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EXPERIMENTAL STUDY

Effects of Chaiyuwendan decoction on endocannabinoids levels in adipose tissue of rats with chronic stress-induced depression

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Abstract

OBJECTIVE: To investigate how Chaiyuwendan decoction (CWD) affects endocannabinoid levels in the adipose tissue of depressed rats.

METHODS: Twenty-four male Sprague-Dawley rats were randomly divided into four groups with six rats in each. One group was randomly selected as the control group. The remaining three groups were subjected to chronic stress to induce depression. Groups were randomly assigned as a model group, CWD group, and amitriptyline group. CWD was given to the CWD group once a day from the second day of modeling. The amitriptyline group was administered amitriptyline intragastrically (10 mg/kg) once a day. After treatment for 21 days, body weight and fat weight were measured and the levels of N-arachidonoylethanolamine (AEA), 2-arachidonoylglycerol (2-AG), and N-palmitoylethanolamine (PEA) in adipose tissue were determined with liquid chromatography-mass spectrometry.

RESULTS: Compared with the control group, body weight, fat weight, AEA, and PEA were significantly lower, and 2-AG was higher, in the model group ($P<0.05$, $P<0.01$). Compared with the model group, body weight, fat weight, the AEA, and PEA levels were significantly higher, and 2-AG level was significantly lower in the CWD group ($P<0.05$). However, the levels did not differ significantly between the CWD group and the amitriptyline group.

CONCLUSION: CWD could regulate the levels of AEA, 2-AG, and PEA in rats with depression induced by chronic stress.

Key words: Adipose tissue; Depression; N-arachidonoylethanolamine; Palmidrol; 2-arachidonoylglycerol; Endocannabinoids; Chaiyuwendan decoction

INTRODUCTION

The clinical features of depression include a significant and persistent low mood, decreased activity, and retarded cognitive function. We have previously found that Chaiyuwendan decoction (CWD), a Traditional Chinese Medicine formula, has significant pharmacological effects on depression.[1-4] Some researchers have found that endocannabinoids regulate the processes of emotion, memory, appetite, and directed behavior.[5] Disordered endocannabinoids can therefore induce depression, phobias, and extreme anxiety.[6] This study used chronic stress to establish a rat model of depression to observe the effects of CWD on regulating endocannabinoids in rat adipose tissue.

MATERIALS AND METHODS

Drug preparation

CWD was prepared with granules of Chaihu (*Radix Bupleuri Chinensis*), Renshen (*Radix Ginseng*), Yujin (*Radix Curcumae Wenyujin*), Banxia (*Rhizoma Pinelliae*), Fuling (*Poria*), Chenpi (*Pericarpium Citri Reticulatae*), Zhishi (*Fructus Aurantii Immaturus*), Zhuru (*Caulis Bambusae in Taeniam*), and Gancao (*Radix Glycyrrhizae*). Their weight ratio was 2∶3∶3∶3∶4∶3∶3∶3∶1. The granules were provided by Jiangyin Tianjiang Pharmaceutical Co., Ltd. (Jiangyin, China). The decoction was prepared with double distilled water at a concentration of 1.25 g/mL. Amitriptyline (H32023764), which was provided by Changzhou Siyao Pharmaceutical Co., Ltd. (Changzhou, China), was also prepared with double distilled water to a concentration of 0.01 g/mL.

Animals, grouping, and model establishment

Twenty-four specific pathogen free Sprague-Dawley male rats, 8-weeks-old and weighing (250±20) g, were supplied by Xipuerbikai Laboratory Co., Ltd. (Shanghai, China) [Certificate of quality No: SCXK (Hu) 2007-0005] and fed in the Laboratory Animal Center in the Medical College of Xiamen University. Rats were randomly divided into four groups by a random number table. Then, one group was randomly selected as the control group, while the rest remaining groups were subjected to stress for model establishment. The depression model was induced by different kinds of chronic stress for 21 days. The methods for generating stress were improved according to Katz *et al* [7] including: 48 h fasting, 24 h dehydration, night-lighting (12 h), swimming in 4℃ water for 5 min, tail-clamping for 1 min, high-level-oscillation (160 Hz) for 10 min, day-night-reversal, wet-feeding, and needling for 5 min. Rats from these three groups were randomly exposed to one stressor every day to avoid adaptation.

Dosage and treatment

Of the three groups subjected to stress, one was randomly selected as a CWD group, one as an amitriptyline group, and one as a model group. CWD (1 mL/100 g) was intragastrically administered to the CWD group once a day from the second day of modeling. The amitriptyline group was administered amitriptyline intragastrically (1 mL/100 g) until the 21st day. The control group and model group were intragastrically administered an equal volume of drinking water at the same time.

Measurement

The levels of AEA (N-arachidonoylethanolamine), 2-AG (2-arachidonoylglycerol), and PEA (N-palmitoylethanolamine) in rat adipose tissue were determined with liquid chromatography-mass spectrometry (LC-MS 1100, Agilent, Shanghai, China). For lipid extraction, 100 mg of tissue were taken and placed into 2 mL methanol-water (1∶1), which had 100 pmol/L fatty acid ethanolamide (FAEs). Then, tissue was homogenized in an ice bath and broken apart by sonication. Homogenate was joined with 4 mL chloroform to extract, and then centrifuged for 10 min at 4℃ and 1500 × *g*. After that, the lower organic layer was removed and dried by nitrogen, then redissolved with 1 mL chloroform and separated with a silica gel column. The silica gel column was washed with chloroform and methanol-chloroform (1∶9). The separated methanol -chloroform (1∶9) components were then collected and dried with liquid nitrogen. Powder was redissolved in 100 μL methanol-chloroform (3∶1). Finally, the contents of AEA, 2-AG, and PEA were measured by LC-MS.

For LC-MS, an Agilent Eclipse XDB-C18 column (LC-MS, Agilent Technologies Co., Ltd., Santa Clara, CA, USA) was used (4.6 mm×150 mm×5 μm). Mobile phase A was methanol, mobile phase B was water elution: gradient elution: 0-5 min 85% B, 5-20 min 100% B. The current velocity was 1 mL/min. Column temperature was 25℃. Atmospheric pressure chemical ionization source and positive ion detection were used. Curtain gas was set at 30 psi. Ion source temperature was 275℃. Source gas was N_2 at 60 psi. The collision gas pressure was 70psi and discharge current (NC) was 3.0 μA.

Statistical analysis

All data were analyzed by using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Quantity data were written as mean standard deviation ($\bar{x} \pm s$). One-way ANOVA was performed to detect the difference among the groups. $P<0.05$ was the significance level.

RESULTS

Body and fat weight of rats in each group

Compared with rats in the control group, the body weight and fat weight of rats in model group were significantly lower ($P<0.01$). Compared with the rats in model group, the body weight and fat weights of rats in the CWD group were significantly lower ($P<0.05$). Compared with rats in the model group, the body weight and fat weights of rats in the amitriptyline group were higher, but the differences were not significant. Compared with rats in the amitriptyline group, the body weight and fat weight of rats in the CWD group were markedly higher, but the differences were not significant (Table 1).

Levels of AEA, 2-AG, and PEA

The spectra of AEA, 2-AG, and PEA are shown in Figure 1. Compared with the control group, AEA and PEA in the model group were significantly lower, while 2-AG was significantly higher ($P<0.05$). Compared with the model group, AEA and PEA in the CWD