Original Research

An Open-Label Pilot Study of a Formulation Containing the Anti-Inflammatory Flavonoid Luteolin and Its Effects on Behavior in Children With Autism Spectrum Disorders

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ABSTRACT

Background: Accumulating evidence suggests an association between autism spectrum disorders (ASD) and inflammation in brain regions related to cognitive function. The natural flavonoid luteolin has antioxidant, anti-inflammatory, mast cell–blocking, and neuroprotective effects. It was shown to improve cognitive performance in a mouse model of ASD, but its effect in humans has not been adequately studied.

Objectives: The goal of this study was to assess the effectiveness and tolerability in white children with ASD of a dietary supplement containing 2 flavonoids (>95% pure), luteolin (100 mg/capsule, from chamomile) and quercetin (70 mg/capsule), and the quercetin glycoside rutin (30 mg/capsule) from the Sophora japonica leaf, formulated in olive kernel oil to increase oral absorption.

Methods: Fifty children (4–10 years old; 42 boys and 8 girls) with ASD were enrolled in a 26-week, prospective, open-label trial at the 2nd University Department of Psychiatry at "Attikon" General Hospital, Athens, Greece. Children were referred for the study by their respective physicians or came from the practice of the senior author. ASD diagnosis by clinical assessment was based on the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision,* symptom list and corroborated by using the Autism Diagnostic Observation Schedule. The dose of the study formulation used was 1 capsule per 10 kg weight per day with food. The primary outcome measures were the age-equivalent scores in the Vineland Adaptive Behavior Scales domains. Secondary outcomes included the Aberrant Behavior Checklist, the Autism Treatment Evaluation Checklist, and the Clinical Global Impression–Improvement score. Data were measured at baseline, week 18, and week 26. Parents were interviewed for any possible improvements they noticed and instructed to report any unusual adverse events.

Results: A total of 40 children completed the protocol. There was a significant improvement in adaptive functioning as measured by using the VABS age-equivalent scores (8.43 months in the communication domain, 7.17 months in daily living skills, and 8 months in the social domain; $P < 0.005$), as well as in overall behavior as indicated by the reduction (26.6%–34.8%) in Aberrant Behavior Checklist subscale scores. Age, sex, and history of allergies had no effect on the results, whereas the initial level of functioning or difficulty did predict the final outcome in most of the measures used. There was a transient (1–8 weeks) increased irritability in 27 of the 50 participants.

Conclusions: These results are encouraging in that the combination of the flavonoids luteolin and quercetin seemed to be effective in reducing ASD symptoms, with no major adverse effects. ClinicalTrials.gov identifier: NCT01847521. (*Clin Ther*. 2013;35:592–602)

Key words: ASD, luteolin, flavonoids, inflammation, brain.

INTRODUCTION

Autism spectrum disorder (ASD) is a life-long condition characterized by marked impairment in social communication and language, as well as repetitive/restricted behaviors.[1,2] A steady increase in its preva-

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lence has been well documented in the past 20 years, with recent findings ranging from 1.13% in the last Centers for Disease Control and Prevention study[3] (in 2008) to 2.64%.[4] The lack of any definitive pathogenesis has prevented the development of effective treatments for the core symptoms of ASD.[5,6] The current use of medications addresses only specific secondary behavioral symptoms of the disorder. Moreover, recent studies have shown that use of selective serotonin reuptake inhibitor antidepressants[7] and some antipsychotic agents[8] may actually worsen ASD symptoms. Although risperidone and aripiprazole are 2 antipsychotic agents approved for use in children with ASD, these medications only address the disruptive, aggressive, and self-mutilative behaviors and not the core symptoms of the disorder. Only recently has research tried to translate behavioral findings to possible targets for pharmacologic agents in an attempt to address the core symptoms of ASD.[9–11]

Increasing evidence indicates that brain inflammation is important in the pathogenesis of neuropsychiatric disorders,[12,13] including at least a significant proportion of subjects with ASD.[14–16] "Allergic" issues, especially food intolerance and eczema, are often present among children with ASD.[17–20] Mast cells, which are implicated in both allergic and inflammatory reactions, are activated in autism,[21] and the prevalence of ASD seems to be 10 times higher among children with mastocytosis.[22]

Natural flavonoids, such as luteolin and quercetin, exhibit potent antioxidant and anti-inflammatory activities,[23] inhibit the release of inflammatory mediators from human mast cells,[24] and reduce maternal interleukin 6–induced autism-like behavioral deficits related to social interactions in mice.[25] However, these flavonoids have not been adequately studied in children. A case series of children with ASD in the United States (37 children, 4–14 years old) who took a dietary supplement containing luteolin and quercetin* for 4 months reported gains in eye contact and improvements in attention in 50% of subjects and social interaction in 25% of subjects.[26] However, no data were reported regarding subject characteristics, no baseline measurements were taken, and the reported gains were solely based on parental impression, with no use of objective instruments. We conducted here an open-label study by using validated instruments to assess the tolerability and effectiveness of the same trial formulation in Greek white children in 2 age groups (4–6 years old and 7–10 years old) to try and establish any correlation with age, severity of symptoms or history of allergic problems.

*Trademark: NeuroProtek® (Algonot, LLC, Sarasota, Florida).

SUBJECTS AND METHODS

Children were referred to the Athens University "Attikon" 2nd Psychiatric Clinic for ASD from various professionals, as well as from the private practice of the senior author, from around Athens, Greece. The study was announced at ASD support groups and at the ASD clinic. No other centers were involved. The parents of all subjects were informed of the study's aims, including risks versus benefits of participating and not participating as well as the inclusion and exclusion criteria. They provided written consent for participation in the study after being informed of all details of the study. The study was approved by the Ethics Committee of "Attikon" General Hospital, Athens, Greece.

Fifty white children (42 boys and 8 girls; 4–10 years of age) with ASD were enrolled consecutively in this 26-week, prospective, open-label trial after meeting the inclusion and exclusion criteria. They were divided into 2 age groups (4–6 years old, n = 25; 7–10 years old, n = 25). Participants had already been diagnosed with ASD based on clinical assessments, and this diagnosis was corroborated at the 'Attikon' clinic by meeting the cutoff scores on both the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision,* symptom list[27] and the Autism Diagnostic Observation Schedule algorithm.[28] All children were medication naive and had never received the study luteolin formulation. None of the referred subjects met any of the exclusion criteria set (ie, any medical condition likely to be etiological for ASD [eg, Fragile X syndrome, tuberous sclerosis], any neurologic disorder involving pathology above the brain stem [other than uncomplicated nonfocal epilepsy], any evidence of probable neonatal brain damage, mastocytosis [including urticaria pigmentosa] and a history of systemic inflammatory diseases).

Children were administered the dietary formulation made by a Good Manufacturing Practices–certified facility (Tishcon Laboratories, Long Island, New York) under contract from Algonot, LLC (Sarasota, Florida; www.algonot.com). The study formulation contains 2 flavonoids (>95% pure), luteolin (100 mg/capsule, from chamomile) and quercetin (70 mg/capsule), and the quercetin glycoside rutin (30 mg/capsule) from the