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Basic Study

Study on the antipyretic effect of pushing Tianheshui in young rabbits: focus on the α-MSH-mediated cAMP/PKA/NF-κB signaling pathway

基于α-MSH介导的cAMP/PKA/NF-κB信号通路探讨清天河水对幼兔的退热机制

HUANG Yumei (黄玉梅)[1], WANG Dejun (王德军)[1], WANG Wei (王伟)[2], GAO Juan (高涓)[1], CHEN Liwei (陈力维)[1]
1 School of Acupuncture-moxibustion, Tuina and Rehabilitation, Hunan University of Chinese Medicine, Changsha 410208, China
2 Nanchang Hongdu Hospital of TCM, Jiangxi University of Chinese Medicine, Nanchang 330006, China

Abstract

Objective: To explore the antipyretic effect and partial mechanism of the pushing Tianheshui manipulation on lipopolysaccharide (LPS)-induced fever in young New Zealand rabbits.

Methods: Thirty 50-day-old New Zealand rabbits were randomly assigned to five groups, including a normal group, a model group, a Tuina (Chinese therapeutic massage) group, a Tuina control group, and a drug group, with 6 rabbits in each group. All groups except for the normal group received LPS injections through the marginal ear vein to induce fever. One hour post-modeling, the Tuina and Tuina control groups received pushing Tianheshui manipulation and pushing manipulation on the medial middle of the hind limbs, respectively, administered every hour for a total of 3 interventions. The drug group was given acetaminophen oral liquid via gavage. Anal temperature was recorded every 30 min for 4.0 h to monitor temperature changes among groups. At 4.0 h post-modeling, hypothalamus samples from each group were analyzed using Western blotting (WB) and real-time quantitative polymerase chain reaction (RT-qPCR) to measure the relative expression levels of α-melanocyte-stimulating hormone (α-MSH), melanocortin 4 receptor (MC4R), cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), nuclear factor-κB p65 (NF-κB p65), and interleukin (IL)-1β proteins and their mRNAs.

Results: Compared to the model group, the Tuina group showed a significant reduction in the anal temperature from 3.5 h to 4.0 h post-modeling (P<0.05). The Tuina control group did not show a significant temperature reduction from 0.5 h to 4.0 h post-modeling (P>0.05). The drug group exhibited a significant temperature reduction from 1.5 h to 4.0 h post-modeling (P<0.05). At 4.0 h post-modeling, compared to the model group, the Tuina group showed significantly increased relative expression of α-MSH and MC4R proteins and mRNAs (P<0.05) and significantly decreased relative expression of cAMP, PKA, NF-κB p65, and IL-1β proteins and mRNAs in the hypothalamus tissue (P<0.05). No significant differences were observed in these parameters in the Tuina control group compared to the model group (P>0.05).

Conclusion: Pushing Tianheshui manipulation demonstrated a significant antipyretic effect, potentially linked to point specificity. Its mechanism may involve the α-MSH-mediated cAMP/PKA/NF-κB pathway.

Keywords: Tuina; Massage; Manual Therapies; Pushing Tianheshui; Lipopolysaccharides; Fever; Rabbits

【摘要】目的: 探讨清天河水推拿对脂多糖(LPS)致热新西兰幼兔的退热效果及部分作用机制。**方法:** 将30只50日龄新西兰幼兔采用随机数字表法分为正常组、模型组、推拿组、对照推拿组和药物组, 每组6只。除正常组外, 其余组经耳缘静脉注射LPS溶液建立发热模型。造模1.0 h后, 推拿组和对照推拿组分别行清天河水的推拿操作和后肢内侧正中推法操作, 每1.0 h干预1次, 共干预3次; 药物组予以对乙酰氨基酚口服液灌胃。每30 min测量一次幼兔肛温, 连续测量4.0 h以比较各组肛温变化。造模4.0 h后取各组幼兔下丘脑样本进行免疫印迹法(WB)和实时荧光定量聚合酶链反应(RT-qPCR)检测, 比较各组α-黑素细胞刺激素(α-MSH)、黑皮质激素4型受体(MC4R)、环磷酸腺苷(cAMP)、蛋白激酶A (PKA)、核转录因子-κB p65 (NF-κB p65)、白介素(IL)-1β蛋白及其mRNA的相对表达量。**结果:** 与模型组比较, 造模后3.5～4.0 h, 推拿组肛温显著下降(P<0.05); 造模后0.5～4.0 h, 对照推拿组肛温无明显下降(P>0.05); 造模后1.5～4.0 h, 药物组肛温显著下降(P<0.05)。造模后4.0 h, 与模型组比较, 推拿组α-MSH、MC4R蛋白及mRNA相对表达量显著升高(P<0.05), cAMP、PKA、NF-κB p65、IL-1β蛋白及mRNA相对表达量显著降低(P<0.05)。与模型组比较, 对照推拿组下丘脑组织α-MSH、MC4R、cAMP、PKA、NF-κB p65、IL-1β蛋白及mRNA相对表达量无显著变化(P>0.05)。**结论:** 清天河水具有显著退热效果, 且存在一定穴位特异性; 其退热机制可能与α-MSH介导的cAMP/PKA/NF-κB通路有关。

First Author: HUANG Yumei, master degree candidate
Corresponding Author: WANG Dejun, M.M., associate professor.
E-mail: 36497062@qq.com

【关键词】推拿; 按摩; 手法治疗; 推天河水; 脂多糖类; 发热; 兔
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Fever results from pyrogens acting on the body to elevate the set point of the thermoregulatory center, leading to an increase in body temperature. It is the most common symptom in pediatric illnesses, with a particularly high incidence in children under 5 years old, who experience an average of 4-6 episodes per year. The frequency decreases gradually in school-aged children[1]. Clinically, it has been shown that excessive or prolonged fever can negatively impact essential organs and immune function[2]. As a result, investigating the antipyretic mechanisms and enhancing the clinical efficacy of fever treatment has become a significant focus in pediatric Tuina (Chinese therapeutic massage) research.

Compared to conventional drug treatments, which often involve notable adverse reactions and potential sequelae, pediatric Tuina offers distinct advantages, including a positive curative effect, simplicity, low cost, non-invasiveness, and absence of adverse reactions, making it highly valuable in clinical settings[2-3]. Pushing Tianheshui manipulation is the most frequently used antipyretic technique in pediatric Tuina, with an application rate of approximately 74.6%[4]. Therefore, selecting the pushing Tianheshui manipulation as an intervention to explore its potential antipyretic mechanism holds great clinical significance.

1 Materials and Methods

1.1 Animal source

This study was approved by the Animal Ethics Committee of Hunan University of Traditional Chinese Medicine (Ethics No. LLBH-202212040001). Thirty 50-day-old New Zealand rabbits, balanced by gender (15 males and 15 females) and weighing between 1.5 kg and 2.0 kg, were supplied by the Animal Experiment Center of Hunan University of Chinese Medicine. The animal use license number was [SYXK (Xiang) 2019-0009], and the animal production license number was [SCXK (Xiang) 2020-0005].

The young rabbits were adaptively housed at the experimental center for 7 d, maintained at a temperature of 24-26 ℃ and a relative humidity of 50%-70%. Following the adaptation period, anal temperatures were recorded each morning and afternoon for 3 consecutive days. A mercury thermometer was inserted 5 cm into the anus for temperature measurement. Rabbits with an anal temperature fluctuation greater than 0.4 ℃ were excluded from the study.

1.2 Main reagents and instruments

1.2.1 Main reagents

Lipopolysaccharide (LPS) derived from *E. coli* 055: B5 (Cat. No. L2880, Sigma, USA) was used. Additional reagents included α-melanocyte-stimulating hormone (α-MSH), melanocortin 4 receptor (MC4R), cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), nuclear factor-κB p65 (NF-κB p65), and interleukin (IL)-1β (Cat. No. A10364, No. A17389, No. DF6523, No. AF0361, No. AF5006, No. AF5103, Jiangsu Qinke Biological Research Center Co., Ltd., China).

1.2.2 Primary instruments

The primary instruments included a cryogenic centrifuge (Sigma, Germany), SDS-PAGE electrophoresis system (Bio-Rad, USA), gel imaging system (UVP, USA), and chemiluminescence imaging system (Clinx Science Instruments, China).

1.3 Modeling method and grouping

The young rabbits were randomly assigned to five groups using a random number table: a normal group, a model group, a Tuina group, a Tuina control group, and a drug group, with 6 rabbits in each group. The normal group received normal saline [0.3 mL/(kg·bw)] injected into the marginal ear vein, while the other groups were injected with LPS aqueous solution (2 μg/mL) at a dose of [0.3 mL/(kg·bw)] through the marginal ear vein to induce fever. Modeling was considered successful if the anal temperature increased by 0.6 ℃ or more 1.0 h later[5-6].

1.4 Intervention methods

The normal group and model group did not receive Tuina intervention.

Tuina group: After successful modeling, the Tuina group received pushing Tianheshui manipulation (Figure 1). According to the *Experimental Acupuncturology*[7], Tianheshui is located on the medial midline of the rabbits' forelimbs, extending in a straight line from the wrist to the elbow joint. A brush was used to push from the midpoint of the "wrist" to the midpoint of the "elbow" on the forelimbs, with room-temperature water as a medium. The pushing frequency was maintained at 200 times per minute, with each limb treated for 5 min, totaling 10 min for both limbs. The intervention was administered once per hour for a total of 3 sessions (i.e., at 1.0 h, 2.0 h, and 3.0 h after modeling).

Tuina control group: In this group, a brush was used to push directly from the "ankle joint" to the "knee joint" along the midline of the inner hind limbs, using room-temperature water as the medium. The frequency was also maintained at 200 times per minute, with each hind limb treated for 5 min, totaling 10 min for both hind limbs. The intervention was initiated 1.0 h after modeling and repeated per hour for a total of 3 sessions (i.e., at 1.0 h, 2.0 h, and 3.0 h after modeling).

Drug group: Acetaminophen oral solution (100 mL:

3.2 g) was administered via gavage at a dose of [150 mg/(kg·bw)] 1 h after modeling[8].

All Tuina manipulations were performed by the same professional.

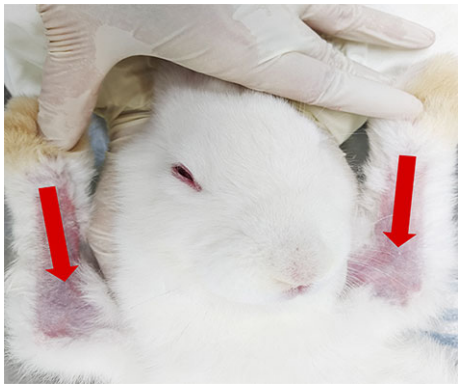


Figure 1 Pushing Tianheshui manipulation

1.5 Specimen collection and detection indicators

1.5.1 Anal temperature detection

Anal temperature was measured using a mercury thermometer, which was lubricated with paraffin oil and inserted 5 cm into the anus. Readings were taken after 5 min and recorded every 30 min for a continuous period of 4.0 h.

The change in anal temperature after modeling was calculated as follows. The baseline anal temperature (recorded prior to modeling) was subtracted from the anal temperature measured after modeling to obtain the post-modeling temperature change value.

1.5.2 Western blotting (WB)

The relative protein expression levels of α-MSH, MC4R, cAMP, PKA, NF-κB p65, and IL-1β in the hypothalamus of young rabbits were analyzed by WB. Four hours post-modeling, after the final anal temperature measurement, animals were anesthetized with 3% sodium pentobarbital and sacrificed by cervical dislocation. The skull and cerebral dura mater were removed, and the entire brain was extracted. The hypothalamic tissue was isolated, placed in cryotubes, and stored in liquid nitrogen for WB analysis. A portion of the hypothalamic tissue was lysed with lysis buffer and centrifuged at 4 ℃ at 12 000 r/min for 15 min. The supernatant was collected, and protein concentration was determined using the BCA protein quantification method. A 10% separation gel and a 5% concentration gel were prepared, with the voltage adjusted to 80 V after sample loading. Electrophoresis was performed, allowing samples to pass through the concentrating gel and separation gel (at approximately 8 V/cm). Membrane transfer occurred at 65 V for 2.0 h, followed by blocking at room temperature with gentle shaking on a shaker for 1.0 h. Primary antibodies (actin 1:3 000, α-MSH 1:500, MC4R 1:500, cAMP 1:500, PKA 1:500, NF-κB p65 1:500, IL-1β 1:500) were incubated with the membrane overnight at 4 ℃. The following day, after three washes, a secondary antibody was added and incubated for 1.0 h. After washing, the membrane was developed with ECL reagent and imaged using the chemiluminescence imaging system. The gray values of bands were analyzed to determine the relative expression levels of the proteins to be tested.

1.5.3 Real-time quantitative polymerase chain reaction (RT-qPCR)

The relative mRNA expression levels of α-MSH, cAMP, PKA, NF-κB p65, and IL-1β in the hypothalamus of young rabbits were measured using RT-qPCR. The total RNA was extracted from the tissue samples using Trizol, and cDNA synthesis was performed using the Superscript Ⅲ reverse transcription kit (Invitrogen). Data were analyzed using the $2^{-\Delta\Delta ct}$ method (Table 1).

Table 1 PCR primers

Name	Upstream primer sequence (5'→3')	Downstream primer sequence (5'→3')	Amplification length/bp
α-MSH	AGTGCTTCCTTATCGGTG	TCACGAAGGAATAGCCAC	130
cAMP	TGGGACCTAAAAGACTGG	ACCCTGGATTTTCTGACC	145
PKA	GCGAGCAAGAGAGTGTCAAAG	GCATAGTGGTTCCCGGTCTC	181
NF-κB p65	AACCCCTTCCAAGTGCCCAT	CAGATCTTGAGCTCGGCAGT	176
IL-1β	GATGGAAAAGCGATTTGTCT	GTTATATCTCGGCCACCAA	130
Actin	ACGACATGGAGAAGATCTGGCAC	AACGTCTCGAACATGATCTGGGT	142

Note: PCR=Polymerase chain reaction; α-MSH=α-melanocyte-stimulating hormone; cAMP=Cyclic adenosine monophosphate; PKA=Protein kinase A; NF-κB p65=Nuclear factor-κB p65; IL-1β=Interleukin-1β.

1.6 Statistical methods

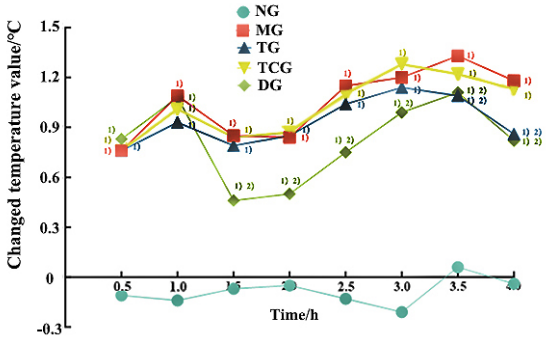
Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Anal temperature data were analyzed using the repeated measures analysis of variance with a simple effects analysis. WB and RT-qPCR data were confirmed to follow a normal distribution with homogeneous variance, and the one-way analysis of variance was applied. Pairwise comparisons between groups were conducted using the least significant difference method. For data that did not meet normality or homogeneity of variance, the non-parametric Kruskal-Wallis *H*-test was used. $P<0.05$ was

considered statistically significant.

2 Results

2.1 Comparison of the anal temperature changes among groups after modeling

After modeling, the model group showed a significant increase in anal temperature from 0.5 h to 4.0 h compared to the normal group, with a statistically significant difference (P<0.05). In the Tuina group, the anal temperature significantly decreased following the pushing Tianheshui manipulation (at 3.5-4.0 h post-modeling), and this reduction was statistically significant (P<0.05). In contrast, the Tuina control group showed no significant change in anal temperature from 0.5 h to 4.0 h after intervention (P>0.05). In the drug group, a significant decrease in anal temperature was observed within 3.0 h after administration, lasting from 1.5 h to 4.0 h post-modeling, with a statistically significant difference (P<0.05). See Figure 2.

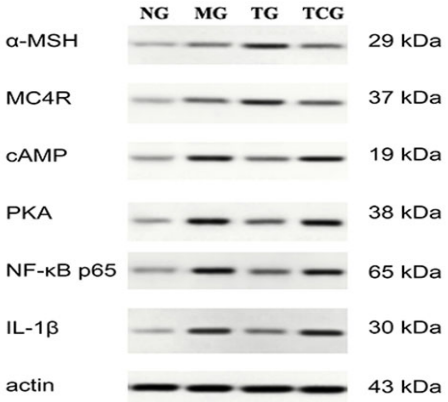


Note: NG=Normal group; MG=Model group; TG=Tuina group; TCG=Tuina control group; DG=Drug group; compared to the normal group, 1) P<0.05; compared to the model group, 2) P<0.05.

Figure 2 Comparison of the anal temperature changes among groups after modeling

2.2 Comparison of the relative expression levels of α-MSH, MC4R, cAMP, PKA, NF-κB p65, and IL-1β proteins in the hypothalamus of rabbits among groups

After 4.0 h of modeling, the model group exhibited significantly increased relative expression levels of α-MSH, MC4R, cAMP, PKA, NF-κB p65, and IL-1β proteins in the hypothalamus compared to the normal group (P<0.05). In the Tuina group, the relative expression levels of α-MSH and MC4R proteins were significantly elevated compared to the model group after 4.0 h (P<0.05), while the relative expression levels of cAMP, PKA, NF-κB p65, and IL-1β proteins were significantly reduced (P<0.05). In contrast, the Tuina control group showed no significant changes in the relative expression levels of α-MSH, MC4R, cAMP, PKA, NF-κB p65, or IL-1β proteins in the hypothalamus compared to the model group after 4.0 h (P>0.05). The details are shown in Figure 3 and Table 2.



Note: NG=Normal group; MG=Model group; TG=Tuina group; TCG=Tuina control group; α-MSH=α-melanocyte-stimulating hormone; MC4R=Melanocortin 4 receptor; cAMP=Cyclic adenosine monophosphate; PKA=Protein kinase A; NF-κB p65=Nuclear factor-κB p65; IL-1β=Interleukin-1β.

Figure 3 Comparison of the relative expression levels of α-MSH, MC4R, cAMP, PKA, NF-κB, and IL-1β proteins in the hypothalamus of rabbits among groups

Table 2 Comparison of the relative expression levels of α-MSH, MC4R, cAMP, PKA, NF-κB p65, and IL-1β proteins in the hypothalamus of rabbits among groups ($\bar{x}$ ±s)

Group	n	α-MSH	MC4R	cAMP	PKA	IL-1β	NF-κB p65
Normal	6	0.22±0.02	0.20±0.03	0.19±0.02	0.18±0.04	0.19±0.04	0.22±0.04
Model	6	0.34±0.01[1)]	0.33±0.04[1)]	0.53±0.01[1)]	0.52±0.05[1)]	0.59±0.01[1)]	0.46±0.03[1)]
Tuina	6	0.49±0.04[1)2)]	0.40±0.02[1)2)]	0.32±0.03[1)2)]	0.32±0.02[1)2)]	0.33±0.04[1)2)]	0.31±0.04[1)2)]
Tuina control	6	0.35±0.03[1)]	0.32±0.04[1)]	0.51±0.05[1)]	0.53±0.04[1)]	0.60±0.04[1)]	0.47±0.03[1)]

Note: α-MSH=α-melanocyte-stimulating hormone; MC4R=Melanocortin 4 receptor; cAMP=Cyclic adenosine monophosphate; PKA=Protein kinase A; IL-1β=Interleukin-1β; NF-κB p65=Nuclear factor-κB p65; compared to the normal group, 1) P<0.05; compared to the model group, 2) P<0.05.

2.3 Comparison of the relative expression levels of α-MSH, cAMP, PKA, NF-κB p65, and IL-1β mRNAs in the hypothalamus of rabbits among groups

Four hours after modeling, the model group showed a significant increase in the relative mRNA expression levels of α-MSH, cAMP, PKA, NF-κB p65, and IL-1β in the hypothalamus compared to the normal group (P<0.05). In comparison to the model group, the Tuina group

exhibited a significant increase in the α-MSH mRNA expression in the hypothalamus at 4.0 h post-modeling (P<0.05). Conversely, the mRNA levels of cAMP, PKA, NF-κB p65, and IL-1β were significantly reduced in the Tuina group (P<0.05). In the Tuina control group, there were no significant changes in the relative expression levels of α-MSH, cAMP, PKA, NF-κB p65, or IL-1β mRNA in the hypothalamus compared to the model group at 4.0 h post-modeling (P>0.05). The data are shown in Table 3.

Table 3 Comparison of the relative expression levels of α-MSH, cAMP, PKA, NF-κB p65, and IL-1β mRNAs in the hypothalamus of rabbits among groups ($\bar{x}$ ±s)

Group	n	α-MSH	cAMP	PKA	NF-κB p65	IL-1β
Normal	6	1.00±0.07	1.01±0.05	1.00±0.10	0.99±0.04	1.00±0.09
Model	6	1.52±0.08[1)]	2.19±0.09[1)]	2.21±0.17[1)]	2.16±0.10[1)]	2.20±0.09[1)]
Tuina	6	2.21±0.11[1)2)]	1.54±0.00[1)2)]	1.53±0.15[1)2)]	1.58±0.12[1)2)]	1.49±0.05[1)2)]
Tuina control	6	1.55±0.10[1)]	2.21±0.19[1)]	2.39±0.13[1)]	2.30±0.19[1)]	2.24±0.04[1)]

Note: α-MSH=α-melanocyte-stimulating hormone; cAMP=Cyclic adenosine monophosphate; PKA=Protein kinase A; NF-κB p65=Nuclear factor-κB p65; IL-1β=Interleukin-1β; compared to the normal group, 1) P<0.05; compared to the model group, 2) P<0.05.

3 Discussion

Tianheshui, also known simply as "Tianhe", is a point characterized by a naturally cooling effect, effective for clearing heat and releasing the exterior. It is particularly useful in addressing heat syndromes of both Wei-defense and Qi phases, making it applicable for treating both deficiency and excess heat conditions. Clinically, Tianhe is commonly used for symptoms such as colds, fever, headache, and sore throat[9-10]. Pediatric Tuina offers advantages in treating fever, especially when decoctions are difficult to administer to children. This non-invasive treatment is simple, environmentally friendly, and safe[11]. In this study, compared to the model group, the Tuina group exhibited a significant reduction in anal temperature following the pushing Tianheshui manipulation, whereas the Tuina control group showed no significant temperature change. This suggests that pushing Tianheshui manipulation has an antipyretic effect, potentially demonstrating point specificity. In contrast, the drug group showed a significant reduction in anal temperature 0.5 h after acetaminophen administration, occurring from 1.5 h to 4.0 h post-modeling. Meanwhile, the Tuina group showed a significant temperature decrease only between 3.5 h and 4.0 h post-modeling, indicating that the antipyretic effect of pushing Tianheshui manipulation has a slower onset than acetaminophen.

In the humoral pathway of fever signal transmission, endogenous pyrogens transmit fever signals to the brain, disrupting the balance of central regulatory mechanisms. This results in the up-regulation of positive regulatory mediators, while negative regulatory mechanisms act to limit the temperature increase. The interaction between these positive and negative mediators determines the degree of fever[12-13]. Positive central regulatory mediators involved in thermoregulation include prostaglandin E_2 (PGE_2), cAMP, and corticotropin-releasing hormone, while negative regulators comprise neuropeptides like arginine vasopressin, α-MSH, β-endorphin, substance P, and taurine[13-15]. Studies suggest that the antipyretic mechanism of pediatric Tuina may involve promoting the synthesis of negative regulatory mediators such as α-MSH and arginine vasopressin in the hypothalamus while inhibiting the production of positive mediators like PGE_2, cAMP, and the TLR4/NF-κB pathway. This process also down-regulates serum neutrophil ratios and peripheral inflammatory factors, including tumor necrosis factor-α (TNF-α) and IL-1β, thereby providing both anti-inflammatory and antipyretic effects[5, 16-19].

α-MSH, a melanocyte-stimulating hormone, possesses significant anti-inflammatory and anti-microbial properties[20-21]. It functions by generating melanocortins, which then bind to melanocortin receptors to modulate the body's immune response. Five main types of melanocortin receptors (MC1R-MC5R) exist, with the MC4R primarily localized in the hypothalamus, spinal cord, and cortex. MC4R mediates most of α-MSH's effects in the central nervous system and is particularly involved in central fever signal transduction[20-22]. α-MSH can significantly inhibit the elevation of cAMP levels in the hypothalamic tissue induced by IL-1β, TNF-α, and other substances, thereby reducing body temperature. However, it has no effect on the body temperature of healthy young rabbits or on cAMP level in their hypothalamic tissue, indicating that α-MSH selectively inhibits cAMP production in the hypothalamic tissue to lower the central set point for temperature regulation, producing an antipyretic effect[23-25]. The results of this experiment showed that, after modeling, the anal temperature of young rabbits in the model group increased compared to the normal group, activating both positive and negative regulatory centers and promoting the production of regulatory mediators. Specifically, the relative expression of cAMP protein in the hypothalamus increased, while α-MSH and MC4R protein levels also rose in a reactive manner.

In the Tuina group, following pushing Tianheshui manipulation, there was an increase in α-MSH and MC4R syntheses in the hypothalamic tissue of young rabbits, which in turn inhibited cAMP synthesis, resulting in a significant reduction in anal temperature (P<0.05). In contrast, in the Tuina control group, there was no significant decrease in anal temperature following the intervention, and the relative expression levels of α-MSH, MC4R, and cAMP proteins in the hypothalamic tissue did not change substantially. These findings suggest that the antipyretic mechanism of pushing Tianheshui manipulation may involve the up-regulation of the hypothalamic α-MSH level, which subsequently down-regulates the cAMP level.

cAMP is a crucial mediator in central fever regulation, while cytokines such as IL-1β, IL-6, and TNF-α serve as important endogenous pyrogens and inflammatory mediators[13-15,26-27]. Numerous studies have shown that peripheral IL-1β can enter the brain and elevate cAMP levels in the hypothalamus, raising the temperature set point and resulting in fever[28-31]. As cAMP production increases, it activates downstream proteins, most notably PKA. In the cytoplasm, NF-κB binds to its inhibitor IκB in an inactive state. When PKA releases an active catalytic subunit that phosphorylates IκB, NF-κB is activated and translocates into the nucleus. Here, NF-κB binds to specific sequences in the promoter regions of target genes, increasing the expression of cytokines, enzymes, and adhesion molecules, and promoting the release of cellular inflammatory factors such as IL-1β and TNF-α. This process completes fever signal transduction, resulting in an elevated body temperature[32]. Thus, inhibiting the cAMP/PKA/NF-κB pathway and subsequently reducing the production of endogenous pyrogens such as IL-1β is essential for fever reduction. In this experiment, compared to the model group, the relative expression levels of cAMP, PKA, NF-κB p65, and IL-1β proteins in the hypothalamic tissue of young rabbits decreased significantly after pushing Tianheshui manipulation in the Tuina group, and the anal temperature also significantly decreased (P<0.05); in the Tuina control group, no significant decrease in anal temperature or change in the hypothalamic protein levels of cAMP, PKA, NF-κB p65, or IL-1β was observed. This suggests that the antipyretic effect of pushing Tianheshui manipulation may be related to the α-MSH-mediated inhibition of the cAMP/PKA/NF-κB pathway in the hypothalamus.

In summary, the results of this experiment demonstrate that pushing Tianheshui manipulation has an antipyretic effect on young febrile rabbits. Compared to the antipyretic effect of acetaminophen, pushing Tianheshui manipulation produces a slower response. However, as an external treatment method, pushing Tianheshui manipulation is natural and safe, and its therapeutic effect may be enhanced by repeated application within a short period. Compared to the Tuina control group, the antipyretic effect of pushing Tianheshui manipulation was significant, supporting a reasonable inference that Tianheshui may have point specificity. The antipyretic mechanism of pushing Tianheshui manipulation may involve the α-MSH-mediated cAMP pathway. By up-regulating α-MSH protein levels in the hypothalamus, pushing Tianheshui manipulation inhibits the cAMP/PKA/NF-κB signaling pathway, thereby lowering the set point of the hypothalamic thermoregulatory center and producing anti-inflammatory and antipyretic effects. The results of this experiment provide theoretical support for using Tuina to treat fever in children. However, this study did not further utilize α-MSH receptor inhibitors or receptor agonists to confirm this signaling pathway, which poses a limitation and warrants further investigation.

Conflict of Interest

The authors declare that there is no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals in this experiment conformed to the ethical criteria.

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