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Antimanic Treatment With Tamoxifen Affects Brain Chemistry: A Double-Blind, Placebo-Controlled Proton Magnetic Resonance Spectroscopy Study

Ayşegül Yildiz, Burç Aydin, Necati Gökmen, Ayşegül Yurt, Bruce Cohen, Pembe Keskinoglu, Dost Öngür, and Perry Renshaw

ABSTRACT

BACKGROUND: The antimanic efficacy of a protein kinase C inhibitor, tamoxifen, has been tested in several clinical trials, all reporting positive results. However, mechanisms underlying the observed clinical effects require further confirmation through studies of biological markers.

METHODS: We investigated the effect of tamoxifen versus placebo on brain metabolites via a proton magnetic resonance spectroscopy study. Scanning was performed in 48 adult manic patients with bipolar disorder type I (mean Young Mania Rating Scale score of 37.8 ± 5.8) at baseline and after 3 weeks of double-blind treatment. We hypothesized that alleviation of manic symptoms would improve the levels of markers associated with brain energy metabolism (creatine plus phosphocreatine [total creatine (tCr)]) and neuronal viability (*N*-acetylaspartate).

RESULTS: The Young Mania Rating Scale scores decreased from 38.6 ± 4.5 to 20.0 ± 11.1 in the tamoxifen group and increased from 37.0 ± 6.8 to 43.1 ± 7.8 in the placebo group ($p < .001$). Proton magnetic resonance spectroscopy measurements revealed a 5.5% ± 13.8% increase in tCr levels in dorsomedial prefrontal cortex in the tamoxifen group and a 5.3% ± 13.1% decrease in tCr in the placebo group ($p = .027$). A significant correlation between the Young Mania Rating Scale score change and tCr percent change was observed in the whole group (Spearman rho = .341, $p = .029$). Levels of both tCr and *N*-acetylaspartate in the responder group were increased by 9.4% ± 15.2% and 6.1% ± 11.7%, respectively, whereas levels in the nonresponder group were decreased by 2.1% ± 13.2% and 6.5%± 10.5%, respectively ($p < .05$).

CONCLUSIONS: Tamoxifen effectively treated mania while increasing brain tCr levels, consistent with involvement of both excessive protein kinase C activation and impaired brain energy metabolism in the development of bipolar manic states.

Keywords: Bipolar mania, Creatine, Dorsomedial prefrontal cortex, MRS, Protein kinase C, Tamoxifen

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Bipolar disorder (BD) has been associated with a wide range of neurobiological models, but pathophysiologic mechanisms underlying the extreme and erratic shifts of mood, thinking, and behavior associated with this complex disorder are yet to be fully elucidated. Nevertheless, in the light of accumulated evidence, a few hypotheses are supported by convergent and replicated evidence. Among these are mitochondrial dysfunction and oxidative damage resulting in altered brain energy metabolism (1), altered arachidonic acid cascade and inflammatory response (2), decreased neuroplasticity (3), and increased protein kinase C (PKC) activation (4). The extent to which these brain processes take part or interact with each other in the development of bipolar mood states requires further research (1,3–6).

Evidence from different types of studies suggests that excessive PKC activation can disrupt prefrontal cortical regulation of thinking and behavior (7,8) and that use of PKC inhibitors, such as tamoxifen, can improve mania-like symptoms (i.e., amphetamine-induced hyperactivity) in animal models (5,9–13). The results from genetic, postmortem, and clinical investigations also support a role for altered PKC signaling in the pathophysiology and treatment of BD (5,13). A polymorphism in the gene encoding phospholipase C, the initial enzyme in the PKC cascade, was identified in a population-based association study of patients with BD who were responders to lithium (14). A genome-wide association study of BD in two independent case-control samples of European origin found the strongest association signal in the gene encoding diacylglycerol kinase eta, a key protein for the PKC pathway (5,15). Although postmortem findings show increased PKC activity in the frontal cortex of patients with BD (16), patients with BD in manic states have accelerated membrane-bound PKC activity in their

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platelets, and treatment with lithium or valproate normalized this abnormal activity within 2 weeks (5,13,17). A few proof-of-concept trials employing tamoxifen as the study drug further investigated the role of PKC inhibition in the treatment of acute mania (13,18–20), and all reported positive results with a rapid onset of action (within 5 days) and response rates for tamoxifen of 63% after 3 weeks of treatment (5).

Mitochondrial pathology has been repeatedly shown to accompany BD and has been considered to result from genetic and environmental causes as well as neurotransmission dysregulation (21). Mitochondrial dysfunction may contribute to BD pathophysiology through structural abnormalities (22) and inefficient energy production and oxidative stress (23), as evidenced by increased neuronal lactate (24) and decreased *N*-acetylaspartate (NAA) (25), creatine plus phosphocreatine (total creatine [tCr]) (26), and pH levels (27) detected in patients with BD by several cerebral magnetic resonance spectroscopy (MRS) studies.

Proof-of-concept clinical trials are based on etiologic molecular models, and they are valuable for developing new target-specific treatments. However, proposed drug mechanisms of action require further confirmation through studies of biological markers in the actual study populations. Cerebral MRS is a noninvasive neuroimaging tool enabling investigation of in vivo neurochemistry. Proton (^{1}H)-MRS is most commonly used in clinical research settings, and it can provide information on the levels of 1) NAA, a marker synthesized in the mitochondria and considered to reflect neuronal density, viability, and function; 2) phosphorylcholine plus glycerophosphocholine (Cho), which participate in membrane phospholipid turnover; and 3) tCr, which is involved in brain energy metabolism and can be used to store high-energy phosphate bonds used to generate adenosine triphosphate (ATP) (1,28).

By adding a longitudinal ^{1}H-MRS study to the largest randomized double-blind, placebo-controlled, antimanic treatment trial of a PKC inhibitor, tamoxifen, we investigated neurochemical changes in the bipolar manic state before and after 3 weeks of treatment with tamoxifen. Based on the above-mentioned molecular mechanisms, we hypothesized a priori that amelioration of manic symptoms via treatment with tamoxifen would improve brain energy metabolism (reflected in increased tCr levels) and neuronal viability (reflected in increased NAA levels).

METHODS AND MATERIALS

Subjects

This longitidunal ^{1}H-MRS study was added to a proof-of-concept study of tamoxifen designed for testing the antimanic efficacy of PKC inhibition (ClinicalTrials.gov Identifier: NCT00411203). The full protocol and results of this trial were previously reported (13). Briefly, patients were 18–58 years old with a diagnosis of DSM-IV BD type I currently in a manic or mixed state with a Young Mania Rating Scale (YMRS) total score >20, with or without psychotic features. The Turkish Ministry of Health Central Review Board and the Local Ethical Committee of Dokuz Eylul University approved the study protocol. Each potential subject and one first-degree relative reviewed the study, and both gave written informed consent for participation.

Treatment and Clinical Assessment

Subjects were randomly assigned 1:1 to receive tamoxifen citrate or identical placebo tablets in a double-blind fashion for 3 weeks. The starting dosage of tamoxifen citrate was 20 mg twice daily (40 mg/day). Daily doses were adjusted gradually in 10-mg increments to achieve 80 mg/day in twice-daily divided doses for all subjects. Concomitant use of oral lorazepam was allowed during the study as clinically indicated, with a maximum dose of 5 mg/day. All subjects were hospitalized throughout the 3-week trial period and beyond until adequate amelioration of manic symptoms could be achieved. Manic symptom severity was evaluated using the YMRS, completed weekly over the 3-week period.

MRS Procedure and Data Analysis

The present ^{1}H-MRS study protocol was a part of longitudinal ^{1}H-MRS studies added to two clinical trials, one for bipolar mania (13) and the other for unipolar depression (29). The clinical trials and ^{1}H-MRS experiments were conducted at the Departments of Psychiatry and Radiology, Dokuz Eylul University, Izmir, Turkey. The Local Ethical Committee of Dokuz Eylul University approved the ^{1}H-MRS study protocol. In this project, baseline ^{1}H-MR spectra from manic patients with BP were obtained right before randomization to tamoxifen or placebo. After 3 weeks of a double-blind treatment period, ^{1}H MR spectra were obtained again (within 12 hours after the last dose of treatment with no concomitant psychotropic medication administered). To enable reliable data acquisition in the manic state, all patients were sedated with midazolam (.03 mg/kg) and fentanyl (2 μg/kg) administration just before both MRS scans (28). As reported previously, the sedation was safe and did not cause any alterations in the measured metabolites of the brain (28). The ^{1}H-MRS measurements were made on a Philips system (Philips Healthcare, Best, The Netherlands) with field strength of 1.5 tesla. Sagittal, axial, and coronal T2-weighted cerebral images were obtained to place voxels, and single voxel short echo time MRS data were collected with a point resolved spectroscopy sequence (echo time = 31 ms, repetition time = 2000 ms, spectral bandwidth 1 kHz, 1024 complex data points, 128 acquisitions) from four different brain regions—dorsomedial prefrontal cortex (DMPFC), right basal ganglia, frontal lobe, and right hippocampus—to create a database for present and future comparisons in patients with mood disorders and healthy volunteers. For the present study, we selected DMPFC as our primary region of interest because it covers the regions most prominently shown to be involved in BD (30–32). The DMPFC voxel dimensions were 2 × 3 × 2.5 cm (15 cm^3) (Figure 1). Specifically trained, blinded experts at the study site made data acquisitions and metabolite measurements.

Unsuppressed water reference spectra were acquired for all acquisitions and used for both eddy current correction and water scaling to estimate absolute metabolite concentrations. The commercial spectral-fitting package LCModel Version 6.1-4E was used to measure individual metabolite peak integrals (28,33). To accept individual metabolite fitting, we