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Basic Study

Effects of moxibustion at different times on prostaglandin and vasopressin levels in uterine tissues of rats with dysmenorrhea due to cold-dampness retention

不同时机艾灸对寒湿凝滞型痛经大鼠子宫组织前列腺素及加压素含量的影响

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Abstract

Objective: To observe the effects of moxibustion at different times on prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and arginine vasopressin (AVP), in the uterine tissues of rats with dysmenorrhea due to cold-dampness retention, and to explore the differences and possible mechanisms of moxibustion at different times in easing pain in dysmenorrhea due to cold-dampness retention.

Methods: Forty-three female Wistar rats were randomly divided into a blank control group (*n*=7), a model group (*n*=9), a pre-moxibustion group (*n*=9), an immediate-moxibustion group (*n*=9) and a pre-moxibustion plus immediate-moxibustion group (*n*=9). Rat models of primary dysmenorrhea due to cold-dampness retention were established using (0±1) ℃ ice water immersion method combined with injection of estradiol benzoate for 10 d, followed by injection of oxytocin on the 11th day. Rats in the 3 intervention groups received moxibustion to Shenque (CV 8) and Guanyuan (CV 4), 10 min for each acupoint, once a day. Rats in pre-moxibustion group were given mild moxibustion, beginning on the 8th day during modeling, for 3 continuous days; rats in immediate-moxibustion group were given one time mild moxibustion, immediately after injection of oxytocin on the 11th day during modeling; rats in pre-moxibustion plus immediate-moxibustion group were given mild moxibustion, beginning on the 8th day during modeling till immediately after injection of oxytocin on the 11th day during modeling, for 4 continuous days. The level of $PGF_{2\alpha}$ in the rat uterine tissues was measured by enzyme-linked immunosorbent assay (ELISA), and the levels of PGE_2 and AVP in rat uterine tissues were measured by radioimmunoassay.

Results: Compared with the blank control group, the levels of $PGF_{2\alpha}$ and AVP, the $PGF_{2\alpha}/PGE_2$ ratio in the model group were significantly increased (P<0.01), and the PGE_2 level was significantly decreased (P<0.01) in the rat uterine tissues in the model group. Compared with the model group, the writhing latency was significantly prolonged, the writhing number and the total writhing score were all decreased in the pre-moxibustion group, the immediate-moxibustion group and the pre-moxibustion plus immediate-moxibustion group (all P<0.01); the levels of $PGF_{2\alpha}$ and AVP, and the $PGF_{2\alpha}/PGE_2$ ratio were all significantly decreased (P<0.05, P<0.01), and the PGE_2 level was significantly increased (P<0.01) in the rat uterine tissues of the 3 treatment groups. Compared with the pre-moxibustion group, the writhing number and the total writhing score were all decreased in the immediate-moxibustion group and the pre-moxibustion plus immediate-moxibustion group (all P<0.01), the writhing latency was significantly prolonged in the pre-moxibustion plus immediate-moxibustion group(P<0.01); the levels of $PGF_{2\alpha}$ and $PGF_{2\alpha}/PGE_2$ ratio were significantly decreased (P<0.05, P<0.01), and the PGE_2 level was significantly increased (P<0.01) in rat uterine tissues in the immediate-moxibustion group and the pre-moxibustion plus immediate-moxibustion group. Compared with the immediate-moxibustion group, the writhing latency was significantly prolonged and the writhing number was

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decreased (all $P<0.05$), and the total writhing score was decreased ($P<0.01$) in the pre-moxibustion plus immediate-moxibustion group; the $PGF_{2\alpha}$ level and the $PGF_{2\alpha}/PGE_2$ ratio were significantly decreased ($P<0.01$), and the PGE_2 level was significantly increased ($P<0.01$) in rat uterine tissues in the pre-moxibustion plus immediate-moxibustion group.

Conclusion: Moxibustion at different times all can produce obvious analgesic effects on dysmenorrhea due to cold-dampness retention in rats, and pre-moxibustion plus immediate-moxibustion ranks the top. The mechanism of this analgesic effect may be via the regulation of abnormal $PGF_{2\alpha}$, PGE_2 and AVP levels, to effectively inhibit the spastic contraction of uterine smooth muscle in dysmenorrhea rat, thereby improving the ischemia and hypoxia in uterus.

Keywords: Moxibustion Therapy; Moxa Stick Moxibustion; Point, Guanyuan (CV 4); Point, Shenque (CV 8); Prostaglandins; Dysmenorrhea; Rats

【摘要】目的：观察不同时机艾灸对寒湿凝滞型痛经大鼠子宫组织前列腺素E_2 (PGE_2)、前列腺素$F_{2\alpha}$ ($PGF_{2\alpha}$)及精氨酸加压素(AVP)含量的影响，探讨不同时机艾灸对寒湿凝滞型痛经大鼠止痛效应的差异及可能的作用机制。**方法：**将43只雌性Wistar大鼠随机分为空白组7只，模型组、预先灸组、即刻灸组和预先即刻灸组，每组9只。采用(0±1) ℃冰水浸泡法结合苯甲酸雌二醇注射法干预10 d建立寒湿凝滞证，再于第11 d注射缩宫素后制备成寒湿凝滞型原发性痛经模型。艾灸干预的3组均取神阙和关元，每穴均灸10 min，均每日1次。预先灸组于造模期间第8 d开始接受温和灸，连续3 d；即刻灸组于第11 d注射缩宫素后即接受温和灸1次；预先即刻灸组于第8 d至第11 d注射缩宫素后均接受温和灸，连续4 d。采用酶联免疫法检测大鼠子宫组织$PGF_{2\alpha}$含量，放射免疫分析法检测大鼠子宫组织PGE_2和AVP含量。**结果：**与空白组比较，模型组大鼠子宫组织$PGF_{2\alpha}$、AVP含量及$PGF_{2\alpha}/PGE_2$比值均显著升高(均$P<0.01$)，PGE_2含量显著降低($P<0.01$)。与模型组比较，预先灸组、即刻灸组及预先即刻灸大鼠的扭体潜伏期明显延长，扭体次数减少，扭体总分降低(均$P<0.01$)；3个治疗组大鼠子宫组织$PGF_{2\alpha}$、AVP含量及$PGF_{2\alpha}/PGE_2$比值均明显降低($P<0.05$, $P<0.01$)，PGE_2含量明显升高($P<0.01$)。与预先灸组比较，即刻灸组和预先即刻灸大鼠扭体次数减少，扭体总分降低(均$P<0.01$)，预先即刻灸大鼠的扭体潜伏期明显延长($P<0.01$)；即刻灸组和预先即刻灸大鼠子宫组织$PGF_{2\alpha}$含量及$PGF_{2\alpha}/PGE_2$比值均明显降低($P<0.05$, $P<0.01$)，PGE_2含量明显升高($P<0.01$)。与即刻灸组比较，预先即刻灸组大鼠的扭体潜伏期明显延长，扭体次数减少(均$P<0.05$)，扭体总分降低($P<0.01$)；预先即刻灸组大鼠子宫组织$PGF_{2\alpha}$含量及$PGF_{2\alpha}/PGE_2$比值明显降低(均$P<0.01$)，PGE_2含量明显升高($P<0.01$)。**结论：**不同时机艾灸对寒湿凝滞型痛经大鼠均有明显的止痛效果，其中以预先即刻灸最佳。其作用机制可能是通过调节$PGF_{2\alpha}$、PGE_2和AVP异常水平，有效地抑制痛经大鼠子宫平滑肌痉挛性收缩，进而改善子宫局部缺血、缺氧状态而起到镇痛作用。

【关键词】灸法；艾条灸；穴，关元；穴，神阙；前列腺素；痛经；大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Primary dysmenorrhea, also known as functional dysmenorrhea, commonly occurs in adolescent women. Modern medicine holds that the occurrence of primary dysmenorrhea is not only related to neuroendocrine, but also to the genetic, immune, metabolic and environmental factors[1]. A large number of literatures reported that moxibustion showed a satisfactory therapeutic effects on primary dysmenorrhea, and was favored by patients[2-4]. In previous clinical trials, our research group found that dysmenorrheal in most patients is attributed to cold-dampness retention. Application of partitioned moxibustion to Shenque (CV 8) and Guanyuan (CV 4) has achieved a good effect[5-7]. However, currently few studies reported the effects of moxibustion at different times on primary dysmenorrhea for pain relief. In this study, we investigated the effects of moxibustion at different times on the prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and arginine vasopressin (AVP) in the uterine tissues due to cold-dampness retention, to explore the differences and possible mechanisms of moxibustion at different times in releasing pain in dysmenorrhea rats due to cold-dampness retention.

1 Materials and Methods

1.1 Experimental animals

A total of 60 clean grade, healthy female Wistar rats, 8-10 weeks old, sex maturation without mating, body mass (200±20) g, were provided by the Animal Experimental Center of Hebei Medical University (certificate number: 1509045). Feeding environment: clean grade animal room, indoor temperature (23±2) ℃, humidity (45±5)%, 12 h light and 12 h dark, free drinking and diet. The Ethics Committee of Hebei University of Chinese Medicine approved the care and use of laboratory animals in this study.

1.2 Main reagents and instruments

PGE_2 radioimmunoassay kit (Beijing Puer Weiye Biotechnology Co., Ltd., China); rat $PGF_{2\alpha}$ ELISA kit (Shanghai Xitang Biotechnology Co., Ltd., China); AVP radioimmunoassay kit (Beijing Huabu Lite Biotechnology Research Institute, China); estradiol benzoate injection, oxytocin injection (Chifeng Boen Pharmaceutical Co., Ltd., China); moxa stick (7 mm × 120 mm, Nanyang Hanyi Moxa Co., Ltd., China); TD10001 electronic balance (Tianjin Balance Instrument

Co., Ltd., China); DP73 digital microscope (Olympus, Japan); HMIAS-2000 model microscopic image analysis system (Wuhan Tongji Medical University, China); TDL-5-A model centrifuge (Shanghai Anting Science Instrument Factory, China); SHB-D-model circulating water vacuum pump (Zhengzhou Great Wall Scientific Industrial and Trade Co., Ltd., China); FJ-2021 model γ-radioimmunoassay counter (Xi'an 262 Factory, China).

1.3 Modeling and grouping

The 60 Wistar female rats were adaptively fed for 7 d, and then screened by vaginal smear[8-9] for 4 d. Rats without diestrus or staying in the same period were excluded. A total of 43 rats in diestrus were selected. According to the random number table method in *Medical Statistics*, rats were randomly divided into a blank control group (n=7), a model group (n=9), a pre-moxibustion group (n = 9), a immediate-moxibustion group (n=9) and a pre-moxibustion plus immediate-moxibustion group (n=9). Rats in the blank control group received subcutaneous injection of saline, once a day, for continuous 10 d (0.5 mL on the 1st and 10th day, 0.2 mL from the second day to the 9th day, for each rat). Rats in the other 4 groups were treated with (0±1) ℃ ice water bath combined with injection of estradiol benzoate to prepare cold-dampness retention dysmenorrhea rat models[10-11]: at room temperature [(23±2) ℃], rat hind limbs and lower abdomen were immerged into (0±1) ℃ ice water mixture for cold stimulation, once a day, 20 min/time. At the same time, rats were subcutaneously injected with estradiol benzoate, once a day for 10 continuous days (0.5 mg on the 1st and 10th day, 0.2 mg from the second day to the 9th day, for each rat). The rats gradually appeared chills, arched back and erect hair, sneeze, curled body and less moving, apathetic, loose stools, reduced food and water intake; pale lips, ears and nose, claws and tails, and so on. These were consistent with the symptoms of cold-dampness retention. On the 11th day, each rat was injected with 2 U oxytocin intraperitoneally. Rat's abdomen was contracted and concave. The trunk and hind legs of rats were stretched, and the buttocks and unilateral limbs were rotated interiorly[12].

1.4 Intervention methods

The blank control group and the model group: Rats in the two groups were put into the self-made rat fixing bags, 10 min/time, once a day, for continuous 4 d, without other interventions.

The pre-moxibustion group: Based on the acupoint selection standard in *Experimental Acupuncture Science*[13-14], the following acupoints were selected. Shenque (CV 8, in the acromphalus, on the midline of the abdomen, at same horizontal line as the midpoint of the two iliac crest line); Guanyuan (CV 4, on the midline of the abdomen, about 25 mm below the umbilical region, at the midpoint of the connection line between the two rat hind limbs). Positioning was marked by dyeing. At 75 min after modeling on the 8th day, rats were put into the self-made rat fixing bags. The investigator conducted warm moxibustion for both acupoints at the same time, by holding fine moxa sticks (7 mm in diameter), 10 min/time, once a day, for continuous 3 d.

The immediate-moxibustion group: One time moxibustion treatment was conducted on the 11th day after injection of oxytocin, with the same acupoints and method used in the pre-moxibustion group, 10 min/time.

The Pre-moxibustion plus immediate-moxibustion group: Moxibustion treatment was started at 75 min after modeling on the 8th day to the 11th day after injection of oxytocin, with the same acupoints and method used in the pre-moxibustion group, 10 min/time, once a day, for continuous 4 d.

1.5 Observation items and testing methods

1.5.1 The writhing number

The writhing number in each group was observed, between 10 min and 30 min after injection of oxytocin. Observation initiated from 10 min after the injection, due to the potential influence of moxibustion treatment on the judge to the writhing.

1.5.2 Writhing latency

The beginning time of writhing reaction was recorded after injection of oxytocin in each group.

1.5.3 Writhing score

The rat writhing status was graded and the writhing score was calculated according to the grades.

Grade 0: Paws were prostrated on the bottom of the box or appeared normal trying behaviors.

Grade 1: The body was sloped to one side.

Grade 2: Hind legs were stretched, hind paws appeared dorsiflexion, rat bodies were stretched with frequent pelvic lateral rotation.

Grade 3: Abdominal muscles were contracted with hind legs extension.

Writhing scores=Grade 0 (writhing numbers) × 0 point + Grade 1 (writhing numbers) × 1 point + Grade 2 (writhing numbers) × 2 points + Grade 3 (writhing numbers) × 3 points[15].

1.5.4 The levels of $PGF_{2\alpha}$, PGE_2 and AVP in rat's uterine tissues

The rapidly removed uterine tissue from the left side of 'Y' shaped uterus was weighed after washing with ice physiological saline. 30% uterine tissue homogenate was prepared by adding physiological saline. Centrifuged for 15 min at 3 000 r/min and 4 ℃ to collect the supernatant. $PGF_{2\alpha}$, PGE_2 and AVP levels in the supernatant were detected using the kits following

the kit instructions. The level of $PGF_{2\alpha}$ in uterine tissues was detected by enzyme-linked immunosorbent assay (ELISA). The levels of PGE_2 and AVP in uterine tissues were detected by radioimmunoassay. Detection was conducted by the Experimental Center of Hebei University of Chinese Medicine.

1.6 Statistical processing

The SPSS 17.0 version statistical software was used for data analysis. Measurement data with normal distribution and homogeneity of variance were presented as mean ± standard deviation ($\bar{x} \pm s$); one-way ANOVA was used for comparisons between groups. The least significant difference *t*-test (LSD-*t*) was used for comparing data before and after treatment within the same group; data unfitting the normal distribution and with heterogeneity of variance were analyzed using the Tamhane's T2 test. $P<0.05$ indicated that the difference was statistically significant.

2 Results

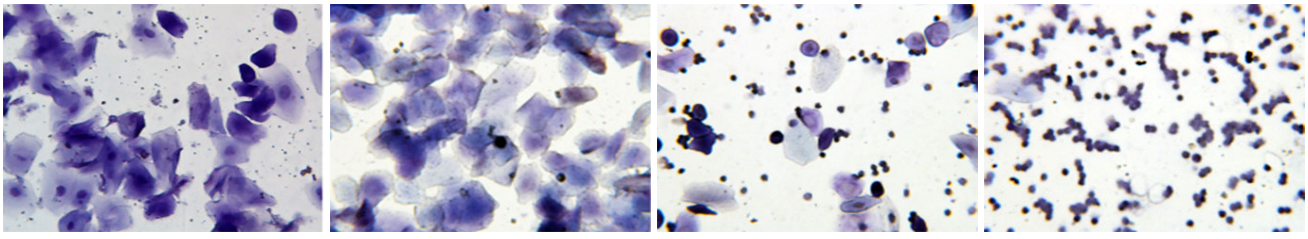
One rat died in the model group during modeling, therefore, 7 rats in the blank control group, 8 rats in the model group and 9 rats in each treatment group were used for the final data analysis.

2.1 Status of rat diestrus screening

Proestrus: vaginal secretions were mostly expanded large oval-shaped flat epithelial cells (lasting 17-21 h). Estrus: nuclei of the expanded epithelial cells disappeared, and became the squamous shedding (keratinized epithelial cells) and aggregated, as if many leaves were stacked together (lasting 9-15 h). Metestrus: vaginal secretions were mostly white blood cells with a small amount of keratinized epithelial cells (lasting 10-14 h). Diestrus: vaginal secretions were mostly the white blood cells, with occasionally smaller epithelial cells containing nuclei (lasting 60-70 h), (Figure 1).

2.2 Comparison of rat writhing behaviors among groups

After the intervention, compared with the model group, writhing latency was significantly prolonged, the writhing number and the total writhing score were decreased in the pre-moxibustion group, the immediate-moxibustion group and the pre-moxibustion plus immediate-moxibustion group (all $P < 0.01$); compared with the pre-moxibustion group, the writhing number and the total writhing score were decreased in the immediate-moxibustion group and the pre-moxibustion plus immediate-moxibustion group (all $P<0.01$), and the rat writhing latency was significantly prolonged ($P < 0.01$) in the pre-moxibustion plus immediate-moxibustion group; compared with the immediate-moxibustion group, the writhing latency was significantly prolonged and the writhing number was decreased (all $P<0.05$), and the total writhing score was decreased ($P<0.01$) in the pre-moxibustion plus immediate-moxibustion group (Table 1).



Proestrus Estrus Metestrus Diestrus

Figure 1. Observation of vaginal smears at different stages during the rat's oestrus cycle (HE, ×40)

Table 1. Comparison of rat's writhing behaviors among groups ($\bar{x} \pm s$)

Group	*n*	Writhing latency (minute)	Writhing numbers (frequency)	Writhing score (point)
Model	8	4.38±1.06	38.75±5.63	86.25±12.33
Pre-moxibustion	9	9.67±1.32[1)]	22.11±1.90[1)]	44.89±4.34[1)]
Immediate-moxibustion	9	11.78±1.30[1)]	16.33±2.96[1)2)]	32.44±6.11[1)2)]
Pre-moxibustion plus immediate-moxibustion	9	15.00±1.22[1)2)3)]	11.78±2.22[1)2)4)]	21.11±2.76[1)2)3)]

Note: Compared with the model group, 1) $P<0.01$; compared with the pre-moxibustion group, 2) $P<0.01$; compared with the immediate-moxibustion group, 3) $P<0.01$, 4) $P<0.05$

2.3 Comparison of $PGF_{2\alpha}$ and PGE_2 levels in rat uterine tissues among groups

Compared with the blank control group, the $PGF_{2\alpha}$ level and $PGF_{2\alpha}/PGE_2$ ratio were all significantly increased (all $P < 0.01$), and the PGE_2 level was significantly decreased ($P<0.01$) in the rat uterine tissues in the model group; the $PGF_{2\alpha}$ level and $PGF_{2\alpha}/PGE_2$ ratio were significantly decreased ($P<0.05$, $P<0.01$), and the PGE_2 level was significantly increased in the rat uterine tissues in the pre-moxibustion group,

immediate-moxibustion group and the pre-moxibustion plus immediate-moxibustion group ($P < 0.01$); compared with the pre-moxibustion group, the $PGF_{2\alpha}$ level and $PGF_{2\alpha}/PGE_2$ ratio were significantly decreased ($P<0.05$, $P<0.01$), and PGE_2 was significantly increased ($P<0.01$) in the rat uterine tissues in the immediate-moxibustion group and the pre-moxibustion plus immediate-moxibustion group; compared with the immediate-moxibustion group, the $PGF_{2\alpha}$ level and $PGF_{2\alpha}/PGE_2$ ratio were significantly decreased (all $P<0.01$), and the PGE_2 level was significantly increased ($P<0.01$) in rat's uterine tissues in the pre-moxibustion plus immediate-moxibustion group (Table 2).

2.4 Comparison of AVP levels in rat uterine tissues among groups

The AVP level of rat uterine tissues in the model group was significantly higher than that in the blank control group ($P<0.01$). Compared with the model group, the AVP level of rat uterine tissues was decreased ($P<0.05$ or $P<0.01$) in the pre-moxibustion group, the immediate-moxibustion group and the pre-moxibustion plus immediate-moxibustion group. Compared with the pre-moxibustion group and the immediate-moxibustion group, the AVP level of rat uterine tissues in the pre-moxibustion plus immediate-moxibustion group showed decrease, while there was no statistically significant difference ($P>0.05$), (Table 3).

Table 2. Comparison of $PGF_{2\alpha}$, PGE_2 levels and $PGF_{2\alpha}/PGE_2$ ratio in uterine tissues among groups ($\bar{x}\pm s$)

Group	n	$PGF_{2\alpha}$ (pg/mg)	PGE_2 (pg/mg)	$PGF_{2\alpha}/PGE_2$
Blank control	7	270.96±47.33	110.06±10.64	2.50±0.58
Model	8	737.64±67.30[1)]	35.64±10.47[1)]	22.60±7.66[1)]
Pre-moxibustion	9	470.14±67.42[2)]	61.38±10.31[2)]	7.83±1.65[3)]
Immediate-moxibustion	9	409.27±53.18[2)5)]	74.71±11.23[2)4)]	5.53±0.69[2)5)]
Pre-moxibustion plus immediate-moxibustion	9	317.74±44.31[2)4)6)]	88.07±10.11[2)4)6)]	3.63±0.49[2)4)6)]

Note: Compared with the blank control group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$, 3) $P<0.05$; compared with the pre-moxibustion group, 4) $P<0.01$, 5) $P<0.05$, compared with the immediate-moxibustion group, 6) $P<0.01$

Table 3. Comparison of AVP levels of rat uterine tissues in each group ($\bar{x}\pm s$, pg/mg)

Group	n	AVP
Blank control	7	2.63±0.29
Model	8	3.97±0.61[1)]
Pre-moxibustion	9	2.81±0.14[2)]
Immediate-moxibustion	9	2.65±0.16[3)]
Pre-moxibustion plus immediate-moxibustion	9	2.64±0.34[3)]

Note: Compared with the blank control group, 1) $P<0.01$; compared with the model group, 2) $P<0.05$, 3) $P<0.01$

3 Discussion

Primary dysmenorrhea is lower abdominal pain before, during or after menstruation. The pain may radiate toward the lumbosacral region. In severe cases, it may cause faint. In Chinese medicine, it falls under the category of 'abdominal pain during menstruation'. The pain can be caused by obstruction of qi and blood in the Thoroughfare and Conception Vessels and uterus. Contributing factors include cold, dampness or stagnation. Alternatively, the pain can be caused by malnourishment of the Thoroughfare and Conception Vessels and uterus. Contributing factors include deficiency of the liver and kidney and insufficiency of qi and blood. In clinical setting, we found that the most common pattern of dysmenorrhea is cold-dampness retention. As a result, warming therapy is often used to warm the meridians, dissipate cold, resolve stasis and alleviate pain[16].

In this study, we applied moxibustion to Shenque (CV 8) and Guanyuan (CV 4), because moxibustion acts to warm the meridians, dissipate cold, and resolve stasis/masses. Studies have proven that an infrared ray generated from moxa burning has strong permeability, which allows the infrared ray to transport energy to the uterus via the meridian system[17]. Shenque (CV 8) and Guanyuan (CV 4) function to warm yang, tonify the kidney, reinforce Yuan-primordial qi and regulate the Thoroughfare and Conception Vessels. Studies have suggested that moxibustion at Shenque (CV 8) and Guanyuan (CV 4) helps to circulate qi and blood in the pelvic to increase the local perfusion, decrease neural excitation, increase the pain threshold, inhibit uterine contractions and thus alleviate uterine spasm-induced pain[18]. Other studies have also indicated that needling Guanyuan (CV 4) helps to regulate the excitation and inhibition of the autonomic nervous system, alleviate the contractions of uterine smooth muscle and thus alleviate pain[19]. Modern studies suggested that the occurrence of primary dysmenorrhea should be closely related to prostaglandins (PGs) and AVP synthesis by endometrium. $PGF_{2\alpha}$ can promote contraction of non-pregnancy uterine smooth muscle[20]. The function of PGE_2 is different from $PGF_{2\alpha}$, which can inhibit uterine contraction to achieve uterine relaxation.

$PGF_{2\alpha}/PGE_2$ ratio reflects the status of uterine smooth muscle contraction and relaxation[21]. Protein lytic enzymes destroy the endometrial cells during the menstrual period. High concentrations of $PGF_{2\alpha}$, produced by the endometrium, will act on the $PGF_{2\alpha}$ receptor on the spiral arterial wall. This will cause abnormal contraction of uterine smooth muscle, ischemia and hypoxia of uterine, and acidity metabolites accumulated in the myometrium, resulting in dysmenorrhea[22-23]. AVP is also a strong uterine contraction agent, which can promote $PGF_{2\alpha}$ synthesis, enhance the sensitivity of uterine smooth muscle to oxytocin and other drugs, so that uterine blood flow is decreased, causing primary dysmenorrhea[24]. It's been confirmed that AVP secretion can enhance uterine contraction and local ischemia, thereby aggravating the symptoms of dysmenorrhea[25].

Based on the previous clinical research, we exploded the differences of moxibustion at different time in the analgesic effect in primary dysmenorrhea and the possible mechanism. The purpose of this study was to provide a reliable experimental basis, for selection of the optimal acupuncture time for the patients with primary dysmenorrhea due to cold-dampness retention. The dysmenorrhea rat model due to cold-dampness retention used in this study was excessive and acute in symptoms. In traditional Chinese medicine, immediate-moxibustion reflects the principle of 'symptomatic treatment for acute conditions'; pre-moxibustion is 'to prevent before sick'; and pre-moxibustion with immediate-moxibustion indicates the 'treatment for both root cause and symptoms'.

The results of this study showed that for the dysmenorrhea rats, moxibustion at different times could significantly reduce the writhing number and writhing score, prolong the latency of writhing reaction, and effectively relieve the symptoms of dysmenorrhea; could regulate the $PGF_{2\alpha}$ and PGE_2 imbalance and abnormal AVP level in the uterine tissues, inhibit the spastic contraction of uterine smooth muscle, increase blood flow, improve local blood circulation, ischemia, and hypoxia, thus to play a role in analgesia. In the regulation of $PGF_{2\alpha}$, PGE_2 and AVP, the effect of pre-moxibustion together with immediate-moxibustion was significantly better than that of the immediate-moxibustion alone and the pre-moxibustion alone, showing a good analgesic effect. Moxibustion intervention at different times in the treatment of rat dysmenorrhea showed different efficacies. The analgesic effect of pre-moxibustion together with immediate-moxibustion was the best, showed a clear mitigation effect on the pain response, while immediate-moxibustion or pre-moxibustion alone showed a less significant analgesic effect, however, the immediate-moxibustion along showed better pain relief than pre-moxibustion alone. Inferred from these results, the dysfunction between pain and analgesic system appeared in vivo during pain. This imbalance could be corrected by immediate-moxibustion to a certain extent, to relieve pain. Pre-moxibustion alone showed a poor local analgesic effect in uterus, and pre-moxibustion with immediate-moxibustion showed the best effect.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

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

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