the BAE score (by 2–6 points) was noted in 8 patients, while the scores of 2 patients did not change after 12 months of treatment ($P = 0.012$). No adverse events (AEs) considered related to treatment were reported. Moreover, no AEs not related to the treatment (apart from classical symptoms of MPS III) were noted.

CONCLUSIONS: This pilot study found some improvements in GAG concentration, hair morphology, and cognitive function in these pediatric patients with Sanfilippo syndrome treated with genistin-rich soy isoflavone extract for 1 year. Clinical trials are needed to evaluate the efficacy and safety of this potential treatment. (*Curr Ther Res Clin Exp.* 2008;69:166–179) © 2008 Excerpta Medica Inc.

KEY WORDS: mucopolysaccharidosis, substrate reduction therapy, genistein, genistin, gene expression.

INTRODUCTION

Mucopolysaccharidoses (MPSs) are genetic diseases that are inherited in an autosomal recessive manner (except MPS II, which is X-linked).[1] Storage of glycosaminoglycans (GAGs) in cells of patients with MPS results in progressive damage of the affected tissues, including the heart, respiratory system, bones, joints, and, in some cases, the central nervous system (CNS).[1] In nearly all cases, except for extremely mild ones (which are rare), the disease is fatal, with an average expected life span of 1 to 2 decades.[1] However, prediction of its severity and clinical progression is usually difficult, even when biochemical and genetic data are available.[2]

Until 2003, no effective, approved treatment was available for all types of MPS. Currently, enzyme replacement therapy (ERT), which includes IV infusion of an active, recombinant form of a deficient enzyme, can be used for treatment of MPS I, MPS II, and MPS VI.[3–7] This therapy is effective in treatment of somatic symptoms (eg, reduction in urinary GAG concentration, decrease in hepatosplenomegaly, improvements in heart and pulmonary functions, increased joint motion).[3–7] However, neurologic symptoms due to GAG accumulation in the CNS cannot be managed by ERT because of inefficient delivery of proteins across the blood-brain barrier. CNS symptoms occur in some MPS I patients (subtype MPS IH), in most MPS II and MPS VII patients, and in all MPS III patients, in whom they are especially severe.[1] Although intrathecal administration of α-L-iduronidase was found to reduce lysosomal GAG storage in the brain and meninges in the canine model of MPS I,[8,9] such a therapy might be problematic as a chronic treatment in humans because of the risk associated with this invasive proce-

dure, which includes the frequency with which it would need to be performed (usually weekly) and the need to immobilize the patient. Pediatric patients with MPS are usually not compliant with medical procedures, and any anesthetic procedure is associated with high risk in these patients due to problems with intubation.[1] Bone marrow and cord blood transplantation were found to some extent to protect against the development of severe CNS symptoms when performed early (up to the second year of life); but such treatments did not restore function that had already been lost.[10]

Sanfilippo syndrome (MPS type III) comprises 4 subtypes (MPS IIIA, B, C, and D) that have similar clinical symptoms and are characterized by lysosomal storage of the GAG heparan sulfate (HS).[1] This condition is associated with severe learning disabilities, behavioral disturbances, and relatively mild somatic involvement. In all affected patients (except very rare cases of extremely mild symptoms), the progressive nature of the disease leads to death in the second (or rarely the third) decade of life.[1] Because this disorder primarily affects the brain and nervous system, attempts to cure patients have not been successful, with treatment being limited to palliative or symptomatic care.[1]

Our previous study[11] found that the isoflavone genistein (4′,5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-[4-hydroxyphenyl]-4H-1-benzopyran-4-one) inhibited GAG synthesis in fibroblasts of MPS I, MPS II, MPS IIIA, and MPS IIIB patients, as measured by incorporating a radioactive precursor. This inhibition led to a decrease in lysosomal GAG storage that was observed after 1 week of incubation of fibroblasts with genistein at concentrations between 10 and 30 μM (determined biochemically by measuring intracellular GAG concentration and in electron microscopic studies by observation and counting atypical intracellular structures).

The rationale for the use of genistein was based on previous observations that maximum synthesis of some GAGs requires either follicle-stimulating hormone or epidermal growth factor (EGF).[12,13] EGF influences expression of certain genes by binding to its transmembrane receptor, which then becomes an active protein kinase, initiating a specific kinase cascade that finally results in regulation of activity of particular transcription factors. This tyrosine-specific protein kinase activity of the EGF receptor is inhibited by genistein.[14,15] Therefore, we proposed to use a potential substrate reduction therapy that is based on the action of genistein.

In a landmark study,[16] genistein was found to cross the blood-brain barrier in rats. A subsequent study[17] found that another inhibitor of GAG synthesis, rhodamine B, appeared to be effective in the treatment of MPS IIIA in mice. After administration of rhodamine B 1 mg/kg, reduction of GAG storage was evident in the brain, although a decrease in urinary GAG concentration was less pronounced compared with untreated mice.[17] The mechanism of action of rhodamine B is unknown, and the use of this compound in clinical practice is unlikely due to possible toxic effects.[17] However, a similar degree of inhibition of GAG synthesis in fibroblasts was achieved using genistein and rhodamine B.[11,17] Genistein has been reported to have good tolerability and a good safety profile.[18–20] Therefore, we decided to perform a pilot clinical study with Sanfilippo patients in which urinary GAG concentration, hair morphology, and cognitive function were examined using previously developed and verified methods[21–26] that were validated in our laboratories.

PATIENTS AND METHODS

PATIENTS

Patients with a biochemically confirmed diagnosis and aged between 3 and 14 years were eligible to enroll in this open-label, pilot study.

Sanfilippo syndrome (MPS IIIA or MPS IIIB) was diagnosed by estimating urinary GAG concentration (considerably elevated [defined as >1 SD]), using semiquantitative turbimetric analysis, and the identification of GAG excretion pattern (ie, presence of HS) as primary indicators for MPS, and measuring the activities of particular lysosomal hydrolases in leukocytes. Deficiency in activity of heparan *N*-sulfatase (mean [SD] control value: 4.1 [1.4] nmol/mg protein for 18 hours) and α-*N*-acetylglucosaminidase (mean [SD] control value [m]: 90 [34] nmol/mg protein for 42 hours) were considered diagnostic criteria for MPS IIIA and MPS IIIB, respectively. This diagnostic procedure was validated in our laboratory.

PILOT CLINICAL STUDY

Patients with MPS IIIA and MPS IIIB were eligible for this open-label, pilot clinical study in which a genistin-rich soy isoflavone extract was administered orally for 12 months. The extract, SE-2000, was provided by Biofarm International, Ltd., Poznan, Poland. SE-2000 consists of genistin and genistein (26.90%), daidzin and daidzein (13.37%), glicitin and glycitein (1.98%), soy proteins, carbohydrates, and lipids (remaining amount). Genistin is a glycan that can be converted to genistein by either an acid environment or intestinal bacteria. The extract was administered orally at a dose corresponding to 5 mg of genistin and genistein kg/d.

Three parameters—urinary GAG concentration, hair morphology, and cognitive function—were estimated at baseline and after 12 months of the treatment. Since patients received the extract at home and visited the clinic for study purposes only at baseline and after 12 months of treatment, the monitoring of adverse events (AEs) was based on reports of the parents, who were instructed to report AEs immediately (by telephone or e-mail, with confirmation of receipt of the information). Parents also provided a written assessment of the AEs every 3 months, even if no AEs were observed.

This pilot clinical study was approved by the Independent Bioethics Committee of the Medical University of Gdansk, Gdansk, Poland. The parents of the children involved in the study provided written informed consent.

Estimation of Urinary Heparan Sulfate Concentration

In all MPS types, elevated urinary GAG concentration is a characteristic feature, which is employed in both diagnostics and monitoring of treatment.[3–7] Because HS is the only GAG that accumulates in MPS III, we measured this compound in urine samples of the patients using a method that was previously reported to be adequate and able to produce results compatible with other commonly used methods.[25,26] The urinary HS concentrations were determined using an HS enzyme-linked immunoassay kit (Seikagaku Corporation, Tokyo, Japan) according to the manufacturer's instruction and were calculated as milligrams of HS per gram of creatinine. Creatinine concentration was determined using a QuantiChrom™ creatinine assay kit (DICT-500, BioAssay Systems,

Hayward, California) for quantitative colorimetric creatinine determination at 510 nm. Urine collections were analyzed at 24-hour intervals.

Hair Morphology Evaluation

Hair morphology is considerably changed in patients with MPS I, MPS II, MPS IIIA, and MPS IIIB and can be visualized using electron microscopic analyses.[21] Moreover, hair morphology has been found to normalize within 12 months of starting ERT in MPS I patients,[24] indicating that it could be used to assess the effectiveness of treatment.

The evaluation we used was based on a previously published method that used a semiquantitative scale (0 = normal hair morphology; 1 = minor changes in hair surface [ie, minor cavity]; 2 = deeper cavity and hilly hair surface; 3 = unambiguous concave and convex areas on the long hair axis with appearance of the hair as flat in some area; 4 = deeper changes of the type characteristic for score 3; and 5 = the most severe hair morphology abnormalities, which include, apart from features described above, characteristic hair twisting) assessing hair morphology in MPS.[21] It has been found to be appropriate for use in clinical studies. During the procedure, a hair sample (each including a hair follicle) was collected from the scalp of each patient. The samples were sterilized prior to examination in the scanning electron microscope by washing with 5% sodium dodecyl sulfate for 20 minutes and then rinsing twice in distilled water for 15 minutes. Air-dried hair samples were attached to an SPI carbon conductive double-sided adhesive disc. The samples were gold-coated (SPI Module™ Sputter Coater; SPI Supplies, West Chester, Pennsylvania) for 120 seconds. Scanning electron micrographs were obtained using a Philips XL30 microscope (FEI Worldwide Corporate, Hillsboro, Oregon) operating at 5 kV-acceleration.

Brief Assessment Examination

Since severe learning disabilities, behavioral disturbances, and neurologic symptoms are the most pronounced problems in Sanfilippo syndrome, assessment of cognitive function in the patients was crucial to estimate the effects of therapy. A modified version of the Brief Assessment Examination (BAE)[22,23] was used to assess cognitive and behavioral changes at baseline and after 1 year of treatment. This test has been validated for children with severe mental disorders. The following 8 parameters were estimated: alertness/activity; obeying commands; pointing at objects; pointing at colors; matching shapes; speech; auditory digit span; and mathematics (score maximum for each parameter was 6 or 7, depending on the individual measurement). The overall BAE scale ranged from 0 to 52 (0 = no contact with the tested child and 52 = normal score for properly developed child at the age of 3 years).

Parents' Observations

The parents of the patients were given a questionnaire on which they were asked to assess 18 parameters regarding changes in the condition of their children from 1 year before treatment to the start of treatment and from the start of treatment to the end of treatment. The parents performed the first assessment prior to the start of treatment

and performed the latter assessment at the end of the study (ie, after 12 months). They were instructed to be objective. Some parents reported they relied, in part, on the opinion of other, uninitiated persons (eg, teachers). Nevertheless, it is worth noting that responses could be subject to recall bias and the results might have been subjective.

STATISTICAL ANALYSIS

Data were analyzed using Statistica software version 7 (StatSoft, Inc., Tulsa, Oklahoma). The Wilcoxon signed rank test[27] was used for comparison between baseline and after a year of treatment. A $P < 0.05$ value was considered statistically significant.

RESULTS

Ten patients (6 girls, 4 boys; mean age, 8 years [range, 3–14 years]; mean weight, 28 kg [range, 17–43 kg]) were included in the study (**Table I**). All patients were clinically diagnosed with Sanfilippo syndrome according to diagnostic criteria. Of the patients included in the study, 5 patients had MPS IIIA and 5 had MPS IIIB. All patients received a genistin-rich soy isoflavone extract (genistein and genistein content = 5 mg/kg/d) administered PO for 12 months.

A significant decrease ($P = 0.028$) in urinary HS excretion was observed in all MPS IIIA patients and 2 MPS IIIB patients treated with the extract (**Figure 1**).

Hair morphology improved significantly ($P = 0.012$) in all 5 MPS IIIA patients and in 3 MPS IIIB patients after 12 months of treatment (**Table II**). **Figure 2** illustrates this improvement in 1 MPS IIIA patient. In the 2 MPS IIIB patients (IIIB-1 and IIIB-2) in whom no changes were noted, the initial abnormality of hair morphology was very mild (score = 1).

Table I. Baseline demographic and clinical characteristics of the study patients (N = 10).

MPS Type	Patient No.	Sex	Age, y	Weight, kg	Residual Enzyme Activity*
IIIA	IIIA-1	Male	6	25	0.3
	IIIA-2	Female	5	18	0.01
	IIIA-3	Male	3	17	0.3
	IIIA-4	Female	6	28	0.12
	IIIA-5	Male	7	30	1.5
IIIB	IIIB-1	Female	11	29	0.1
	IIIB-2	Male	14	36	3.0
	IIIB-3	Female	6	24	8.5
	IIIB-4	Female	13	31	2.6
	IIIB-5	Female	9	43	0.01

MPS = mucopolysaccharidosis.

*Residual activities of enzymes deficient in MPS IIIA and MPS IIIB were measured in leukocytes, and were determined as heparan *N*-sulfatase (MPS IIIA) in nmol/mg of protein · 18 h (controls, 4.1 [1.4]) or as α-*N*-acetylglucosaminidase (MPS IIIB) in nmol/mg of protein · 42 h (controls, 90 [34]).

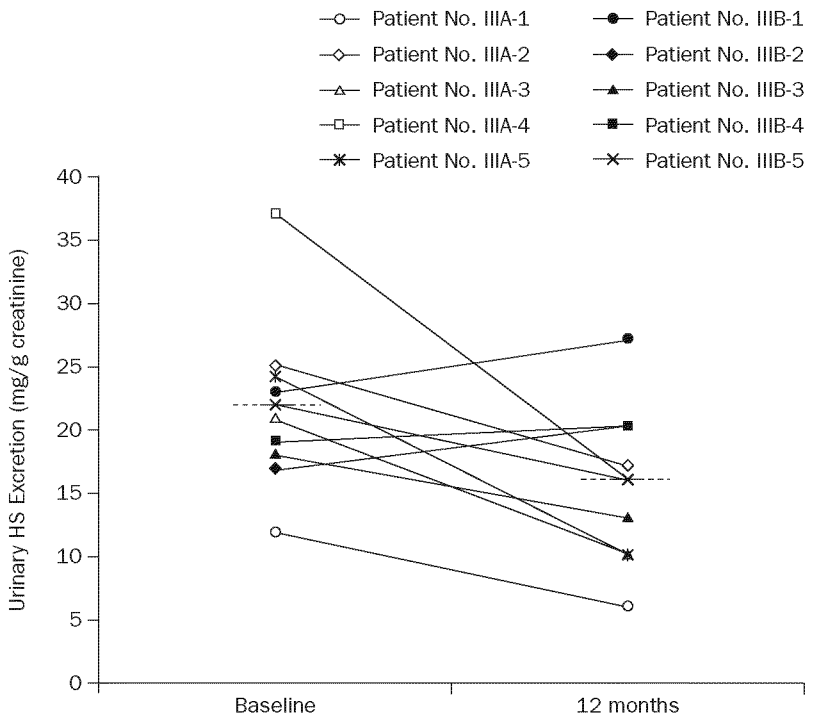

Figure 1. Urinary heparan sulfate (HS) concentrations in patients with mucopolysaccharidosis (MPS) IIIA or IIIB treated with a genistin-rich isoflavone extract. (Median values are shown by the dashed horizontal bars at baseline and 12 months.) *P* = 0.028 versus baseline. Significant changes were found in patients IIIA-1, IIIA-2, IIIA-3, IIIA-4, IIIA-5, IIIB-3, and IIIB-5.

A significant increase in the BAE score (by 2–6 points) was noted in 8 patients, while the scores of 2 patients did not change after 12 months of treatment ($P = 0.012$) (Table II).

Of the 18 variables assessed in the questionnaire regarding changes in the condition of their children, the parents reported deterioration in many parameters in the period from 1 year before treatment to the start of the study, while they described improvement in these parameters from baseline to after 12 months of treatment. After 12 months of treatment, the parents reported subjective improvements in hair structure (assessed by touching), stool frequency and consistency, and the frequency and severity of infections in 8 patients; improvement in sleep habits in 7 patients; improvements in speech comprehension, general behavior, and skin elasticity (assessed by touching) in 6 patients; and improvements in speech performance and dyspeptic symptoms in 5 patients (Table III).

No AEs considered to be related to the isoflavone extract were noted during the study period. Moreover, no AEs that were not related to the treatment (apart from classical symptoms of MPS III) were reported.

Table II. Hair morphology assessed using electron microscopy and cognitive function assessed using the Brief Assessment Examination (BAE) at baseline and after 12 months of treatment with genistin-rich isoflavone extract.[21–23]

	Hair Morphology Score*		BAE Score†	
Patient No.	Baseline	12 Months	Baseline	12 Months
IIIA-1	1	0‡	19	23‡
IIIA-2	1	0‡	14	19‡
IIIA-3	2	1‡	36	36
IIIA-4	2	1‡	0	4‡
IIIA-5	3	1‡	11	17‡
IIIB-1	1	1	9	12‡
IIIB-2	1	1	33	36‡
IIIB-3	2	1‡	12	12
IIIB-4	3	2‡	4	6‡
IIIB-5	3	1‡	27	32‡

*Scale: 0 = normal to 5 = most abnormal.
† Scale: 0 = no contact with the tested child to 52 = normal score for properly developed child at the age of 3 years.
‡P = 0.012 versus baseline.

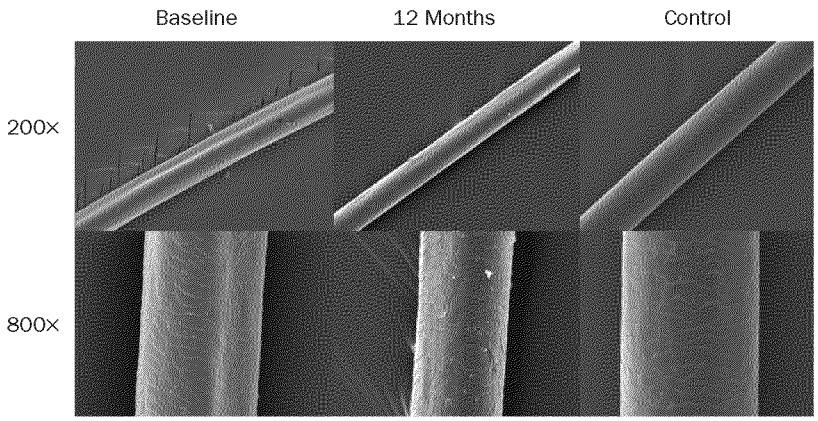

Figure 2. An example of hair morphology in a healthy child and a patient with mucopolysaccharidosis IIIA at baseline and after 12 months of treatment with a genistin-rich isoflavone extract (electron microscopy, original magnification 200X and 800X). Morphology scale: 0 = normal hair morphology; 1 = minor changes in hair surface (ie, minor cavity); 2 = deeper cavity and hilly hair surface; 3 = unambiguous concave and convex areas on the long hair axis with appearance of the hair as flat in some area; 4 = deeper changes of the type characteristic for score 3; 5 = the most severe hair morphology abnormalities which include, apart from features described above, characteristic hair twisting.

Table III. Scores given by parents in assessing their children's condition.*

	Patient No.									
Variable	IIIA-1	IIIA-2	IIIA-3	IIIA-4	IIIA-5	IIIB-1	IIIB-2	IIIB-3	IIIB-4	IIIB-5
Speech performance	–2/–1	0/1	–1/2	0/1	–1/1	–2/0	0/0	0/0	0/0	–1/2
Speech comprehension	–1/2	0/0	–1/2	0/1	–1/1	–2/0	0/1	0/0	0/0	–1/2
Activity	–2/2	0/1	–1/0	0/0	–1/1	0/0	0/0	0/0	0/0	0/1
General behavior	–1/2	0/0	0/2	0/0	–1/1	0/0	0/1	0/1	0/0	–1/2
Sleep habits	–2/2	0/0	0/2	0/0	–1/1	–2/1	–1/1	0/1	0/0	–2/2
Pain	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	–2/2
Walking	–2/2	0/1	–1/0	0/0	0/1	0/0	–1/0	–1/–1	–2/–2	–2/1
Manual dexterity	–2/2	0/0	0/0	0/1	–1/1	0/0	0/0	0/0	0/0	0/1
Joint mobility	–2/2	0/0	–1/0	0/1	–1/1	0/0	0/0	–1/–1	–2/–1	–1/1
Breathing problems during the day	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	–1/1	0/0
Breathing problems at night	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/1	0/0	–1/2
Infections	–2/2	–1/2	–2/2	2/2	0/1	0/0	0/2	0/1	0/0	–1/2
Vision	0/0	0/0	0/0	0/0	0/0	0/0	–1/0	0/0	0/0	0/0
Hearing	0/2	0/0	–1/0	0/2	0/0	0/0	0/0	0/1	0/0	0/0
Skin elasticity	0/2	0/1	–1/–1	0/1	0/0	–1/1	0/0	0/0	0/1	–1/2
Hair structure	0/2	0/1	–1/–1	0/1	–1/1	0/2	0/0	0/1	0/2	–1/2
Dyspeptic symptoms	0/2	0/0	–2/1	0/0	–1/1	0/0	–1/0	0/1	0/0	–1/2
Stool frequency and consistency	–2/2	0/0	–2/1	0/–1	–1/1	–1/2	0/1	0/2	–1/2	–1/2

Scale: –2 = considerably deteriorated; –1 = somewhat deteriorated; 0 = no change; 1 = somewhat improved; 2 = considerably improved.
*The first number is the score for the comparison of changes in a particular child from 1 year before treatment to the start of treatment; the second number is the score for the comparison of changes from baseline to 1 year after the start of treatment.

DISCUSSION

In this report we described results of an open-label, pilot clinical study in 10 patients with 2 types of Sanfilippo syndrome (MPS IIIA or MPS IIIB). The aim of this study was to perform a preliminary assessment of the effects of treatment with a genistin-rich soy isoflavone extract. This was based on results of our previous study[11] in which we found that genistein inhibited synthesis of GAGs in cultured fibroblasts, and that prolonged incubation of cells of MPS patients with genistein resulted in a decrease in storage material.

Patients were treated for 12 months with genistin-rich isoflavone extract 5 (genistin and genistein content = 5 mg/kg/d) and 3 main variables were measured—urinary GAG concentration, hair morphology, and cognitive function. In all cases, statistically significant improvements were found between values measured at baseline and after 12 months of treatment.

Urinary GAG concentration decreased significantly in 7 patients after 12 months of the treatment compared with baseline. The absence of a decrease in HS concentration in 3 patients might be due to high day-to-day variability in urinary GAG concentration, as reported by others.[25] Residual enzyme activity was different among all 10 patients, with no correlation between this parameter and HS levels. Therefore, this variable could not be crucial in the changes in urinary HS excretion. The 3 patients whose urinary HS excretion did not change were the oldest patients in this study (ages 11, 13, and 14). It is possible that a longer time might be required to observe significant changes in urinary GAG concentration in older patients.

Hair morphology improved significantly in 8 patients after 12 months of treatment. This finding, although of little importance for patients' quality of life, was a good preliminary indicator of the effectiveness of the MPS therapy.[21,24,26] The dysmorphology of hair strands of MPS I patients was found to improve during ERT[24,26]; the changes in hair morphology characteristic of MPS I also were found in MPS II, MPS IIIA, and MPS IIIB.[21] Therefore, improvement in hair morphology in the MPS III patients in our study might reflect generally positive effects of treatment with this genistin-rich isoflavone extract.

Since severe learning disabilities, behavioral disturbances, and neurologic symptoms occur in Sanfilippo syndrome, we assessed cognitive function of all patients at baseline and after 12 months of treatment. We used a modified version of the BAE that allowed measurement of cognitive and behavioral functions in severely disabled children.[22,23] Significant improvement was found in 8 patients, while there was no change in the BAE score in the other 2 patients. Gradual mental deterioration occurs in Sanfilippo patients after they are aged 3 to 5 years.[1] The condition of each patient in this study was deteriorating when treatment was started. Worsening mental status was not found in any of the study patients after 1 year of treatment compared with baseline. These findings might suggest that treatment had positive effects on mental and cognitive functions in these patients with MPS IIIA and MPS IIIB. However, temporal changes in children cannot be excluded, since there was no control group and the study design was not blind. This might be considered as a limitation in interpretation of our results. Some parents reported subjective improvements in speech performance (5 patients),

speech comprehension (6 patients), activity (4 patients), and general behavior (6 patients) after 12 months of treatment compared with baseline, while all parents reported deterioration or no change in these variables from 1 year before treatment to the start of treatment. This seems to be compatible with the remainder of our findings and supports the preliminary positive results of this therapy.

The potential subjectivity of the parents' opinions in the 18 variables regarding their children's condition before and after treatment must be considered, although the parents were instructed to be objective. There were some indications that the parents attempted to complete the questionnaire in an objective manner. First, as there are generally no serious problems with vision or breathing in patients with MPS III, these questions were designed to be control questions. In fact, most parents indicated there were no changes in these variables in either period. If the opinions were subjective, the parents might have reported improvement in these variables. Second, patients IIIA-1 and IIIA-3 were siblings, as were patients IIIB-1 and IIIB-4. Although both pairs of siblings were assessed by the same pairs of parents, the answers provided in the questionnaire differed considerably from one sibling to the other, which would not be expected in the case of subjective opinions. Furthermore, some parents reported they depended on the opinion of other, uninvolved persons (eg, teachers). This might have decreased the level of subjectivity in the assessment of particular variables.

Sanfilippo syndrome is a lysosomal storage disease (LSD) that is difficult to treat because the neurologic symptoms associated with the disease cannot be effectively managed using ERT, which is associated with inefficient delivery of the enzymes across the blood-brain barrier. Recent clinical studies[28–30] of substrate reduction therapy in Niemann-Pick type C disease found some improvement, particularly in horizontal saccadic eye movement velocity, swallowing capacity, and stable auditory acuity, in patients with this neurodegenerative LSD. Positive effects of substrate reduction therapy on cognitive and behavioral functions might also be suggested on the basis of results presented in this preliminary report. To our knowledge, this is the first report that found positive effects in patients with MPS III following 1 year of pharmacologic treatment. Clinical trials are needed to evaluate the efficacy and safety of this potential treatment.

CONCLUSIONS

This open-label pilot study found some improvements in GAG concentration, hair morphology, and cognitive function in these pediatric patients with Sanfilippo syndrome treated with genistin-rich soy isoflavone extract for 1 year.

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The use of genistein in treatment of MPS is a subject of patent application (International Patent Application No. PCT/PL2006/000064). The authors declare that they have no other competing interests.

E. Piotrowska, MSc, analyzed HS concentrations and contributed to the study design and organization, data interpretation, and writing. J. Jakóbkiewicz-Banecka, PhD, contributed to the study design, data interpretation, and writing. A. Tylki-Szymanska, MD, PhD, DSc, and A. Liberek, MD, PhD, were responsible for the design of the clinical part of the study and contributed to data interpretation. A. Maryniak, PhD, DSc, performed psychological tests. M. Malinowska, MSc, performed most of the electron microscopic analyses of hair morphology. B. Czartoryska, PhD, performed biochemical diagnostic studies. E. Puk, PhD, was responsible for preparation of the genistin-rich soy isoflavone extract. A. Kloska, MSc, participated in electron microscopic analyses of hair morphology. T. Liberek, MD, PhD, DSc, contributed to data interpretation and writing, and performed the statistical analyses. S. Baranska, PhD, participated in electron microscopic analyses of hair morphology. A. Wegrzyn, PhD, DSc, contributed to the study design. G. Wegrzyn, PhD, DSc, was the principal investigator and provided scientific leadership on study design, data interpretation, and writing.

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