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Basic Study

Effect of electroacupuncture on c-IAP1 mRNA and c-IAP2 mRNA in synovial tissues of rats with adjuvant arthritis

电针对佐剂性关节炎大鼠滑膜组织 c-IAP1 mRNA 和 c-IAP2 mRNA 的影响

Ai Kun (艾坤)[1], Li Yan-ling (李艳玲)[1], Qi Fang (祁芳)[1], Liu Li (刘梨)[2], Chang Xiao-rong (常小荣)[1], Yu Zhao-an (余兆安)[2]
1 Hunan University of Chinese Medicine, Changsha 410208, China
2 The First Hospital of Hunan University of Chinese Medicine, Changsha 410021, China

Abstract
Objective: To observe the therapeutic effect of electroacupuncture (EA) at Zusanli (ST 36), Guanyuan (CV 4) and Ashi points on adjuvant arthritis rats, and explore the mechanism of EA treatment of rheumatoid arthritis (RA).
Methods: Sixty male rats were randomly divided into a normal group, a model group, a methotrexate group and an EA group, with 15 rats in each group. Rats in the normal group and the model group were routinely raised and did not receive treatment; rats in the methotrexate group received methotrexate at a dose of 0.35 mg/(kg·bw), twice a week for 3 weeks; rats in the EA group received acupuncture at Zusanli (ST 36), Guanyuan (CV 4) and Ashi points, and the bilateral Zusanli (ST 36) and Ashi points were connected to EA apparatus, once a day for 3 weeks. The general status, the swelling degree of the toe, the arthritis index (AI) score, the pathological morphology of the ankle joint, and the mRNA expressions of cellular inhibitor of apoptosis protein (c-IAP) 1 and c-IAP2 in joint synovial tissue cells of the rats in each group were observed.
Results: The swelling degree of the toe, AI score and mRNA expressions of c-IAP1 and c-IAP2 in the model group were significantly higher than those in the normal group (all $P<0.05$). Compared with the model group, the swelling degree of the toe, AI score and mRNA expressions of c-IAP1 and c-IAP2 in the methotrexate group and the EA group improved ($P<0.01$ or $P<0.05$); the expressions of c-IAP1 mRNA and c-IAP2 mRNA in rat synovial tissues in the EA group were significantly higher than those in the methotrexate group ($P<0.01$).
Conclusion: EA alleviates joint swelling in rats with adjuvant arthritis. The mechanism may be related to suppressing mRNA expressions of c-IAP1 and c-IAP2, thus to induce apoptosis of synoviocytes.
Keywords: Acupuncture Therapy; Electroacupuncture; Arthritis, Rheumatoid; Arthritis, Experimental; Inhibitor of Apoptosis Proteins; Rats

【摘要】目的：观察电针刺激足三里、关元和阿是穴对佐剂性关节炎大鼠的治疗作用，探讨电针治疗类风湿性关节炎(RA)的作用机理。**方法：**将 60 只雄性大鼠随机分为空白组、模型组、甲氨蝶呤组和电针组，每组 15 只。空白组、模型组不予治疗，常规饲养；甲氨蝶呤组按 0.35 mg/(kg·bw)进行甲氨蝶呤灌胃，每周 2 次，连续给药 3 周；电针组针刺足三里、关元及阿是穴，双侧足三里和阿是穴接电针，每日 1 次，连续治疗 3 周。观察并检测各组大鼠的一般状态、足跖肿胀度、关节炎指数(AI)评分、踝关节病理形态学及关节滑膜组织细胞凋亡抑制蛋白(c-IAP) 1 mRNA 和 c-IAP2 mRNA 的表达情况。**结果：**模型组大鼠足跖肿胀度、AI 评分及 c-IAP1 mRNA 和 c-IAP2 mRNA 表达均较空白组显著升高(均 $P<0.05$)。与模型组比较，甲氨蝶呤组和电针组大鼠足跖肿胀度、AI 评分及 c-IAP1 mRNA 和 c-IAP2 mRNA 表达均有改善($P<0.01$ 或 $P<0.05$)；电针组大鼠滑膜组织细胞 c-IAP1 mRNA 和 c-IAP2 mRNA 表达较甲氨蝶呤组显著增高($P<0.01$)。**结论：**电针疗法能够减轻佐剂性关节炎大鼠关节肿胀度，其作用机理可能与抑制凋亡抑制蛋白 c-IAP1 mRNA 和 c-IAP2 mRNA 表达，从而诱导滑膜细胞凋亡有关。
【关键词】针刺疗法；电针；关节炎，类风湿；关节炎，实验性；凋亡抑制蛋白；大鼠
【中图分类号】R2-03　　**【文献标志码】**A

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by joint synovium hyperplasia, articular cartilage destruction and subchondral bone erosion[1]. The main pathological change is synovitis, which is characterized by decreased synovium cell apoptosis, tumor-like growth and massive secretion of inflammatory mediators[2-3]. This leads to the destruction of articular cartilage and bone erosion, which eventually leads to irreversible destruction of the joint and disability[4]. Fibroblast-like synoviocytes (FLS) are rapidly proliferated with decreased apoptosis, which

Author: Ai Kun, M.M., associate professor
Joint Corresponding Authors: Chang Xiao-rong, bachelor, professor, doctoral supervisor. E-mail: xrchang1956@163.com;Yu Zhao-an, M.M., attending physician. E-mail: 125184382@qq.com

is the main cause of synovium proliferation and joint swelling[2-3,5]. In the family of inhibitor of apoptosis proteins (IAP), the cellular IAP (c-IAP) 1 and c-IAP2 are major anti-apoptotic proteins. They can significantly increase the anti-apoptotic ability of FLS to show some characteristics of xenome cells[6]. The decrease in the expressions of c-IAP1 and c-IAP2 alleviates FLS proliferation to some extent.

Acupuncture-moxibustion therapy, such as acupuncture, electroacupuncture (EA), moxibustion, warm needling moxibustion, fire needle and other methods, all can achieve satisfactory therapeutic effects for RA[7-8].

In this study, a rat model of adjuvant-induced arthritis (AA) was established. Modern molecular biotechnology was used to observe c-IAP1 mRNA and c-IAP2 mRNA expressions in synovial tissue cells of AA rats intervened by EA at Zusanli (ST 36), Guanyuan (CV 4) and Ashi points, thus to explore the fundamental mechanism of EA in relieving RA joint swelling and provide experimental basis for acupuncture treatment of RA.

1 Materials and Methods

1.1 Experimental animals and grouping

A total of 70 healthy adult, male SPF-grade Sprague-Dawley (SD) rats (body weight: 80-100 g) were provided by Hunan Slack Jingda Experimental Animal Co., Ltd. [license number: SYXK (Xiang) 2013-0005]. The rats were adapted for 7 d in the SPF laboratory of Hunan University of Chinese Medicine. They were fed with ultraviolet disinfected food and distilled water, at 20-25 ℃ with humidity of 50%-70%, air exchange at 15-20 times/h and pressure gradient of 20-50 Pa. The laboratory was disinfected every night with ultraviolet radiation. After 7 d, the rats were examined and their body weight was measured. Those unqualified rats were excluded. Sixty rats were randomly selected from those weighing 120-150 g and without obvious abnormality or disease. The rats were labeled with a marker pen at the tail and randomly divided into a normal group, a model group, a methotrexate group and an EA group according to the body weight, with 15 rats in each group and 5 rats per cage.

1.2 Materials and instruments

Heat-killed mycobacterium tuberculosis H37Ra (Difco Laboratories, USA); mineral oil (Sigma, USA); AIMS animal tattoo identification, isoflurane inhalation anesthetic, and wetting compound solution (Shenzhen RWD Life Science Co., Ltd., China); CDS9000 small animal respiratory anesthesia machine (Shanghai Kufeng Industrial Co., Ltd., China); 250 mL Hamilton micro- injector (Beijing Envta Technology Co., Ltd., China); Terumo 27G needle and 37140 foot swelling measuring instrument (MGO, Italy); SDZ-II EA instrument and acupuncture needle (Suzhou Medical Products Factory Co., Ltd., China); Eppendorf TGL-18R desktop refrigerated centrifuge (Shenzhen Keli Yixiang Instrument Equipment Co., Ltd., China); reverse transcription kit (Fermentas, Canada); trizol (Invitrogen, USA); primer (Nanjing Genscript Biotechnology Co., Ltd., China); dNTP (Shenzhen Huinuo Biotechnology Co., Ltd., China); agarose (Beijing Wobison Technology Co., Ltd., China); and PIKO REAL 96 fluorescence quantitative polymerase chain reaction (PCR) instrument (Thermo Corporation, USA).

1.3 Model preparation[9]

1.3.1 Adjuvant preparation

The inactivated bacilli calmette-guerin (BCG) was accurately weighed and put into a high temperature sterilized dry mortar. The mineral oil was accurately added into the mortar using pipette to make a 1.25 mg/mL solution by fully grinding. The solution was mixed well and shaken up until the mineral oil became clear without obvious suspended impurities. After dispensing into the EP tube with a pipette, the solution was mixed thoroughly with an oscillating device for use.

1.3.2 Arthritis induction

A surgical towel was spread before the rats were lightly anesthetized using a small animal respiratory anesthesia machine. The adjuvant was mixed again with the shaker and 0.1 mL of adjuvant was injected subcutaneously at 2 cm from the end of the tail with 250 L microsyringe. A white hillock was seen after successful injection (the adjuvant was remixed with a vibrator and a new needle was replaced before each adjuvant injection). The injection site was pressed with the cotton ball when the needle was taken out. The above operations were all carried out in a fume hood. The paws appeared red and swollen from 9 d to 12 d after the model establishment, and the secondary symptoms of the whole body and joints appeared. The arthritis index (AI) >5 indicated successful modeling. Specific manifestations included arthritis nodules in the tail end, auricle, toe joint and ankle joint, and also rat activity disorder. The peak period of inflammation was 19-22 d after modeling. The normal group was injected with mineral oil in the same way.

1.4 Intervention according to groups

The rats in the normal group did not receive treatment and were routinely reared.

Rats in the EA group were fixed on a self-made rat fixator (the rats with slightly struggling or without struggling), and Zusanli (ST 36), Guanyuan (CV 4) and Ashi points were selected. The acupoint localization was based on the standard acupoint pattern of rats in the *Experimental Acupuncture Science*[10], and anthropomorphic control was performed. Ashi points were the most obvious sites for swelling of bilateral ankles. The hair around the acupoints was shaved and the acupoints were labeled with the animal tattoo identification.

Acupuncture was performed with filliform needles (0.30 mm in diameter and 25 mm in length), and the insertion depth was 0.2-0.3 cun. After the needle was inserted, the SDZ-II type EA apparatus was connected. The same side Zusanli (ST 36) and Ashi point were a group, and both sides were connected with EA apparatus. The sparse-dense wave (10 Hz/50 Hz) was used with the intensity inducing slight needle handle shaking, and the needles were retained for 20 min. Rats in the EA group started to receive treatment from the second day of modeling, once a day, 7 times as a course of treatment, and 3 courses in total (21 times).

The methotrexate group was set as a positive drug control group and intragastrically administered with methotrexate at 0.35 mg/(kg·bw), twice a week for 3 continuous weeks. The entire experimental procedure was carried out in accordance with the regulations of experimental animal ethics.

1.5 Experimental materials

The swelling degree of the toe was measured, the body was weighed, and the AI score was rated for rats in each group on the 21st day of the experiment, within 24 hours after the last treatment. 10% chloral hydrate was intraperitoneally injected at 3 mL/(kg·bw) for anesthesia. The rat was fixed in a supine position. The hind paw was quickly removed and the skin was peeled off to rinse with cold normal saline, and placed on ice. The joint capsule was opened along the back of the ankle joint, and smooth light yellowish synovial tissues were seen. The synovial layer and the fibrous layer of the joint capsule were bluntly separated by ophthalmic straight tweezers, and the synovial tissue was completely removed. Finally, the center of the synovial tissue was gently grasped and the tissue was completely cut with ophthalmic scissors. The moisture adhered on the sample surface was blotted with filter paper, the samples were immediately placed in the cryotube, labeled, and frozen in liquid nitrogen for PCR and other experiments.

1.6 Observation items

1.6.1 General observation

The mental status, diet, body hair, activity, local inflammation and systemic delayed allergy of rats in each group before and after modeling were observed.

1.6.2 Body weight

The body weight of each rat was weighed by an electronic balance every 3 d before and after modeling.

1.6.3 Swelling degree of the toe

Prepared wetting compound solution (700 μL) was added into 0.032 g NaCl to make 70 mL solution by adding double distilled water, which was freshly prepared before each measurement of the swelling degree of the toe. On the day of modeling, a small animal tattoo instrument was used to drill a hole outside the hind paw joints of the rats, which was used as a point of water immersion during the measuring of the volume of the toe; and then the paw swollen measuring instrument was used to measure the volume of rat toe in each group before the modeling. The measurement was performed twice in succession and the average value was obtained. Swelling degree of the hind paw on both sides of rats in each group was measured every 3 d from the day of modeling. Change in the average total volume of the hind legs on both sides was used to assess the swelling status of rat toe.

1.6.4 AI score

The degree of systemic joint disease in rats was evaluated according to the recorded joint swelling, color and joint activity. It was assessed once every 3 d for a total of 4 times. No redness and swelling was 0 point; little toe joint swelling was 1 point; swelling in toe joint and toe was 2 points; toe swelling below the ankle joint was 3 points; all foot joint including the ankle joint swelling and weight-bearing disability was 4 points. Each limb was scored separately, and the cumulative total was AI score (based on the average score measured by two laboratory staff).

1.6.5 Pathological observation of ankle joints

The left hind paw of each group was cut at about 1 cm above the ankle joints and placed into 10% formaldehyde solution for 24 h, 5% nitric acid decalcification for 5 d, 5% sodium sulphate neutralization for 24 h, then washed with water for 12 h and embedded in dehydrated paraffin on the 21st day of the experiment, within 24 h after the last treatment. Paraffin sections were stained with hematoxylin-eosin (HE), and pathological conditions such as inflammatory cell infiltration, synovial cell proliferation and cartilage destruction were observed by a pathologist who had no access to grouping and intervention under a light microscope.

1.6.6 Reverse transcription polymerase chain reaction (RT-PCR) for detection of c-IAP1 mRNA and c-IAP2 mRNA expressions in synovial tissues of ankle joints

Total RNA was extracted using the lysate: RNA purity OD260/OD280 was between 1.85 and 2.00.

Agarose gel electrophoresis of RNA: 1% denaturing agarose gel was prepared. 2 μL extracted RNA was mixed at a ratio of 1:5. Electrophoresis was conducted at 170 V and stopped when the bromophenol blue was migrated to 2/3 of the total gel length, and then observed under a gel imaging system.

RNA reverse transcription: cDNA was reversely transcribed using total mRNA as a template, and the obtained cDNA was frozen at –20 ℃. The primer sequences are shown in Table 1.

Quantitative PCR amplification procedure: Pre-denaturation at 95 ℃ for 10 min, denaturation at 95 ℃ for 10 s, annealing at 59 ℃ for 50 s, 40 cycles; melting curve acquisition at 60-95 ℃.

RT-PCR product analysis: CT values were automatically calculated by computer; the relative mRNA expression levels of c-IAP1 and c-IAP2 were calculated according to the obtained Ct values using the $2^{-\Delta\Delta Ct}$ method. ΔCt = Target ΔCt – Internal reference ΔCt, –ΔΔCt = Average value of ΔCt in the control group – ΔCt value of each sample. The testing was performed by Weier Biotechnology Co., Ltd.

Table 1. Primer sequences for RT-PCR assay

Name	Amplification length (bp)	Primer sequence
GAPDH internal reference	107	Upstream: 5'-CATCCTGCGTCTGGACCTGG-3' Downstream: 5'-TAATGTCACGCACGATTTCC-3'
C-IAP1	191	Upstream: 5'-TGC TGGACAACTGGAAACA-3' Downstream: 5'-GAGGGCAGGCTGGAAT-3'
C-IAP2	154	Upstream: 5'-TGCTGTGATGGTGGGCTAA-3' Downstream: 5'-GCTGCTCAAGTAGATGAGG GTAA-3'

1.7 Statistical methods

All data were processed using SPSS 17.0 software. The data were multi-dependent variable, single-independent variable and multi-group comparison measurement data. The data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and measured using the Hotelling T2 test if each group satisfied the normality; rank sum test (K independent samples) or multivariate analysis of variance (Wilk's lambda test) was used for those not meeting normal distribution and heterogeneity of variance. $P<0.05$ indicated statistical significance.

2 Results

2.1 General observation of rats

During the feeding period, rats in the normal group showed normal diet and drinking, shiny coat and normal stool. Rats in the model group were the same as those in the normal group before the model was established. After the model was replicated, the diet and drinking were decreased while dysphoria occurred with significantly increased body temperature on the third day; the inflammatory rats showed obvious less diet, loose and dull fur, and diarrhea or sticky stool, lethargy, different degrees of redness and swelling on both sides of the front and hind paws with progressive aggravation, severely restricted activity, ulceration and pus in the severe area, and more obvious inflammatory nodules, on the 12th day. The rats in the methotrexate group and the EA group were generally better than those in the model group. Compared with the model group in the same period, the local inflammation was reduced, the systemic multiple arthritis appeared better, and the tail and ear inflammatory nodules were not obvious in the EA group and the methotrexate group.

2.2 Comparison of body weight, swelling degree of the toe and AI score

2.2.1 Effect of EA on body weight of RA rats

There was no significant difference in the body weight of the rats before the model establishment ($P>0.05$). On the 12th day after model establishment, the body weight of the model group was significantly lower than that of the normal group ($P<0.01$), suggesting that the modeling affected the growth of rat body weight, which was consistent with the general condition of rats. After the 15th day of modeling, the body weight of the EA group was significantly higher than that of the model group ($P<0.01$, $P<0.05$); there was no significant difference between the EA group and the methotrexate group ($P>0.05$), but the overall trend of body weight was higher in the EA group than that of the methotrexate group, indicating that EA can reduce the effect of immune inflammatory response on rat body weight. The effect of EA was slightly better than that of methotrexate. The detail is shown in Figure 1.

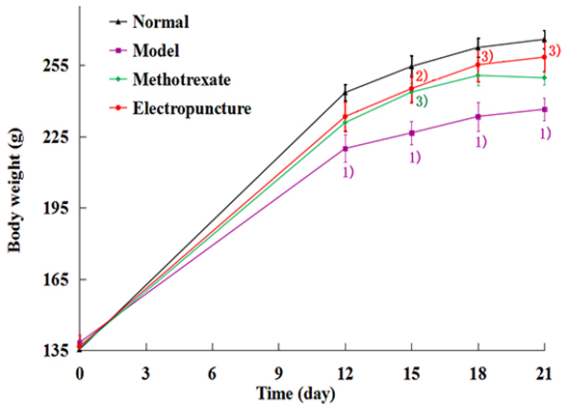


Figure 1. Comparison of rat's body weight among groups

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

2.2.2 Effect of EA on swelling of the toe in RA rats

On the 15th day after model establishment, the toe volume of the model group was significantly higher than that of the normal group (P<0.01). After treatment, the toe volume of the EA group was significantly lower than that of the model group (P<0.05); there was a significant difference between the EA group and the methotrexate group (P<0.05), suggesting that EA treatment can improve joint swelling in RA rats, but the curative effect was lower than that of methotrexate. The detail is shown in Figure 2.

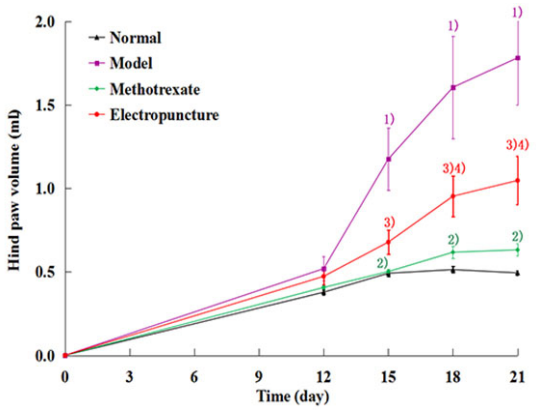


Figure 2. Comparing swelling of the toe among groups

Note: Compared with the normal group, 1) P<0.01; compared with the model group, 2) P<0.01, 3) P<0.05; compared with the methotrexate group, 4) P<0.05

2.2.3 Effect of EA on AI score of RA rats

On the 12th day after model establishment, rats in the model group first appeared small joint redness and swelling, and the AI score was significantly higher than that of the normal group (P<0.01). After treatment, the AI score of the EA group was significantly lower than that of the model group (P<0.01, P<0.05); there was no significant difference between the EA group and the methotrexate group (P>0.05), but the overall trend of the AI score was higher than that of the methotrexate group, suggesting that EA treatment can alleviate the inflammation level of RA rats. Its efficacy was slightly lower than that of methotrexate. The detail is shown in Figure 3.

2.3 Comparing c-IAP1 mRNA and c-IAP2 mRNA expressions in rat synovial tissues

Compared with the normal group, the mRNA expressions of c-IAP1 and c-IAP2 in the synovial tissues of the model group were significantly higher than those in the normal group (P<0.01), suggesting that the synovial cell apoptosis in the model group was significantly lower than that in the normal group. Compared with the model group, the mRNA expressions of c-IAP1 and c-IAP2 in the synovial tissues of the methotrexate group and the EA group were significantly lower than those in the model group (P<0.01, P<0.05). Compared with the methotrexate group, the expressions of c-IAP1 and c-IAP2 mRNA in the synovial tissues of the EA group were relatively higher (P<0.01). It is suggested that EA and methotrexate can inhibit the expressions of c-IAP1 mRNA and c-IAP2 mRNA in synovial tissues of RA rats. However, methotrexate was superior to EA (Table 2).

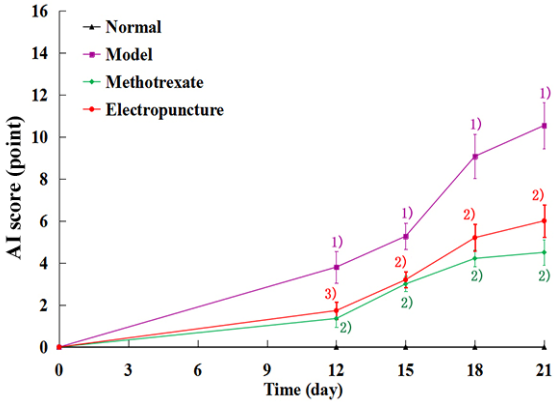


Figure 3. Comparing AI scores of rats in each group

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

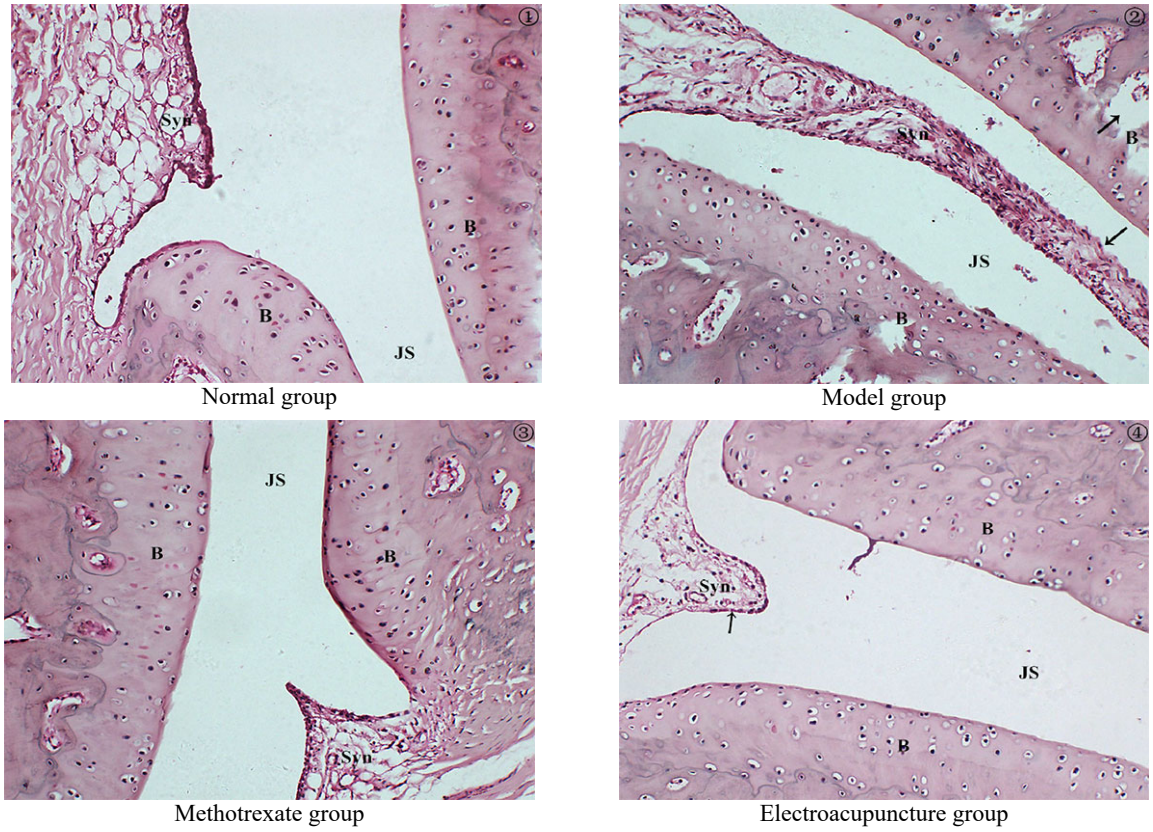
Table 2. Comparing mRNA expressions of c-IAP1 and c-IAP2 in synovial tissues of rats ($\bar{x}$ ±s)

Group	n	c-IAP1 mRNA	c-IAP2 mRNA
Normal	15	0.1007±0.0384	0.0839±0.03586
Model	15	7.0131±3.09568[1)]	4.2071±0.94957[1)]
Methotrexate	15	0.5959±0.29341[2)]	0.2440±0.10496[2)]
EA	15	4.2808±2.32886[3)4)]	1.3878±0.67378[3)4)]

Note: Compared with the normal group, 1) P<0.01; compared with the model group 2) P<0.01, 3) P<0.05; compared with the methotrexate group, 4) P<0.01

2.4 Comparing pathological changes of toe tissues in rats among groups

The pathological sections of the ankle joints of each group were observed under light microscope. The results showed that the synovial membrane of the rats in the normal group was smooth and flat, and the perichondrium was flat without abnormalities. In the model group, different degrees of inflammatory cell infiltration occurred in the synovial membrane and cartilage of the joint, the synovial hyperplasia was obvious, the perichondrium was partially destroyed, and the surface was irregular. In the methotrexate group, the synovial tissue hyperplasia was not obvious, and no inflammatory cell infiltration was observed. Rats in the EA group showed mild synovial hyperplasia, while the synovitis and cartilage destruction were not obvious. It is suggested that the pathological morphology of the rats in the methotrexate group and the EA group improved. The detail is shown in Figure 4.



Normal group　Model group　Methotrexate group　Electroacupuncture group

Figure 4. Pathomorphological changes in rats of each group (HE, ×200)

Note: Syn=Synovial tissue; B=Articular cartilage; JS=Joint space

3 Discussion

The main pathological feature of RA is inflammation and synovium hyperplasia. At present, the available data indicate increased numbers of synovial and infiltrating inflammatory cells, and reduced apoptosis, that is, less apoptosis than proliferation, may be one of the important reasons causing synovial hyperplasia[11]. The FLS, that forms the synovial lining layer, plays an important role in RA synovial hyperplasia, synovial inflammatory response, cartilage and bone damage destruction[2-3,5]. Activated FLS have the characteristics tumor cells, proliferating rapidly in the synovial membrane filled with toxic substances, but rarely causing apoptosis. They can result in the thickness growth of synovial tissues from the normal 1-2 layers of cells to 10-20 layers of cells[12]. At the same time, activated proliferating FLS continuously secrete inflammatory cytokines and matrix metalloproteinases in the synovial membrane, which in turn exacerbates the inflammatory response and causes damage and destruction of cartilage and bone tissues[13]. Therefore, promoting FLS apoptosis during the treatment of RA has positive significance for the relief of RA.

The anti-apoptotic genes of c-IAP1 and c-IAP2 belong to the IAP family. The common structure of IAP is to contain at least one baculovirus IAP repeat domain (BIR), which plays an important role in anti-apoptosis. c-IAP1 and c-IAP2 have similar structures, and they exert strong anti-apoptotic effects by inhibiting the cascade of caspases[14], but the anti-apoptotic effects of c-IAP1 and c-IAP2 are not only exerted by caspases. c-IAP1 and c-IAP2 also activate the nuclear factor κB (NF-κB) pathway through the E3 ubiquitin ligases-dependent manner to increase the expressions of other downstream anti-apoptotic genes[15]. c-IAP1 and c-IAP2 can be regulated by the NF-κB pathway, which in turn can positively feedback the NF-κB pathway, thereby promoting the expressions of other anti-apoptotic genes. Decreased expressions of c-IAP1 and c-IAP2 can reduce the activation of NF-κB pathway to a certain extent, thereby alleviating FLS proliferation and inflammation.

EA treatment of RA has a good effect. Previous studies have confirmed that EA at Zusanli (ST 36), Guanyuan (CV 4) and Ashi points can alleviate the inflammatory response of AA rats, relieve joint swelling and correct the body's immune disorder[16]. The results of this experiment showed that the swelling degree of the toe in the model group was significantly higher than that in the normal group (P<0.01). The results of HE staining showed that there were different degrees of inflammatory cell infiltration in the synovial membrane and cartilage of the model group, and the synovial hyperplasia was obvious. The expressions of c-IAP1 and c-IAP2 mRNAs in the synovial tissues of the model

group were significantly higher than those in the normal group (P<0.01), suggesting that the high mRNA expressions of c-IAP1 and c-IAP2 in the model group may be the cause of synovial hyperplasia and joint swelling. The results of HE staining showed that the EA group had mild hyperplasia of synovium, while the synovitis and cartilage destruction were not obvious, suggesting that the pathological morphology of the EA group was improved. The mRNA expressions of c-IAP1 and c-IAP2 in the synovial tissues of the EA group were significantly lower than those in the model group (P<0.05), suggesting that EA down-regulated the expressions of c-IAP1 and c-IAP2 in the synovial tissues, thereby achieving inhibition of synovial hyperplasia and reducing joint swelling.

In Chinese medicine, RA falls under the category of Bi-impediment syndrome. Its etiological factors can be external or internal. Internally, this condition is mainly caused by deficiency of the liver and kidney and general deficiency of qi and blood. External pathogenic factors include wind, cold and damp heat. In summary, the root cause of RA is deficiency, whereas its manifestations appear excessive[17-18]. This actually matches with the understanding of modern medicine: a long-term autoimmune and inflammatory disorder.

The treatment principles of RA vary from doctor to doctor, but they all agree with a general treatment principle of strengthening the healthy qi to eliminate pathogenic factors[19-20]. In the ancient and modern literatures and researches on acupuncture treatment of RA, the choice of acupoints mainly follows local point selection, combined with special acupoints that regulate immune function, such as Zusanli (ST 36), Qihai (CV 6), Guanyuan (CV 4), Mingmen (GV 4), Ganshu (BL 18) and Shenshu (BL 23). In general, it reflects the treatment principles of strengthening the body resistance and dispelling exogenous pathogen to relieve the symptoms. In this experiment, Ashi acupoints, Zusanli (ST 36) and Guanyuan (CV 4), which can enhance the immune function of the body, were selected. Among them, Ashi acupoints directly work on the affected region to make the local qi and blood smooth by adjusting local meridians. Zusanli (ST 36) and Guanyuan (CV 4) can benefit qi and solidify the body, adjust the immune function, and resist the evil outside, and produce the therapeutic effect of eliminating pathogen and strengthening healthy qi[21-22].

Furthermore, in this experiment, EA and methotrexate both improved the toe swelling, AI score, and mRNA expressions of c-IAP1 and c-IAP2 in synovial tissues of RA rats, methotrexate was better than EA in suppressing the expressions of c-IAP1 and c-IAP2 mRNAs (P<0.01). This difference suggested that EA and methotrexate may have different mechanisms in treating RA. The specific mechanism remains to be further studied.

Methotrexate is recognized as the option for the treatment of RA, but its long-term adverse reactions are relatively significant. In our experimental results, although methotrexate was better than EA in improving the swelling of toe and AI score, in terms of the body weight, the effect of EA was slightly better than that of methotrexate, which may be related to adverse drug reactions. The results of this study indicated that EA should be effective in the treatment of RA, and it is easy to operate and has no adverse reactions. EA can be used as a practicable method for the treatment of RA and deserves further study.

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.

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Translator: Yang Yan-ping (杨燕萍)