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Microtubule associated protein 2 in bipolar depression: Impact of pregnenolone

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ABSTRACT

Background: Pregnenolone, and related neurosteroids, may have antidepressant properties. Preclinical research proposes that microtubule associated protein 2 (MAP2) binding may be a mechanism for antidepressant properties of pregnenolone. Thus, MAP2 might be a novel target for antidepressant therapy. This clinical study is the first to examine serum MAP2 levels in people with bipolar depression and controls, and whether pregnenolone treatment is associated with a change in MAP2 levels.

Methods: Blood samples from a previously published clinical trial of pregnenolone for adult bipolar depression were analyzed at baseline and week 6 of treatment with pregnenolone or placebo for serum MAP2 levels using Western Blot. MAP2 levels from healthy controls were also obtained.

Results: MAP2 levels in the bipolar depressed patients (n = 11) tended to be higher than in controls (n = 4) (p = 0.062). MAP2 levels decreased non-significantly from baseline to week 6 in placebo (n = 5) and pregnenolone-treated patients (n = 6). MAP2 level changes correlated positively with change in self-reported depressive symptom scores in the pregnenolone group (r = 0.771, p = 0.072) but not in the placebo group (r = 0.000, p = 1.000).

Limitations: This study, exploring relationships between MAP-2 in humans with mood disorders, is limited by the small sample size. Thus, the findings must be viewed with great caution.

Conclusion: These findings suggest possible differences in serum MAP-2 levels between bipolar depressed persons and controls and a relationship between changes in depressive symptoms and MAP-2 levels during pregnenolone therapy. Findings suggest additional research is needed on MAP-2 in mood disorders.

1. Introduction

Microtubules are tubular cytoskeletal structures made from the globular protein, tubulin (Mohri, 1968). Tubulin allows microtubules to assist in cellular locomotion and determine cell shape (Chakraborti et al., 2016). Microtubule associated proteins (MAPs) assemble and stabilize microtubules and are essential for determining neuronal shape (Murakami et al., 2000). There are two main types of MAPs: structural and end binding +TIP proteins. The +TIP proteins interact with the growing ends of microtubules to form dynamic interactions with other complex proteins. Structural MAPs bind to microtubules for assembly (Mohan and John, 2015). Within the structural category, MAP2, located in the dendrites, is a neural protein that accelerates microtubule growth by stabilizing microtubules (Tucker, 1990).

Microtubules and MAPs are implicated in mood and other psychiatric disorders (Marchisella et al., 2016). In humans, MAP2 gene is associated with the mood dimension of anhedonia (van Veen et al., 2012), while in animal models a flattening of the glucocorticoid diurnal rhythm, as is observed in mood disorders, is associated with changes in MAP2b mRNA expression (Gartside et al., 2003). Transcranial ultrasound, a neuromodulation technique thought to act through microtubules, appears to improve mood (Hameroff et al., 2013).

MAP2 can be measured both in the central nervous system and blood and may serve as a blood marker for neuronal differentiation (Maccioni and Cambiazo, 1995). To our knowledge two prior studies have reported MAP2 levels in serum. Park et al. used western blotting to

Abbreviations: BBB, blood-brain barrier; BPD, bipolar disorder; CSF, cerebrospinal fluid; HRSA, Hamilton Rating Scale for Anxiety; HRSD, Hamilton Rating Scale for Depression; IDS-SR, Inventory of Depressive Symptomatology-Self Report; MAP, microtubule associated protein; NOS, not otherwise specified; SCID, structured clinical interview; TBI, traumatic brain injury; YMRS, Young Mania Rating Scale

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report MAP2 levels in the cerebrospinal fluid (CSF) and serum in rats following middle cerebral artery occlusion and subsequent reperfusion. This study demonstrated increases in serum MAP2 levels within 30 min following ischemia (Park et al., 2012). These results suggest that MAP2 levels can be detected in the serum following brain injury. Mondello et al. reported higher serum MAP2 levels in humans with traumatic brain injury (TBI; n = 16) six months post-injury compared to controls (n = 16) (Mondello et al., 2012).

Pregnenolone, a naturally occurring neurosteroid formed from cholesterol in the adrenal gland and brain (Baulieu and Robel, 1990), appears to have an effect on both mood and MAP2. Due to its lipophilicity, pregnenolone readily crosses the blood-brain barrier (BBB) and enters the brain (Baulieu and Robel, 1990). Lipoidal derivaties of pregnenolone have also been identified in the brain and plasma (Bélanger et al., 1992; Liere et al., 2004). In fetal rat brains, pregnenolone administration induced a large and dose-dependent increase in MAP2-induced microtubule assembly (Murakami et al., 2000), while a pregnenolone-derivative (MAP4343) that binds MAP2 was reported to have more rapid and persistent antidepressant properties than fluoxetine in an animal model of depression (Bianchi and Baulieu, 2012).

In humans, neurosteroids may have antidepressant and mood stabilizing properties. Women with major depressive disorder (MDD) or bipolar disorder have higher plasma levels of progesterone and 3alpha-hydroxy-5alpha-pregnan-20-one than controls with higher levels observed in those with bipolar disorder than MDD (Hardoy et al., 2006). Women with bipolar disorder have low plasma levels of allopregnanolone when depressed and elevated levels after recovery (Carta et al., 2012). Two placebo-controlled clinical trials suggest that pregnenolone may be effective for bipolar depression. In an 8-week clinical pilot trial of pregnenolone in patients with bipolar and unipolar depression, Osuji et al. reported a statistically significant improvement in depressive symptom severity in the pregnenolone treatment group (n = 18) compared to the placebo group (Osuji et al., 2010). In a more recent study, Brown et al. replicated these findings in a larger sample of patients (n = 80) with bipolar depression (Brown et al., 2014), observing a several fold increase in pregnenolone and several downstream neurosteroids following pregnenolone administration. In the current report, serum MAP2 levels were assessed at baseline and following pregnenolone or placebo therapy in a subset of participants from this bipolar depression study (Brown et al., 2014) and in healthy controls. The study was an exploratory, secondary analysis, designed to test feasibility for future research. However, given the literature linking progenenolone and its derivatives with both mood disorders and microtubules, we hypothesized baseline differences in MAP2 levels between participants with bipolar disorder and controls, as well as changes in MAP2 levels associated with pregnenolone administration.

2. Methods

2.1. Participants

A total of 80 participants were randomized, 40 of whom received pregnenolone (titrated to 250 mg twice daily at week 4) and 40 received placebo. Inclusion criteria comprised of individuals 18–75 years of age diagnosed with bipolar I, II, or not otherwise specified (NOS) disorder with a depressive episode at the time of the baseline evaluation. The study was registered on clinicaltrials.gov (NCT01409096). For detailed information about the study see our former report (Brown et al., 2014).

2.2. Clinical Assessments

Participants completed a structured clinical interview (SCID) (First et al., 1995) at the baseline visit. Mood assessments, including the Hamilton Rating Scale for Depression (HRSD, clinician-rated depression) (Hamilton, 1960), Inventory of Depressive Symptomatology-Self Report (IDS-SR, self-rated depression) (Rush et al., 2000), Hamilton Rating Scale for Anxiety (HRSA, clinician-rated anxiety) (Hamilton, 1959), and Young Mania Rating Scale (YMRS, clinician-rated manic symptoms) (Young et al., 1978), were administered at baseline and biweekly.

2.3. Blood Analysis

Blood samples were drawn at baseline, week 6, and week 12 for routine laboratory analysis and for neurosteroid levels. Participants with baseline and week 12 levels were used in an analysis of neurosteroid levels which were previously published (Brown et al., 2014). Thus, week 12 blood samples were not available. Current analyses used the remaining baseline and week 6 blood samples of participants who completed the baseline and week 6 assessments, but not the week 12 assessment. In addition, four healthy controls without psychiatric illness history were recruited from the community, as a reference standard for MAP2 levels in blood samples. All participants provided UT Southwestern IRB-approved written informed consent.

The blood samples were analyzed using western blot at the Laboratory of Toxicology at the College of Veterinary Medicine and Research Institute of Veterinary Medicine at Chungbuk National University in Cheongju, Republic of Korea. Human serum albumin often accounts for greater than 60% of total protein present in serum, thus high concentration of albumin interferes with the detection of many proteins of biological interest. Serum albumin was eliminated using Pierce™ Albumin Depletion kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The kit includes optimized buffer and microcentrifuge spin columns to remove albumin quickly and conveniently from 10 to 50 µl samples. In brief, albumin-containing blood samples were incubated with resuspended resin in a spin column for 1–2 min at room temperature, and centrifuged at 12,000 × *g* for 1 min. After repeating such procedures for maximum binding of albumin, the column was washed to release unbound proteins by adding Binding/Wash buffer, and recentrifuged at 12,000 × *g* for 1 min. The retained protein fractions were analyzed by SDS-PAGE analysis or by protein concentration determination.

Albumin-depleted serum samples were separated by 10% SDS-PAGE. After electrophoresis, proteins were transferred onto polyvinylidene fluoride membranes, and the membranes were blocked with 5% bovine serum albumin in Tris-buffered saline solution containing 0.1% Tween-20. The membrane was immunoblotted with a primary antibody specific for MAP2 (Millipore, Billerica, MA, USA), followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG (Cell Signaling Technology, Danvers, MA, USA). Blots were developed using an ECL solution (Pierce ECL western blotting substrate, Thermo Scientific) and exposed to X-ray film (Amersham Bioscience, Piscataway, NJ, USA). The experiments were performed three times, and representative bands and mean densities were normalized to GAPDH (Cell Signaling Technology).

2.4. Statistical analysis

MAP2 levels were analyzed in serum samples at baseline and week 6 in the bipolar depressed participants and at a single time point in controls. Due to the small sample size the data were analyzed with non-parametric statistical tests. A Wilcoxon rank-sum test compared MAP2 levels between the controls and the bipolar depressed patients at baseline. The Wilcoxon signed-rank test was used to assess baseline to week 6 changes in MAP2 levels among patients with bipolar depression, who were treated with either pregnenolone or placebo. Spearman rank correlations were calculated to determine the degrees of association between variables at baseline and week 6.