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Abdominal Massage to Improve Motor Dysfunction in Rats with Cerebral Palsy

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Abstract

Cerebral palsy (CP) is a disease with a high disability rate and morbidity. The clinical symptoms of cerebral palsy are motor dysfunction and abnormal posture development, often accompanied by cognitive impairment. Massage, a traditional Chinese Medicine therapy, can coordinate Zang and Fu, regulate Qi and blood, make the viscera work more smoothly, and calm Yin and Yang. Furthermore, it has been an effective method for CP in clinical. This paper summarizes a set of simple and standardized manipulations of massage for young rats with CP, which is easy to follow. The procedure follows: first, massage of four limb acupoints, including Quchi (LI11) and Zusanli (ST36); second, massage of the abdomen acupoints Zhongwan (RN12), Tianshu (ST25), Guanyuan (CV4), and Qihai (CV6); and finally, massage of the abdomen of the rats. This set of massage methods considerably improved the motor function of young rats with CP and is simple, standardized, and easy to follow. We adapted this set of manipulation methods in animal models to promote the internationalization and standardization of massage.

Introduction

Cerebral palsy (CP) is a disease with a high disability rate and morbidity. Epidemiological investigation of cerebral palsy shows that 3-5 infants have cerebral palsy in every 1,000 infants[1]. The typical symptoms of cerebral palsy are persistent central motor and posture development disorder[2], activity limitation with sensory[3], perception, cognition[4], communication[5], behavioral disorder, epilepsy, and secondary musculoskeletal problem[1]. So far, there is no cure for this lifelong disabling disease. The current treatment methods include exercise therapy[6], hydrotherapy[7], functional electrical stimulation[8], muscle exoskeleton[9], umbilical cord blood cell therapy[10], botulinum toxin injection, and surgery[11]. However, clinical rehabilitation takes a long time and consumes much energy, and rehabilitation requires active cooperation from patients, making it difficult to replicate in animal models. Surgery has limitations, indications, and

irreversibility. Oral medication has poor efficacy in treating motor function in children with cerebral palsy[12]. Muscle exoskeleton therapy and umbilical cord blood therapy have high economic costs and are difficult to afford, making it critical to find suitable treatment strategies.

Massage is a non-invasive, side effect-free, highly accepted, cost-effective treatment method for children. It has been proven in both clinical[13] and animal experiments[14] to improve the dysfunction of CP. Traditional Chinese medicine believes massage can improve CP dysfunction by regulating the organs and unblocking meridians[15]. As an effective TCM treatment method, massage requires modern medical research methods to explain its mechanism of action to promote it to the world. However, there are objective research limitations and ethical limitations in the study of relevant mechanisms in children with CP. Therefore, studying the relevant massage mechanisms to improve CP in animals is more convenient. Although animals are different from children, investigating the effects of massage on animals requires adherence to TCM's fundamental theories. From the perspective of clinical and experimental zoology, we will replicate this technology from the human body to the animal body to study the effectiveness of massage, which will help promote the application of massage therapy.

First, traditional Chinese medicine believes that CP belongs to the symptoms of "five lateness" and "five softness"[16]. The causes of CP are congenital fetal dysfunction, liver and kidney deficiency, insufficient essence and qi, or acquired nutritional deficiency[15]. The kidney dominates the growth, development, or regeneration and repair of human organs and tissues, and these substances are crucial for the growth, development, tissue repair, and resurrection of the body. The Essential Qi of the kidney is the foundation of growth and development. Insufficient kidney essence can lead to bone marrow failure, disorders of qi and blood, and a lack of nutrition in the internal organs of the body, muscles, and bones. Children with CP are prone to delayed growth and development, decreased joint mobility of the limbs, and increased muscle tension. In addition, long-term illness can lead to excessive deficiency and blood stasis, causing meridian obstruction, leading to intellectual disability, slow response, motor disorders, language ambiguity, swallowing weakness, and symptoms of drooling, such as limb movement disorders.

The spleen governs the circulation and transformation, serving as the source of qi and blood biochemistry and the foundation of acquired ability. The human meridians connecting the viscera, arms, and legs are pathways that circulate qi and blood, communicate up and down, and regulate yin and yang. Massaging the Ren meridian can enhance kidney function and regulate qi and blood. The use of acupoints on the Spleen Meridian of the Foot Tai-Yin, Stomach Meridian of the Foot Yang-Ming, and Large Intestine Meridian of the Hand Yang-Ming in massage techniques improve spleen and stomach function, which helps to promote the circulation of Qi and blood. Massage utilizes meridians, specific parts, and acupoints to provide benign physical stimulation to the body of the child, causing reactive changes from the surface to the inside and from the outside to the inside, thereby improving local and overall blood circulation, restoring normal excitability and inhibition of the brain, and achieving corresponding balance in visceral and tissue blood function. According to this theory, we mainly use massage on acupoints such as the abdomen, Zhongwan (RN12), Tianshu (ST25), Guanyuan (CV4), and Qihai (CV6). Abdominal massage can improve the clinical symptoms of children with CP[17,18,19], and it is highly likely to promote brain injury

repair through the brain-gut pathway[20]. Because the spleen and stomach are the foundation of postnatal development, they can promote brain injury recovery by strengthening the spleen, benefiting the stomach, and replenishing qi and blood.

We replicated this technique on rats to simulate the intervention of TCM on CP as much as possible and found that abdominal massage can improve the dysfunction of CP rats. In summary, we present a set of methods for studying abdominal massage to investigate the relevant mechanisms of improving CP through abdominal massage. As the existence of many massage genres makes it very difficult to study massage mechanisms, it is necessary to standardize massage methods.

Protocol

The Animal Ethics Committee of Jinshan Hospital, affiliated with Fudan University in Shanghai, approved this study. Young Sprague Dawley (SD) rats were raised in an SPF animal room with light from 08:00 to 20:00, darkness from 20:00 to 08:00, temperature controlled at 22 °C, and indoor relative humidity of 40%-50%. They had free access to food and water. All experimental operations on animals comply with the welfare ethics of experimental animals and animal experimental safety regulations.

1. Neonatal hypoxic-ischemic encephalopathy animal model [21]

1. Randomly divide 18 Sprague Dawley (SD) rat male pups, 7 days after birth (P7), into the following groups: sham (no massage), model, and Massage (model + massage group) (n = 6 for each group).
2. Anesthesia
    1. Use a small animal gas anesthesia machine to administer 5% sevoflurane gas, adjust the oxygen flow rate to 4, and use a 4% induction fraction to anesthetize the young rats. Confirm the animal is fully anesthetized by waiting for the point when the young rat is lying on its back, its limbs stop moving, there is no response when touched, and its muscles relax.
    2. Add pet eye ointment and eye drops to the anesthetized young rats to prevent eye dryness.
3. Microscopic surgical process
    1. First, disinfect the neck skin of rats with iodophor once, then with 75% ethanol once (perform this procedure three times), and make a longitudinal incision ~0.5 cm long in the middle of the neck of the young rats. Separate the right subcutaneous tissue passively and expose the common carotid artery.
    2. Model and massage groups: Separate the common carotid artery and the vagus nerve carefully (**Figure 1A**). After splitting the common carotid artery, coagulate the common carotid artery with an electric coagulation gun. After confirming the absence of bleeding, suture the skin layer by layer and disinfect the surgical incision again (**Figure 1C**).
    3. Sham group (No CP, no massage): Separate only the common carotid artery without any treatment of blood vessels. Suture the skin layer by layer and disinfect the surgical incision again. When observing the unrestricted crawling of the young rat, place the young rat back beside the mother rat.
4. Disinfect with iodine after surgery. Place the rats that have completed the surgery on a 37 °C insulation pad

and constantly observe whether the wound is bleeding and whether there is inflammation in the eyes.

5. Place the model and massage groups of young rats in an open hypoxia chamber and observe for 1 h until they awaken.

   NOTE: When the young rats can freely crawl, they are ready to undergo hypoxia treatment.

6. Hypoxia treatment

   1. After they recover from anesthesia, place the young rats in a closed anoxic device at 37 °C with 92% N_2 and 8% O_2 gas conditions (2 L/min) for 3 h of hypoxia. After the hypoxic treatment, place the awake young rat back beside the mother rat.

   2. Disinfect the surgical instruments and wipe the hypoxic chamber with alcohol. After the surgery, disinfect the young rat with iodophor, monitor the wound daily for signs of infection, and use sterile padding. Complete the entire surgical process in the SPF animal room and keep the animals in the SPF animal room.

   3. Raise the model group normally after the successful establishment of the model without any intervention measures. Subject the massage group to manual intervention after the completion of the modeling.

      NOTE: The sham group was fed normally and did not undergo hypoxia treatment.

7. Place each group of rats next to the mother rats on sterile and high-temperature sterilized bedding. Observe them for 1-2 h until the mother rats feed the offspring rats. Observe them every day to check whether the wound is bleeding and whether there is inflammation in the eyes; observe until the wound is completely healed.

2. Massage technique parameters

NOTE: The core techniques of massage include pressing and rubbing as the primary intervention techniques. Before the experiment, the researchers drew on the operation of a technique tester who collected techniques from the Tianjin abdominal massage expert Wang Jingui.

1. Set the forces of pressing and rubbing to 6 N and 3 N, respectively[22,23,24,25].

3. Massage manipulation

NOTE: Ensure that the researcher's thumb pressure on the acupoints is stable and long-lasting.

1. On the second day after the operation (P8), take the young rats out of the cage without disturbing the maternal rats. In the massage group, massage the rats from the 8^{th} day of birth to the 40^{th} day.

2. Before massaging the young rats, rub hands slightly to warm them up to almost the same temperature as the skin of the young rats.

3. Follow this sequence of Massage: Limb Acupoints → Abdomen Acupoints → Abdomen.

4. Place the young rat in the palm of the left hand, and gently touch the back of the young rat from head to tail with the thread surface of four fingers for 1 min to make it quiet.

5. Perform the following rubbing steps.

   1. Rub the abdomen of the right thumb and forefinger back and forth.

   2. Rub the forelimb, from the proximal end to the distal end for ~30 s. Press Quchi (LI11), Waiguan (SJ5), and Hegu (LI4) points 10x for 1 s each time.

3. Rub the hind legs, from the proximal end to the distal end for ~30 s, and press Yanglingquan (SP9), Zusanli (ST36), Sanyinjiao (SP6), and Taichong (LR3) points 10x for 1 s each time for a total period of ~5 min.

6. Use the right thumb to press Zhongwan (RN12), Tianshu (ST25), Guanyuan (CV4), and Qihai (CV6). Press each acupoint 10x, each time for ~1 s, lasting for 1 min.

7. Keep the young rat prone, and press and rub the Zhongwan point on the abdomen with the right thumb. Take the navel of the young rat as the center and use the thumb to move clockwise 120x/min for ~5 min.

8. Finally, use the thread surface of the four fingers of the right hand to gently touch the head of the young rat to its tail, make it quiet, then place it back in the cage, and allow the mother rat to feed it.

4. Acupoint localization

NOTE: For rats born within 2 weeks, the weight and length of the rats are the same as those of the mice. Therefore, we used mouse massage methods in relevant literature to massage the rats during the 8-15 days of the rats[24].

1. Two weeks to 40 days later, treat the rats with regular rat massage using the following acupoints: LI11, SJ5, LI4, SP9, ST36, SP6, LR3, RN12, ST25, CV4, and CV6[26].
   NOTE: See **Table 1** and detailed descriptions of rat acupoint localization elsewhere[26] and **Figure 2** for the specific positions of the acupoints.

5. Weight and behavioral test

1. Weigh the young rats daily from P7 to P40.

2. Conduct behavioral experiments on the balance beam at P37 to P40. Test the motor balance function of young rats using a balance beam (35 cm long, 1.5 cm wide, and 100 cm high).
   1. Place the young rat on the balance beam and let it run into the black box placed at the other end of the balance beam.
   2. Conduct balance beam training for the young rats on P37-P39, 3x a day, and conduct a test on P40. Record the time when the three groups of young rats pass the balance beam and the number of times their hind limbs slip (**Figure 1D**).
      NOTE: Use the carbon dioxide suffocation method to euthanize rats.

Representative Results

After the completion of ischemia and hypoxia modeling[27,28,29,30] (the experiment was completed in the SPF animal room), the brains of young rats were taken after 48 h of survival to look for edema, liquefaction, and other phenomena, as shown in **Figure 3**. The weight changes of the young rats in the sham and massage groups were compared with those of the model group (**Supplemental Table S1**). The weight increase of the model group was slower than that of the massage group (**Figure 4**), which showed that massage improves the growth and development of young rats with CP. The balance beam test results showed that the model group took longer to cross the beam than the sham and massage groups (**Figure 5A** and **Supplemental Table S2**).

Moreover, the number of hind limb slips in the model group significantly increased compared with the sham group (**Supplemental Table S3**). In contrast, the number of hind limb slips significantly decreased in the massage

group compared with the model group (**Figure 5B** and **Supplemental Table S3**). Massage intervention significantly improved CP rats' growth and motor function.

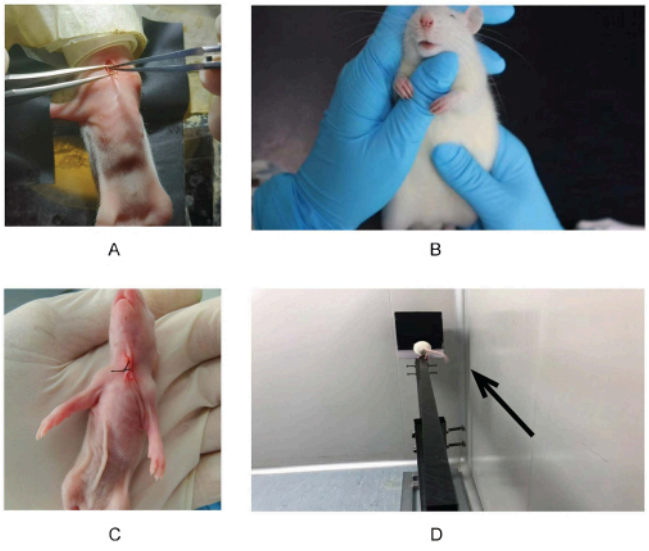

Figure 1: Animal modeling process and related behavioral testing. (**A**) Stripping of vagus nerve during modeling. (**B**) Massage 5-week-old rats. (**C**) Rats after ischemia completion. (**D**) Balance beam test. Please click here to view a larger version of this figure.

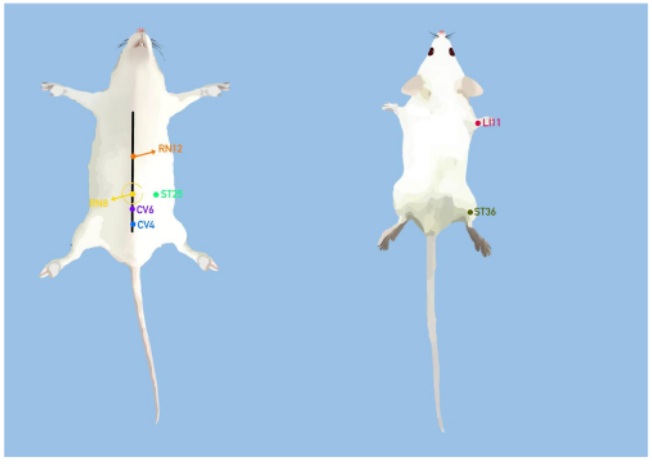

Figure 2: Display of some acupoints related to abdominal massage[26]. Please click here to view a larger version of this figure.

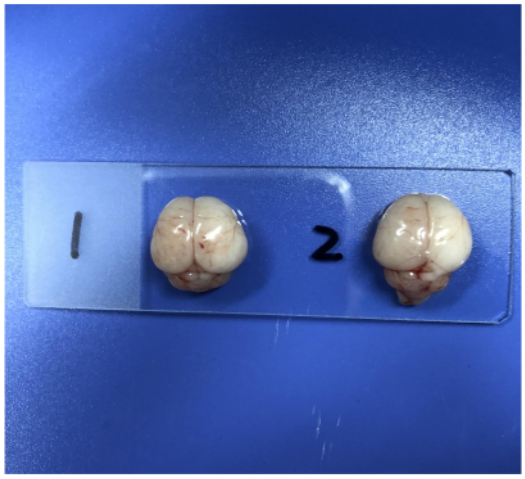

Figure 3: Edema on one side of the brain after 48 h post model establishment. Please click here to view a larger version of this figure.

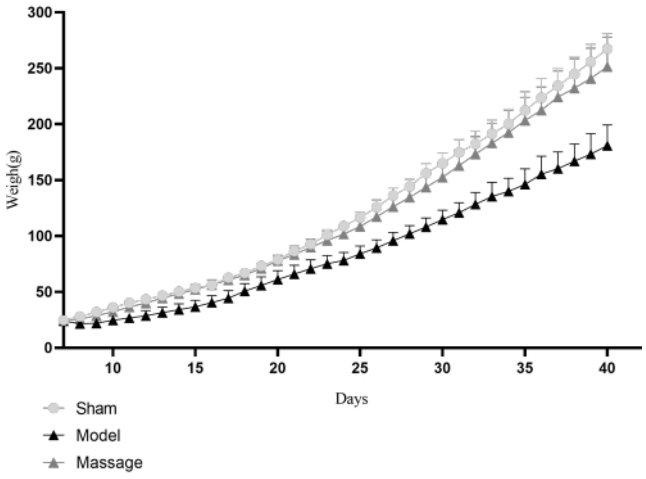

Figure 4: Effects of massage therapy on weight. The average weights of the rat pups (n = 6) were measured from P7 to P40, and hypoxia-ischemia intervention was performed on P7. After HIE modeling, the growth rate of body weight in the model group decreased significantly, and massage therapy significantly improved it ($p < 0.05$). Data are presented as the mean ± SD; #$p < 0.05$ Massage group compared with the Model group. *$p < 0.001$ model group compared with the sham group. Please click here to view a larger version of this figure.

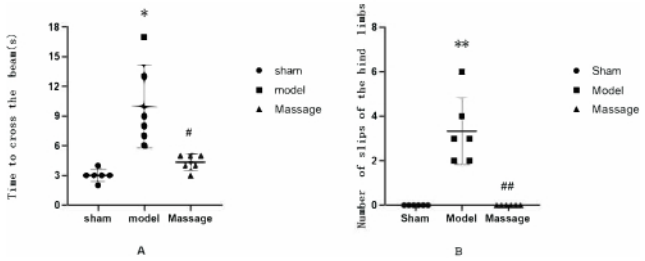

Figure 5: Effects of massage treatment on the motor dysfunction of cerebral palsy rats. (**A**) Time required for the rats to cross the beam (n = 6). (**B**) Number of slips of the hind limbs of the rats. Based on the results of **Figure 4A,B**, the motor performance of the massage group was significantly better than the model group ($p < 0.05$). Data are presented as the mean ± SD; *$p < 0.001$ Model group compared with the sham group. #$p < 0.01$ Massage group compared with the model group. **$p < 0.0001$ Model group compared with the sham group. ##$p < 0.0001$ Massage group compared with the model group. Please click here to view a larger version of this figure.

LI11	The inner depression of the radial extensor carpi muscle and the
SJ5	outer depression of the elbow at the radioulnar depression of the distal 5/6 point on the imaginary
LI4	line connecting wrist and elbow, posterior to the extensor digitorum communis tendon
SP9	on the first dorsal interossei, medial to the middle of the second metacarpal bone, in the forefoot
ST36	at the depression inferior to the medial condyle of the tibia, between tibia and gastrocnemius
SP6	at the proximal one fifth point on the line from ST35 to the anterior side of ankle crease
LR3	at the proximal endpoint of the distal one fifth on the imaginary line connecting SP9 and the medial malleolus of tibiofibular
CV4	at the proximal end of the depression between the first and second metatarsal bones
ST25	at the point of 3/5 down the ventral midline connecting the umbilicus to the pubic tubercle
CV6	lateral to the umbilicus, at the midpoint from the umbilicus to the nipple line at the point of 3/ 10 down the ventral midline connecting the umbilicus to the pubic tubercle

Table 1: The anatomical positions of various acupoints[26].

Supplemental Table S1: Raw data of weights of all three groups of rats. Please click here to download this File.

Supplemental Table S2: Raw data of time to cross the balance beam for all three groups of rats. Please click here to download this File.

Supplemental Table S3: Raw data of hind limb slips of all three groups of rats. Please click here to download this File.

Discussion

The neonatal rat model of ischemia and hypoxia is the most classic model for studying cerebral palsy[27,28,29,30]. We intervened in rats from the 8th to the 40th day, which is approximately equivalent to interfering in humans from 3 months to 11.5 years old, the golden period for human growth

and development[31]. The results indicate that massage is an effective method to ameliorate CP dysfunction. Previous animal experiments have shown that massage can reduce inflammation and improve motor and cognitive functions in young rats with CP[14,32]. Some clinical studies have shown that back and bladder meridian massage improves motor function in children with CP[33]. These results also show that massage can shorten the time for young rats with CP to pass over the balance beam and reduce the number of slips of their hind legs. Thus, it can be seen that abdominal massage can improve the motor function of young rats with CP.

Traditional Chinese medicine believes that CP belongs to the five delayed and five soft symptoms, with congenital kidney qi deficiency as the fundamental factor and acquired spleen and stomach weakness as the promoting factor. The kidney governs the bone and generates marrow, so that when the kidney is full of qi, the marrow becomes abundant, and intelligence remains normal. The spleen controls the muscles and limbs, so if the spleen is healthy, the muscles will be strong, and the joints will move freely. Therefore, it is necessary to replenish the spleen and strengthen the kidneys, improve gastrointestinal function, move limbs and joints, and improve function. First, TCM theory holds that the abdomen is the root of all diseases. Abdominal massage can clear up and down, separate yin and yang, remove the old and make new, enrich the five internal organs, drive out external evil, and eliminate internal diseases. Second, research showed that children with CP have lower body weight and are prone to malnutrition, including excessive or unbalanced intake, energy, or nutritional status[34,35,36,37]. Growth retardation and malnutrition are common in children with CP and are directly related to more severe motor dysfunction[37]. The risk of malnutrition is closely related to the prognosis of patients with CP[34,38,39]. Nowadays, the main research area of abdominal massage is to improve gastrointestinal symptoms such as functional dyspepsia, constipation, diarrhea, and infant anorexia[33,40,41,42]. Eventually, numerous studies show that stimulating Zhongwan, Tianshu, Guanyuan, and Qihai can treat gastrointestinal diseases, promote absorption, and improve nutritional status[43,44,45]. Abdominal points can treat gastrointestinal-related diseases, comb the central energizer's qi mechanism, replenish the primary qi's power, and promote the biochemistry of qi and blood. Therefore, we introduced abdominal massage into the experimental method to improve motor dysfunction in young rats with CP.

Abdominal massage is mainly combined with abdominal points, supplemented by limb points, such as Quchi and Zusanli. The external collaterals and joints of the four limb acupoints are connected to the internal organs, and abdominal massage is used to achieve the best therapeutic effect. On the one hand, experimental animal studies show that acupuncture Zusanli of newborn rats with hypoxic-ischemic CP could improve the function of young rats with CP[46]. On the other hand, clinical research shows that acupuncture at Hegu[47] and other acupoints can effectively enhance the development of CP. Quchi and Zusanli are the acupoints in the Yang-Ming large intestine of Hand meridian[48] and Foot-Yang-Ming Meridian on somatostatin[49], which are the most commonly used acupoints in the clinical treatment of stroke in China to improve limb paralysis[50,51]. In addition, Quchi and Zusanli are the confluence points of the large intestine and stomach meridians, respectively. From this point, the meridian qi reaches the viscera and is connected to the upper and lower parts and the meridians and collaterals. In addition, acupuncture and moxibustion plus massage showed a better effect on improving children with

CP in clinical research results[52]. To summarize, TCM therapy can help improve the dysfunction of patients with CP.

Although there have been many studies on how massage can improve CP, the diversity of massage techniques has become an enormous challenge. There are many massage schools, and the differences in strength, direction, and other factors pose numerous challenges. We have learned a variety of massage methods and explored a set of simple and easy massage techniques. However, we cannot simulate a variety of massage techniques. Second, the time of massage is an important stage in the development of young rats. Its weight and the rapid growth of various body systems lead to the constant change of our finger strength for rats at different stages. Efforts must be continued to study the stability of massage.

Disclosures

The authors have no conflicts of interest to disclose.

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None

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