EFFECTS OF XINFENG CAPSULE ON LIPOPROTEIN METABOLISM IN ADJUVANT ARTHRITIS RATS BASED ON VASCULAR STRESS IMBALANCE

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ABSTRACT

Objective: To observe the effect of Xinfeng Capsule (XFC) on protein metabolism and cytokines in adjuvant arthritis (AA).

Methods: 60 rats were randomly divided into normal control group (NC) group, model control group (MC) group, methotrexate (MTX), tripterygium wilfordii polyglycoside (TPT) group and XFC group. To the rats except the NC group, the right hind foot plantar intradermal injection of 0.1ml inflammation / G), apolipoprotein A1 (Apo-A1), Apolipoprotein B (Apo-B), apolipoprotein A1 / B (APOA1 / B), cytokines, GMP-140, CD40L were observed after 30 days of administration.

Results: Compared with NC group, the levels of PA, ALB, A / G, APOA1, APOA1 / B and IL-10 were decreased in MC group, and the levels of GLO, IL-1β , ANCA, VEGF and E IL-1β , ANCA, VEGF, E-selectin, PAF, GMP-140 and CD40L in MTX, TPT and XFC groups were significantly lower than those in MC group (P <0.05) . While the XFC group in the rise of PA, ALB, APOA1, APOA1 / B and other aspects was significantly better than other treatment groups. (P <0.05 or P <0.01). Compared with TPT and MTX group, the body weight of XFC group increased (P <0.05), and the arthritis index of XFC group was significantly lower than that before treatment , VEGF, E-selectin, PAF decreased, IL-10 was significantly increased (P <0.05).

Conclusion: Xinfeng Capsule can reduce the arthritis of adjuvant arthritis rats and improve the body weight and regulate protein metabolism. The mechanism is that by down-regulating the expression of cytokines IL-1β , ANCA, VEGF, E-selectin, GMP-140, CD40L, upregulates the expression of cytokines IL-10, inhibits inflammatory effects, enhances anti-inflammatory effects, inhibits vasculitis formation, improves microvascular circulation, regulates protein metabolism in vivo.

Keywords: adjuvant arthritis, Xinfeng capsules, lipoprotein, metabolism, vascular stress.

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic synovial inflammation of the joint(1-3). The lesion invades the tissues of the whole body. The basic pathology is vasculitis, which affects the effects of protein Metabolism, the occurrence of lipoprotein metabolism disorders(4-7). The basic pathogenesis of RA for the spleen dampness Sheng, phlegm and blood stasis, the use of spleen and dampness through traditional Chinese medicine Xinfeng capsule (XFC) treatment of RA good results, used in clinical satisfaction Efficacy. To further observe the effect of XFC on RA and its lipoprotein metabolism, adjuvant arthritis (AA) rat model was established by Freund’s complete adjuvant (CFA). The effects of XFC on AA serum Protein metabolism, vascular endothelial ultrastructure and cytokines, and further explore the mechanism of Xinfeng Capsule to improve lipoprotein disorder in RA.

Materials and methods

Experimental animals
60 male SD rats, aged (7 ~ 8) months, body weight (200 ± 20) g, by the Nanjing Medical University Experimental Animal Center, license number: SCXK (Su) 2012-0004.
**Drugs and reagents**
XFC from the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine Preparation Center, hospital lot number: 20120801; methotrexate (methotrexate, MTX) 2.5mg / tablets, produced by the China Xinyi Pharmaceutical, batch: 2012071505; Tripterygium polyglycoside (IL-1β), interleukin-10 (IL-10), interleukin-10 (IL-10), interleukin-10 (IL-10), interleukin-10 (IL-10), interleukin-10 (IL-10), The vascular endothelial growth factor (VEGF) and E-selectin ELISA kit were purchased from R & D Co., Ltd., USA. The batches were respectively available from the United States SIGMA Corporation.

**Instruments and equipment**

**Model replication and administration**
Forty male Wistar rats were randomly divided into 5 groups: normal control group (NC) group, model control group (MC) group, MTX group, TPT group and XFC group (n = 12). In addition to the NC group, 0.1 ml of CFA was injected into the rat right foot plantar lesion and replicated into adjuvant arthritis (AA) rat model. 19 days after the start of administration, the dose is equivalent to 10 times the clinical amount. The dose of each group was as follows: XFC group: XFC removal capsule into fine, add saline made of suspension, 0.3g / ml, according to 1ml / 100g dose gavage once a day; TPT group: 1mg MTX group: 0.3mg / ml, according to the dose of 1ml / 100g dose gavage once a week, no administration of the experimental day, given saline irrigation, 1ml / 100g, once a day; NC group and MC group to normal saline, 1ml / 100g, once a day. The course of each group were 30 days.

**Indicators of detection**
Arthritis index (AI) calculation
On the 12th day after inflammation, the degree of generalized joint disease was observed and recorded every three days. The overall lesion was evaluated by grade 5 score, and AI was calculated based on the cumulative score of the remaining 3 limbs of the non-injected adjuvant. 2 points: toe joints and foot swelling; 3 points: the ankle below the foot swelling; 4 points: including the ankle joints, including all the feet swollen. Accumulate the points of each joint, that is, the AI of each rat.

**Determination of cytokines**
According with ANCA, IL-1β, IL-10, VEGF, E selectin kit instructions.

**Observation of Ultrastructure of Vascular Endothelium in Rats by Electron Microscopy** Respectively, according to the blood vessels, fixed, gradient dehydration, soaking, embedding, slicing, electronic staining and radiography and other steps to operate, do a good job observation records, selected range of film, accurate record of the film number and the corresponding content.

**Statistical processing**
Using SPSS11.5 statistical software, the number of measurement data were expressed as mean ± standard deviation, the two groups were compared between the independent sample t test or paired t test, correlation analysis using Spearman analysis to P <0.05 or P <0.01 For statistical significance.

**Results**
**Rat body mass, toe swelling, arthritis index changes**
Compared with NC group, the body weight of MC rats was significantly lower than that of NC group (P <0.01 or P <0.05). Compared with MC group, the body weight of the other groups increased significantly (P <0.01 or P <0.05). Compared with MTX group and TPT group, the quality of XFC group increased (P <0.01 or P <0.05).

Compared with the control group, the swelling group and arthritis index of the MC group were significantly higher than those of the control group (P <0.01 or P <0.05). Compared with the MC group, there was no significant difference between the two groups (P> 0.05). Compared with the control group, the swelling group and arthritis index of the MC group were significantly higher than that of the control group (P > 0.05) (P <0.01). Compared with the MC group, the arthritis index of each treatment group was significantly higher than that of the control group (P > 0.05) (P <0.01). Compared with XFC group, the arthritis index of MTX group increased (P <0.05) (See Table 1).
Changes in protein metabolism in rats
Compared with NC group, PA, ALB, A / G, APOA1, APOA1 / B were significantly decreased, and GLO and ANCA were increased in MC group (P <0.05 or P <0.01) (P <0.05 or P <0.01). The PA in the TPT group increased, PA and APOA1 / B increased in the XFC group (P <0.05 or P <0.01). Compared with the XFC group, PA was decreased in the MTX group and APOA1 / B in the TPT group (P <0.05) (See Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Before inflammation</th>
<th>Inflammation for 18d</th>
<th>Administration for 30 days</th>
<th>Inflammation for 18d</th>
<th>Administration for 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>179.0±11.07</td>
<td>234.1±11.79</td>
<td>302.7±5.88</td>
<td>3.6+1.89</td>
<td>7.5±1.70</td>
</tr>
<tr>
<td>MC</td>
<td>184.5±13.55</td>
<td>211.5±12.26</td>
<td>254.5±11.69</td>
<td>29.2±8.471</td>
<td>17.2±13.021</td>
</tr>
<tr>
<td>MTX</td>
<td>190.5±16.63</td>
<td>222.0±13.57</td>
<td>275.7±12.02</td>
<td>24.3±6.04</td>
<td>12.8±7.471</td>
</tr>
<tr>
<td>TPT</td>
<td>188.3±19.40</td>
<td>215.9±16.58</td>
<td>283.3±12.94</td>
<td>27.4±10.40</td>
<td>11.9±8.181</td>
</tr>
<tr>
<td>XFC</td>
<td>189.0±16.28</td>
<td>219.4±11.53</td>
<td>302.5±11.62</td>
<td>24.3±6.46</td>
<td>11.4±5.851</td>
</tr>
</tbody>
</table>

Table 2: changes in protein metabolism in each group of rats (n = 12, ±s).

Observation of vascular endothelial ultrastructure
Electron microscopy showed that the normal control group: mitochondria rare swelling and degeneration, uniform distribution, no increase in cell gap, rough endoplasmic reticulum rich, clear structure of the nuclear membrane, ridge normal (Figure 1A); model control group: mitochondrial swelling (Figure 1B); Tripterygium polyglycoside group: mitochondrial mild swelling and degeneration, mitochondria, mitochondria, mitochondria, mitochondria, mitochondria, mitochondria, mitochondria, (Figure 1C); methotrexate group: mitochondrial swelling, vacuolization, cristae destruction or disappearance, nuclear membrane structure damage, the nucleus appears wrinkled (Figure 1D); Xinfeng capsule group: most of the mitochondria intact, a small number of swelling, nuclear membrane clear structure is complete, slightly increased cell gap, no swelling bubble (Figure 1E).

Effects of XFC on GMP-140 and CD40L in Peripheral Blood of AA Rats
Compared with NC group, the levels of serum GMP-140 and CD40L in MC group were significantly higher than those in MC group (P <0.01), and the levels of GMP-140 and CD40L in MTX group, TPT group and XFC group were significantly lower than those in MC group (P <0.05). Compared with XFC group, GMP-140 in MTX and TPT groups increased, but there was no significant difference (P > 0.05) (Figure 2-3).

Analysis of the Correlation between Protein and Cytokines and Joint Swelling in AA Rats
Correlation analysis showed that PA was negatively correlated with toe swelling, VEGF, E-selectin, PAF and CD40L, ALB was negative
ly correlated with IL-1β, Apo-A1 and arthritis index, E-selectin and GMP-140 (P <0.05 or P <0.01). There was a negative correlation between APO-B and ANCA, VEGF and PAF, and APOA1 / B was negatively correlated with IL-1β (P <0.05 or P <0.01). PA, Apo-B and IL-10 were positively correlated (P <0.05) (See Table 4).

Discussion

RA is autoantibody-mediated immune response to autoantigen, RA has different protein expression in the development process, PA, Apo-A1 is one of them[10-11]. This study showed that the model group rats than the normal group compared to PA, ALB, A / G, APOA1, APOA1 / B and other significant reduction in GLO increased. Indicating that there is abnormal lipoprotein metabolism in RA[12-14]. PAB was negatively correlated with the degree of toe swelling, VEGF, E-selectin and PAF, ALB was negatively correlated with IL-1β, Apo-A1 was negatively correlated with arthritis index and E-selectin, APO-B was negatively correlated with ANCA and VEGF, and APOA1 / B was negatively correlated with IL-1β. PA and Apo-B were positively correlated with IL-10.

It is suggested that the abnormalities of immune function, especially the expression of inflammatory cytokines and vascular endothelial factors, cause the metabolic changes of lipoprotein, and the inflammation of blood vessels can cause PA, ALB, A / G, Apo-A1, APOA1 / B Such as the change. In recent years, the study found that Apo-A1 can inhibit the inflammatory response, with anti-inflammatory effect. Can block the activation of activated T cells for macrophage activation, inhibition of macrophage activation and release of IL-1β, ANCA and other inflammatory factors, in the acute inflammation Apo-A1 levels decreased, the inflammatory response has a tendency to chronic[15-18].

Compared with the normal group, the levels of IL-1β, ANCA, PAF, VEGF and E in the serum of
Xinfeng capsule to Qi and spleen, dampness Tongluo common method, the side of the drugs have different degrees of anti-inflammatory analgesic effect, can be swelling and pain\(^{(21-22)}\), which Tripterygium has immunosuppressive effect, can inhibit the RA Abnormal immune response in patients; Astragalus, Coix Seed and Tripterygium on the one hand can regulate immunity, on the other hand can prevent the immune response of Tripterygium wilfordii caused by the immune function is too low. Astragalus can also reduce gastric acid, gastric mucosal protection Effect\(^{(23-24)}\).

Xinfeng capsule from the overall regulation of immune function, because the formula in the formula of Astragalus can protect the gastric mucosa, inhibition of Tripterygium's gastrointestinal adverse reactions, thereby promoting protein absorption, effectively improve the AA rats body weight, the Group AA rats than the other two groups of body weight, the results and a large number of clinical studies are consistent\(^{(25)}\).

The results showed that Xinfeng Capsule not only had the same anti-inflammatory effect as MTX and TPT, but also improved the activity of IL-1\(\beta\), ANCA, PAF, VEGF and E-selectin in the whole function of AA rats. 10, thus inhibiting the cytokine proinflammatory effect, enhance the anti-inflammatory effect of cytokines, inhibit vasculitis formation, improve microvascular circulation, in this study found that Xinfeng capsule can significantly improve vascular endothelial mitochondria, improve the overall nuclear membrane structure. This may be one of the mechanisms by which the drug reduces the arthritis index, eliminates swelling, and reduces the vascular inflammatory response.

A large number of experimental studies have shown that\(^{(22-27)}\), XFC can significantly improve the swelling of the foot muscles of AA rats, and can significantly reduce the AA rats with joint synovial, thy-

### Tab. 4: Correlation analysis of lipoprotein markers with other markers of AA rats (n=60)\(^r\)

<table>
<thead>
<tr>
<th>PA (mg/L)</th>
<th>ALP (g/L)</th>
<th>IL-1(\beta) (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>VEGF (pg/mL)</th>
<th>E-Selectin (pg/mL)</th>
<th>PAF (pg/mL)</th>
<th>GMP-140 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.328*</td>
<td>-0.107</td>
<td>0.143</td>
<td>0.345*</td>
<td>-0.423**</td>
<td>-0.357*</td>
<td>-0.571**</td>
<td>0.126</td>
</tr>
<tr>
<td>0.099</td>
<td>-0.113</td>
<td>-0.09</td>
<td>-0.328*</td>
<td>0.023</td>
<td>0.085</td>
<td>-0.123</td>
<td>0.063</td>
</tr>
<tr>
<td>0.378*</td>
<td>0.085</td>
<td>0.051</td>
<td>-0.191</td>
<td>-0.081</td>
<td>-0.115</td>
<td>0.083</td>
<td>0.014</td>
</tr>
<tr>
<td>-0.096</td>
<td>-0.019</td>
<td>0.162</td>
<td>0.049</td>
<td>-0.137</td>
<td>-0.015</td>
<td>-0.009</td>
<td>-0.234</td>
</tr>
<tr>
<td>-0.044</td>
<td>-0.335*</td>
<td>-0.186</td>
<td>0.06</td>
<td>0.131</td>
<td>-0.374*</td>
<td>-0.119</td>
<td>-0.375*</td>
</tr>
<tr>
<td>0.026</td>
<td>0.017</td>
<td>-0.422**</td>
<td>-0.092</td>
<td>0.392**</td>
<td>-0.387*</td>
<td>0.012</td>
<td>-0.383*</td>
</tr>
<tr>
<td>-0.146</td>
<td>-0.097</td>
<td>-0.025</td>
<td>-0.397</td>
<td>0.097</td>
<td>-0.037</td>
<td>-0.085</td>
<td>0.084</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01
mus, spleen and gastric mucosal mitochondrial disease rate, the immune organs and the digestive tract have a protective effect; can reduce the AA rats too high plasma IL-1 and ANCA levels, regulation of low plasma IL-10, IL-4 levels, the regulation of cytokines.

In conclusion, the levels of lipoprotein metabolism, PA, ALB, APO-A1 and APO-B in AA rats were highly correlated with plantar swelling, arthritis index, inflammatory factors and vascular endothelial cells. The abnormality of lipoprotein metabolism was not only one of the clinical features of RA, but also can be used as an indicator of clinical efficacy of RA: XFC of traditional Chinese medicine to improve the relevant indicators of lipoproteins, etc., the possible mechanism is XFC can reduce the inflammation of the joints, improve the degree of vascular inflammation, regulate lipoprotein metabolism balance.

References

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