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Omega-3 fatty acids combined with aripiprazole and lithium modulates activity of mitochondrial enzymes and acetylcholinesterase in methylphenidate-induced animal model of mania

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ABSTRACT

Many studies have shown the alterations of mitochondrial complexes in bipolar disorder (BD) patients. However, changes in the Krebs cycle enzymes have been little studied.The animal model of mania induced by psychostimulants like amphetamine and methylphenidate (MPD) has been widely used to study the manic phase of bipolar disorder. The aim of the present study is to assess the changes in the activity of Krebs cycle enzymes and acetylcholinesterase (AChE) in an animal model of mania induced by MPD. wiss albino mice were first administered intraperitonially with MPD (5 mg/kg) or saline for 14 days, upon changes in the behavioral activity, animals were treated with lithium (50 mg/kg), omega-3 fatty acids (1.5 ml/kg) given orally and aripiprazole administered intraperitonially (1.5 mg/kg) from 8th day onwards. Activity of Krebs cycle enzymes and AChE were measured in brain. Administration of MPD inhibited the activity of Krebs cycle enzymes and AChE in brain. Treatment with lithium, aripiprazole and omega-3 fatty acids significantly reversed MPD induced mitochondrial dysfunction and AChE activity. This finding suggest that lithium, aripiprazole and omega-3 fatty acids exert protective effect against MPD induced impairment of Krebs cycle enzymes in brain of mice; further supporting the hypothesis that mitochondrial dysfunction may be associated with the pathophysiology of BD.

1. Introduction

Bipolar disorder (BD) is a common psychiatric disorder that leads to serious health problems, little is known about its pathophysiology [1]. It is multifactorial illness and has diverse symptoms such as feelings of heightened energy, creativity, and euphoria, fatigue, appetite or weight changes, including recurrences of mania, depression and mixed states. Several studies have suggested that dysfunctional cellular energy metabolism has a central role in BD, mainly in the mitochondria [2]. Abnormalities in energy metabolism were found in functional assays [3,4]. Lithium is the classic mood stabilizer and it was the first drug approved by the Food and Drug Administration (FDA) in 1974 for the treatment of BD; Valvassori and his research team revealed that lithium prevented amphetamine-induced mitochondrial dysfunction, and stabilizes mitochondrial function in the pathophysiology of BD [5].

Aripiprazole is a second-generation antipsychotic, which has been demonstrated to reduce symptoms of mania, it differs from all currently marketed atypical antipsychotics and its primary mechanism of action involves both postsynaptic dopamine (DA) D2/D3 receptors and presynaptic DA autoreceptors. Furthermore, several studies have demonstrated relatively selective and unique D2 partial agonist properties of aripiprazole. Many reports revealed that aripiprazole and omega-3 fatty acids reduces oxidative stress and shown neuroprotective effects in both *in vivo* and *in vitro* studies; there is lack of studies appropriate to the Krebs cycle enzyme changes induced by MPD [6,7]. Moreover, few studies evaluated changes in the Krebs cycle enzymes in the manic state of BD [8,9]. Dysfunction in the Krebs cycle can be capable of altering the rate of brain metabolism and the production of free radicals. After glycolysis, pyruvate was decarboxylated to acetyl CoA by the pyruvate dehydrogenase. The conversion of acetyl CoA to CO_2 in the Krebs cycle results in the production of NADH for the electron transport chain and subsequent production of ATP. In Krebs cycle, malate dehydrogenase (MDH) catalyzes the dehydrogenation of L-malate to oxaloacetate [10]. Succinate dehydrogenase (SDH) is one of the most important markers of the mitochondrial ability to supply an adequate amount of ATP [11]. Additionally, α-KGDH could significantly contribute to oxidative stress in mitochondria [12].

Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine (ACh) at the synaptic cleft of cholinergic synapses

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and neuromuscular junctions [13]. Altered AChE activity, usually in relation to a second neurotransmitter, has been suggested to underlie several pathologic neuropsychiatric disorders [14]. Patients with manic state, schizophrenia are proposed to have in common reduced transmission in certain brain ACh pathways [15]. In addition, the drugs that are considered first line for BD are lithium, omega-3 fatty acids and more recently aripiprazole. They show reasonable protection against recurrent mood episodes and have a modest antidepressant and antimanic property [16–18]. Previous studies revealed that lithium, aripiprazole and omega-3 fatty acids have direct action on neurons, including protection from cell death or enhancement of neurogenesis [6,7]. Our previous findings clearly demonstrated that behavior and oxidative stress abnormalities induced by MPD were reduced by combined treatment of omega-3 fatty acids, lithium and aripiprazole [19,20].

Thus, we have designed the present study to examine the activity of mitochondrial enzymes and AChE in brain of mice undergoing treatment with the manic agent MPD and to evaluate the effect of lithium, aripiprazole and omega-3 fatty acids in this context.

2. Materials and methods

2.1. Animals and drug administration

The male adult Swiss albino mice (weighed 25–30 ± 5 g) were housed in wire-topped plastic cages, as six animals per cage. Control and experimental mice were received a standard diet of rodent chow (12–15 g/day) and water *ad libitum*. All mice were kept on an alternating 12-h light and 12-h dark cycle. All the experiments were performed at the same time every day and in the light period (9:00–11:00 A.M.). All the experimental procedures were approved, and animals were taken care according to the Institutional Animal Ethical Committee of Rajah Muthiah Medical College and Hospital, Annamalai Nagar, Tamil Nadu, India (Reg No. 160/1999/CPCSEA, Proposal number 933). After 7 days of acclimatization period, the mice were randomly assigned to eight groups consisting of six mice per group. MPD was procured from Ipca pharmaceutical company and the study group was administered 5 mg/kg/day of MPD intraperitoneally (i.p.), whereas the control group was administered with distilled water. The dosage of MPD administration to mice was similar to that of Barbosa et al. [21]. 1.5 ml of 0.1% fish oil (FO) with a homogenous 1% Tween suspension [22] contained 120–180 mg eicosapentaennoic acid (EPA)/docosahexaeonoic acid (DHA), and lithium carbonate (50 mg/kg) given orally [23] aripiprazole (1.5 mg/kg) [17] was kindly provided by Sun Pharma, Karnataka, India, and dissolved in water and administered intraperitoneally (i.p.). All other chemicals used in this study were of analytical grade obtained from HiMedia Laboratories, Mumbai, India. The dose of lithium, aripiprazole, and omega-3 fatty acids were chosen based on previous literature [21–23,17] and their combinatorial effect on behavior and oxidative stress studies were confirmed [19–21].

The experimental design of the present study was as follows; each of the following groups consists of six animals.

Group I: Vehicle control

Group II: Vehicle control + lithium (50 mg/kg) + aripiprazole (1.5 mg/kg) + omega-3 fatty acids (1.5 ml/kg)

Group III: MPD-treated animals (methylphenidate (5 mg/kg))

Group IV: MPD + lithium (50 mg/kg)

Group V: MPD + aripiprazole (1.5 mg/kg)

Group VI: MPD + omega-3 fatty acids (1.5 ml/kg)

Group VII: MPD + lithium (50 mg/kg) + aripiprazole (1.5 mg/kg)

Group VIII: MPD + lithium (50 mg/kg) + aripiprazole (1.5 mg/kg) + omega-3 fatty acids (1.5 ml/ kg).

2.2. Isolation of mitochondria

The animals were sacrificed by decapitation immediately after the behavioral analysis (OFT, actophotometer test, FST); datas were published in Arunagiri et al. [19,20] mice brain tissues were stored at −80 °C until used for the biochemical analysis. The mice brain tissues were thawed, weighed, and then mitochondrial fraction of the brain tissue was isolated by the standard method of Takasawa et al. [24] The brain tissue was put into ice-cold 50 mM Tris–HCl buffer; pH 7.4 containing 0.25 M sucrose and homogenized. The homogenates were centrifuged at 700*g* for 20 min and then the supernatants obtained were centrifuged at 9000*g* for 15 min. Then, the pellets were washed with 10 mM Tris–HCl buffer (pH 7.8) containing 0.25 M sucrose and finally resuspended in the same buffer.

2.3. Activity of Krebs cycle enzymes

2.3.1. Assay of isocitrate dehydrogenase

The activity of isocitrate dehydrogenase (ICDH) in the brain mitochondrial fraction was assayed by the method of King [25]. The incubation mixture contained 0.4 ml of Tris-HCl buffer, 0.2 ml of substrate, 0.2 ml of manganese chloride, 0.2 ml of nicotinamide adenine dinucleotide phosphate (NADP+), and 0.2 ml of mitochondrial fraction. The NADP+ was replaced by 0.2 ml of saline in tubes labeled as control. A suitable aliquot of enzyme preparation was added and mixed well. The tubes were then incubated at 37 °C for 60 min. At the end of the incubation period, 1.0 ml of the coloring reagent and 0.5 ml of EDTA were added. The contents of the tubes were mixed well and allowed to stand at room temperature for 20 min and 10 ml of 0.4 N NaOH was added and the color intensity was read at 420 nm after 10 min in a UV-spectrophotometer.

2.3.2. Assay of succinate dehydrogenase

The activity of succinate dehydrogenase (SDH) in the brain mitochondrial fraction was assayed by the method of Slater and Borner [26]. The reaction mixture contained 1.0 ml of phosphate buffer, 0.1 ml of EDTA, 0.1 ml of sodium cyanide, 0.1 ml of bovine serum albumin, 0.3 ml of sodium succinate, 0.2 ml of potassium ferricyanide, and made up to 2.8 ml with distilled water. The reaction was initiated by the addition of 0.2 ml of mitochondrial fraction. The change in optical density was recorded at 15 s intervals for 5 min at 420 nm.

2.3.3. Assay of malate dehydrogenase

The activity of malate dehydrogenase (MDH) in the brain mitochondrial fraction was assayed by the method of Mehler et al. [27]. The reaction mixture contained 0.75 ml of phosphate buffer, 0.15 ml of reduced nicotinamide adenine dinucleotide (NADH), and 0.75 ml of oxaloacetate. The reaction was done at 25 °C and was started by the addition of 0.2 ml of mitochondrial fraction. The control tubes contained all reagents except NADH. The change in optical density at 340 nm was measured for 2 min at an interval of 15s in a Systronics UV–vis spectrophotometer.

2.3.4. Assay of α-ketoglutarate dehydrogenase

The activity of α-ketoglutarate dehydrogenase (α-KGDH) in the brain mitochondrial fraction was assayed by the method of Reed and Mukherjee [28]. The incubation mixture contained 0.1 ml of phosphate buffer, 0.1 ml of thiamine pyrophosphate, 0.1 ml of magnesium sulfate, 0.1 ml potassium α-ketoglutarate, 0.1 ml of potassium ferricyanide and distilled water to a final volume of 1.4 ml. A suitable aliquot of the mitochondrial fraction was added in test, while it was replaced by distilled water in the control. The mixture was then incubated at 30 °C for 30 min. At the end of this