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Predicting relapse in schizophrenia: Is BDNF a plausible biological marker?

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ABSTRACT

Understanding the biological processes that underlie why patients relapse is an issue of fundamental importance to the detection and prevention of relapse in schizophrenia. Brain Derived Neurotrophic Factor (BDNF), a facilitator of brain plasticity, is reduced in patients with schizophrenia. In the present study, we examined whether decreases in plasma BDNF levels could be used as a biological predictor of relapse in schizophrenia. A total of 221 patients were prospectively evaluated for relapse over 30 months in the Preventing Relapse in Schizophrenia: Oral Antipsychotics Compared to Injectables: eValuating Efficacy (PROACTIVE) study. Serial blood samples were collected at a maximum of 23 time points during the 30-month trial and BDNF levels were measured in plasma samples by ELISA. Receiver Operating Characteristic (ROC) curve analysis indicated that BDNF was not a significant predictor of relapse, hospitalization or exacerbation. Regardless of treatment group (oral second generation antipsychotic vs. long-acting injectable risperidone microspheres), baseline BDNF value did not differ significantly between those who experienced any of the adverse outcomes and those who did not. While contrary to the study hypothesis, these robust results offer little support for the use of plasma BDNF alone as a biomarker to predict relapse in schizophrenia.

1. Introduction

Schizophrenia is, by any estimate, a disorder of major public health significance. Although treatment options for this disabling condition have increased (and improved) over time, it is sobering that schizophrenia is still characterized by persistent functional impairment and recurrent psychotic relapses for most/many patients. Wyatt (1991) proposed that relapse in schizophrenia was a biologically toxic event, leading successively to deteriorations in course of illness and overall functioning. Complementarily, Loebel et al. (1992) showed that each successive relapse among first episode schizophrenia patients was more prolonged than the prior relapse. A longitudinal MRI study of patients with schizophrenia found that relapse was associated with progressive loss of cortical tissue and overall brain volume (Andreasen et al., 2013). Ample and compelling evidence, largely from antipsychotic withdrawal and maintenance studies with a placebo-controlled condition (Gilbert et al., 1995; Kane, 1996; Schooler et al., 1997) shows that patients with schizophrenia have an inordinately high risk of relapse over time if they are not receiving treatment with antipsychotic medications. More recent evidence suggests that second-generation antipsychotic (SGA) medications reduce the risk of relapse when compared to first generation antipsychotics (FGAs) (Leucht et al., 2003). Thus, it is plausible that relapse is more than an expression of worsening

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psychopathology but may represent some fundamental pathobiological event and/or follow some discrete neurobiological trajectory. However, the underlying neurobiological mechanisms that drive these effects are not clear. We have previously proposed that brain derived neurotrophic factor (BDNF) might be a biological marker underlying a cascade of events in relapse in schizophrenia (Pillai and Buckley, 2012).

There is intense interest in the discovery and application of biomarkers to major mental illnesses (Arnow et al., 2015; Ivleva et al., 2016; Weickert et al., 2013). BDNF plays a critical role in neurodevelopment and neuronal and synaptic plasticity (Lu et al., 2014). A number of preclinical, clinical, as well as imaging studies indicate a potential role of BDNF in the pathophysiology of schizophrenia (Ahmed et al., 2015; Buckley et al., 2011; Harrisberger et al., 2015). For example, post-mortem studies show that schizophrenia patients have lower BDNF mRNA levels in the prefrontal cortex and hippocampus (Reinhart et al., 2015; Thompson Ray et al., 2011; Weickert et al., 2003). Concentrations of BDNF in cord blood from infants exposed to obstetric complications who later developed schizophrenia were lower than in cord blood from exposed infants who did not develop the disorder (Cannon et al., 2008). Serum and plasma BDNF levels in adult schizophrenia patients are generally reduced compared with healthy subjects, including never-medicated, first-episode patients (Buckley et al., 2007; Pillai, 2008; Pandya et al., 2013). In animal experiments, the acquisition and maintenance of spatial memory are impaired when BDNF signaling is decreased (Mizuno et al., 2000a, b; Linnarsson et al., 1997); however, brain BDNF is increased when rodents perform a spatial learning task (Mizuno et al., 2000a, b) or are housed in cognitively stimulating environments (Ickes et al., 2000). Indeed, Vinogradov et al. (2009) found that schizophrenia subjects who participated in 10 weeks of neuroplasticity-based computerized cognitive training showed a significant increase in serum BDNF compared with carefully matched control subjects. Together, these studies indicate that peripheral BDNF levels may serve as a biomarker for clinical outcomes in schizophrenia subjects.

The PROACTIVE (Preventing Relapse Oral Antipsychotics Compared to Injectables eValuating Efficacy) study was a clinical trial evaluating relapse and clinical outcomes over 30 months in patients who were randomly assigned to receive the long-acting SGA, risperidone microspheres (LAI-R), or oral SGAs. Serial plasma samples collected from the patients enrolled in PROACTIVE were examined to test the hypothesis that a decrease in plasma BDNF levels could predict relapse in schizophrenia. Also, the relationship between plasma BDNF and hospitalization or exacerbation was examined at baseline and follow up visits. Based upon the prevailing findings, albeit from smaller and often cross sectional studies of BDNF, this study was funded by the National Institute of Mental Health (clinicaltrials.gov NCT00330863) as a translational component to the already funded PROACTIVE study to allow a rigorous and longitudinal evaluation of BDNF and relapse in schizophrenia.

2. Methods and materials

2.1. Study design and subjects

The study is described in detail in an earlier publication (Buckley et al., 2015) and was approved by Institutional Review Boards at all sites and at the coordinating center. In brief, 305 patients with a DSM IV-TR diagnosis of schizophrenia or schizoaffective disorder were enrolled at eight U.S. academic centers and randomly assigned to receive either LAI-R or physician's choice of oral SGA medication. Following randomization, clinical teams on-site were not blinded to treatment assignment; however, subjects were evaluated every three months by masked raters at another location via a live and secure two-way video connection. Of the 305 subjects in the PROACTIVE study, 221 are included in the present analysis because the BDNF assessment was added after enrollment in the RCT had started.

2.2. Primary outcome

Relapse, adapted from criteria first used by Csernansky et al. (2002), was defined by at least one of the following: 1) psychiatric hospitalization for worsening symptoms but not for social reasons; 2) increase in level of psychiatric care (e.g. significant crisis intervention to avert hospitalization, emergency room visit, increase in frequency of contact to maintain outpatient status); 3) substantial clinical deterioration as indicated by a score of 6 (much worse) or 7 (very much worse) on the Clinical Global Impressions-Improvement (CGI-I) scale, or a sustained increase in psychotic symptoms as rated by either site or a masked Master Rater; and 4) deliberate self-injury or suicidal or homicidal ideation that was judged clinically significant as determined by the investigator, or violent behavior resulting in clinically significant injury to another person or property damage. Relapses were independently adjudicated by a Relapse Monitoring Board (RMB) of schizophrenia experts, masked to treatment assignment. The RMB also identified less severe episodes of symptom exacerbation defined by substantial and sustained increases in psychotic symptoms that lasted at least four weeks (three biweekly visit ratings).

2.3. Plasma BDNF levels

Blood sample was collected at every six weeks or during relapse visit. The samples were collected in vacutainers containing EDTA and centrifuged at 3000 rpm for 30 min at 4 °C. Plasma was stored at −80 °C, until used for analyses. Plasma levels of BDNF were determined by using an enzyme-linked immunosorbent assay (ELISA) (BDNF Emax Immunoassay System, Promega, USA), according to the manufacturer's instructions as described previously (Pillai et al., 2012). Briefly, 96-well flat bottom immunoplates were coated with an anti-BDNF monoclonal antibody and incubated at 4 °C overnight. After blocking by non-specific binding with Block & Sample Buffer, standards and samples were added to the plates and incubated and shaken for 2 h at room temperature. After washing with TBST wash buffer, plates were incubated for 2 h with anti-human BDNF polyclonal antibody. The last incubation required the addition of anti-immunoglobulin Y-horse-radish peroxidase conjugate. In the last step of the assay, TMB One solution was added in order to develop the color. After stopping the reaction with HCl 1 N, the absorbance was read at 450 nm on a microplate reader and BDNF concentrations were determined automatically according to the BDNF standard curve (ranging from 7.8 to 500 pg/ml purified BDNF). The sensitivity of the assay was 15.6 pg/ml. All the samples were analyzed in duplicate at the end of the study by an investigator blind to treatment arm and clinical status. The mean intra- and inter-assay coefficients of variation were 4.2 and 6.8%, respectively.

2.4. Statistical analysis

To examine BDNF as a predictor of adverse outcomes, "baseline BDNF" for each patient was defined to be the first BDNF value obtained for that patient, regardless of visit number. Table 1 provides information on the timing of the "baseline BDNF" value according to this definition. As can be seen from this table, less than 50% of the subjects had their first BDNF determination at the true baseline of the study because the BDNF assessment was added after the RCT had started. "First relapse"

Table 1
Timing of "baseline" BDNF value for all subjects and by treatment group.

Timing of "baseline" BDNF value[a]	All subjects number (%)	LAI-R group number (%)	Oral group number (%)
At true baseline	98 (44)	54 (50)	44 (39)
Within 28 weeks of true baseline	64 (29)	28 (26)	36 (32)
Later than 28 weeks from true baseline	59 (27)	26 (24)	33 (29)

[a] See text for definition.

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