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EXPERIMENTAL STUDY

Yiguanjian cataplasm attenuates opioid dependence in a mouse model of naloxone-induced opioid withdrawal syndrome

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Abstract

OBJECTIVE: To investigate the effect of Yiguanjian (YGJ) cataplasm on the development of opioid dependence in a mouse model of naloxone-induced opioid withdrawal syndrome.

METHODS: One hundred Swiss albino mice, of equal male to female ratio, were randomly and equally divided into 10 groups. A portion (3 cm^2) of the backside hair of the mice was removed 1 day prior to the experiment. Morphine (5 mg/kg) was intraperitoneally administered twice daily for 5 days. YGJ cataplasm was prepared and pasted on the bare region of the mice immediately before morphine administration on day 3 and subsequently removed at the end day 5. On day 6, naloxone (8 mg/kg) was intraperitoneally injected to precipitate opioid withdrawal syndrome. Behavioral observation was performed in two 30-min phases immediately after naloxone injection.

RESULTS: The YGJ cataplasm significantly and dose-dependently attenuated morphine-naloxone-induced experimental opioid withdrawal, in terms of withdrawal severity score and the frequencies of jumping, rearing, forepaw licking, and circling behaviors. However, YGJ cataplasm treatment did not alter the acute analgesic effect of morphine.

CONCLUSION: YGJ cataplasm could attenuate opioid dependence and its associated withdrawal symptoms. Therefore, YGJ cataplasm could serve as a potential therapy for opioid addiction in the future.

Key words: Yi Guan Jian; Paste; Morphine; Naloxone; Opioid dependence

INTRODUCTION

Opioid drugs are used primarily for pain control and can produce a state of euphoria or well-being during their use. Owing to these mood-enhancing effects, opioid drugs have become the most commonly abused psychoactive substances worldwide. Continuous administration of opioid drugs can elicit opioid dependence, while their abrupt withdrawal may result in the precipitation of an abstinence syndromes.[1] Opioid dependence is usually associated with serious medical, le-

gal, social problems, and is associated with comorbid psychiatric disorders. In 2002, the cost of opioid dependence to society was approximately 181 billion US dollars.[2]

Until now, several approaches have been proposed for the management of opioid dependence. Opioid replacement therapy is an effective method in preventing acute withdrawal and maintaining long-term abstinence; however, acceptance of this therapy has been rather slow.[3] Other treatment approaches, such as the administration of α_2-adrenergic agonists, were also examined in previous studies.[4] Unfortunately, none of these therapies have promised to conclusively treat opioid dependence and its related abstinence syndromes. Therefore, new approaches for the management of opioid dependence are urgently needed.

Traditional Chinese Medicine (TCM) has been applied in medical care throughout East Asia for thousands of years.[5,6] Although the active ingredients in most mixed herbal formulas/medications have not been fully identified, it has been widely recognized that synergism among all components within the prescription is an important characteristic.[7,8] The Yiguanjian recipe (YGJ) is a traditional Chinese herbal formula that possesses the medical function of nourishing liver-*Yin* and dispersing stagnated liver-*Qi*. It has been widely used in China for the treatment of diseases with liver-*Yin* deficiency.[9-11] We found that liver-*Yin* deficiency was a common clinical syndrome of TCM in opioid dependence in clinical examinations. Therefore, we examined the use of YGJ for the treatment of opioid dependence and showed good effect.

Transdermal drug delivery refers to the topical application of drugs to healthy intact skin, either for localized treatment or systemic therapy.[12] The potential of skin to serve as a pathway for drug administration has already been demonstrated previously.[13] Compared with conventional drug administration routes, the transdermal drug delivery system (TDDS) has several additional advantages. It can maintain constant drug levels in the blood, while minimizing first-pass metabolism associated with gastro-intestinal administration of drugs.[14-16] It is more convenient and has increased patient compliance compared with oral administration routes. To date, the transdermal drug delivery of traditional herbal medicine has been used to treat many diseases.[17]

In this study, we established a mouse model of naloxone-induced opioid withdrawal syndrome and analyzed the effectiveness of YGJ cataplasm in treating opioid dependence.

MATERIALS AND METHODS

Preparation of YGJ cataplasm

The YGJ formula consists of Dihuang (*Radix Rehmanniae*), Beishashen (*Radix Glehniae*), Danggui (*Radix Angelicae Sinensis*), Maidong (*Radix Ophiopogonis Japonici*), Gouqizi (*Fructus Lycii*), Dangshen (*Radix Codonopsis*) and Baizhu (*Rhizoma Atractylodis Macrocephalae*). All crude herbs were the products of Jianlian TCM Co., Ltd. (Jinan, China).[7] The herbs were chopped finely and extracted twice with 10-fold 75% ethanol (Tianjin Guangcheng Chemical Reagent Co., Ltd., Tianjin, China) under reflux. The 75% ethanol extract was then filtered through absorbent gauze, and the filtrate was concentrated under reduced pressure to remove the ethanol and produce the dried extract powder. Then, the extract powder, artificial moschus (Beijing Lianxin Pharmaceutic Co., Ltd., Beijing, China), emodin (Shanghai Yantuo Biosciences Co., Ltd., Shanghai, China) and borneol (Hebei Jinmu Pharmaceutic Group Co., Ltd., Hebei, China) were mixed into a cream with excipients, including plasdone K-90, polyplasdone XL-10, and viscomate NP700 (Guangzhou Biours Biosciences Co., Ltd., Guangzhou, China), according to the requirements of the medicinal paste detailed in the Pharmacopoeia of the People's Republic of China.[17] The cream was then coated onto rubberized fabric with herb extract powders at a concentration of 1 mg/cm^2 and the resulting plaster was cut into 1 cm^2, 2 cm^2 and 3 cm^2 pieces, which were stored in desiccators until use.[18] The procedure generally corresponded to the pharmaceutical quality standards currently under the guidance of pharmaceutical experts from Qilu Hospital of Shandong University. The manufacturing procedure of placebo cataplasm was the same as that of YGJ cataplasm. The placebo cataplasm contained all of the excipients that were used in YGJ cataplasm without the herb extracts.

Animals

One hundred Swiss albino mice of specific pathogen-free grade (50 males and 50 females), aged 7 weeks old, weighing (25 ± 2) g, were used in this study. The mice were obtained from the Laboratory Animal Center of Shandong University (Certificate of quality number: SCXK [Lu] 20090001). The mice were fed on standard laboratory diet and tap water and were housed in the Laboratory Animal Center of Shandong University. The mice were divided into 10 groups using a random number table method. The experiments were conducted in a semi-soundproof laboratory. The mice were exposed to a 12 h/12 h light/dark cycle, with the light period between 08:00 to 20:00 h. Behaviors were observed using a 30 cm^3 large transparent Perspex observation chamber. Two observers who were blind to the treatment schedule simultaneously observed the withdrawal measurements. The mean value of both observations was recorded. The experimental protocol was performed in accordance with the Institutional Animal Ethics Committee. The study was approved by the local Research and Ethics Committee at Qilu Hospital of Shandong University, in accordance with the guidelines of the 1975 Declaration of Helsinki.

Drugs and chemicals

Morphine was obtained from Shandong Public Security Bureau, Jinan, China. Naloxone (Bejing Kewin Technology Share-holding Co., Ltd., Beijing, China) was obtained from Qilu Hospital of Shandong University. Naloxone and morphine were diluted in normal saline (China Otsuka Pharmaceutical Co., Ltd., Tianjin, China) immediately before use.

Induction of opioid withdrawal syndromes in mice

A 3 cm^2 section of hair was removed on the backside of the mice, using hair removal cream, 1 day prior to the initiation of the experiment. Morphine (5 mg/kg) was intraperitoneally administered twice daily (at 08：00 and 20：00 h) for 5 days. Cataplasm was pasted on the bare backside of the mice immediately before morphine administration on day 3 and removed at the end of day 5. On day 6, 8 mg/kg naloxone was intraperitoneally injected at 10：00 h to precipitate withdrawal syndrome. Behavioral observation was performed in two 30-min phases immediately after injecting naloxone.[18-20] The first 30-min observation period, immediately after naloxone administration, was used to assess jumping frequency and withdrawal severity score. The second 30-min observation period was used to assess the frequency of rearing, forepaw licking, and circling behaviors.

Assessment of jumping frequency

Repeated jumping behavior precipitated by naloxone was considered as a predominant sign of opioid withdrawal syndrome in the mice.[20,21] A jumping response was defined as a spring or leap off the surface by a muscular effort of the legs and feet.

Assessment of withdrawal severity score (WSS)

WSS was used to quantify the withdrawal syndrome in each mouse, which included the following behaviors: forepaw tremors, wet dog shake, straightening, ptosis and sneezing.[22-24] The severity of each behavior was recorded in terms of both magnitude and frequency during the observation period. The scores were recorded as follows: 0, no change; 1, mild increase; 2, moderate increase; and 3, severe increase. A cumulative withdrawal severity score of the five component scores was obtained.

Assessment of rearing, forepaw licking and circling frequency

Rearing, forepaw licking and circling frequency observations were observed as a measure of the behavioral aberration severity ascribed to experimental opioid withdrawal in mice.[25-27] Rearing was defined as repetitive rising of the frontal aspect of the mouse body while balancing the body on the hind limbs. Forepaw licking was defined as the mouse licking both of its forepaws at least once. Circling was defined as the completion of a complete circle of the entire observation chamber.

Measurement of the effect of YGJ cataplasm on nociceptive threshold using the tail-flick test

The tail-flick test was used to measure the nociceptive threshold in mice.[20,21,28] The tail-flick latency was regarded as the time between tail exposure to radiant heat and tail withdrawal. Electrically-heated nichrome wire was used as the radiant heat source in our study. The intensity of radiant heat was modulated to obtain pretreatment latency between 2-3 s. The cut-off latency time was fixed at 10 s. Tail-flick latency was expressed as a percentage of the maximum possible effect (MPE), as follows:

MPE (%) = 100 × (posttreatment latency-pretreatment latency) / (cut off time – pretreatment latency)

The peak time for morphine was 30 min after administration. Therefore, we observed tail-flick latency immediately before and at 30 min after morphine administration.

Experimental protocol

The experimental protocols are shown in the following section. Ten groups of mice were employed in our study, with each group comprising of 10 animals (5 male, 5 female).

Group Ⅰ (vehicle-vehicle control): vehicle for morphine (saline, 10 mL/kg, i.p.) twice daily + placebo cataplasm (2 cm^2) for 3 days + vehicle for naloxone (saline, 10 mL/kg, i.p.) on day 6.

Group Ⅱ (vehicle-naloxone control): vehicle for morphine (saline, 10 mL/kg, i.p.) twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6.

Group Ⅲ (morphine-naloxone control): morphine (5 mg/kg, i.p.) twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6.

Group Ⅳ (YGJ cataplasm + vehicle-naloxone control): vehicle for morphine (saline, 10 mL/kg, i.p.) twice daily + YGJ cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6.

Group Ⅴ-Ⅶ (YGJ cataplasm + morphine-naloxone): morphine (5 mg/kg, i.p.) twice daily + YGJ cataplasm (1, 2, 3 cm^2 for groups Ⅴ, Ⅵ, and Ⅶ, respectively) for 3 days + naloxone (8 mg/kg, i.p.) on day 6.

Group Ⅷ (morphine analgesia group): morphine (5 mg/kg, i.p) only.

Group Ⅸ (vehicle-morphine analgesia group): Each mouse was treated with placebo cataplasm (2 cm^2) for 3 days prior to morphine administration (5 mg/kg, i.p).

Group Ⅹ (YGJ cataplasm-morphine analgesia group): each mouse was treated with YGJ cataplasm (2 cm^2) for 3 days prior to morphine administration (5 mg/kg, i.p).

Although Groups Ⅱ and Ⅳ were not involved in the statistical analysis, we still considered it necessary to include them as control groups to ensure the rigorousness of study and the accuracy of data compared with several previous studies.[20,21]

Statistical analyses All results were expressed as means ± standard deviation ($\bar{x} \pm s$). The data were analyzed us-

ing SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA). One-way analysis of variance followed by Tukey's post-hoc analysis was used to analyze the difference among variables between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Jumping behavior

There was significant difference ($P < 0.01$) in jumping frequencies between Group Ⅲ (16.50 ± 2.64) and Group Ⅰ (0.00 ± 0.00). Meanwhile, YGJ cataplasm (1, 2, 3 cm^2) significantly (all $P < 0.01$) and dose-dependently (all $P < 0.05$) decreased jumping frequencies in opioid-dependent mice (Figure 1).

WSS

There was a significant difference ($P < 0.01$) in WSSs between Group Ⅲ (12.60 ± 1.71) and Group Ⅰ (0.00 ± 0.00). Meanwhile, YGJ cataplasm (1, 2, 3 cm^2) significantly (all $P < 0.01$) and dose-dependently (all $P < 0.05$) decreased the WSSs in opioid-dependent mice (Figure 2).

Rearing frequency

There was a significant difference ($P < 0.01$) in rearing frequencies between Group Ⅲ (26.20 ± 1.81) and Group Ⅰ (0.00 ± 0.00). Meanwhile, YGJ cataplasm (1, 2, 3 cm^2) significantly (all $P < 0.01$) and dose-dependently (all $P < 0.05$) decreased the rearing frequency in opioid-dependent mice (Figure 3).

Forepaw licking frequency

There was a significant difference ($P < 0.01$) in forepaw licking frequencies between Group Ⅲ (9.00 ± 1.16) and Group Ⅰ (0.00 ± 0.00). Meanwhile, YGJ cataplasm (1, 2, 3 cm^2) significantly (all $P < 0.01$) and dose-dependently (all $P < 0.05$) decreased the forepaw licking frequency in opioid-dependent mice (Figure 4).

Circling frequency

There was significant difference ($P < 0.01$) in circling frequencies between Group Ⅲ (24.60 ± 6.35) and

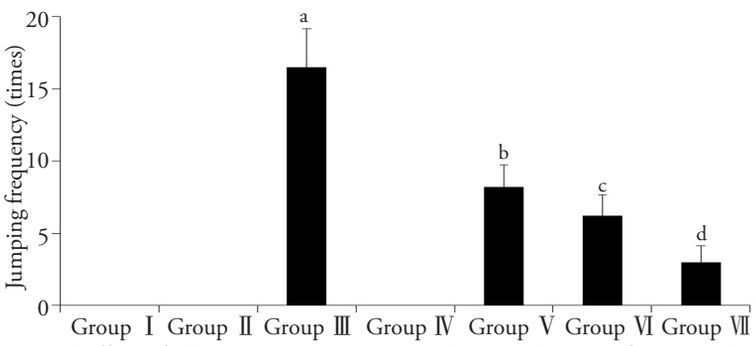

Figure 1 Effect of YGJ cataplasm on naloxone-induced jumping frequency in different groups

Group Ⅰ (vehicle-vehicle control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + saline, 10 mL/kg, i.p. on day 6. Group Ⅱ (vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅲ (morphine-naloxone control): morphine (5 mg/kg, i.p.) twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅳ (YGJ cataplasm + vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + YGJ cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅴ-Ⅶ (YGJ cataplasm + morphine-naloxone): morphine (5 mg/kg, i.p.) twice daily + YGJ cataplasm (1, 2, 3 cm^2 for groups Ⅴ, Ⅵ, and Ⅶ, respectively) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. YGJ: Yiguanjian. $^aP < 0.05$ *vs* Group Ⅰ; $^{b,c,d}P < 0.05$ *vs* Group Ⅲ. The jumping frequencies of Group Ⅰ, Ⅱ, and Ⅳ were 0.

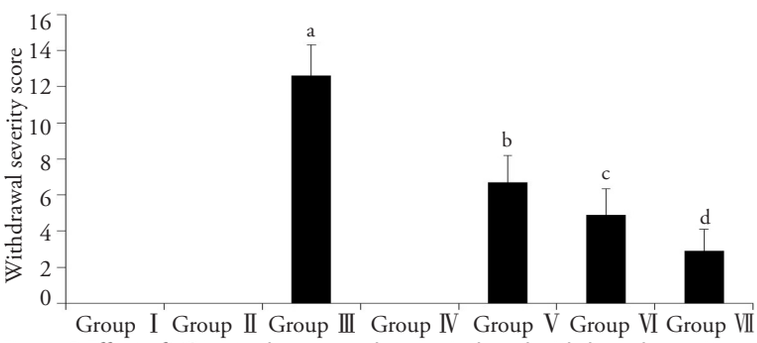

Figure 2 Effect of YGJ cataplasm on naloxone-induced withdrawal severity score in different groups

Group Ⅰ (vehicle-vehicle control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + saline, 10 mL/kg, i.p. on day 6. Group Ⅱ (vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅲ (morphine-naloxone control): morphine (5 mg/kg, i.p.) twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅳ (YGJ cataplasm + vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + YGJ cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅴ-Ⅶ (YGJ cataplasm + morphine-naloxone): morphine (5 mg/kg, i.p.) twice daily + YGJ cataplasm (1, 2, 3 cm^2 for groups Ⅴ, Ⅵ, and Ⅶ, respectively) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. $^aP < 0.05$ *vs* Group I; $^{b,c,d}P < 0.05$ *vs* Group Ⅲ. YGJ: Yiguanjian. The withdrawal severity scores of Group Ⅰ, Ⅱ, and Ⅳ were 0.

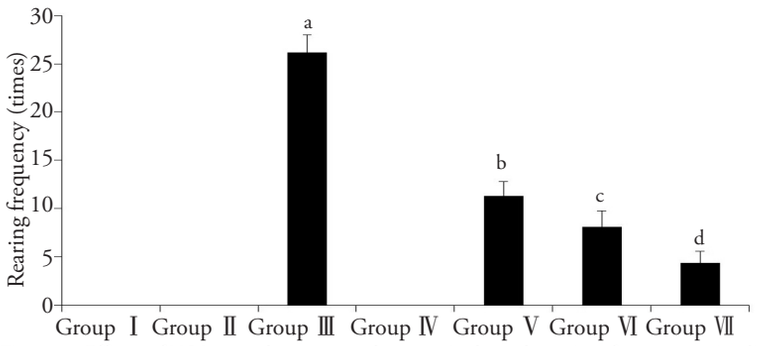

Figure 3 Effect of YGJ cataplasm on naloxone-induced rearing frequency in different groups

Group Ⅰ (vehicle-vehicle control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + saline, 10 mL/kg, i.p. on day 6. Group Ⅱ (vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅲ (morphine-naloxone control): morphine (5 mg/kg, i.p.) twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅳ (YGJ cataplasm + vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + YGJ cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅴ-Ⅶ (YGJ cataplasm + morphine-naloxone): morphine (5 mg/kg, i.p.) twice daily + YGJ cataplasm (1, 2, 3 cm^2 for groups Ⅴ, Ⅵ, and Ⅶ, respectively) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. YGJ: Yiguanjian. $^{a}P < 0.05$ *vs* Group I; $^{b,c,d}P < 0.05$ *vs* Group Ⅲ. The rearing frequencies of Group Ⅰ, Ⅱ, and Ⅳ were 0.

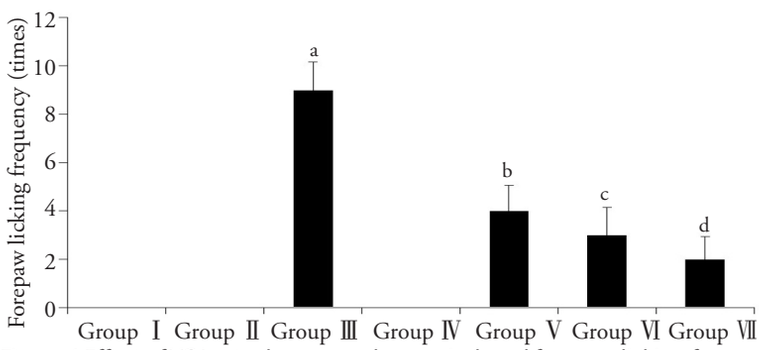

Figure 4 Effect of YGJ cataplasm on naloxone-induced forepaw licking frequency in different groups

Group Ⅰ (vehicle-vehicle control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + saline, 10 mL/kg, i.p. on day 6. Group Ⅱ (vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅲ (morphine-naloxone control): morphine (5 mg/kg, i.p.) twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅳ (YGJ cataplasm + vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + YGJ cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅴ-Ⅶ (YGJ cataplasm + morphine-naloxone): morphine (5 mg/kg, i.p.) twice daily + YGJ cataplasm (1, 2, 3 cm^2 for groups Ⅴ, Ⅵ, and Ⅶ, respectively) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. YGJ: Yiguanjian. $^{a}P < 0.05$ *vs* Group I; $^{b,c,d}P < 0.05$ *vs* Group Ⅲ. The forepaw licking frequencies of Group Ⅰ, Ⅱ, and Ⅳ were 0.

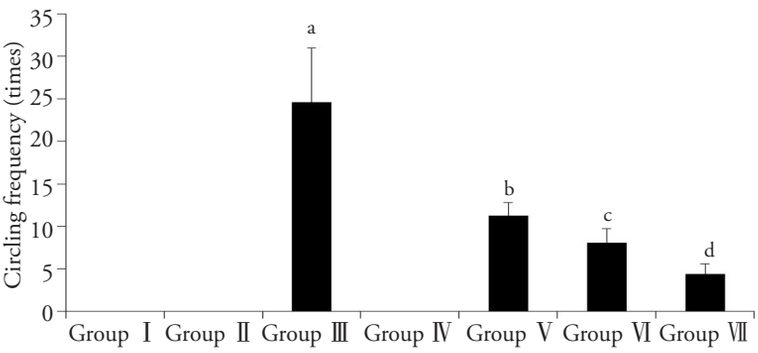

Figure 5 Effect of YGJ cataplasm on naloxone-induced circling frequency in different groups

Group Ⅰ (vehicle-vehicle control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + saline, 10 mL/kg, i.p. on day 6. Group Ⅱ (vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅲ (morphine-naloxone control): morphine (5 mg/kg, i.p.) twice daily + placebo cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅳ (YGJ cataplasm + vehicle-naloxone control): saline, 10 mL/kg, i.p. twice daily + YGJ cataplasm (2 cm^2) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. Group Ⅴ-Ⅶ (YGJ cataplasm + morphine-naloxone): morphine (5 mg/kg, i.p.) twice daily + YGJ cataplasm (1, 2, 3 cm^2 for groups Ⅴ, Ⅵ, and Ⅶ, respectively) for 3 days + naloxone (8 mg/kg, i.p.) on day 6. YGJ: Yiguanjian. $^{a}P < 0.05$ *vs* Group Ⅰ; $^{b,c,d}P < 0.05$ *vs* Group Ⅲ. The circling frequencies of Group Ⅰ, Ⅱ, and Ⅳ were 0.

Group Ⅰ (0.00 ± 0.00). Meanwhile, YGJ cataplasm (1, 2, 3 cm^2) significantly (all $P < 0.01$) and dose-dependently (all $P < 0.05$) decreased the circling frequency in opioid-dependent mice (Figure 5).

Morphine-induced analgesia

We performed the tail-flick test to confirm that YGJ cataplasm did not possess antinociceptive effects. There were no significant difference (all $P > 0.05$) in the MPE in among Groups Ⅷ (53.96 ± 2.79), Ⅸ (50.89 ± 4.80) and Ⅹ (52.45 ± 5.05).

DISCUSSION

In our study, we analyzed the effect of YGJ cataplasm for treating opioid dependence in a mouse model of naloxone-induced opioid withdrawal syndrome. We found that YGJ cataplasm significantly and dose-dependently decreased the jumping frequency, WSS, rearing frequency, forepaw licking frequency and circling frequency in opioid-dependent mice. Furthermore, the tail-flick test showed that YGJ cataplasm did not alter the antinociceptive activity of morphine.

Tolerance refers to the decreased effect of a drug after repeated administration, requiring higher doses to receive similar effects. Withdrawal syndrome is characterized by a series of emotional, physical, and behavioral changes after discontinuing certain drugs.[29] The emergence of tolerance and withdrawal symptoms is common when opioid analgesics and other illicit opiates are used. Recently, the treatment of opioid dependence has mainly depended on opiate replacement therapy and symptomatic treatment of withdrawal signs.

TCM has been practiced in China for more than 2000 years, and for the past 200 years it has been used in the treatment of drug addiction. Until now, at least 10 Chinese medicines have been approved for the treatment of opiate addiction by the Chinese State Food and Drug Administration.[30,31] We found that liver-*Yin* deficiency was a common clinical syndrome of TCM in opioid dependence in clinical examinations. YGJ was established by Wei Zhixian in the Qing Dynasty (AD 1722-1772) and was first recorded in medical practice in Liu Zhou, a classic liver-*Yin* tonifying herbal formula, while YGJ is commonly used for treating diseases with liver-*Yin* deficiency.[9-11] Therefore, we examined the efficacy of YGJ cataplasm in treating opioid dependence in this study. After analysis, we found that YGJ cataplasm could significantly and dose-dependently attenuate opioid withdrawal syndrome in mice, in terms of WSS, and the frequencies of jumping, rearing, forepaw licking, and circling behaviors.

In our study, we used TDDS for the administration of drugs. TDDS had many advantages over alternative methods of drug administration. It had better patient compliance than conventional drug administration routes, such as oral delivery. It can distribute certain drugs in the systemic circulation in a more convenient and effective way, and maintain constant blood drug levels for long time periods. Meanwhile, drugs can be delivered directly to blood circulation without undergoing first-pass metabolism through TDDS.

Our study also had several limitations. We demonstrated that YGJ cataplasm might attenuate opioid dependence and its withdrawal syndrome; however, we did not reveal the detailed mechanism underlying how this was achieved. This mechanism might be elucidated in our future studies, in which we might also compare the effect of YGJ cataplasm and other recently-developed drugs for treating opioid dependence and its withdrawal syndrome. As a famous TCM formula, the YGJ cataplasm has been used in clinical applications for many years in China. Therefore, we might analyze the efficacy of YGJ cataplasm for treating opioid dependence in clinical studies in the future.

In conclusion, we found that YGJ cataplasm might attenuate opioid dependence and its withdrawal syndromes in a mouse model of naloxone-induced opioid withdrawal syndrome. YGJ cataplasm might serve as a potential therapy for combating opioid addiction in the future.

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