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Research Progress on Molecular Mechanism of Moxibustion in the Treatment of Ankylosing Spondylitis

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Abstract

As an important part of traditional Chinese medicine (TCM) nursing technology, moxibustion plays a unique role in improving the symptoms of ankylosing spondylitis (AS). The research on the mechanism of moxibustion intervention in AS mainly focuses on anti-inflammatory effects, immune regulation, bone metabolism regulation, intestinal flora regulation, and so on. Molecular medicine is of great significance to further clarify the mechanism of moxibustion intervention in AS. However, there are still some problems in the research on the molecular mechanism of moxibustion intervention in AS: the existing biomedical research methods only explore from a specific field and lack the exploration of moxibustion-targeted molecules based on biomedical network. In the future, the molecular network effect of moxibustion on AS can be discussed comprehensively and systematically with the help of omics technology and the construction of biological information interaction network between omics. The effect of moxibustion on upstream osteogenic transcription factors and related signaling pathways such as WNT, β-catenin, and BMP/Smads is not yet clear. Future research can focus on the relevant signal targets of bone reconstruction and clarify the mechanism of moxibustion against the new bone formation. In addition, there is a lack of research on the molecular mechanism of moxibustion in the treatment of AS from the perspective of metabolites. It is necessary to further explore the mechanism of moxibustion in the treatment of AS with the help of metabonomics technology.

Keywords
- ankylosing spondylitis
- moxibustion
- molecular mechanism
- anti-inflammatory
- bone metabolism
- intestinal flora

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Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease. It is typically characterized by inflammatory back pain, sacroiliac joint stiffness, spinal deformity, limited thoracic movement, low extremity synovitis, knee and heel swelling, and fatigue.[1] Some patients also have extra-articular manifestations, such as uveitis, inflammatory bowel disease, and psoriasis. The prevalence rate of AS in the United States is estimated to be 0.55% with an average age of onset being less than 40 years and in China it is likely to be approximately 0.2 to 0.3%.[2,3] Evidence from the Assessment of SpondyloArthritis International Society/European League Against Rheumatism international guidelines recommended that pharmacotherapies including nonsteroidal anti-inflammatory drugs (NSAIDs) and the U.S. Food and Drug Administration-approved biological agents were conventional and mainstream treatments for AS. However, clinically, there are some adverse events and deficiencies related to both traditional NSAIDs and novel biological agents.[4] As an important part of TCM nursing intervention, moxibustion has the function of warming yang qi, dispersing cold, regulating meridians, especially has a significant effect in tonifying kidney qi. To further explore the molecular mechanism of moxibustion in the treatment of AS, the literature of recent 10 years was searched and summarized from the aspects of anti-inflammatory effects, immune regulation, bone metabolism regulation, intestinal flora regulation, and so on.

Anti-Inflammatory Mechanism of Moxibustion in Ankylosing Spondylitis

Cytokines play important roles in the pathogenesis of AS, which not only amplify the effect of the "immune–inflammation" reaction but also stimulate the production of vascular endothelial growth factors. The above-mentioned pathological reaction may result in vascular infiltration and tissue regeneration in joints, as well as the ossification of AS ligament.[5] The cytokines involved in the pathological process of AS mainly include proinflammatory factor (TNF-α, IL-1 β, IL-6, IFN-γ, PGE, and IL-17) and anti-inflammatory factors (IL-10 and TGF-β).

TNF-α is mainly produced by activated macrophages and monocytes and is considered to be the master orchestrator of systemic immune-inflammatory response.[6] Mice overexpressing membrane-bound TNF have shown typical bamboo-like changes in the spine and new bone formation at the vertebral angle[7]; IL-1 β could be secreted by all nucleated cells, especially macrophages, and produce inflammation at the attachment site of AS by recruiting other relevant immune cells.[8] In addition, IL-1 β can activate osteoclasts, enhance bone resorption, and induce the production of a large number of proteases involved in bone and joint destruction[8]; IL-6, a cytokine mainly secreted by the monocyte and macrophage, may activate Th cells, maintain the growth and differentiation of B cells, and produce immunoglobulin.[9] Moreover,by binding to membrane surface receptor IL-6R,; IL-6 can directly act on T lymphocytes and neutrophils, which may induce persistent chronic inflammation, cartilage metaplasia and calcification, connective tissue hyperplasia, etc,[10]; IFN-γ is mainly secreted by natural killer cells and $CD8^+$ T cells and plays an important role in activating macrophages of AS.[11] Recent clinical studies have shown that compared with healthy volunteer people, the levels of IFN-γ of $CD4^+$ T cells (Th1) in peripheral blood mononuclear cells of AS patients are increased.[12] PEG_2 is an important component of arachidonic acid cyclooxygenase metabolites. Previous studies suggested that the infiltration of immune cells (such as macrophages and $CD8^+$ T cells) into the joint, the increased expression of proinflammatory cytokine PEG_2, and the immune–inflammation microenvironment formation might be the reasons for sacroiliitis and enthesitis in patients with AS.[13] In this inflammatory microenvironment, Th17 cells can recruit neutrophils, macrophages, and epithelial cells by secreting IL-17 and release other proinflammatory cytokines (such as IL-1β, IL-6, and TNF-α), which may form continuous positive feedback to result in the inflammatory injury to the ligament of AS.[14]

At present, many studies have explored the anti-inflammatory mechanism of moxibustion in AS, which are summarized below. To provide a theoretical basis for hyperthermia therapy in the treatment of AS, Chen et al[15] explored the effect of moxibustion therapy on cytokines in patients with AS. The results showed that two courses of moxibustion therapy could up-regulate the expression levels of IL-10 and TGF-β and down-regulate the expression levels of TNF-α and IL-6, which exerted the anti-inflammatory effect to a certain extent, so as to reduce the inflammatory response of AS. Lu et al[16] explored the effect of moxibustion on the expression of Th1, Th2, and Th17 cells and serum cytokines in patients with AS. The results showed that 4-week moxibustion intervention could effectively reduce serum levels of IFN-γ and the ratio of IFN-γ/IL-4 and increase the serum levels of IL-10. Kong[17] explored the effectiveness of moxibustion intervention in AS patients with kidney yang deficiency. The results showed that moxibustion could significantly reduce the inflammatory cytokines levels of IL-17 and IL-1β and acute inflammatory response protein expression of CRP in AS patients with kidney yang deficiency. Li et al[18] observed the clinical efficacy and serum cytokine expression of moxibustion in the treatment of AS patients with kidney yang deficiency. The results suggested that three courses of moxibustion therapy could effectively reduce serum cytokines levels of IL-17 and IL-1β and serum expression levels of acute inflammatory markers CRP and ESR in AS patients with kidney yang deficiency. Jiang et al[19] found that ginger garlic separated moxibustion could reduce serum cytokines IL-6, TNF-α, and ESR expression in patients with AS, and improve physical function and reduce the disease activity for patients with AS. Tang and Li[20] found that 8-week moxibustion could reduce serum levels of IFN-γ and up-regulate the serum levels of TGF-β in AS patients with cold dampness, which could improve the mobility of the spine in patients with AS. Zuo et al[21] explored the anti-inflammatory mechanism of moxibustion in the treatment of AS with early and medium stages. The results suggested that moxibustion could inhibit inflammatory cytokine levels of TNF-α, which might improve the symptoms of AS with early and medium

stages. Based on the above findings, moxibustion can reduce the inflammatory injury and ameliorate the disease progress of AS by down-regulating the expression of inflammatory cytokines and up-regulating the expression level of anti-inflammatory cytokines.

Immunomodulatory Mechanism of Moxibustion in Ankylosing Spondylitis

Dysregulation of T cell subsets, abnormal immunoglobulin expression, and immune response caused by HLA-B27 gene misfolding play a critical role in the development of AS. Tang and Li[20] found that the ratio of $CD4^+/CD8^+$ in peripheral blood of AS patients was significantly lower than that of healthy people, suggesting the dysregulation of immune function in AS patients. The activation of immunoglobulin is involved in the immune response mediated by B cells and is closely related to the disease activity of AS.

Colbert et al[22] showed that *HLA-B27* gene misfolding during the endoplasmic reticulum (ER) assembly might lead to the unfolded protein response (UPR). The misfolded *HLA-B27* gene further accumulates in the endoplasmic reticulum, which finally leads to ER stress. UPR and ER stress can induce proteasome to degrade MHC class I molecules (including B27 molecules), resulting in arthritis peptides. The antigen-antibody complex leads to nuclear factor through specific binding with $CD8^+$ T lymphocytes κ B(NF-κ B), which actives autoimmune response, induces many inflammatory cytokines at the enthesitis of spine, such as TNF-α, IL-23, and IFN-γ, and finally induces the immune–inflammation response of AS.

At present, many studies have investigated the immunomodulatory mechanism of moxibustion in AS, which is summarized below. Liu et al[23] applied flow cytometry to explore the effect of moxibustion on peripheral blood T lymphocyte subsets in patients with early and medium stage AS. The results showed that moxibustion could significantly reduce the percentage of $CD8^+$ cells in peripheral blood of AS patients, increase the percentage of $CD4^+$ cells and the ratio of $CD4^+/CD8^+$, regulate the dysfunction of T cell subsets in AS patients, and effectively modulate the state of immune deficiency of AS patients. Deng[24] performed moxibustion on patients with AS at Shenshu (BL 23) acupoint. The results showed that moxibustion could increase the number of $CD3^+$ and $CD4^+$ cells in patients' blood and balance the immune function of AS patients. Fan[25] found that moxibustion could effectively up-regulate the expression of $CD3^+$ and $CD4^+$, down-regulate the expression of $CD8^+$, and increase the ratio of $CD4^+/CD8^+$ in the serum of AS patients, so as to regulate the T cell subsets dysfunction and balance the immune status in AS patients. Luo[26] observed the effect of mild moxibustion on AS patients with kidney deficiency and cold coagulation. The results showed that mild moxibustion could effectively reduce the expression levels of IgA and IgG and improve the expression of IgM, so as to regulate the immune status and ameliorate the disease progress of AS. Sun[27] explored the effect of ginger-garlic-separated moxibustion on immunoglobulin in AS patients with kidney deficiency and cold coagulation type. The results suggested that moxibustion could significantly reduce the serum levels of IgG and modulate the immune dysfunction of AS. Treg is a kind of T cell subsets that control the immune response in vivo. It can inhibit the function of Th cells and antigen presenting cells through direct contact between cells or secretion of anti-inflammatory cytokines, so as to reduce the secretion of inflammatory cytokines and antibodies, and finally exert an immune effect. The study found that the ratio of $CD4^+CD25^+$ Treg cells in peripheral blood of patients with AS was significantly lower than that of healthy people. Meanwhile, the imbalance in the number and function of Th17 and Treg cells has been proved to be closely related to the disease progress of AS.[28] Zuo et al[29] found that moxibustion could regulate the immune dysfunction of AS by increasing the serum mRNA expression of Foxp3 and T-bet and the ratio of $CD4^+CD25^+$ Treg and $CD4^+$ Th1 cells. Zuo et al[30] found that after four courses of moxibustion therapy on patients with AS, the ratio of $CD4^+$ Th17 cells and the expression of inflammatory cytokines such as IL-17 and IL-23 in serum of AS patients were significantly decreased, while the ratio of Treg cells and $CD4^+$ Th1 cells increased. It suggested that moxibustion could regulate the immune dysfunction of AS patients by mediating the balance of Th17/Treg/Th1. Wang and Wang[31] discussed the effect of moxibustion intervention on the immune mechanism of AS patients with kidney yang deficiency. The results showed that moxibustion could regulate the balance of Th17/Treg by up-regulating the ratio of Treg cells and down-regulating the proportion of Th17 cells, so as to reduce the mRNA expression levels of inflammatory cytokines such as IL-23 and IL-17. Based on the above-mentioned findings, moxibustion can correct the imbalance of Th17/Treg/Th1 and exert the immunomodulatory effect of moxibustion on AS by up-regulating the number of $CD3^+$ and $CD4^+$ cells, down-regulating the expression of $CD8^+$, reducing the expression level of immunoglobulin IgA and IgG, and increasing the ratio of $CD4^+$ $CD25^+$ Treg cells and the mRNA expression of Foxp3 and T-bet.

Bone Metabolism Regulation Mechanism of Moxibustion in Ankylosing Spondylitis

Bone metabolism markers mainly include bone formation biomarkers (osteocalcin and bone alkaline phosphatase) and bone resorption biomarkers (type I collagen C-terminal peptide and type I collagen N-terminal peptide). The balance between bone formation and bone resorption is the key to the regulation of bone metabolism in patients with AS.[32]

Among bone metabolic factors, receptor activator of NF-κ B (RANK)-RANK ligand (RANKL) is not only a critical component involved in the bone immune response but also a key signal system of regulating osteoclast activity and participating in bone metabolic balance.[33,34] RANKL is mainly expressed in osteoblasts and fibroblasts.[33,34] The differentiation and maturation of osteoclasts and the balance of bone homeostasis need to depend on the interaction between RANKL and RANK.[35] The imbalance of RANK–RANKL system may directly lead to bone remodeling or bone erosion in AS.[36] In addition, serum osteocalcin is a vitamin K-dependent protein, which is mainly

synthesized by mature osteoblasts and is associated with the rate of bone mineralization. The serum expression of osteocalcin can reflect osteoblast activity. Alkaline phosphatase is also a specific biomarker reflecting osteoblast activity and bone formation. Compared with healthy volunteers, the serum levels of osteocalcin and alkaline phosphatase in patients with AS were significantly increased.[37]

At present, many original pieces of research have explored the bone metabolism regulation mechanism of moxibustion intervention in AS, which are summarized below. Xu et al[38] revealed that moxibustion at acupoints of Shenshu (BL 23), Zusanli (ST36), and Mingmen (GV4) of AS mice could effectively inhibit the overexpression of ALP and OCN, reduce the new bone formation, and improve the mobility of the spine in AS mice. Lin and Wu[39] explored the effect of medicine-separated moxibustion on the pain and bone metabolism of AS. The results showed that moxibustion could reduce the deformity and remodeling of the bone structure of AS via down-regulating the expression of alkaline phosphatase. Zhang et al[40] found that moxibustion inhibited nonspecific endochondral ossification of AS by down-regulating the expression of CTX-I, ALP, and OCN proteins pertaining to pathological osteogenesis. Ma[41] explored the bone metabolism of moxibustion in patients with AS of kidney yang deficiency. The results showed that moxibustion could repair muscle lesions, inhibit bone destruction, and prevent ligament sclerosis and calcification by inhibiting the overexpression of ALP and creatine kinase. Zhu et al[42] found that moxibustion could effectively inhibit the overexpression of tartrate-resistant acid phosphatase and OCN, regulate the balance of bone metabolism, and improve spinal stiffness of AS. Matrix metalloproteinases (MMP) are a group of hydrolases that can degrade the extracellular matrix. MMP participate in the degradation of cartilage matrix and bone resorption and play an important role in the pathogenesis of bone destruction. MMP-3 is secreted by fibroblasts, synovial cells, and chondrocytes. It is considered to be the most important protease leading to cartilage degradation. Liu et al[43] and Tang and Li[20] found that moxibustion could inhibit bone destruction and new bone formation in AS by down-regulating the expression of MMP-3 and tissue inhibitor of metalloproteinase (TIMP). Zhu[44] and Ma and Wu[45] explored the mechanism of moxibustion on bone metabolism in patients with AS. The results showed that moxibustion could inhibit the osteogenic metabolism and improve the activity of the waist and spine by down-regulating the expression of MMP-3.

Intestinal Flora Regulation Mechanism of Moxibustion in Ankylosing Spondylitis

The intestinal flora-mediated gut-bone axis plays an important role in the pathogenesis of AS. Specifically, *Faecalibacterium prausnitzii* flora decreased in the feces of AS patients compared with healthy people. At the same time, there was an increase in the number of *Dialister* flora in ileal and colon biopsies of AS patients, which was positively correlated with AS disease activity score.[46] Intestinal T lymphocytes activated by intestinal pathogenic microbial antigens migrate to joints and induce inflammation.[47] In the inflammatory state, the release of various cytokines will cause vasodilation and increase vascular permeability, resulting in increased leukocyte infiltration. Leukocyte at the site of intestinal inflammation further bind to synovial vessels and enter joints through a variety of adhesion molecules.[48]

At present, there are few studies that have explored the mechanism of moxibustion on the intestinal flora of AS. The relevant results are summarized below. Ye et al[49] explored the effect of meridian moxibustion on intestinal microorganisms in AS patients with kidney deficiency and cold coagulation. The results showed that moxibustion could achieve the balance of intestinal flora by down-regulating the number of sulfate-reducing bacteria and *Bacteroides fragilis* in intestinal microorganisms. Xu et al[38] found that moxibustion could reduce the permeability of the intestinal tract and improve the mechanical barrier function of colon by down-regulating the L-phenylalanine of intestinal flora and up-regulating the expression of short-chain fatty acid and intestinal mucosal synthetic protein L-threonine. Moreover, moxibustion could prohibit the inflammatory signaling pathway, up-regulate the protein expression of Foxp3 and IL-10, and finally improve the intestinal defense against inflammation.

Summary

As a chronic rheumatic disease, AS not only leads to the dysfunction of physical activity but also reduces the quality of life of patients and even results in mental health problems such as anxiety and depression and disability. Until now, there is no special Western medicine to intervene in AS. Moxibustion, as an important part of TCM nursing techniques, has a unique effect on improving the symptoms of AS. At present, the research on the mechanism of moxibustion intervention on AS mainly focuses on anti-inflammatory effects, immune modulation, bone metabolism regulation, intestinal flora adjustment, and so on. The molecular medicine methods are of great significance to further clarify the mechanism of moxibustion intervention in AS. At the same time, the prospect of the molecular mechanism of moxibustion intervention in AS is as follows: first, moxibustion is to regulate the Yin and Yang balance of AS based on the concept of TCM. However, the current biomedicine research methods only explore from a certain and single field (inflammation, immunity, or bone metabolism) and lack the exploration of moxibustion-targeted molecules based on the biomedicine network. In the future, we can further explore the molecular network effect of moxibustion on AS from a whole and systematic view with the help of omics techniques and the construction of biological information interaction network between omics; second, previous studies only discussed the effect of moxibustion on the expression of downstream osteogenic markers CTX-I, ALP, and OCN, but the effect of moxibustion on the upstream osteogenic transcription factor (DKK1) and bone remodeling pertaining signal pathways (Wnt β-catenin and BMP/Smads) remains unclear. Therefore, future research should focus on the relevant signal targets of

bone remodeling and further clarify the mechanism of moxibustion against new bone formation; third, the increase of serum fatty acid metabolites will induce ectopic fat deposition, which aggravates the "immune–inflammation" response of AS. However, previous studies have not clarified the molecular mechanism of moxibustion on AS from the view of metabolites. In the future, with the help of metabonomics technology, the mechanism of moxibustion against AS can be better explored from a systematic perspective.

Credit Authorship Contribution Statement

Min Yan: Conceptualization, writing-original draft. **Jiangshan Huali:** Validation and visualization. **Linyun Wu:** Validation and visualization. **Xiao Zhou:** Validation and visualization. **Qing Yang:** Validation and visualization. **Qinfeng Wu:** Conceptualization, investigation, methodology, and writing - review & editing. **Xi Liu:** Methodology. **Hongyuan Wang:** Validation and visualization. **Xiao Xu:** Conceptualization, funding acquisition, methodology, resources, software, validation, visualization, and writing - review editing.

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Conflict of Interest

The authors declare no conflict of interest.

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