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Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com

RESEARCH ARTICLE

Laser Acupuncture Improves Behavioral Disorders and Brain Oxidative Stress Status in the Valproic Acid Rat Model of Autism

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Available online 19 June 2015

Received: Jan 15, 2015

Revised: Jun 4, 2015

Accepted: Jun 5, 2015

KEYWORDSautism;
laser acupuncture;
oxidative stress;
valproic acid**Abstract**

The therapeutic strategy against autism, a severe neurological development disorder, is one of the challenges of this decade. Recent findings show that oxidative stress plays a crucial role on the pathophysiology of autism, and laser acupuncture at Shenmen (HT7) can improve oxidative status in many neurological disorders. Therefore, we aimed to assess the effect of laser acupuncture at HT7 on behavior disorders and oxidative stress status in the cortex, striatum, and hippocampus of the valproic acid rat model of autism. Laser acupuncture was performed once daily during postnatal day (PND) 14–PND 40. Behavioral tests including rotarod, open-field, learning and memory, and social behavior tests were performed during PND 14–PND 40. At the end of study, brain oxidative status including malondialdehyde levels and the activities of superoxide dismutase, catalase, and glutathione peroxidase were determined in the cortex, striatum, and hippocampus. Laser acupuncture at HT7 significantly improved autistic-like behaviors. Decreased malondialdehyde levels were observed in all areas mentioned above, however, increased glutathione peroxidase activity was observed only in the striatum and hippocampus. No changes in superoxide dismutase and catalase activities were observed in any investigated area of the brain. Therefore, our study suggests that laser acupuncture at HT7 partly mitigates autistic-like symptoms via improved oxidative status.

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1. Introduction

Autism, a severe and pervasive heterogeneous neurodevelopment disorder [1], is characterized by impaired social interaction and communication, repetitive behavioral patterns, and restricted interests [2]. The etiology of autism is still unclear, but it has been regarded as a multi-etiology disorder that is influenced by numerous factors, including genetic, environmental, and immunological factors, as well as oxidative stress [3]. Several lines of evidence have demonstrated that patients with autism show elevated levels of malondialdehyde (MDA) [4], a lipid peroxidation product, together with decreased levels of main scavenger enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) [5,6], in serum. Oxidative stress levels in the cortex and the cerebellar vermis are also elevated [7]. A proposal has been made that an elevation of oxidative stress in parts of the brain can impair or disturb brain development, resulting in the clinical manifestation of autism [4].

Recently, a randomized control trial study demonstrated that a 4-week electroacupuncture treatment at eight acupoints, Sisheengong (Ex-HN1), Yintang (EX-NH3), Neuguan (PC6), Shenmen (HT7), Taichong (LR3), Ear Naotean (AT3), Ear Shenmen (TF4), and Sanyinjiao (ST6), could improve many symptoms in autism spectrum disorder, including social initiation, receptive language, motor skills, coordination, and attention span [8]. Our previous work also clearly demonstrated that stimulation at HT7 could improve the status of oxidative stress in the brain and improve cognitive function in an animal model of Alzheimer's disease [9]. Based on this information and the crucial role of oxidative stress in autism pathophysiology, as mentioned earlier, laser acupuncture at HT7 has been thought to have a beneficial effect on various symptoms of autism. To the best of our knowledge, no scientific evidence concerning this issue has been available until now. Therefore, the current study aimed to determine the effect of laser acupuncture at HT7 on behavior disorders and on the status of oxidative stress in the cortex, striatum, and hippocampus of a valproic acid (VPA) rat model of autism.

2. Materials and methods

2.1. Animals

Pregnant female Wistar rats were obtained from the National Laboratory Animal Center, Salaya, Nakorn Pathom, Thailand. Rat pups, both male and female, were housed

together in cages maintained in a 12-hour light:dark cycle and given *ad libitum* access to food and water. The experimental protocols were approved by the Institutional Animal Care and Use Committee, Khon Kaen University, Thailand (AEKKU 56/2556).

2.2. Experimental protocol

Rat pups were randomly divided into four groups of 10 rats (5 females and 5 males) per group: control (naïve intact) group; VPA group; VPA plus laser acupuncture at HT7 group; and VPA + sham laser acupuncture. Rat pups not in the control group were treated with VPA and showed abnormal neurodevelopment. Laser acupuncture was performed once daily during postnatal days (PND) 14–40. Autism-like behaviors were observed using the rotarod, open-field, Morris water maze, and social behavior tests. On PND 41, the striatum, hippocampus, and cortex were isolated and oxidative stress markers including MDA level and the activities of SOD, CAT, and GSH-Px enzymes were assessed as shown in Fig. 1.

2.3. Autism-like condition induction

Rat pups at age 14 days with body weights of 18–30 g were injected with sodium valproate (Sigma Aldrich, St Louis, MO, USA) at a dose of 400 mg/kg body weight via a subcutaneous route. Autism-like symptoms were confirmed by using the reduced weight gain measured in weekly intervals, as well as the impaired olfactory discrimination on PND 9, delayed eye opening on PNDs 13 and 14, and the impaired motor development (swim performance) on PNDs 8 and 12 as initial activities reflecting abnormal neuron development in pups [10].

2.4. Laser acupuncture treatment

Five minutes prior to laser acupuncture treatment, rat pups were separated and housed individually. A blue laser beam (Xinland International Limited, Xi'an, Shaanxi, China) with a wavelength of 405 nm, a power output of 100 mW (0.100 J/second), and a diameter of 500 μ m was continually administered at HT7 on both the left and the right sides. The blue laser treatment was performed once daily for 10 minutes from PND 14 to PND 40. In this study, the sham laser acupuncture group was treated with laser acupuncture at a location 2–4 mm lateral to HT7, as shown in Fig. 2.

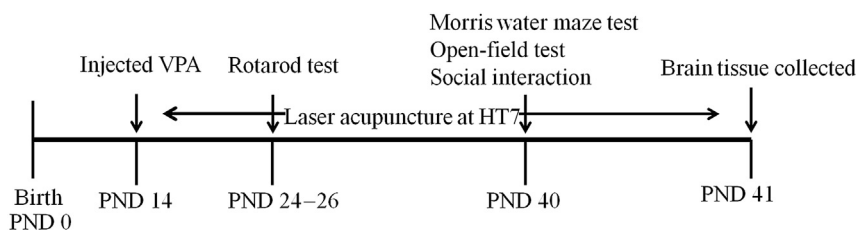


Figure 1 Schematic diagram for experimental protocol. PND = postnatal day; VPA = valproic acid.

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