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Basic Study

Effect of electroacupuncture at Huantiao (GB 30) and Weizhong (BL 40) on serum IgG and IgM in rabbits with lumbar intervertebral disc herniation

电针环跳和委中对腰椎间盘突出兔血清IgG和IgM的影响

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Abstract

Objective: To observe the effect of electroacupuncture (EA) at Huantiao (GB 30) and Weizhong (BL 40) on thigmesthesia, gait function, and expression levels of serum immunoglobulin G (IgG) and immunoglobulin M (IgM) in rabbits with lumbar intervertebral disc herniation (LIDH).

Methods: Forty healthy New Zealand rabbits were randomly divided into a blank control group, a model group, an EA at acupoint group and an EA at non-acupoint group, with 10 rabbits in each group. The LIDH pathological model of rabbit was established using the self-made LIDH model maker. The thigmesthesia and gait function of rabbits were recorded by Siegal method. The serum IgG and IgM expression levels were detected by enzyme-linked immunosorbent assay.

Results: EA at Huantiao (GB 30) and Weizhong (BL 40) could improve the clinical symptoms of thigmesthesia and gait function, and inhibit the expressions of serum IgG and IgM in the LIDH rabbits, which were significantly different compared with those in the model group and EA at non-acupoint group.

Conclusion: EA at Huantiao (GB 30) and Weizhong (BL 40) can improve the clinical symptoms of LIDH rabbits, which is associated with inhibition of the serum IgG and IgM expressions and reduction of the immunoinflammatory factor release. This may be one of the mechanisms of EA at Huantiao (GB 30) and Weizhong (BL 40) in the treatment of LIDH.

Keywords: Acupuncture Therapy; Electroacupuncture; Point, Huantiao (GB 30); Point, Weizhong (BL 40); Intervertebral Disc Displacement; Low Back Pain; Immunoglobulins; Rabbits

【摘要】目的：观察电针环跳、委中对家兔腰椎间盘突出症(LIDH)的触觉、步态功能、血清免疫球蛋白G(IgG)、免疫球蛋白M(IgM)表达水平的影响。**方法：**将40只健康新西兰家兔随机分为空白对照组、模型组、电针穴位组和电针非穴点组，每组10只。采用自制的LIDH造模器建立家兔LIDH病理模型，家兔触觉、步态功能采用Siegal法来记录，血清IgG、IgM表达水平采用酶联免疫吸附法检测。**结果：**电针环跳、委中穴可改善LIDH家兔的触觉、步态功能等临床症状,抑制LIDH家兔血清中IgG、IgM的表达，与模型组、电针非穴点组比较有显著性差异。**结论：**电针环跳、委中穴对LIDH家兔的临床症状的改善是良性作用，电针环跳、委中穴能减轻临床症状与抑制血清中IgG、IgM的表达、减少免疫炎症因子释放有关，这可能是电针环跳、委中穴治疗LIDH的作用机制之一。

【关键词】针刺疗法；电针；穴，环跳；穴，委中；椎间盘移位；下背痛；免疫球蛋白；兔
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Lumbar intervertebral disc herniation (LIDH) is a common and frequently-occurring disease with clinical symptoms of low back pain and/or sciatica[1]. Clinical and experimental studies have shown that electro-acupuncture (EA) has confirmed therapeutic effects on LIDH. LIDH patients showed improved or alleviated clinical symptoms, such as lumbar and leg pain, limited motion and sciatica, after EA treatment[2-4], but the underling mechanism is still controversial. The clinical symptoms, physical signs, degree of intervertebral disc herniation and the nerve root compression are not exactly the same in LIDH patients. Therefore, the theory of nervous mechanical compression cannot fully explain the mechanism of LIDH[5]. Serum immunoglobulin G (IgG) and immunoglobulin M (IgM) are important products of the immune response in the body. The occurrence or increase of IgG and IgM in LIDH patients may be the results of autoimmune reaction in the prominent intervertebral disc[6]. Immunoassay for LIDH patients showed increased positive rate of IgG, suggesting that the immune response might be an important cause of LIDH[7]. In this study, we focused on

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the autoimmune reaction of LIDH to observe the changes of thigmesthesia function, gait function, expression levels of serum IgG and IgM in experimental rabbits with LIDH after EA at Huantiao (GB 30) and Weizhong (BL 40), thus to explore the related immunological mechanisms of EA for LIDH .

1 Experimental Materials

1.1 Experimental animals

A total of 40 healthy adult New Zealand purebred rabbits were provided by the Experimental Animal Center of Hunan Provincial People's Hospital Clinical Research Center (general level), half male and half female with body weight of 1.5-2.0 kg. Each rabbit was housed separately in the second laboratory of Experimental Animal Center in Hunan Provincial People's Hospital Clinical Research Center. Rabbits were fed at the temperature of 20-25 ℃, and humidity of 50%-70%, with sufficient light and free access to standard diet and drinking water. The handling of animals during the experiments was in line with the requirements of the *Guiding Opinions on the Treatment of Experimental Animals* issued by the Ministry of Science and Technology of the People's Republic of China[8].

1.2 Materials and reagents

Hwato Brand acupuncture needles of 0.30 mm in diameter and 25 mm in length (Suzhou Medical Appliance Factory, China); G6805-2 mode EA instrument (Qingdao Hua Qing Instrument Factory, China); IgG and IgM ELISA kit (Wuhan Xinqidi Biological Technology Co., Ltd., China).

1.3 Statistical methods

All data were analyzed using SPSS 19.0 software for Windows. First of all, normality test and homogeneity of variance test were performed. The data in normal distribution were presented as mean ± standard deviation ($\bar{x} \pm s$). Paired *t*-test was used for intra-group comparison before and after intervention. One-way ANOVA was used to compare the differences among multiple groups. The least significant difference (LSD) was used when the variance was homogeneous and Tamhane's T2 method was used when the variance was not homogeneous. $P<0.05$ indicated a statistical significance.

2 Experimental Methods

2.1 Animal groups

Using the quadratic random method, the rabbits were divided into a blank control group, a model group, an EA at acupoint group and an EA at non-acupoint group according to random number table method, with 10 rabbits in each group. All rabbits were fasted for 1 day, while with free access to water. Except for rabbits in the blank control group, rabbits in the other three groups were subjected to LIDH modeling[9]. Lumbar intervertebral disc CT examination was performed for the randomly selected rabbit samples after the successful modeling. Intervention started according to the grouping from the second day after modeling.

2.2 Modeling method and standards for identification of the successful modeling

2.2.1 Modeling method

A LIDH model maker, reformed from a maxillary sinus puncture needle of 1.6 mm in diameter according to the literature, was used to establish the LIDH models[9]. The median incision next to the left lower abdomen was performed under aseptic conditions to expose both the anterior longitudinal collateral ligament before the intervertebral space and the area behind the intervertebral space.

The LIDH model maker was inserted by 7.80-8.00 mm to the right with a 45° angle at the L_6-L_7 intervertebral space. Syringe was fixed and the intervertebral disc tissue in the syringe was push to the front of the posterior longitudinal ligament with a caput planum wick-in-needle, resulting in the right side LIDH model at L_6-L_7. Stitched the wounds, and the rabbits were sent back to the animal feeding room.

2.2.2 Standards to determine the success of models

Observation of lower limb nervous function: The lower limb nervous function of rabbits was observed and compared before and after modeling. Rabbit gait was scored according to the recommended neurological function criteria in the literature[10].

There were no statistically significant differences in neurological function scores of the lower extremities among groups before modeling. Except for the blank control group, the neurological function scores of rabbits' lower extremity in the other three groups after modeling were significantly lower than those before modeling ($P<0.01$), indicating that the gait function in rabbits was weakened after successful modeling.

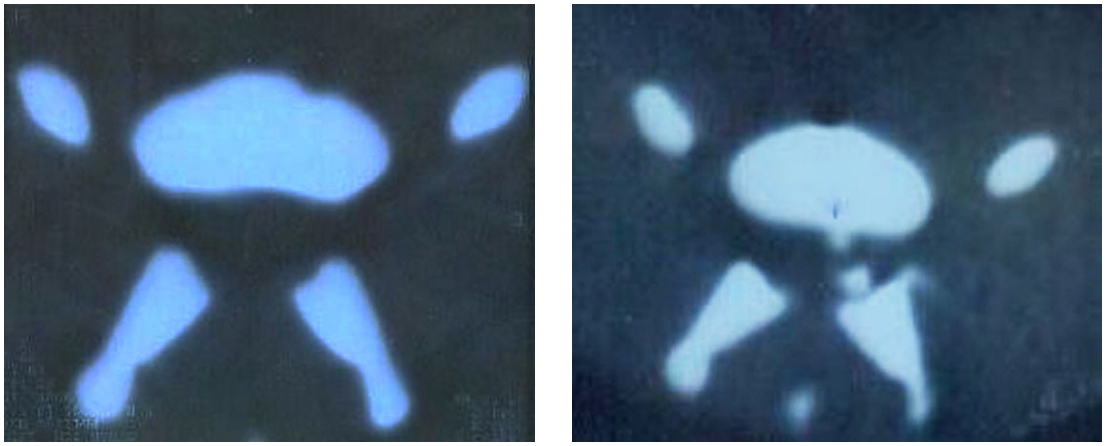
CT examination: Lumbar CT examination for modeling rabbits was performed after anesthesia before and after modeling, respectively (Figure 1).

Rabbit's nucleus pulposus herniation to the internal spinal canal after modeling indicated the intervertebral disc herniation and successful modeling.

2.3 Point positioning

Point positioning was performed according to the commonly-used animal point positioning method in the *Experimental Acupuncture Science*[11].

Huantiao (GB 30): At the 1/3 middle and outside intersection of the line between the highest point of the femur greater trochanter and the sacral fissure of rabbit.



Before modeling After modeling

Figure 1. CT findings before and after modeling

Control point of Huantiao (GB 30): Non-acupoint, 2 cm above Huantiao (GB 30).

Weizhong (BL 40): In the depression of the posterior knee joint.

Control point of Weizhong (BL 40): Non-acupoint, 0.5 cm medial to Weizhong (BL 40).

2.4 Treatment methods for rabbits in each group

Blank control group: Rabbits were fixed for 20 min using the rabbit fixing box without acupuncture treatment.

Model group: Rabbits were fixed for 20 min using the rabbit fixing box without acupuncture treatment.

EA at acupoint group: Rabbits were fixed using the rabbit fixing box. Huantiao (GB 30) and Weizhong (BL 40) on the affected side were selected. After shearing and sterilizing, acupuncture needles of 0.3 mm in diameter and 25 mm in length were inserted directly by a depth of 1 cm. A group of output wires of the G6805-2 EA instrument were respectively connected to Huantiao (GB 30) and Weizhong (BL 40). Negative pole was connected to Huantiao (GB 30), and positive pole was connected to Weizhong (BL 40). Sparse-dense wave was used. The frequency of sparse wave was 30 Hz and the dense wave was 100 Hz. The current of 0.5-1.0 mA was used to keep the hind limbs of rabbits slightly tremulous. Each stimulus lasted for 20 min.

EA at non-acupoint group: Control points of Huantiao (GB 30) and Weizhong (BL 40) were selected. Negative pole was connected to the control point of Huantiao (GB 30), and positive pole was connected to the control point of Weizhong (BL 40). Acupuncture methods and EA parameters were the same as those in the EA at acupoint group.

2.5 Specimen collection and treatment

All rabbits were anesthetized with 20% urethane [4 mL/(kg·bw)]. Four milliliter of carotid artery blood was collected and kept at room temperature for 2-3 h, then centrifuged at 3 000 r/min for 15 min at 4 ℃. The supernatant was stored at −20 ℃ after aliquoted in EP tubes for later measurement.

2.6 Observed items

2.6.1 Thigmesthesia function test for the limbs

Thigmesthesia function score was conducted by gently touching the affected toes with a cotton swab, according to the neurological function standards in the literatures[10]. 0 point: no any reaction; 1 point: slight reaction of affected limbs; 2 points: affected limbs had flexion or extension reaction, but the reaction was slow. 3 points: reaction of affected limbs was quicker, but slightly worse than the healthy side; 4 points: thigmesthesia of the affected side was sensitive and showed no difference compared with the healthy side.

2.6.2 Determination of limb gait function

The gait of rabbit was observed for gait score with reference to the literature[10]. 0 point: affected limbs were panplegia without autonomic activities; 1 point: affected limbs were semiplegia with muscle contraction and slight joint movement; 2 points: affected limbs showed poor strength, slow joint movement and walking instability; 3 points: affected limbs only showed interphalangeal joint dyskinesia; 4 points: recovered to normal exercise.

2.6.3 Serum IgG and IgM tests

Serum IgG and IgM were detected by enzyme-linked immunosorbent assay according to the kit instructions.

3 Results

3.1 Results of thigmesthesia function

There was no significant difference in thigmesthesia score among groups before modeling (P>0.05). The thigmesthesia function scores of the same group before and after modeling were compared: except for the blank control group, the thigmesthesia scores of the other three groups after modeling were significantly lower than those before modeling (all P<0.01), indicating that the thigmesthesia function of the rabbits

after modeling was weakened. Comparing of the thigmesthesia function scores before and after treatment in the same group: thigmesthesia function score was significantly improved in the EA at acupoint group after treatment (P<0.01), while there was no statistically significant difference in other groups before and after treatment (all P>0.05). Comparison of thigmesthesia score differences after treatment among groups: the EA at acupoint group was significantly higher than the blank control group, model group and EA at non-acupoint group (all P<0.01), suggesting that the thigmesthesia function was significantly improved by EA at Huantiao (GB 30) and Weizhong (BL 40), thus showing a positive therapeutic effect of EA at Huantiao (GB 30) and Weizhong (BL 40) on dysfunction of thigmesthesia function in LIDH rabbits (Table 1).

3.2 Results of gait scores

There was no significant difference in gait score among groups before modeling (P>0.05). The gait scores before and after modeling in the same group were compared: except for the blank control group, the gait scores of rabbits in the other three groups after modeling were significantly lower than those before modeling (all P<0.01), indicating that the walking function was weakened after modeling. Comparison of the gait scores before and after treatment in the same group: the gait score after treatment in EA at acupoint group was significantly higher than that before treatment (P<0.01), but no statistically significant intra-group differences in gait scores were found in other groups (all P>0.05). Comparison of gait score difference after treatment among groups: EA at acupoint group was significantly higher than the blank control group, model group and EA at non-acupoint group (all P<0.01), suggesting that the walking function of LIDH rabbits was significantly improved by EA at Huantiao (GB 30) and Weizhong (BL 40), thus showing a positive therapeutic effect on dysfunction of walking function in LIDH rabbits (Table 2).

Table 1. Comparison of rabbits' thigmesthesia function scores ($\bar{x}$ ±s, point)

Group	n	Before modeling	After modeling	After treatment	Difference before and after treatment
Blank control	10	3.89±0.31	3.61±0.11	3.98±0.04	0.37±0.09
Model	10	3.87±0.32	1.80±0.42[1)]	2.11±0.47	0.31±0.11
EA at acupoint	10	3.87±0.32	1.75±0.35[1)]	3.63±0.46[2)]	1.88±0.20[3)4)5)]
EA at non-acupoint	10	3.90±0.31	1.76±0.42[1)]	2.14±0.47	0.38±0.11

Note: Compared with the score before modeling in the same group, 1) P<0.01; compared with the score after modeling in the same group, 2) P<0.01; compared with the blank control group, 3) P<0.01; compared with the model group, 4) P<0.01; compared with the EA at non-acupoint group, 5) P<0.01

Table 2. Comparison of rabbits' gait scores ($\bar{x}$ ±s, point)

Group	n	Before modeling	After modeling	After treatment	Difference before and after treatment
Blank control	10	3.92±0.16	3.61±0.11	3.98±0.04	0.37±0.09
Model	10	3.90±0.18	1.95±0.44[1)]	2.24±0.40	0.29±0.07
EA at acupoint	10	3.93±0.16	1.87±0.32[1)]	3.81±0.38[2)]	1.94±0.18[3)4)5)]
EA at non-acupoint	10	3.93±0.16	1.91±0.20[1)]	2.30±0.21	0.39±0.10

Note: Compared with the score before modeling in the same group, 1) P<0.01; compared with the score after modeling in the same group, 2) P<0.01; compared with the blank control group, 3) P<0.01; compared with model group, 4) P<0.01; compared with the EA at non-acupoint group, 5) P<0.01

3.3 Serum IgG and IgM levels

Serum IgG and IgM levels in the model group were significantly higher than those in the blank control group (all P<0.01). The serum levels of IgG and IgM in the EA at acupoint group were significantly lower than those in the model group and EA at non-acupoint group (P<0.05). Serum IgG and IgM levels in EA at non-acupoint group were not statistically different compared with those in the model group, suggesting that the EA at Huantiao (GB 30) and Weizhong (BL 40) could inhibit serum IgG and IgM expressions in LIDH rabbits (Table 3).

Table 3. Comparison of the levels of IgG and IgM expressions ($\bar{x}$ ±s, μg/mL)

Group	n	IgG	IgM
Blank control	10	8.04±1.15	6.72±1.39
Model	10	12.37±3.85[1)]	10.33±3.14[1)]
EA at acupoint	10	9.01±2.83[2)3)]	7.44±1.76[2)3)]
EA at non-acupoint	10	11.89±2.79	9.98±2.87

Note: Compared with the blank control group, 1) P<0.01; compared with the model group, 2) P<0.01; compared with the EA at non-acupoint group, 3) P<0.01

4 Discussion

LIDH falls under the category of 'Bi-impediment syndrome', 'low back and leg pain' or 'Wei-flaccidity syndrome' in Chinese medicine. It can be caused by either 'obstruction' or 'malnourishment'. Obstruction occurs when exogenous wind, cold and dampness or traumatic injuries causes qi stagnation or blood stasis. Malnourishment occurs when long-lasting pain damages the liver and kidney or the failure of kidney qi to nourish the muscles or sinews due to constitutional liver and kidney deficiency. Consequently, the onset, development and treatment of LIDH are closely associated with meridians, qi, blood and functions of the Zang-fu organs.

Studies have found that the degree of lumbar disc herniation and nerve root compression, severities of clinical symptoms and physical signs in LIDH patients are not exactly the same[5]. Therefore, some people have questioned the opinion that compression and stimulationto the nerve root by the herniated nucleus pulposus are the main cause of low back and leg pain in LIDH patients[12]. It is believed that the mechanism of LIDH is not completely explained by mechanical compression of the nerve root alone, which may be closely related to the inflammatory response caused by biochemical substances in diseased intervertebral disc tissues[13] and autoimmune reactions[14-15]. Autoimmune response is also an important factor that causes low back pain and sciatica[14]. Intervertebral disc is an avascular tissue in the body. The nucleus pulposus is surrounded by the fibrous ring to make it isolated from the outside, thus has certain auto-immunogenicity, so the nucleus pulposus can be called 'hidden antigen'. If the fibrous ring is ruptured, the nucleus pulposus will bulge from the ruptured fibrous ring and expose to the body's immune system, which can cause autoimmune reactions, lead to low back and leg pain, and other symptoms[14,16]. A study found that during the early phase of nucleus pulposus herniation in the non-compressive LIDH rats, T cell-mediated immune response caused nerve root injury, leading to radicular pain[17]. After LIDH modeling, IgG level in the body of rats was increased. Scraping treatment inhibits the autoimmune response induced by the nucleus pulposus and the inflammatory response mediated by autoimmune response, therefore, the immune abnormalities recover to the normal[18]. IgG and IgM are immunoglobulins in the body that reflect the body's immune status. The severity of LIDH is closely related to the expression level of IgG and IgM in the body[19-22]. IgM shows stronger antigen binding ability and is the earliest appeared antibody during the initial humoral immune response. IgG is synthesized and secreted by the plasma cells in spleen and lymph nodes, and is the main antibody produced during the secondary humoral immune response. It is a high-affinity antibody that plays an important role in immune defense. Serum IgG and IgM are significantly elevated in LIDH patients, and acupuncture combined with herb-partitioned moxibustion can modulate humoral immunity in LIDH patients, leading to the IgG and IgM levels in patients to the normal[23]. Increased IgG and IgM levels in LIDH patients are thought to be the result of autoimmune response in intervertebral disc tissue[18]. Acupuncture can effectively reduce the levels of blood IgG and IgM in LIDH patients, decrease the secretion of immunoglobulin, and reduce the deposition of immune complexes in the lumbar intervertebral discs and surrounding tissues, thereby reducing the local inflammatory response in the intervertebral discs[24].

Area of the low back and leg pain caused by LIDH is similar to paths of the Bladder Meridian and Gallbladder Meridian. The two branches from the lumbus and back of the Bladder Meridian both meet at Weizhong (BL 40). Weizhong (BL 40) is the He-Sea point of the Bladder Meridian, and the common acupoint used in the treatment of low back and leg pain. Huantiao (GB 30) is a crossing acupoint of the Bladder Meridian and Gallbladder Meridian, and can be used to treat low back and leg pain related to the Bladder Meridian and Gallbladder Meridian. Gallbladder controls bone-induced diseases, and bladder controls tendon-induced diseases. Acupuncture at Huantiao (GB 30) and Weizhong (BL 40) has the role to smooth the tendons and help the joints, used in the treatment of low back and leg pain, bones and muscles pain of lower limbs and other related diseases.

There are a lot of records about the treatment of low back and leg pain by Weizhong (BL 40) and Huantiao (GB 30) in the TCM classics from different ages. Therefore, Huantiao (GB 30) and Weizhong (BL 40) are the commonly used acupoints in the treatment of low back and leg pain. In this study, we observed the effects of EA at Huantiao (GB 30) and Weizhong (BL 40) on the expression of serum IgG and IgM in LIDH rabbits. We found that the thigmesthesia and walking functions of rabbits were decreased, and the levels of IgG and IgM in serum were increased significantly after modeling. After EA at Huantiao (GB 30) and Weizhong (BL 40), thigmesthesia and walking functions of rabbits were improved significantly, and serum IgG and IgM levels were significantly lower than those in the model group and EA at non-acupoint group; thigmesthesia and walking functions, and serum IgG and IgM levels in EA at non-acupoint group had no significant difference compared with those in the model group, suggesting that the autoimmune reaction may be related to the condition of LIDH, which is consistent with that being reported in domestic and foreign literatures[16,18,24], indicating that EA at Huantiao (GB 30) and Weizhong (BL 40) in regulation of LIDH rabbit serum immune

response has a relative acupoint specificity effect. Based on the results above, it is concluded that the improvement of clinical symptoms, including thigmesthesia and gait score, of rabbits after treatment indicates that EA at Huantiao (GB 30) and Weizhong (BL 40) benifits LIDH rabbits. Prominent nucleus pulposus in LIDH rabbits can cause autoimmune response. EA at Weizhong (BL 40) and Huantiao (GB 30) can regulate the abnormal autoimmune reactions caused by autoantigen exposure due to the prominent nucleus pulposus in LIDH rabbits. Inhibition of the excessive immune response to nucleus pulposus autoantigen, thereby inhibiting of the initiation and progression of various inflammatory responses and alleviating clinical symptoms, may be one of the mechanisms during the treatment of LIDH. However, due to the small sample size, the limited experimental observation time (two courses) to detect the LIDH rabbit serum IgG and IgM levels in this trial, the real effect of EA at Huantiao (GB 30) and Weizhong (BL 40) on serum IgG and IgM levels in LIDH rabbit with a large sample size or under different experimental observation times remain to be further explored.

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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