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Long-term curcumin treatment antagonizes masseter muscle alterations induced by chronic unpredictable mild stress in rats

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ABSTRACT

Objective: To investigate the correlation between psychological stress and masseter muscle (MM) alterations, and explore the therapeutic agents for restoring the impaired masticatory muscle.

Design: We established a chronic unpredictable mild stress (CUMS) animal model and observed the changes of ultrastructure, redox homeostasis and energy metabolism in MM in rats with and without curcumin treatment.

Results: The depressive-like behavior in stressed rats was confirmed by the evidences of altered behaviors in sucrose preference test and open field test; while these phenomena were eased by curcumin. Except for the pathological changes in ultrastructure, decreased SOD, GSH-Px, CAT, Na^+-K^+ATPase, and Ca^{2+}-Mg^{2+}ATPase activities as well as increased MDA and LD content and LDH activity were also observed in MM in stressed rats. However, curcumin was capable of reversing CUMS-induced MM disorder by improving the activities of the examined anti-oxidant enzymes and energy metabolism enzymes. Additionally, the increased MDA content, LD content, and LDH activity in stressed rats were reduced by curcumin.

Conclusion: All the findings indicate the adverse effects of CUMS on MM function in rats, and raise the possibility of developing curcumin as a potential therapeutic agent for psychological stress-induced masseter dysfunction.

Introduction

Temporomandibular disorders (TMD) is a heterogeneous array of pathological changes that affects the temporomandibular joint (TMJ), the jaw muscles, or both.[1] The etiology of this disease is considered to be multifactorial, which involved both psychological and physiological components, including mental stress, occlusion disorder, trauma, and autoimmune diseases. In recent years, the role of psychosocial factors in TMD has been intensively investigated, and the findings suggested that it was associated with abnormal physiology of masticatory muscle, and considered an important risk indicator for the development of TMD.[2,3]

Psychological stress is ubiquitous and virtually all diseases, are affected by this phenomenon.[4] Anxiety and

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depression are the most common stress-related psychological disorders. In our previous studies, we found that anxiety caused by psychological stress induces oxidative damage and up-regulates the expression of HSP70 in the masseter muscle (MM) of rats; these molecular changes are associated with behaviors that resemble anxiety, which induced by a communication box.[5] However, the communication box only tended to result in anxious state in rats,[5] while depression is also a commonly seen emotional disorder. And the single stressor of communication box has a potential to cause adaption of animals more easily over time, consequently weakens the effect on animal model. Therefore, in this study, we used a CUMS procedure to produce depressive stated in rats. Multiple stressors in this kind of procedure could also prevent habituation, which can occur rapidly if a single stressor is presented repeatedly.[6] The MM is the largest and strongest muscle among the masticatory muscles in rats,[7] and is very sensitive at perceiving the changing state of the stomatognathic system, for it contains abundant proprioceptors, such as muscle spindles.[8] However, it is unknown whether the depression caused by psychological stress will affect the structure and function of the MM.

Anti-depressants improve the clinical symptoms of depression by enhancing or adjusting the functions of monoamine neurotransmitters or receptors. The commonly used chemical anti-depressants, such as tricyclic anti-depressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), atypical anti-depressant drugs, and other compounds, have been reported to produce several unwelcome reactions, including orthostatic hypotension, blurred vision, dry mouth, sinus tachycardia, various gastrointestinal reactions, hepatotoxicity, and even suicidal intentions.[9] Therefore, a novel anti-depressant with improved safety and fewer side effects is needed. Curcumin, a *Curcuma longa* extract, which has been used to effectively manage depression-related disorders in China,[10] successfully piqued our interest. Actually, the anti-depressant effects of curcumin has been proved in various animal models of depression, such as forced swim, tail suspension, unpredictable mild stress, olfactory bulbectomy, and chronic fatigue model of depression.[11] In addition, curcumin has great potential in stimulating muscle regeneration after traumatic injury, including masseter, and tibialis anterior muscles,[12] and the protective effects of curcumin against ischemia-reperfusion injury in rat skeletal muscle have been revealed, due to its great antioxidant ability.[13] Moreover, the usage of curcumin has not been reported to cause the aforementioned side effects that can be produced by commonly used chemical anti-depressants to our knowledge.

Thus, the present study sought to characterize the ultrastructure, oxidative level, and energy metabolism in the MMs of rats with depressive-like behavior that had been subjected to psychological stress induced by the chronic unpredictable mild stress (CUMS) procedure. Furthermore, curcumin was administered as an anti-depressant to assess whether it plays a protective role in this process. We hypothesized that CUMS caused alterations in the MM that are fully or partially reversible through the administration of curcumin.

Methods and materials

An animal model of CUMS[14]

The rats were subjected to 7 various and repeated unpredictable stressors during the administration of a CUMS procedure that lastedfor 12 weeks; the duration of this procedure were determined by preliminary experiment. The stressors were as follows: (i) damp sawdust for 12 h, (ii) food deprivation for 12 h, (iii) water deprivation for 12 h, (iv) inversion of the light-dark cycle, (v) immersing in 4 °C cold water for 5 min, (vi) immersing in 45 °C hot water for 5 min (The temperature was determined by Yang's research.[15] To avoid physically stimulating the MM of rats, we put the rats in a cage with 8 cm depth of preheated or cooled water. The rats' head was above water from beginning to the end.), and (vii) 1 h of restraint.[16] (For this last stressor, the rats were placed in a restraining device that was composed of inflexible wire mesh; during the stress procedure, the rats were not allowed to move freely, but their bodies were not constricted.) Over the course of each week of the CUMS procedure, one of the 7 stressors was applied each day, started at 9:00 AM on a random schedule. The same stressor was never applied for 2 consecutive days.

Experimental groups and drug administration

A total of 48 male Sprague–Dawley rats (8 weeks old, provided by the Laboratory Animal Center of the Fourth Military Medical University, Xi'an, China) with a mean weight of 230 ± 10 g were housed in groups of 4 in a temperature-controlled room (24 ± 1 °C) with a 12 h light-dark cycle (light on from 08:00 to 20:00 h). Twice each week, the cages were cleaned, and new bedding was provided. The animals were acclimated to the laboratory conditions for 1 week prior to the experiment, with food and water available *ad libitum*. The animals were randomly divided into the following 6 groups, with 8 rats in each group: a blank control group, a group of rats with CUMS, groups of rats pretreated with curcumin (10, 40, and 80 mg/kg of curcumin) and then treated with CUMS, and a group of rats pretreated with fluoxetine and then treat with CUMS. Curcumin (Sigma–Aldrich Co. St. Louis, MO, USA) was dissolved in 1 ml of peanut oil and administered to the rats once daily by gavage. The doses of curcumin were determined based on the report of Sivalingam et al., with minor modification.[17] Fluoxetine (5 mg/kg, Eli Lilly, Indianapolis, IN, USA) was dissolved in saline solution and administered to the rats by gavage. The dose of fluoxetine was determined based on the report of Bonilla-Jaime et al.[18] The rats in control group received 1 ml of peanut oil but did not receive CUMS. All the drugs were administered to the rats 0.5 h before they experienced CUMS.

This study was performed in strict accordance with the recommendations in the guide for the care and use of laboratory animals of the National Institutes of Health, and was approved by the committee on the ethics of animal research of the Fourth Military Medical University (Xi'an, China). All of the surgeries were performed under anesthesia, and every effort was made to minimize the animals' suffering.