Basic Investigation

Effects of cold-damp and hot-damp environment on VEGF and IL-1 expression in joint cartilage cells in adjuvant arthritis in rats

BAI Yun-jing, JIANG De-xun, AN Na, SHEN Hong-bo, HU Yin-qi

Abstract

OBJECTIVE: To study the effects of environmental factors on the degree of injury and expression of vascular endothelial growth factor (VEGF) and interleukin-1 (IL-1) in cartilage cells of the joint in a rat model of adjuvant arthritis (AA).

METHODS: SD rats aged 10 months were randomly divided into 4 groups that varied by temperature and humidity housing conditions and induction of AA: a control group, a model group, a cold-damp group, and a hot-damp group. All groups except the control group were induced with AA. After 4 w, VEGF and IL-1 expression in cartilage cells of ankle joints of hind limbs were observed.

RESULTS: Mean area, optical density, and numbers of VEGF- and IL-1-positive cells in the model group, the cold-damp group, and the hot-damp group were significantly higher than that of the control group (all P<0.05). Optical density and positive cell numbers in the cold-damp group and the hot-damp group were significantly higher than that of the model group (all P<0.05). Optical density and positive cell numbers in the cold-damp group and the hot-damp group were significantly higher than that of the model group (all P<0.05).

CONCLUSION: Environmental factors such as high humidity combined with either high or low temperature increase the severity of damage and expression of VEGF and IL-1 in cartilage cells of joints in rats induced with AA.

INTRODUCTION

Rheumatoid arthritis (RA) belongs to the arthralgia syndrome category in traditional Chinese medicine (TCM). It is thought to be caused by an accumulation of wind, cold, and damp pathogens in the limbs, joints, channels, and collaterals, obstructing flow of Qi and blood circulation. Su Wen: Bi Lun Pian states that “wind, cold and dampness pathogens induce arthralgia-syndrome, excessive wind causing migratory arthralgia, excessive cold causing arthralgia, and excessive dampness causing fixed arthralgia”. Under this theory environmental factors play the most important role in the occurrence and development of the arthralgia syndrome. Basic pathological changes of RA include chronic inflammation and proliferation of articular.
synovium, forming pannus and invading articular cartilage, subcartilaginous bone, ligament, muscles, tendons, and so on, inducing destruction of articular cartilage, bone, and joint capsules and finally leading to malformation and functional loss of the joint. IL-1 plays an important role in bone invasion and cartilage destruction in RA by regulating the expression of many cytokines, cell adhesion molecules, immunoregulatory molecules, and pro-inflammatory mediators. VEGF strongly promotes vascular proliferation and vascular permeability, also playing a key role in the formation of synovial pannus. We performed this study to probe the relationship between environmental factors and expression of VEGF and IL-1 in cartilage cells in a rat model of RA.

MATERIALS AND METHODS

Experimental animals
SD rats (n = 32), SPF grade, aged 10 months, weighing (100 ± 20 g), were supplied by Beijing Weitong Lihua Experimental Animal Technique Co. Ltd, SCXK (Jing) 2002-0003.

Experimental instruments
The color image analyzer was a model Mis-2000SP microimage analysis system (PLR Ecommerce, LLC, Minnetonka MN, USA) fitted with a Nikon microscope with Polaroid MDC (micro-digital camera). Image analysis software was Pixelpro, Ver. 4.0. The artificial climate box was an FPQ multi-segment artificial climate box (Ningbo Laifu Science and Technology Co. Ltd, China).

Modeling and grouping
SD rats were divided into 4 groups of 8 rats each according to a random number table. The groups were a control group, a model group, a cold-damp group, and a hot-damp group.

In the model group complete Freund’s adjuvant (0.1 mL/rat) was injected into the intracutaneous part at the cross of the middle and internal 1/3 of the tail. For the cold-damp group the inoculation method was the same as that in the model group, and from the second day after inoculation rats were placed in an artificial climate box with relative moisture 95%-100% and temperature 7-10°C for 2 h each day. The hot-damp group received the same procedure as the cold-damp group except that the temperature was 30-33°C. The control group rats did not receive any treatment and were fed in the envirment with temperature 22±2°C and relative moisture 50%.

Twenty-eight days after modeling rats were sacrificed and bilateral ankle joints were excised and fixed with 40 g/L paraformaldehyde, decalified for 5 days with hydrochloric acid-formic acid decalcification liquid, and then underwent routine dehydration, embedding, sectioning, HE staining, and immunohistochemical staining. Pathological changes of articular cartilage were observed under a microscope and image analysis performed.

Detection of VEGF and IL-1
Areas, optical densities, and numbers of VEGF- and IL-1-positive cartilage cells were observed. The articular surfaces of ankle joints on the tibial side were selected from all the rats. Three visual fields in the center and bilateral sides of the articular surface were observed. The mean of the number of positive cells from the 3 visual fields was taken as the positive cell number in the ankle joint; the mean optical density of positive cells of the 3 visual fields was taken as the mean optical density (MOD) of the positive cells in the ankle joint; and the mean area of the positive cells of the 3 visual fields in the ankle joint was also observed.

Statistical analysis
Data were analyzed with SAS 8.0 statistical software. ANOVA was used for normally distributed data, and the Wilcoxon test for data not conforming to the normal distribution. P<0.05 was taken as significant.

RESULTS

Observation of pathological changes of joints
As revealed by HE staining, we observed normal-appearing articular structures, bone, and cartilage in the control group. In the model group, bone trabeculae were sparse; in the cold-damp group, bone trabeculae were sparse and the marrow cavity was enlarged; in the hot-damp group, bone trabeculae were sparse, breaking with an irregular arrangement.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Area(μm²)</th>
<th>Optical density</th>
<th>Cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>608.19±144.85</td>
<td>0.152±0.026</td>
<td>27.58±8.31</td>
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<tr>
<td>Model</td>
<td>8</td>
<td>816.16±173.71*</td>
<td>0.198±0.020*</td>
<td>34.50±7.06*</td>
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<tr>
<td>Cold-damp</td>
<td>8</td>
<td>835.10±140.65*</td>
<td>0.252±0.061*</td>
<td>40.59±3.99*</td>
</tr>
<tr>
<td>Hot-damp</td>
<td>8</td>
<td>947.11±10478*</td>
<td>0.289±0.015**</td>
<td>47.18±2.35**</td>
</tr>
<tr>
<td>F value</td>
<td>7.80</td>
<td>22.98</td>
<td>16.01</td>
<td></td>
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<tr>
<td>P value</td>
<td>&lt;0.0006</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Compared with the control group, *P<0.05; Compared with the model group, **P<0.05; Compared with the cold-damp group, ***P<0.05.
Comparison of the area, optical density, and number of IL-1-positive cells among the groups

At the end of the fourth week of the experiment, the area, optical density, and numbers of IL-1-positive cells in the model group, the cold-damp group, and the hot-damp group were significantly higher than that of the control group (all \( P < 0.05 \)). The optical density and positive cell numbers in the cold-damp group and the hot-damp group were higher than that of the model group (all \( P < 0.05 \)). The optical density in the hot-damp group was higher than that of the cold-damp group (\( P < 0.05 \)) (Table 2 and Figures 9-12).

<table>
<thead>
<tr>
<th>Group</th>
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<th>Area (( \mu m^2 ))</th>
<th>Optic density</th>
<th>Cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>1814.84±64.32</td>
<td>0.151±0.025</td>
<td>15.63±2.36</td>
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<tr>
<td>Model</td>
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<td>1925.51±126.92*</td>
<td>0.221±0.024*</td>
<td>20.67±4.24*</td>
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<tr>
<td>Cold-damp</td>
<td>8</td>
<td>1931.92±95.13*</td>
<td>0.26±0.031**</td>
<td>24.67±3.72**</td>
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<tr>
<td>Hot-damp</td>
<td>8</td>
<td>2004.34±70.52*</td>
<td>0.298±0.014**</td>
<td>26.00±3.18**</td>
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<tr>
<td>F value</td>
<td>5.71</td>
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<td>53.64</td>
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<tr>
<td>P value</td>
<td>0.0035</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
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</table>

Table 2 Comparison of the area, optical density, and number of IL-1-positive cells among the groups (±s)

Notes: Compared with the control group, \( ^* P < 0.05 \); Compared with the model group, \( \bullet P < 0.05 \); Compared with the cold-damp group, \( \circ P < 0.05 \).

DISCUSSION

The concept of viewing a clinical situation as a whole is one of main characteristics of the TCM theoretical system, which considers the human body as an organic entirety in close interaction with the environment. As conceived in the Su Wen: Bao Ming Quan Xing Lun mankind lives in the natural world in close linkage with natural environments. Thus changes of the natural environment produces corresponding changes in the human body. As stated in Ling Shu: Xie Ke Lun, “[the] human being corresponds to heaven and earth”. Normal environmental factors are necessary conditions for life, yet excessive or insufficient factors can induce disease. Formation of the arthralgia syndrome is chiefly caused by “wind, cold and dampness” pathogens invading the organism. This indicates that environmental factors are closely related with attacks of the arthralgia syndrome.

Based on modern literature, Du et al.\(^6\) probed distributive characteristics of TCM syndromes of RA and found that the most frequent main RA syndrome types were: syndrome of stagnation of wind-cold-dampness, wind-dampness-heat arthralgia syndrome, arthralgia syndrome of stagnation of dampness-heat, simultaneous occurrence of cold and heat syndromes. These indicate that the exopathogens—such as wind, cold, dampness and heat—are the most important in the pathogenesis of RA. Also, based on modern literature, Yan\(^7\) made a frequency analysis of RA syndromes and found that the frequencies of both cold syndrome and heat syndrome were the highest of the syndromes surveyed. This suggested that cold-heat syndrome differentiation plays a guiding role in RA syndrome classification, with the two large classes of cold and heat syndromes passing through the beginning and end of arthralgia diseases. Yu\(^8\) and our previous study\(^9\) found that cold-damp and hot-damp environmental factors are closely related to expression of auto-antibodies, such as serum rheumatic factor, anti-cyclic citrulline antibody, anti-keratin antibody, and anti-perinuclear factor, further indicating that cold-damp and hot-damp environmental factors obviously influence the attacks and pathogenic advance of adjuvant arthritis (AA) in the rat. At present, the pathogenesis of RA is not very clear, although modern medicine views it to be an autoimmune disease characterized pathologically mainly by synovial inflammation and dysfunction of immune cells\(^10\). Vascular proliferation and formation of pannus can be seen at early stages of RA and pass through the whole pathological course. These are the main factors inducing joint deformity and influencing the prognosis of RA. Many inflammatory factors and growth factors, such as VEGF, IL-1, TNF-\(\alpha\), and IL-6, are involved in the development of synovial lesions. VEGF possibly plays a key role in the development of synovial inflammation and the formation of pannus\(^11,12\). For example, in synovial pannus formation in RA, high levels of VEGF expression were found\(^13\). IL-1 is produced in large amounts by synovial mononuclear leukocytes and macrophages, and can activate vascular endothelial cells, increase expression of VEGF, and promote angiogenesis\(^14\). Because of these important roles for VEGF and IL-1 in the occurrence and development of RA, we chose to study expression of these molecules in the articular cartilage cells of a rat model of RA. In addition, a large number of studies show that AA has similar pathological characteristics to human RA, thus making it an ideal animal model for study of RA\(^15\).

Therefore,
we used AA to model RA.
Optical microscopy showed that in the control group, the articular structures of joints, the bone, and cartilage were normal. In the model group, on the other hand, bone trabeculae were sparse; in the cold-damp group, bone trabeculae were sparse and the marrow cavity was enlarged; in the hot-damp group, bone trabeculae were sparse, breaking with an irregular arrangement. At the end of the fourth week of the experiment, the area, optical density, and number of VEGF- and IL-1-positive cells in the model group, the cold-damp group, and the hot-damp group were significantly higher than that of the control group. The optical density and number of positive cells in the cold-damp group...
and the hot-damp group were significantly higher than those of the model group, and values of these parameters in the hot-damp group were higher than those of the cold-damp group. 

The above results indicate that environmental factors, such as ambient temperature and a high relative humidity, significantly influence the promotion, pathological course, and severity of AA in the rat. This includes increased expression of VEGF and IL-1 in articular cartilage. As has been discussed, environmental factors play an important role in attacks of RA. We found that rat joints in the hot-damp group were the most severely destroyed. This group, of the groups studied, had the highest optical density and number of VEGF- and IL-1-positive cells, indicating that a hot and damp environment has the most pronounced effects on autoimmune responses and arthritis in the AA rat model. We also found that in the cold and damp environment the rats crowded together for warming, possibly partially relieving the effect of the low ambient temperature.

REFERENCES

15. Yi JF. Preliminary study on effects and mechanisms of combined brufen and dihydro-arteannuin for treatment of adjuvant arthritis in the rat. Chin J Experimental Pharmacology of TCM Formulae 2011;17: 177-180