Basic Investigations

Effects of Preventive Administration of *Juanbi* Capsules on TNF-α, IL-1 And IL-6 Contents of Joint Fluid in the Rabbit with Knee Osteoarthritis

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**Objective:** To probe into the mechanism of the Chinese herbs with functions of reinforcing kidney and supplementing qi for preventing knee osteoarthritis of the rabbit.

**Methods:** Totally 72 healthy Japan long-ear white rabbits, aged 4 months, were randomly divided into 6 groups, blank group (A), model group (B), high dose Chinese herb group (C), middle dose Chinese herb group (D), small dose Chinese herb group (E), aminoglucose hydrochloride capsule control group (F), 12 rabbits in each group. All the rabbits in the groups, except the group A, were fixed with plaster cast for six weeks to establish rabbit knee osteoarthritis. At the same time of modeling, the different doses of *Juanbi* Capsules and aminoglucose hydrochloride capsule were administrated intragastrically in the group C, D, E, F, respectively, for 4 weeks, for preventive treatment. In the group B, the rabbit was administrated intragastrically with equal volume of normal saline to the medication groups, twice each day, in the morning and the evening, and in the group A, nothing was administrated. After modeling for 6 weeks, the joint fluid was taken and TNF-α, IL-1 and IL-6 contents were detected with ELISA method, and the articular cartilage was taken for macroscopic and microscopic examinations.

**Results:** In all the preventive treatment groups, the articular cartilage color changed to varying degrees with formation of osteophyte and bone cyst, superficial erosion on the chondral articular surface, and the cartilage defect reached to the mid layer in a part of specimens with cartilage exfoliation, but which in the extent were significantly lower than those in the model group. There were significant differences between the group A and B in TNF-α, IL-1 and IL-6 contents in the joint fluid (P<0.05), indicating that the modeling is successful; and there were significant differences as group B compared with the group C, D, E, F, showing that TNF-α, IL-1 and IL-6 contents are decreased in all the medication groups; and significant differences between group C, D, E suggests that the increase of Chinese herb doses strengthened the effect of reducing TNF-α, IL-1 and IL-6 contents in joint fluid.

**Conclusion:** The *Juanbi* Capsule prevents osteoarthritis possibly through decreasing serum TNF-α, IL-1 and IL-6 contents.

**Keywords:** method for reinforcing kidney and supplementing qi; osteoarthritis; prevention; TNF-α; IL-1 and IL-6

Osteoarthritis (OA), i.e. proliferative arthritis, pathologically is characterized mainly by progressive retrograde degeneration of articular cartilage, proliferation of joint edge and reactive change of subchondral bone substance. The morbidity raises along with increase of age, and it is a commonly-seen osteoarthropathy. At present, there is not effective method for reversing and blocking the disease, only symptoms and signs may be alleviated and pathologic changes may be delayed. Therefore, the study on
prevention of the disease gradually attracts attention of medical circle. Traditional Chinese medicine always pays great attention to prevention of diseases, and the thinking of “treatment of pre-disease” has played an important guiding role in clinic of Chinese medicine for long time. Thus, the studies on prevention and early treatment of OA are very necessary.

The authors hold that deficiency of both liver and kidney, insufficiency of both qi and blood, and no-strong muscle and bone are main pathogenesis of OA, and clinically, the method for reinforcing kidney and supplementing qi are adopted for treatment of early OA, with the compound of Chinese herbs, Juanbi Capsules (蠲痹胶囊 Capsules for treatment of arthralgia-syndrome) used, achieving obvious effects. In order to explore the mechanism of this recipe for prevention and early treatment of OA, the study was designed.

MATERIALS AND METHODS

Experimental Animals

Seventy-two male health Japan long-ear rabbits, aged 4 months, weighing 1.8–3.5 kg, were provided by Experimental Animal Center, Medical College of Xi’an Communication University (License No: SCXK 陕 2008-008). Before the experiment, the rabbits were raised and free access to food and water for one week in the animal room, at room temperature.

Drugs and Reagents

Juanbi Capsule (蠲痹胶囊) was composed of Shu Di Huang (熟地黄 Radix Rehmanniae Preparata), Sheng Huang Qi (生黄芪 Radix Astragali), Yin Yang Huo (淫羊藿 Herba Epimedii) and Gu Sui Bu (骨碎补 Rhizoma Drynariae), 15 portions for each; Rou Cong Rong (肉苁蓉 Herba Cistanchis) and Niu Xi (牛膝 Radix Achyranthis Bidentatae), 10 portions for each; and Gan Cao (甘草 Radix Glycyrrhizae) 6 portions, which were provided by the Pharmacy of Chinese Herbs, the Affiliated Hospital of Shaanxi College of Traditional Chinese Medicine, and were decocted in 10 folds of water and extracted twice, and then the twice extracts were put together and concentrated with reducing pressure into a relative density of 1:1 at 50 ℃, which was precipitated with alcohol in a final concentration of 60%. The supernatant was taken and the alcohol was recovered with reducing pressure, and the sediments were dried by reducing pressure and were grinded into fine powder for use. Just before use, the drug powder of the corresponding dose were added with water and dissolved for administration. Animoglucose hydrochloride capsules were made by Zhejiang Chengyi Pharmaceutical Co, Ltd (Batch No: 20090503, Instrument of ratification: H20060748). TNF-α, IL-1 and IL-6 ELISA kits for rabbits were supplied by R & D Company, USA.

Experimental Instruments

HB-2 Olympus microscope, CBQ-II ultramicrotome and DL-8R low temperature high speed centrifuge, ELX808IU enzyme labeling analyzer were supplied by FCC Compliance, USA..

Grouping of Animals, Preparation of Model and Administration

The 72 healthy Japan long-ear white rabbits, aged 4 months, were randomly divided into 6 groups, blank group (A), model group (B), high dose Chinese herb group (C), middle dose Chinese herb group (D), low dose Chinese herb group (E), aminoglucose hydrochloride control group (F), 12 rabbits in each group. All the rabbits in all the groups, except the group A, were fixed with plaster cast for six weeks to establish rabbit knee osteoarthritis. At the first day of modeling, different doses of Juanbi Capsules (20, 10 and 6 folds corresponding to the clinical adult dose) and aminoglucose hydrochloride Capsules (5 folds corresponding to the clinical adult dose) were administrated intragastrically in the group C, D, E, F, respectively, for 4 weeks. In the model group, the rabbit was administrated intragastrically with the equal volume of normal saline (2 ml/d) to the medication groups, twice a day, in the morning and evening, and in the black group, nothing was administrated. After modeling for 6 weeks, the joint fluid was taken and TNF-α, IL-1 and IL-6 contents were detected with ELISA method, and the articular cartilage was taken for gross and microscope examinations.

Gross Inspection

General state of the animal was observed, including body weight, mental state, hair brightness, urine and stool, and gross form of articular cartilage of knee joins.

Histomorphological Observation

Histological changes of the articular cartilage and synavium were observed by light microscope.
Detection of TNF-α, IL-1 and IL-6 contents in Joint Fluid

After administration for 4 weeks and the knee joints at straightening position were fixed with plaster cast for 6 weeks, the rabbit was anesthetized with injection of 10% urethane (0.5 g/kg) into the marginal vein and then fixed on a table for rabbits. Before the rabbit was killed, 0.1 ml saline was injected into the left knee joint and repeatedly pushed and pulled the injector, finally 0.6 ml joint fluid was taken and centrifuged at 4000 r/min, for 10 min. Then about 0.3 ml supernatant was kept at -20℃. TNF-α, IL-1 and IL-6 contents were detected with ELISA method.

Statistical Analysis
The data were expressed as $\bar{x} \pm s$, and analysis of variance was used for comparison between two groups.

RESULTS

General State of the Rabbit and Gross Form of the Articular Cartilage of Knee Joint
In the rabbit of the normal control group, the mental state was good with no abnormal changes of hair color, and it was more sensitive to external stimulation, and the plaster support for the modeling of knee joint was fixed reliably; In the model group, the hair color was withered with no brightness, and spontaneous activity and the sensitivity to external simulation markedly reduced, and the plaster support for the modeling of knee joint was fixed reliably; In the Chinese herb treatment groups and the aminoglucose hydrochloride group, the mental state was better, hair color was brighter, and more sensitive to external stimulation, and the spontaneous activity increased as compared with the model group, and the plaster support for the modeling of knee joint was fixed reliably.

Comparison of Microscopic Structures of Rabbit Knee Joint among the Groups

After modeling for 6 weeks, the rabbits in all the groups were killed and the left knee joint was dissected, and then morphological changes of the articular cartilage and synovium were observed with the naked eye. In the group A, rabbit synovium was normal; In the group B, the knee joint cartilage had obvious morphological changes, the articular cartilage markedly lost original brightness, becoming yellow, opaque; the cartilage surface was incomplete, rougher and softer, and there were erosion and ulcerative faces on the loading regions with deeper basis reaching to the whole layer of the cartilage, cartilage exfoliation of different extents, subcartilaginous bone appeared, osteophyte formed, cartilage synovium had swelling and congestion in varying degrees; In all the preventive treatment groups, the articular cartilage had color changes of different extents, osteophyte and bone cyst formed, with superficial erosion on the cartilage joint face, and the cartilage defect in a part of samples reached to the mid lay of the cartilage, with exfoliation of cartilage, but which in the extent were markedly lower than those in the group B.

Effects of Juanbi Capsules on TNF-α Content in Knee Joint Fluid (Table 1)
There were significant differences in the TNF-α content as the group B compared with the group C, D, E, F, suggesting that the TNF-α content in knee joint fluid was significantly decreased in all the treatment groups; There was a significant difference between the group C, D, E, showing that the TNF-α content was decreased more greatly along with the increase of medical dose in the Chinese herb prevention groups; There were significant differences as the group F compared with the group C and E, indicating that the effect of aminoglucose hydrochloride capsules in decreasing the TNF-α content is superior to the low dose Chinese herb group and lower than the high dose Chinese herb group. There was no significant difference between the group F and D, indicating that the effect of aminoglucose hydrochloride capsules in decreasing the TNF-α content is same as that of the middle dose Chinese herb group.

Effects of Juanbi Capsules on IL-1 Content in Knee Joint Fluid (Table 1)
There were significant differences in the IL-1 content as the group B compared with the group C, D, E, F, suggesting that the IL-1 content in knee joint fluid was significantly decreased in all the treatment groups. The significant difference in the IL-1 content between the group F and E shown the effect of aminoglucose hydrochloride capsules in decreasing the TNF-α content is superior to the low dose Chinese herb group, and the significant differences between the group C, D, E indicated that the IL-1 content was decreased more greatly along with increase of the medical dose in the Chinese herb prevention groups; There were no
Effects of Chinese herb group.

Knee joint fluid is same as that of low or middle dose Chinese herb group.

**Effects of Juanbi Capsules on IL-6 Content in Knee Joint Fluid (Table 1)**

There were significant differences in IL-6 content as the group B compared with the group C, D, E, F, suggesting that the IL-6 content in knee joint fluid was significantly decreased in all the medication groups. There was a significant difference between the group C, D, E, showing that the IL-6 content was decreased more greatly along with the increase of the drug dose in the Chinese herb prevention groups; There were significant differences as the group F compared with the group C, E, indicating that the effect of aminoglucose hydrochloride capsules in decreasing the IL-6 content was superior to the low dose group and lower than the high dose group. There was no significant difference between the group F and D, indicating that the effect of aminoglucose hydrochloride capsules in decreasing the IL-6 content is same as that of the middle dose Chinese herb group.

**Table 1. Effects of Juanbi Capsule on TNF-α, IL-1 and IL-6 contents in knee joint fluid of rabbit (X±σ, n=12)**

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α(ng/L)</th>
<th>IL-1(ng/L)</th>
<th>IL-6(ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.001±0.0000</td>
<td>8.604±0.900</td>
<td>0.096±0.125</td>
</tr>
<tr>
<td>B</td>
<td>16.136±2.336</td>
<td>321.583±6.050</td>
<td>249.958±37.199</td>
</tr>
<tr>
<td>C</td>
<td>1.587±0.657</td>
<td>91.213±4.321</td>
<td>47.446±17.374</td>
</tr>
<tr>
<td>D</td>
<td>5.023±0.969</td>
<td>124.767±1.780</td>
<td>98.950±11.943</td>
</tr>
<tr>
<td>E</td>
<td>11.785±2.052</td>
<td>158.125±11.191</td>
<td>157.879±16.333</td>
</tr>
<tr>
<td>F</td>
<td>5.018±0.949</td>
<td>113.338±10.512</td>
<td>98.329±12.309</td>
</tr>
</tbody>
</table>

Notes: Compared with the group B, \*P<0.05; Compared with the group E, \*P<0.05; Compared with the group C, \*P<0.05; Compared with the group F, \*P<0.05.

**DISCUSSION**

In recent years, it has been proved that IL-1, IL-6 and TNF-α are important mediators involved in pathogenic process of osteoarthritis (OA), and also are important regulators for inflammatory response, and they are of an important role for development of synovitis and destruction of cartilage matrix.\(^1\) TNF-α contents in both serum and joint fluid obviously increase along with deterioration of OA with significant correlation between them, indicating that the TNF-α levels in serum and joint fluid have a certain significance in reflecting serious degree of OA.\(^2\)

Kim SJ, et al. find that the form and metabolism of cartilage cells, distribution and structure of proteoglycan in cartilage, and metabolism of collagenous fibers have obvious changes in the early stage of OA and before destruction of the network frame structure of collagenous fibers.\(^3\) At present, it is held that these changes are related with effects of some resolutive cytokines. Among them, TNF and IL-1 and IL-6 play the most important role. They can directly damage collagenous fibers, inducing their fragmentation; at the same time, a large number of proteoglycan lose for hydrolysis of proteinase or osmotic action of cartilage surface, causing collapse of the network frame structure of collagen. Experimental study finds that the damage of joint in OA can be repaired by selective antibodies of IL-1, IL-6 and TNF-α and antagonistic proteins of cytokine receptors, soluble cytokine receptor molecules, showing that IL-1, IL-6 and TNF-α are closely correlated with destroying of cartilage.\(^4\)

OA belongs the category of “rheumatism involving the bone” in TCM, which is caused mainly by deficiency of both liver and kidney with affection by extrapathogen or by trauma, and reinforcing and supplementing liver and kidney should be a basic therapeutic principle. In Neijing (Internal Classic) it was firstly raised that when kidney yang was weak, cold-dampness would invade the bone”, which is a key pathogenesis of osteoarthropathy.\(^5\) After supplement and development of medical experts in the Song and Yuan Dynasties, the principle for treatment of osteoarthropathy based on liver and kidney was established till the Ming and Qing Dynasties. In physiology, both liver and kidney are homologous, “essence and blood transform each other”; In pathology, diseases in liver and kidney are concordance; In
treatment, reinforcing kidney as main is usually used for reinforcing liver. The kidney is the origin of congenital constitution, and bone is in charge of regeneration of marrow; the liver is an organ for storing blood, being in charge of the tendon and controlling the bones and normalizing the function of joints.

Prof. LI Kan-yin of Shaanxi College of Traditional Chinese Medicine, a famous expert of orthopedics and traumatology in TCM in China, holds syndrome differentiation of OA is within category of arthralgia-syndrome, but deficiency of both liver and kidney, insufficiency of both qi and blood, weakness of muscle and bone are main pathogenesis of OA, because the patient usually is the aged, chronic disease and OA most occurs in the joints of body weight-carrying or more activities, and the method for reinforcing the kidney and supplementing qi should used as the therapeutic principle, with the recipe Juanbi Capsule used. Clinically, it has been applied for 30 years, achieving satisfactory clinical therapeutic effects.

In the study, in the model group, breaking and exfoliation on cartilage surface, proliferation and adhesion of synovium, aggregation and necrosis of cartilage cells were found, but in the high-, middle- and low-dose Juanbi preventive groups, the pathological changes of the cartilage and synovium were significantly alleviated as compared with those in the model group, the cartilage structures being more complete, and the form of cartilage cells little changing, indicating that Juanbi Capsule can relieve degeneration of cartilage cells and degradation of cartilage matrix. Also, it was found that Juanbi Capsule can electively improve the symptoms and signs of knee OA in the rabbit, and obviously decrease the TNF-α level in synovial fluid of the knee joint. It is inferred that Juanbi Capsule prevents and early treat OA possibly through decreasing TNF-α level in joint fluid and delaying degeneration of articular cartilage.

REFERENCES

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