Original Article

Acupuncture inhibits the activation of spinal microglia in the acute uterine cervical distension rats

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Background: Although labor pain is treated clinically by the administration of local anesthetics alone or with opioids in the epidural or spinal spaces, however, the mechanisms of labor pain mechanisms have not been fully elucidated during to the lack of animal models, and the side effects of drugs still existed. Spinal microglia can be activated or mobilized under several pain states, and we want to explore the activation of spinal microglia is involved in the acute uterine cervical distension rats or not.

Methods: (I) The electromyographic (EMG) response to uterine cervical distension (UCD) was observed. Twenty-four Sprague-Dawley rats were randomly divided into three groups: standard group, sham group, and UCD group (n=8). EMG response to UCD was recorded at 30, 60 and 120 min after distension, respectively. The activation of microglia in the spinal cord at UCD 60 min was seen. Grouping following the first part (n=4), four rats were executed perfusion after distension of 60 min, the T12 to L2 spinal cord segments were removed for immunohistochemical analysis. (II) After successfully implantation of the intrathecal catheter, 36 Sprague-Dawley rats were randomly divided into the PBS group, minocycline group and UCD group (n=12). EMG response to UCD was recorded before distension and after 30, 60, and 120 min after distension (n=8). Four rats of each group were executed perfusion at 60 min after distension, the T12 to L2 spinal cord segments were removed for immunohistochemical analysis. (III) Thirty-six Sprague-Dawley rats were randomly divided into an electrical acupuncture group, non-acupuncture group, and UCD group (n=12). EMG response to UCD was recorded at 30, 60, and 120 min after distension. Four rats of each group were executed at 60 min after distension, and the T12 to L2 spinal cord segments were removed for immunohistochemical analysis to observe the effect of Hegu and Sanyinjiao acupuncture electric stimulation in the activation of spinal microglia.

Results: (I) EMG based value of sham group, standard group, and the UCD group were no statistical significance ($P>0.05$). After UCD, the EMG of the UCD group were increased at 30, 60, 120 min. Compared with fundamental values ($P<0.05$), which the most apparent EMG change at 60 min after UCD ($P<0.05$). Sixty min after UCD, compared with the sham group and the standard group, the EMG of the UCD group was higher ($P<0.05$), but no difference was observed between standard group and sham group ($P>0.05$). Compared with the sham group, the number of Iba1 (microglia markers)positive cells in thoracic, lumbar spinal cord (T12 to L2) was higher at 60 min after UCD ($P<0.05$), the most Iba1 labeled cells expressed in IV–V layer and X layer of lumbar spinal cord dorsal horn. (II) EMG based value of UCD group, PBS group, and minocycline group had no significant difference ($P>0.05$). Sixty min after UCD, compared with the PBS and UCD group, the EMG of the minocycline group was decreased significantly ($P<0.05$), but there was no difference between the PBS group and UCD group ($P>0.05$). At 30 and 120 min after UCD, the difference of EMG among the UCD group, PBS group, and minocycline group was no statistical significance ($P>0.05$). Compared with the PBS and UCD group, the number of Iba1 positive cells at the

thoracic, lumbar spinal cord in the minocycline group decreased significantly (P<0.05). But no difference was observed between the PBS group and minocycline group (P>0.05). (III) In the fourth part of the study: EMG based value of electrical acupuncture group, non-acupuncture group, and UCD group were no different (P>0.05). Sixty min after UCD, compared with non-acupuncture and UCD group, the EMG of the acupuncture group was decreased significantly (P<0.05), but no difference was observed between the UCD group and non-acupuncture group (P>0.05).

Conclusions: The activation of spinal microglia is involved in the formation of acute visceral pain induced by uterine cervical distension, Electrical acupuncturing Hegu, and Sanyinjiao alleviate pain, and the possible mechanism is inhibiting the activation of spinal microglia in the acute uterine cervical distension rats.

Keywords: Electrical acupuncturing; acute uterine cervical distension; microglia; spinal

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Introduction

Although labor pain is treated clinically by the administration of local anesthetics alone or with opioids in the epidural or spinal spaces (1-4), however, the mechanisms of labor pain mechanisms have not been fully elucidated during to the lack of animal models, and the side effects of drugs still existed. For these reasons, new, more straight forward approaches to the treatment of labor pain are essential. Advances in understanding and treatment for labor pain care, in general, hampered by our limited understanding of visceral pain because most laboratory work has focused on somatic pain. Recently, an acute uterine cervical distension (UCD) model in the lightly anesthetized rat has been used in several reports to study the physiology of acute visceral nociception induced by the distending of the uterine cervix (5,6). UCD produces a stimulus-dependent increase in afferent nerve and reflex abdominal muscle construction. Besides, local infiltration of the cervix with lidocaine can inhibit the expression of Fos in the spinal cord induced by UCD (7). For protein, the product of c-fos immediate early gene (IEG), has been used as a marker for neuronal activation in center nerve system, further suggesting that UCD is a noxious stimulus and reflects noxious input from the uterine cervix rather than traction on other visceral or somatic structures by deformation of the tissue during parturition. Therefore, this acute UCD model can be used as a visceral model related to labor pain.

Spinal microglia can be activated or mobilized under several pain states (8-11) and plays a vital role in the transmission of nociceptive signals by releasing many inflammatory mediators (IFMs), such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2), and chemokines. Saab *et al.* found activated microglia in a rat model with chronic visceral hyperalgesia induced by neonatal colon irritation by repeated colorectal distension using an angioplasty balloon. Using minocycline to interrupt microglia activation and its associated release of proinflammatory mediators would reverse the visceral pain behavior and restore the standard processing of neuronal information along the visceral pain pathway in adulthood. The content of inflammatory cytokines in cerebrospinal fluid increased significantly during parturition, suggested microglia may be involved in the labor pain signals integration as well. However, the exact role of spinal microglia in acute uterine cervical distension is the primary unknown.

The current labor analgesia method can be divided into non-pharmacological analgesia and pharmacological analgesia. The side effects of ordinarily used drugs, local anesthetics, and opioids, include itching, respiratory inhibition of maternal and infant, hypotension, disturbance of parturient, and motor blockade. Non-drug analgesia such as acupuncture point stimulation, ramaze therapy, has smaller trauma, no drug side effect, less disturbance of parturient. But the analgesic effect of acupuncture point stimulation is not ideal, but existing pieces of evidence show acupuncture point stimulation can reduce perioperative opioid consumption. So, the analgesia methods may improve the labor analgesia effect and reduce the side effect by reducing the consumption of anesthetics.

In our study, we investigated the effects of acupuncture point stimulation in labor analgesia and the activation of

spinal microglia in acute uterine cervical distension rats.

We present the following article in accordance with the ARRIVE reporting checklist (available at http://dx.doi.org/10.21037/apm-20-693).

Methods

Animals

Sprague-Dawley adult virgin female rats, weighting 220–280 g at the time of testing, were purchased from the Laboratory Animal Center, Academy of Medical Sciences of Zhejiang Province. All animals were kept in separate cages at 12/12 h with circadian rhythm inverted, and the temperature was 24–28 ℃, the relative humidity was 50%±5%. Food, but not water, was withheld for 12 h before the study. The experimental animal ethics committee approved all procedures of Wenzhou Medical University and consistent with the ethical guidelines for the investigation of experimental pain in animals.

Experimental protocol

The study was divided into three parts. UCD was applied topically with a distension force of 75 g. In the first part, to investigate the activation of microglia in the spinal cord, 24 rats were randomly divided into the standard group, sham group, and UCD group. The effect of time of UCD was studied by UCD distension of 30 min (UCD 30, n=8), 60 min (UCD 60, n=8) or 90 min (UCD 90, n=8). The T12 to L2 spinal cord segments were removed for immunohistochemical analysis (n=4 at each time point).

The second part of the study aims to detect whether microglia activation contributes to acute uterine cervical distension. After implantation of the intrathecal catheter, 16 rats were randomly divided into two groups. The rats received an intrathecal injection of PBS or minocycline before UCD (n=8 per group). The T12 to L2 spinal cord segments were removed for immunohistochemical analysis (n=4 at each time point).

In the third part of the study, to study the role of acupuncture affect the activation of microglia in the spinal cord by acute uterine cervical distension in rats. Sixteen rats were randomly divided into the acupuncture group and non-acupuncture group (n=8 per group). The T12 to L2 spinal cord segments were removed for immunohistochemical analysis (n=4 at each time point).

Uterine cervical distension

Animals were anesthetized with intraperitoneal chloral hydrate 300 mg/kg and spontaneous ventilation. A small low abdominal laparotomy was performed to expose the uterus, and two fine metal rods were inserted through both uterine cervical losses. The rods entered from the uterus and left through the vaginal wall, both as near as possible to the cervix. With one rod attached to a metal stand by a silk suture, UCD was applied to the cervix by hanging various standard weights on another rod through a silk suture and pulley. The sham operation group included rats with only an abdominal incision and two metal rods inserted through cervical losses but without distension (0 g). All skin incisions were anesthetized with 1% lidocaine for local infiltration. Body temperature was supported in the range of 37–38 ℃ throughout the experiment with a circulating-water heating pad (11).

The visceromotor response induced by UCD was recorded by quantifying electromyographic (EMG) activity with a concentric needle electrode inserted into the right rectus abdominis superior to the inguinal ligament. Through the Medlab-4C501 (Anhui Zhenghua biological instrument equipment Co. Ltd., Anhui, China) biological signal collection system, EMG activity was continuously amplified (10,000 gain), filtered (high pass, 500–5,000 Hz), digitized and stored for off-line analysis. Rats were subjected to UCD stimulus (25, 50, 75, and 100 g, respectively, for 10 s) or no distension (sham group, 0 g) (n=8 for each distension force) following a 5-min basal EMG recording. Each UCD force was only applied once for each individual rat. The raw EMG data over the 10 s just before (baseline) and during UCD was rectified and quantified by calculating the area under the curve. For data analysis, the percentage of are under the curve of distension to baseline was used as there sponses to UCD for each animal. The relevant data in the sham group also were calculated.

Intrathecal drug administration

Intrathecal catheters were inserted according to the method of Yaksh and Rudy (5) with a slight modification. In brief, a polyethylene PE10 catheter was implanted in the lumbar subarachnoid space of each rat during deep pentobarbital sodium (40 mg/kg, i.p.) anesthesia. Animals were allowed 5–7 days recovery and were euthanized at once if they showed any signs of motor deficit.

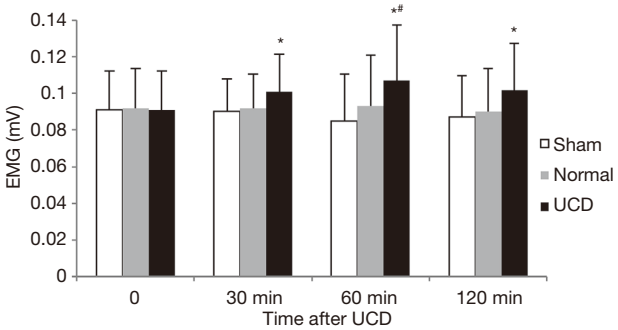

Figure 1 EMG based value of shame group, normal group, and the UCD group was no statistical significance (P>0.05). After UCD, the EMG of the UCD group were increased at 30, 60, 120 min. Compared with basic values (*, P<0.05), which the most apparent EMG change at 60 min after UCD (#, P<0.05). EMG, electromyographic; UCD, cervical, uterine distension.

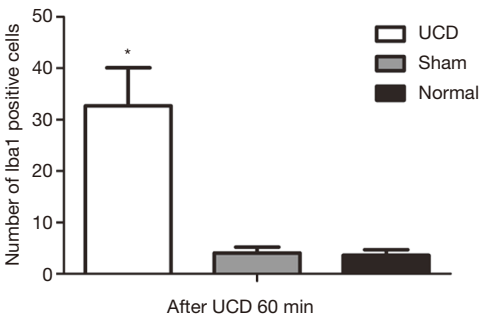

Figure 2 Compared with the sham group, the number of Iba1 (microglia markers) positive cells in the thoracic, lumbar spinal cord (T12 to L2) at 60 min after UCD was higher (*, P<0.05). UCD, uterine cervical distension.

Statistical analysis

Data were expressed as mean ± standard error of means (SEM). In the first part and the second part, two sets of data were compared with the *t*-test (paired or unpaired when proper). In the third part, the statistical analysis was performed using one-way ANOVA with the use of a factorial design with SPSS19.0, and the statistical figures were all performed using Graph Pad Prism 5.0 software. For all analyses, the values of P<0.05 were statistically significant.

Results

EMG response to UCD, and IBA1 expression (*Figures 1*,*2*).

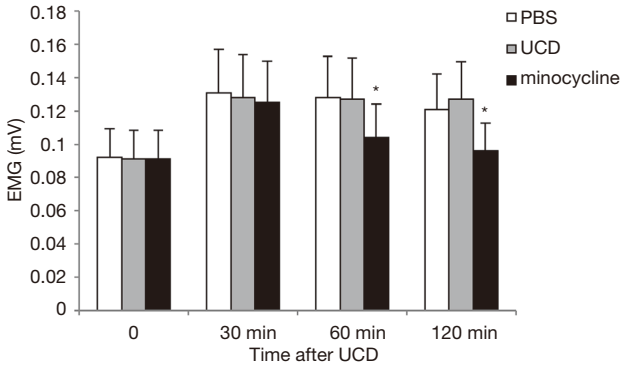

Figure 3 EMG based value of the UCD group, PBS group, and minocycline group had no significant difference (P>0.05). Sixty min after UCD, compared with the PBS and UCD group, the EMG of the minocycline group was decreased significantly (*, P<0.05), but there was no difference between the PBS group and UCD group (P>0.05). At 30 and 120 min after UCD, the difference of EMG among the UCD group, PBS group, and minocycline group was no statistical significance (P>0.05). EMG, electromyographic; UCD, uterine cervical distension.

Sixty min after UCD, compared with the sham group and the standard group, the EMG of the UCD group was higher (P<0.05), but no difference was observed between standard group and sham group (P>0.05).

The effects of minocycline and PBS on cervical, uterine distension to study EMG and IBA1 expression (*Figures 3*,*4*).

The effects of acupuncture and non-acupuncture on cervical, uterine distension to study EMG and IBA1 expression (*Figures 5*,*6*).

Discussion

Our study found that UCD accompanied by activation of spinal microglia, and the duration of UCD exert the number of activated microglia significantly. Intrathecal injection of minocycline and acupuncture point stimulation depressed the UCD induced enhancement of EMG and reduced the activation of spinal microglia.

The role of microglia in somatic pain has been extensively studied, while the data about the visceral pain are quite limited. Our study found that UCD activated microglia. Our results are consistent with the result of earlier studies showing the inflammation, colorectal distension, and chronic psychological stress, which induced chronic visceral pain, can activate the spinal microglia as well. Notably, UCD induced acute visceral pain but not

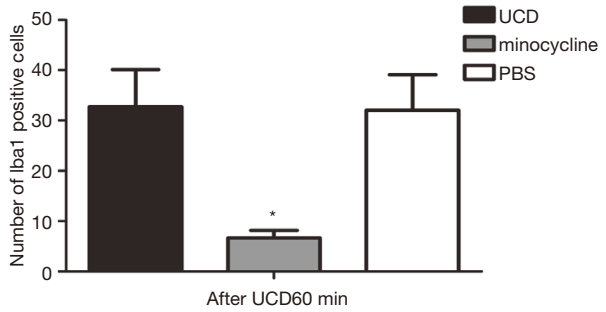

Figure 4 Compared with the PBS and UCD group, the number of Iba1 positive cells at the thoracic, lumbar spinal cord in the minocycline group decreased significantly (*, P<0.05). But no difference was seen between the PBS group and the minocycline group (P>0.05). EMG, electromyographic; UCD, uterine cervical distension.

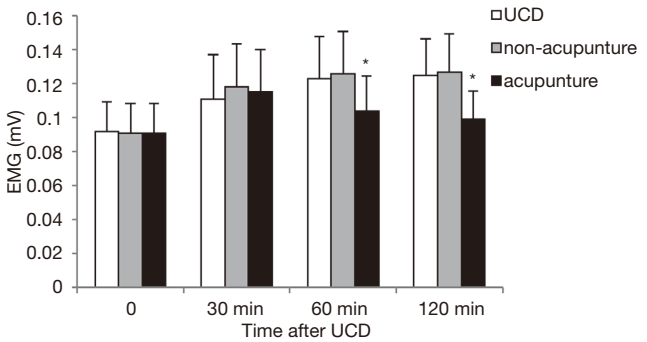

Figure 5 EMG based value of electrical acupuncture group, non-acupuncture group, and UCD group were no different (P>0.05). Sixty min after UCD, compared with non-acupuncture and UCD group, the EMG of the acupuncture group was decreased significantly (*, P<0.05), but no difference was observed between the UCD group and non-acupuncture group (P>0.05). EMG, electromyographic; UCD, uterine cervical distension.

chronic visceral pain. Although the pain processes between chronic and acute nociception have much in common, they also have several significant differences. UCD produces a force-dependent increase in neural activity in the hypogastric nerve, evoke abdominal muscle reflex electromyographic activity (12) reminiscent of the sacral neural activity and reflex electromyographic activity evoked by noxious colorectal distension (13). Systemic opioids inhibit UCD-induced reflex electromyography and afferent fiber firing in doses thought to be antinociceptive in rats. Our study also found that the stimulus intensity of UCD was strictly related to the activation of microglia, and intrathecal

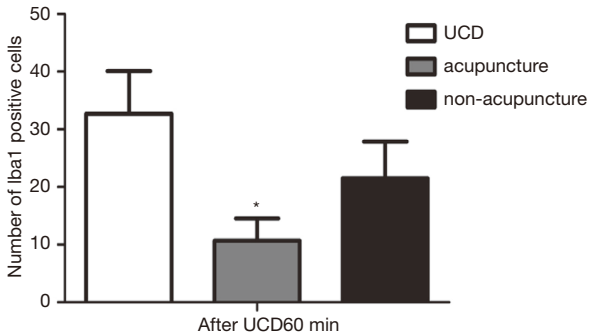

Figure 6 Compared with the non-acupuncture group and UCD group, the number of Iba1 positive cells at the thoracic, lumbar spinal cord in the minocycline group was lower (*, P<0.05). But the difference between the non-acupuncture group and the UCD group was no statistical significance (P>0.05). UCD, uterine cervical distension.

injection of minocycline effectively blocked UCD induced activation of microglia and acute visceral pain. Thus, these results suggest activation of spinal microglia also contributes to the initiating of acute visceral pain.

Our study suggests an increase in spinal microglia expression with increasing duration of UCD, although we cannot exclude the influence of more significant time from onset of UCD to perfusion in the UCD 60-min group compared with the UCD 30-min group to increase microglia expression.

The mechanism of microglia contribute to the acute visceral pain is still unknown. UCD is a model for analogous labor pain; the previous study has shown that the content of inflammatory cytokines in cerebrospinal fluid increased significantly during parturition in human beings. It is well known that the role of microglia in pain signals integration mainly rely upon releasing many IFMs, such as IL-1, TNF-α, PGE2, and chemokines, and the activated microglia is considered a primary source of the inflammatory cytokines. Under chronic pain conditions, P-p38 MAPK and ERK5 are activated in spinal microglia, and the hyperalgesia can be reversed by minocycline, p38 inhibitor, or knockdown of ERK5. It has been shown that p-p38 MAPK and ERK5 pathway can be activated in an hour by the nociceptive stimulus and plays a pivotal role in regulating the expression of IFMs in spinal (14). In the current study, activation of spinal microglia peaked at 60 min, with a slow decline but remained increased at 120 min after UCD, suggests that UCD induced activation of microglia may release IFMs, and modulate the visceral pain

signals in the spinal cord.

The mechanism of acupuncture regulates visceral pain: spinal cord is the first level of pain signals after entering the central integration center, is the nociceptive information transmission from relay station, which can direct modulation of pain, and receives signals central downward adjustment. The spinal cord is a vital part of the pain stimulus to adjust to the visceral pain. It is believed that the spinal cord dorsal horn accepted by many pain downward modulation systems composed of pulp in the central regulation of visceral injury information. By multiple neural spinal cords, such as PAG, amygdala, descending pain modulation system composed of cingulate, the dorsal horn of spinal cord conduction of pain information has a strong inhibitory effect, and acupuncture may be activated descending pain modulation system and exert its analgesic effect. Recent research shows that through the spinal cord dorsal horn neurons, message to the pulp in the central, acupuncture letter to activate pain downward modulation system, and then to the dorsal horn of c-Fos, P38, NRI and 5-HT receptor expression, which in turn have an analgesic effect. Further study shows that the body noxious stimulation and acupuncture point acupuncture inhibitory effect on visceral nociceptive information transmission (15,16).

Participate in electric acupuncture treatment of visceral pain-related neurotransmitters are opioid, 5-HT, and other neurotransmitters and conditioning. A lot of research has shown that acupuncture to relieve visceral pain effect, most likely by activating endogenous opioid system and play a role, this is because the application of naloxone after acupuncture effect on visceral pain was canceled.

Acupuncture has the spinal good curative effect in the treatment of visceral sensitivity, and this may be through the spinal cord, the thalamus, the brain stem, and/or the cortex. The interaction between the senior center may also play a role by nerve endocrine immunity network.

Conclusions

In summary, this study supplies novel evidence that UCD stimulates spinal cord microglia expression, and acupuncture affects the activation of microglia. So, acupuncture can relieve labor pain, and that acupuncture might be useful in the treatment of such pain.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at http://dx.doi.org/10.21037/apm-20-693

Data Sharing Statement: Available at http://dx.doi.org/10.21037/apm-20-693

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-20-693). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The experimental animal ethics committee approved all procedures of the Wenzhou Medical University and consistent with the ethical guidelines for the investigation of experimental pain in animals (No. wydw2019-0927).

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