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Artemisinin reduces the level of antibodies to gliadin in schizophrenia

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ABSTRACT

Objective: To investigate if adjunctive artemisinin, an anti-malarial compound with in vivo activity against *Toxoplasma gondii*, reduces symptoms or antibodies in schizophrenia.
Method: N = 66 outpatients with schizophrenia were randomized to receive 100 mg of artemisinin twice a day or placebo for 10 weeks after a 2 week placebo run-in in addition to their usual psychiatric medications. Symptoms were assessed biweekly. Antibodies to toxoplasma and to gliadin, a food antigen, were assessed at the beginning and end of the trial.
Results: A total of 57 participants (26 in the artemisinin arm and 31 in the placebo arm) completed the 12 weeks of the trial. The medication was well tolerated and there were no significant side effects associated with the treatment regimen. There was no significant difference in the change of positive, negative, general, or total PANSS symptoms between groups for all of the randomized patients or for just the completers. However, individuals in the artemisinin arm but not in the placebo arm had significant decreases in the levels of antibodies to gliadin ($p<.0005$, $p>.2$, respectively by paired t-test). Neither group had significant changes in antibodies to *T. gondii*.
Conclusions: The study did not demonstrate clinical benefit of adjunctive artemisinin for schizophrenia symptoms. The finding of reduced levels of antibodies to gliadin in the artemisinin group merits further study.

1. Introduction

Schizophrenia is a pervasive human brain disease of unknown etiology. In recent years, evidence has accumulated indicating that some cases of schizophrenia are associated with central nervous system infection (Yolken et al., 2000; Yolken, 2004; Torrey et al., 2007). *Toxoplasma gondii* is a coccidial protozoan of the phylum *Apicomplexa* which is capable of infecting the human brain. Recent studies have indicated that infections with *T. gondii* may contribute to the symptoms of schizophrenia in some individuals. For example, increased levels of antibodies to *T. gondii* have been found in individuals with recent onset schizophrenia in several different study populations (Yolken et al., 2001; Torrey et al., 2007). Increased levels of antibodies to *T. gondii* have been measured in mothers of infants who develop schizophrenia in later life (Mortensen et al., 2007). In addition, several medications commonly used for the treatment of schizophrenia have been found to inhibit the replication of *T. gondii* in cell culture; these medications include haloperidol, clozapine, and olanzapine (Jones-Brando et al., 2003).

Increased immune sensitivity to gluten has also been reported in schizophrenia. In a recent study we found that individuals with a recent onset of psychosis and those with multi-episode schizophrenia have increased levels of IgG class antibodies to gliadin as compared to control individuals without a history of psychiatric disorder (Dickerson et al., 2010). The immune response to gliadin differed from that found in individuals with other forms of gluten sensitivity such as celiac disease (Samaroo et al., 2010). While the mechanism associated with the increased level of antibodies to gliadin in individuals with schizophrenia is not known with certainty, recent studies indicate that experimental infection of the gastrointestinal tract with toxoplasma can result in increased levels of intestinal inflammation and absorption of antigens from the intestinal tract (Bereswill et al. 2010).

Recently, a number of new compounds have been developed for the inhibition of the related protozoa which cause malaria. Most notable of these is artemisinin. Artemisinin (*qinghaosu*) is the antimalarial principle isolated by Chinese scientists from *Artemisia annua L.* (Sweet Wormwood plant), which has been in use in China for more than 2000 years as an herbal tea against fever and malaria and with a low level of recorded side effects (van Agtmael et al., 1999; Gordi and Lepist, 2004). In the United States, artemisinin is sold as a nutritional supplement in health food stores and is regulated by the Food and Drug Administration per the Dietary Supplement Health and Education Act of 1994 (http://www.cfsan.fda.gov/~dms/supplmnt.html).

Toxoplasma and malaria are both apicomplexan protozoa and share a number of metabolic pathways (Chaudhary et al. 2006;

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Gopalakrishnan and López-Estraño, 2010). Because of this, anti-malarial compounds can also have activity against toxoplasma and other apicomplexan organisms (Debierre-Grockiego, 2010; Anquetin et al., 2005). Indeed artemisinin and related compounds have been shown to inhibit the replication of toxoplasma organisms in cell culture (Jones-Brando et al., 2006). Artemisinins have also been shown to inhibit the replication of viral agents in cell culture (Efferth et al., 2008). Exposure to some of these agents, such as cytomegalovirus (CMV) (Arav-Boger et al., 2010) and human herpesvirus 6 (HHV6) (Milbradt et al., 2009) have been associated with increased risk of schizophrenia in some populations.

This clinical trial was undertaken to test the hypothesis that symptoms of schizophrenia may be reduced by the anti-malarial compound artemisinin when used in addition to standard antipsychotic medications. A secondary aim was to investigate the effect of artemisinin on levels of antibodies to the food antigen, gliadin.

2. Methods

2.1. Sample and setting

Participants were recruited from outpatient and rehabilitation programs affiliated with the Sheppard Pratt Health System and other psychiatric agencies in the Baltimore, Maryland area. Inclusion criteria were: ages 18–65; primary DSM-IV Axis I diagnosis of schizophrenia, any type, or schizoaffective disorder; outpatient status; psychotic symptoms which were at least moderately severe as evidenced by one or more PANSS positive symptom scores, and/or PANSS negative symptom scores of 4 or more, or a total PANSS score of 50 or more containing at least three positive or negative items with scores of 3 or more at screening; conformance to PORT treatment recommendation about maintenance antipsychotic medication dose (Lehman et al., 2004); receiving antipsychotic medication for at least 8 weeks prior to starting the study with no medication changes within the previous 21 days and no planned medication changes for the duration of the trial. Exclusion criteria were: diagnosis of mental retardation; history of intravenous drug use; any serious medical condition that affects brain or cognitive functioning or clinically-significant or unstable medical disorder; HIV infection or other immunodeficiency condition; primary diagnosis of substance abuse or dependence within the previous three months; participation in any investigational drug trial in the past 30 days; and pregnancy or planning to become pregnant during the study period. Although *T. gondii* was the target organism for the artemisinin treatment, persons who were toxoplasma negative as well as toxoplasma positive were included in the study in order potentially to determine the relationship between toxoplasma seropositivity and response to artemisinin in the study population.

2.2. Study compound

The artemisinin compound used in the study was distributed by Holley Pharmaceuticals and manufactured in factories which are certified by Good Manufacturing Practices (GMP) and which meet pharmaceutical grade standards (http://holleypharma.com). The manufacturer claims that each batch of artemisinin is assayed for purity using high performance liquid chromatography and tested for in vitro bioactivity. In order to be certain about the quality and purity of the compound, we had the Holley Pharmaceuticals artemisinin 100 mg compound independently tested in a university chemistry laboratory by NMR (Nuclear magnetic resonance) chromatography and found to contain 98.9 mg of artemisinin per tablet, in very good agreement with the posted value of 100 mg artemisinin per tablet; no impurities or identifiable contaminants were found (G Posner, personal communication, 02/28/07). The recommended dosing, per Holley Pharmaceuticals, is 1–2 mg per kg body weight twice a day which translates to 136–272 mg per day for a person who weighs 150 lb. Twice a day dosing is consistent with the relatively short half life of the compound (van Agtmael et al., 1999).

2.3. Study procedures

After a physical and psychiatric evaluation and complete blood count to establish eligibility, each participant was assessed at baseline with the Positive and Negative Syndrome Scale (PANSS), a brief cognitive battery (Repeatable Battery for the Assessment of Neuropsychological Status) (RBANS; Randolph, 1998), and functional performance with the University of California Performance-Based Skills Assessment (UPSA; Patterson et al., 2001; Mausbach et al., 2007). Participants then started a 2 week lead-in period in which they received inactive medication. At week 2, patients were randomized to receive 100 mg of artemisinin twice a day or placebo capsules twice per day. The placebo capsules were indistinguishable from the artemisinin capsules. Artemisinin or placebo was administered in addition to the participants' regularly-prescribed psychiatric medications. Participants were seen weekly to assess adherence to medication and any adverse events. Symptom evaluations were performed at weeks 0, 2, 4, 6, 8, 10, and 12. The RBANS and UPSA were administered at baseline and week 12. Blood samples were drawn at the beginning and end of the study from which antibodies to toxoplasma and antibodies to gliadin were measured.

Written informed consent was obtained from all study participants and the study was approved by Institutional Review Boards of the Sheppard Pratt Health System and the Johns Hopkins School of Medicine. The study was monitored by a Data Safety Monitoring Board.

2.4. Data analysis

Statistical analysis was performed by repeated measures analysis of variance (ANOVA) of PANSS scores during the double-blind phase, week 2 to week 12, both for all participants and for completers only, defined as those who completed the double-blind phase with no more than one missed study visit. Paired t-tests were used to compare antibodies to toxoplasma and antibodies to gliadin between groups for these markers which were obtained at the beginning and end of the study; these analyses included only completers. Paired t tests were also performed to compare the RBANS and UPSA scores by group at the beginning and end of the study.

3. Results

A total of n = 66 patients were enrolled, completed the baseline visit, and were randomized. A flow chart of patient participation is shown in Fig. 1. A total of n = 57 participants, 86% of those randomized, completed the full 12 weeks of the study, n = 26 in the adjunctive artemisinin group and n = 31 in the adjunctive placebo group. There was not a statistically significant difference between completers and non-completers by treatment group. The characteristics of the n = 66 patients who were randomized are shown in Table 1. All of the study individuals were receiving anti-psychotic medication and n = 58 (87.8%) were receiving a second generation anti-psychotic. In terms of individual anti-psychotic medications, a total of n = 21 (32%) were receiving risperidone, n = 8 (12%) olanzapine, n = 17 (26%) clozapine, n = 12 (18%) quetiapine, n = 8 (12%) aripiprazole, n = 8 (12%) ziprasidone; and n = 11 (17%) haloperidol. Several of the individuals were receiving more than one anti-psychotic agent. In terms of additional medications, a total of n = 11 (17%) were receiving valproic acid and n = 24 (36%) an anticholinergic medication. There were no significant differences between the artemisinin and the placebo groups in terms of any demographic or clinical variable including age, gender, education, age of onset, baseline PANSS score, baseline RBANS score, percent