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EXPERIMENTAL STUDY

Effect of Pingan Fang, a Traditional Chinese Medicine compound, on behavioral sensitization and conditioned place preference induced by ethanol in mice

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Abstract

OBJECTIVE: To observe the effect of Pingan Fang (PG) on behavioral sensitization and conditioned place preference (CPP) induced by ethanol in mice, and to determine the intervention mechanism of PG on alcohol addiction.

METHODS: A behavioral sensitization mouse model induced by ethanol was established to observe the effect of PG on the development and expression of behavioral sensitization induced by ethanol by recording the spontaneous activity of mice. The resident time of mice in a white box was measured to evaluate the effect of PG on developing CPP induced by ethanol. Concentrations of dopamine (DA), Glutamate (Glu), and ã-aminobutyric acid (GABA) in the corresponding mesolimbic region of mice were determined by enzyme-linked immunosorbent assay.

RESULTS: Although PG did not alter spontaneous activity in mice, it reduced the growth of spontaneous activity stimulated by ethanol. The residence time in the white box after-ethanol-training of mice in CPP experiments was decreased.

CONCLUSION: Our data suggested that PG blocked the development and expression of behavioral sensitization induced by ethanol and the development of CPP in mice. The mechanism might be related to the decreased content of DA and Glu and increased content of GABA in the mesolimbic dopamine system. This suggests that PG might be useful for the prevention and treatment of alcohol addiction.

Key words: Ethanol; Pingan Fang; Behavioral Sensitization; Conditioned place preference; Dopamine; Glutamic acid; Aminobutyrates

INTRODUCTION

Alcohol dependence (AD) as defined by the American Psychiatric Association in the Diagnostic and Statistical Manual of Mental Disorders,[1] is characterized by increased alcohol tolerance, impaired ability of control over drinking, and drinking regardless of consequences. Alcohol dependence is a grievous public health problem that often results in social,medical and eco-

nomic consequences throughout the world.[2] Alcoholism affects nearly 10 percent of the population and results in social problems,considerable morbidity and mortality, and high health care costs.[3,4]

People have an increasing interest in drug therapy for alcohol dependence,[5,6] and the most important rationale for this therapy is based on the growing understanding of the neurobiology of AD. Advances in neurobiology have identified which neurotransmitter systems initiate and maintain the drinking of alcohol, including dopamine (DA), gamma-amino butyric acid (GABA), glutamic acid (Glu), cholinergic systems, 5-hydroxytryptamine systems and endogenous opioid peptide system.[7,8] Pharmacologic modification of these neurotransmitters or their receptors may modify dependence and these systems are potential targets for drug therapy in the treatment of AD. However, currently in the USA, there are four medications approved by the Food and Drug Administration (FDA) to treat AD: disulfiram, naltrexone, and acamprosate. Previous data suggest that their overall efficacy is modest.[9] Therefore, there is a critical need to identify new medications that may be effective in treating AD individuals.

AD is a neurodegenerative disease and studies have shown that brain damage is a common and potentially severe consequence of long-term and heavy alcohol consumption; even mild-to-moderate drinking can adversely affect cognitive functioning.[10] Persistent cognitive impairment can contribute to poor job performance in adult alcoholics, and can interfere with learning and academic achievement in adolescents with an established pattern of chronic heavy drinking.[11] Chinese herbal medicines are the most commonly used Traditional Chinese Medicines. Tianma (*Rhizoma Gastrodiae*) is a well-known herb that has been used to treat neurological disorders in East Asian countries for centuries.[12] Tianma (*Rhizoma Gastrodiae*) has been shown to increase extracellular GABA levels in Sprague-Dawley rats, thus enhancing GABA ergic neurotransmission and decreasing glutamate levels.[13] Gouteng (*Ramulus Uncariae Rhynchophyllae cum Uncis*) prescribed as a Traditional Chinese Medicine is used to treat ailments of the central nervous system. Research shows that Rhynchophylline, an active components of Gouteng (*Ramulus Uncariae Rhynchophyllae cum Uncis*), reduced the spontaneous activity and DA concentration in the cortex, amygdala, and spinal cord and protected neurons from damage induced by DA.[14,15] Baishao (*Radix Paeoniae Alba*),[16] which has been used for over 1500 years in China because of its effects on nourishing yin, replenishing blood of the liver, nourishing liver-yin to calm the liver and suppressing hyperactivity of yang. Therefore, we used Tianma (*Rhizoma Gastrodiae*), Gouteng (*Ramulus Uncariae Rhynchophyllae cum Uncis*) and Baishao (*Radix Paeoniae Alba*) to form a new compound called "Pingan Fang" (PG), which has been used for ten years to treat AD in the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine.

The behavioral sensitization model and the Conditioned Place Preference (CPP) model are two widely used models used for drug addiction research. The behavioral sensitization animal model was found to be closely related to drug addiction and drug craving in humans[17,18] and the CPP model is an indicator of the rewarding effects of drugs. Research showed that ethanol induces a long duration of behavioral sensitization in mice,[19,20] and alcoholics and offspring of alcoholics exhibit reduced behavioral sensitization by ethanol.[21] The above evidence indicates that behavioral sensitization is an important experimental model for the study of alcohol addiction. CPP is a classic model to observe material reward and spiritual dependence, as shown by changes of natural preference after drug training. In this study, we used behavioral sensitization and CPP induced by ethanol in animals to observe the influence of PG on ethanol-induced behavioral sensitization and CPP, to evaluate the effects of PG in the prevention and treatment of alcohol addiction and provide a theoretical basis of PG for treating AD.

MATERIALS AND METHODS

Animals

Three-month-old male Kunming mice weighing (22 ± 3) g were provided by Chengdu Dashuo Animal Experimental Company (Chengdu, China) and were maintained in a specific pathogen free environment. Mice were housed under standard conditions of a 12 h light/dark cycle (lights on from 7: 00 to 19: 00), 22-26 ℃ and 40%-70% humidity. Food and drink were provided ad libitum. All efforts were made to minimize animal suffering and to keep the number of animals used to a minimum. This study was approved by the Chengdu University of Traditional Chinese Medicine Ethics Review Committee and conducted in accordance with the internationally accepted principles for laboratory animal use and care according to the US guidelines (NIH National Institutes of Health Publication No. 85-23, revised in 1985).

Drugs

Saline and ethanol (concentration 96%) solutions were prepared with saline (15% , v/v in 0.9% NaCl) and stored at 4 ℃ . Intragastric (i.g.) administration of PG = 18 g/kg (M g/60 kg × 9 g/kg, M refers to dose of Chinese medicine and 60 kg is the human adult standard weight). Ethanol i.g. administration dose was 2.2 g/kg. Preliminary experiments were used to observe the effect of doses of 1.8, 2.0, 2.2 and 2.4 g/kg. We found 2.2 g/kg of ethanol had a minimum effect on the spontaneous activity of mice.

Reagents and instruments

The following instruments and reagents were obtained: ZZ-6 independent activity tester (Chengdu Thai Union Technology Co., Ltd., Chengdu, China); CPP