PILOT STUDY

Oral Tolerability of Cysteine-Rich Whey Protein Isolate in Autism–A Pilot Study

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ABSTRACT

Purpose: To examine the tolerability of non-denatured whey protein isolate (NWPI) in children with autism. Many children with autism are low in glutathione and have higher levels of oxidative stress. NWPI can raise glutathione levels and reduce oxidative stress. However, anecdotal reports suggest that NWPI may be problematic in children with autism because it contains cysteine and other sulfurated amino acids.

Methods: A 6-week open-label trial was conducted, supplementing 10 children with autism or autism spectrum disorder (ASD), 3-15 years of age, with NWPI (Immunocal®). To measure possible side effects, procedures that examined the frequency, intensity, and types of side effects, as well as behavioral measures, were completed at baseline, and at days 3, 14, 30, and 45.

Results: Seven of the ten children took the supplement over the six-week trial and tolerated it well. Two children discontinued after two weeks due to possible side effects: one due to gastrointestinal disturbance and one due to being less responsive to parents. Another child discontinued due to difficulty of administering the product.

Conclusion: This study suggests that NWPI can be used as a supplement for this small population of children with autism without high rates of side effects, which means that further studies to determine its safety and efficacy in larger populations might yield the same promising result. Larger studies are planned to determine its efficacy in raising glutathione levels.

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INTRODUCTION

Five recent studies showed that oxidative stress and/or lipid peroxidation are increased in autism,[1-6] including research by James et al[4,5] that suggests that glutathione (GSH) is lower in children with autism than in control children, and that a higher fraction of their glutathione is oxidized. For a more complete review of the possible role of oxidative stress and toxicity in the pathology of autism, see Kern and Jones.[7]

A safe supplement that can normalize glutathione levels and reduce oxidative stress could potentially be benefi-

cial in autism. To raise glutathione levels, humans require the building blocks or precursors of glutathione because oral glutathione is considered ineffective.[8,9] Glutathione (GSH), or 2-amino-5-{[2-[(carboxymethyl)amino]-1-(mercaptomethyl)-2-oxoethyl]amino}-5-oxopentanoic acid, is a small protein made up of three amino acids: glycine, cysteine, and glutamic acid. GSH is a thiol and thus contains sulfur.[10,11] The side-chain sulfhydryl residue (-SH) that is in the cysteine part of the molecule is what provides most of its physiological properties.[10,11] Cysteine is the rate-limiting substrate for GSH production.[12]

Non-denatured whey protein isolate is cysteine-rich in the form of cystine (two cysteine molecules linked by a disulfide bond). The term "non-denatured" is preferable to the less precise "un-denatured" to describe a protein that has been preserved in its native state and has retained its original physical characteristics and conformation. Changing a milk protein's native conformation can alter its biological activity.[13] Cystine (reduced cysteine) is much more stable than cysteine and accounts for 90% of the amino acid in the plasma; once it crosses the cell membrane, it is oxidized to cysteine and then used to make glutathione.[10,14] Non-denatured whey protein concentrates and isolates have been shown to increase glutathione levels in many diseases and disorders such as acquired immune deficiency syndrome (AIDS), cystic fibrosis, lung disease, chronic fatigue syndrome, hepatitis B, and cancer (e.g., colon, liver, breast, pancreas), without any toxicity or adverse events.[10,14-26] There have been only occasional cases of gastrointestinal upset reported.[14]

Non-denatured whey protein isolate use has not been examined in autism; and although reports are conflicting, some anecdotal reports suggest that some children with autism may have problems with ingestion of cysteine-rich or any sulfur-rich compound, such as whey protein. Whey protein concentrates (>70% protein) which contain the milk protein caseine can be further problematic. The problems anecdotally reported are worsening of behavior and gastrointestinal disturbance with dysbiosis (specifically, an increase in yeast). In this population, food intolerances and GI disturbance are commonly reported.[27] Because of the anecdotal reports that suggest that children with autism may not tolerate sulfur-rich compounds, combined with the evidence that non-denatured whey protein isolate is safe and effective in raising glutathione levels and reducing oxidative stress in other disorders and diseases, it was determined that a trial using non-denatured whey protein in autism to examine its tolerability in this population was necessary. This study used Immunocal®, a non-denatured whey protein isolate (>90% protein), which is a medically recognized option for raising glutathione levels.

The study was conducted to determine if: (1) the children would take this supplement, which is a bland-tasting powder, and (2) the supplement would be tolerated even though it is a sulfur-rich compound.

METHODS

Design: The study, which used Immunocal®, was a six-week, open-label clinical trial with ten children previously diagnosed with autism or ASD. Informed consent and HIPAA forms were obtained from every child's parent. Children were always in the presence of one or both parents. At baseline, information regarding demographics, formal diagnosis, age at diagnosis, age of apparent onset, information regarding delay or regression, any current medical issues, medications, and allergies was obtained on each child. A Childhood Autism Rating Scale (CARS)[28] was also completed at baseline. After a stool specimen was collected (by the parent at home) for analysis, each child was started on Immunocal®. The stool specimen was completed on seven of the ten children before the treatment to acquire a baseline for dysbiosis (three parents were not able to obtain usable specimens). Other measures examining behavior and side effects were used as repeated measures and were completed periodically by the parents to examine any clinical change in the child. These measures are listed in the measures section.

Location: This study took place at the Mood Disorders Research Program and Clinic at the University of Texas Southwestern Medical Center (UTSW) in Dallas, Texas. The study protocol received Institutional Review Board (IRB) approval from the University of Texas Southwestern Medical Center. All parents signed a consent and Health Insurance Portability and Accountability Act (HIPAA) form and all received a copy.

Subjects: Subjects for this study were recruited from autism societies and physicians from the Dallas/Fort Worth area, and via the Internet (by posting on the UTSW study site). The children were three to fifteen years of age. Six had a formal diagnosis of autism and four had a formal diagnosis of ASD. The CARS ranged from 30 to 43 with a mean of 35.4 (SD= 4.4), thus ranging from mild to severe autism. There were nine males and one female. Eight children were Caucasian and two were African-American. The exclusion criteria were: (1) already taking whey protein or starting any new drug or therapy; and (2) a comorbid diagnosis of Fragile X disorder, tuberous sclerosis, phenylketonuria (PKU), Lesch-Nyhan syndrome, fetal alcohol syndrome, or a history of maternal illicit drug use.

MEASURES

Comprehensive Parasitology (CP3): The CP3 panel includes a standard microbiology (bacterial culture), yeast culture and speciation, microscopic evaluation for yeast (KOH stain), and parasites. This test was completed before treatment to acquire a baseline for possible dysbiosis.

Childhood Autism Rating Scale: The CARS is a 15-item behavioral rating scale developed to identify autism as well as to quantitatively describe the severity of the disor-

der.[28] Independent reports on CARS indicate that it has high validity. Eaves and Milner[29] found that it correctly identified 98 percent of autistic subjects and correlated (r = 0.67) with the Autism Behavior Checklist. In another similar study, 92 percent of subjects were correctly classified, and the CARS correlated with the Real Life Rating Scale.[30] The CARS was completed by the Principal Investigator by observing the subjects and interviewing parents at baseline. The CARS was also completed by a parent on study days 1, 3, 14, 30, and 45.

Frequency and Intensity of Side Effect Rating (FISER)/Global Rating of Side Effect Burden (GRSEB)/: The FISER/GRSEB surveys include global measures, each using a 7-point Likert-type scale rated 0 to 6. One rate is anchored for frequency, another rates the intensity of side effects encountered in the prior week that the caregivers believe were due to the treatment, and the third asks caregivers to estimate the overall burden or degree of interference in day-to-day activities and function due to side effects attributable specifically to the treatment.[31] The survey was completed by a parent on study days 3, 14, 30, and 45.

Patient Rated Inventory of Side Effects (PRISE)—modified: The PRISE lists a variety of possible side effects to choose from and a scale to rate the specific side effect. The list also includes gastrointestinal side effects. In addition, the measure has a place to list any side effects not previously listed. The survey was completed by a parent on days 3, 14, 30, and 45.

Aberrant Behavior Checklist, Subscales I to V (ABC): The ABC scale rates inappropriate and maladaptive behavior.[32] The ABC was designed to monitor the behavioral effects of psychotropic drugs. The scale was completed by a parent at baseline, and at days 3, 14, 30, and 45.

Clinical Global Impression Scale (CGI): The CGI three-item scale asks the caregiver to mark the patient as better (1), the same (2), or worse overall (3). The scale was completed by the parent at baseline and at days 3, 14, 30, and 45.

Treatment Adherence Measure (TAM): The TAM is a ten-item, Morisky-type self-report on treatment adherence that asks specific questions regarding the dose and frequency of use. The TAM was used to determine the level of adherence to the treatment. Morisky-type adherence measures have been used widely and have shown good reliability for a self-report measure.[33] The measure was completed by a parent at days 3, 14, 30, and 45.

Product Used: A medically recognized option for raising glutathione levels, Immunocal® is a bovine milk serum protein or non-heated non-denatured whey protein isolate manufactured in a way that preserves the native protein configuration of the whey. It is a white powdery substance that comes in an airtight and humidity resistant envelope. Immunocal® contains <1 percent lactose, <1 percent fat, and minimal amounts of caseine (*Physician's Desk Reference; PDR*).

Dosing and Administration: Recommended dosing for children is 10g per day for those over 40 pounds and 0.5 grams per kilo (2.2 pounds) of body weight for children under 40 pounds. Due to reports of intolerance of sulfur-rich foods in this population, the dosing of the non-denatured whey protein isolate was cautiously titrated. Dosing began at one-fourth dose for 5 days, then one-half dose for 5 days, then three-fourths dose for 5 days, and finally a full dose by approximately the third week. Immunocal® was taken with a light meal or on an empty stomach once or twice per day. The powder was mixed with fruit juice or food. It was not heated or blended in a power blender. Parents gave the children Immunocal® mixed in a variety of ways (e.g., orange juice, Hershey's chocolate syrup, ice cream, chocolate milk, Carnation Instant Breakfast, applesauce, peanut butter and jelly, yogurt, and Boston Chicken's Caesar salad dressing).

RESULTS

Seven out of the ten children took the supplement over the full six-week trial and tolerated the supplement. Three children discontinued after two weeks of the trial. One child discontinued because of gas and bloating. This occurred at a higher dose level, but not at a lower dose level. This child's baseline bowel flora was within normal limits, but the child did have a history of chronic diarrhea. No other children had any gastrointestinal changes, even the two children who had dysbiosis at baseline. One child discontinued the study due to being less responsive to parents. Another discontinued because one parent had difficulty administering the whey product. Of the completers, two parents verbally reported that their children became more hyperactive initially; however, the hyperactivity resolved in a few weeks.

Data were collected on a number of behavioral measures, the Childhood Autism Rating Scale, the Aberrant Behavior Checklist, and a Clinical Global Impression measure. Means for the three non-completers and seven completers are presented below (Table 1). These measures were used to examine any clinical change in the child that we would need to be concerned about based on anecdotal reports. The data were insufficient for statistical analysis. It is worth noting that the changes in the behavioral measures appear to be in a positive direction (Figure 1). Caution should be used in interpreting these findings since any meaningful test would require a larger study and a control group.

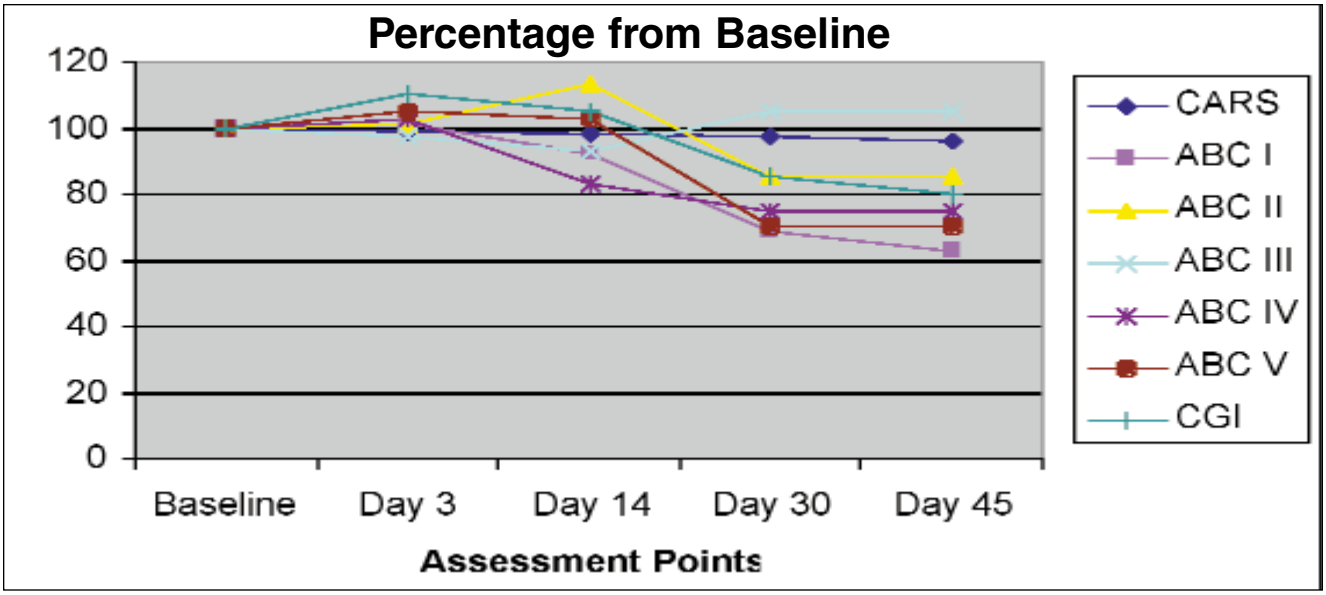
Treatment adherence in the completer group was good, with most participants taking their doses 75 to 100 percent of the time. Parents were allowed to mix the whey with anything as long as it was not blended with a power blender or heated (to avoid denaturization). Some parents had more

Table 1.

Measures	**Baseline** Mean (SD) n=10	**Day 3** Mean (SD) n=10	**Day 14** Mean (SD) n=9	**Day 30** Mean (SD) n=7	**Day 45** Mean (SD) n=7
CARS	33.1 (6.6)	33.4 (6.36)	32.6 (4.8)	32.1 (5.3)	31.7 (5.5)
ABC I	10.6 (7.6)	10.7 (7.5)	9.8 (6.6)	7.3 (5.4)	6.7 (5.0)
ABC II	8.2 (8.5)	8.3 (8.4)	9.3 (8.3)	7.0 (7.3)	7.0 (7.3)
ABC III	4.1 (3.2)	4.0 (3.2)	3.8 (3.3)	4.3 (3.9)	4.3 (3.9)
ABC IV	16.5 (13.3)	17.0 (13.5)	13.7 (9.6)	12.4 (11.2)	12.4 (11.2)
ABC V	3.7 (3.1)	3.9 (2.8)	3.8 (3.1)	2.6 (1.6)	2.6 (1.6)
CGI*	2	2.2 (0.4)	2.1 (0.3)	1.7(0.5)	1.6 (.5)

This table shows the mean and standard deviation (SD) of the parent-rated measures on all participants (completers and non-completers), pre-treatment (baseline), and post-treatment. A decrease in score represents improvement. *Note that all participants started as a 2 on the CGI.

Figure 1.



This figure shows the percentage change (lower is better) over time on CARS, ABC I-V, and CGI.

difficulty getting their child to take the whey than others, with some parents having to try several different ways to mix it and some having no difficulty at all.

A stool specimen (CP3) was completed on seven of the ten children (three parents were not able to obtain usable specimens) before the treatment to acquire a baseline for dysbiosis. Two children had dysbiosis at baseline: one child had moderate yeast overgrowth and one child had *Staphylococcus Aureus* (a bacterium). Neither of these two children had any GI changes with treatment.

DISCUSSION

The study suggests that, for the most part, children with autism do not have problems tolerating cysteine in this form (cystine/whey protein isolate). The results are limited due to fact that the study was conducted on very small sample and for a relatively short period of time. Therefore, a larger and longer study would be necessary to get specific estimates of tolerability and to measure the possible benefits.

As mentioned, one child discontinued the study because of gas and bloating. A variety of medical conditions is often reported in children with autism, including different GI symptomatology.[34] In this study, for example, four children were reported to have GI function within normal limits, three were reported to have chronic constipation, and three were reported to have chronic diarrhea (prior to the start of the study and did not change). This variation suggests that children with autism may tolerate some supplements or treatments better than others. It can be hypothesized that reactions to whey proteins in these individuals may be due to the presence of casein, another milk protein that can be present in whey concentrates or other whey products. Casein-elimination diets have been reported as beneficial in ASD.[35]

Also, as mentioned, one child discontinued the study due to being less responsive to his parents. Overall, the parental reports and measures suggest that, generally, there was no worsening of behavior. Interestingly, four parents reported improvements in their children's ability to communicate and willingness to interact with others, and the CARS, ABC, and CGI all showed a trend toward the positive. Figure 1 shows that trend. However, since the trial was not a blinded trial, it is difficult to determine what was placebo effect and what was treatment effect.

The main purpose of this study was to establish some level of understanding of the safety and tolerability in the use of a cystine-rich (sulfur-rich) compound in the treatment of autism. Ultimately, it is important to know whether non-denatured whey protein can be used to raise GSH levels in these children.

As mentioned in the Introduction, non-denatured whey protein isolate has been shown to increase glutathione levels and improve the healing process in many diseases and disorders, such as cystic fibrosis, lung disease, AIDS and other types of immune deficiencies, chronic fatigue, hepatitis B, and cancer, with no toxicity or adverse events.[14-26] Immunocal®, a non-denatured whey protein isolate, has been shown to augment GSH levels and to improve clinical parameters.

For example, a study by Grey et al.[26] showed that, compared to casein, Immunocal®, 10 g twice a day, increased glutathione levels in patients with cystic fibrosis. An overabundance of oxidants relative to antioxidants is associated with cystic fibrosis.[26] Glutathione functions in the lung as a major frontline defense against oxidative stress.

A Canadian clinical trial using Immunocal® was conducted in children (8 months to 15 years old) with AIDS and wasting syndrome. Immunocal® was administered for six months. All of the children experienced weight gain. Six of the ten patients had improved anthropometric parameters (skin-folds/triceps/mid arm circumference). The GSH-promoting activity of the whey supplement showed in six out of ten subjects.[14]

Watanabe et al.[20] showed that Immunocal® improves GSH levels, as well as liver and immune function, in hepatitis B patients. Serum alanine aminotransferase activity decreased, lipid peroxide levels significantly decreased, and interleukin 2 levels and natural killer activity increased.

A study of performance enhancement with Immunocal was conducted with 10 healthy males and 10 healthy females by Lands et al.[36] Lymphocyte GSH was significantly higher in the treatment group. Likewise, the supplemented subjects had a significantly improved peak power and 30-second work activity as compared to placebo.

Because this product is safe and effective in raising glutathione levels in other disorders and diseases, it is possible that it can be used in autism. This current trial suggests that non-denatured whey protein isolate can be used as a dietary supplement for children with autism without high rates of GI disturbance or other side effects. However, because autism involves a heterogeneous population, parents and clinicians should observe each child individually and understand that it may be better tolerated in some children than others. Since it shows acceptable tolerability, further research is needed and planned to determine its efficacy in raising GSH levels in those with autism and its effect on behavior in autistic children.

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POTENTIAL CONFLICTS OF INTEREST

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