The Molecular Mechanism of Cheng's Juanbi Decoction in Regulating the PI3K/Akt/mTOR Pathway for Rheumatoid

Mu He[1], Kejia Cao[1], Chaoyong Li[1], Yue Cao[2], Le Ding[1*]

[1] School of Traditional Chinese Medicine, Changsha Medical University, Changsha 410219, Hunan, China

[2] Hangzhou Siningkang Animal Hospital Co., Ltd. Hangzhou 310000, Zhejiang, China

Abstract: Cheng's Juanbi Decoction (CJD) is a traditional Chinese medicinal formula widely used in the treatment of rheumatoid arthritis (RA). Recent studies have suggested that the therapeutic effects of CJD are closely linked to the regulation of several key signaling pathways, particularly the PI3K/Akt/mTOR pathway. This pathway plays a crucial role in cellular processes such as inflammation, immune response, and cartilage degradation, all of which are central to the pathogenesis of RA. The molecular mechanism by which CJD exerts its effects on this pathway remains poorly understood. This review aims to explore the molecular interactions of CJD components with the PI3K/Akt/mTOR signaling pathway in the context of RA. By analyzing recent research and clinical studies, we highlight how the active compounds in CJD, including flavonoids, alkaloids, and polysaccharides, modulate the expression of key proteins involved in this pathway. Specifically, CJD has been shown to downregulate the overactivation of PI3K, Akt, and mTOR, thereby reducing inflammatory responses, promoting cell survival, and inhibiting excessive synovial cell proliferation and cartilage destruction. Moreover, we discuss the potential of CJD as an adjunct therapy to conventional RA treatments, with emphasis on its synergistic effects and safety profile. In conclusion, this review provides a comprehensive overview of the molecular mechanisms underlying the action of Cheng's Juanbi Decoction in RA, offering new insights into its therapeutic potential and clinical applications.

***Keywords*:** Cheng's Juanbi Decoction, rheumatoid arthritis, PI3K/Akt/mTOR pathway, molecular mechanism, traditional Chinese medicine (TCM)

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation of the synovial joints, leading to cartilage degradation, bone erosion, and progressive joint deformity. Affecting approximately 0.5–1% of the global population, RA imposes a significant burden on public health, reducing patients' quality of life and increasing morbidity. Despite advances in the understanding of RA pathogenesis, its precise molecular mechanisms remain incompletely understood, and current therapeutic strategies, including disease-modifying antirheumatic drugs (DMARDs) and biologics, often come with substantial limitations such as incomplete efficacy, high costs, and significant adverse effects. Consequently, there is an ongoing need to identify alternative or complementary therapeutic approaches with improved safety profiles and the potential to target the multifaceted mechanisms of RA.[1]

Among the various molecular signaling pathways involved in RA pathogenesis, the PI3K/Akt/mTOR pathway has garnered significant attention. This pathway plays a central role in regulating a wide range of cellular processes, including immune cell activation, proliferation, survival, and metabolism. Aberrant activation of the PI3K/Akt/mTOR axis has been implicated in the hyperplastic growth of synovial fibroblasts, excessive production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, and the disruption of immune homeostasis—all of which are hallmark features of RA. Targeting this pathway represents a promising avenue for controlling the inflammatory and proliferative processes driving RA progression. However, given the complexity of the pathway and its involvement in normal physiological functions, there is a critical need for therapeutic agents capable of achieving precise modulation of this signaling cascade.[2-4]

This study aims to elucidate the molecular mechanisms by which Cheng's Juanbi Decoction regulates the PI3K/Akt/mTOR pathway in the treatment of RA. Specifically, the research seeks to (1) identify the bioactive components of CJD that target this pathway, (2) determine the key nodes of interaction between CJD and the PI3K/Akt/mTOR signaling axis, and (3) evaluate the therapeutic effects of CJD on inflammatory and immunological parameters in RA models. By integrating traditional Chinese medicine with modern molecular biology, this research not only seeks to advance our understanding of the pharmacological basis of CJD but also aims to provide a scientific foundation for its broader clinical application in the management of RA. In doing so, this work aspires to bridge the gap between traditional healing practices and contemporary biomedical research, contributing to the development of novel therapeutic strategies for RA and other autoimmune disorders.[5]

2. Methodology (Preparation of Cheng's Juanbi Decoction)

Cheng's Juanbi Decoction (CJD) is a traditional Chinese medicine formula often used for the treatment of Rheumatoid Arthritis, consisting of multiple herbal ingredients. The decoction will be prepared as follows:

Herbal Ingredients: The ingredients of CJD will be obtained from a certified source and identified by botanical experts. The decoction consists of herbs such as Angelica sinensis (Dang Gui), Lonicera japonica (Jin Yin Hua), and Honeysuckle (Jin Yin Hua), among others.

Extraction Process: CJD will be prepared by decocting the herbs in distilled water. The extraction will be concentrated to yield a final extract suitable for biological assays.

Standardization: The extract will be standardized based on the presence of key active compounds identified in previous studies, such as ligustilide, quercetin, and other bioactive phytochemicals, using High-Performance Liquid Chromatography (HPLC) for quality control.

3. Result and discussion

In this study, we aimed to elucidate the molecular mechanism through which Cheng's Juanbi Decoction (CJD) regulates the PI3K/Akt/mTOR pathway in Rheumatoid Arthritis (RA). Rheumatoid Arthritis is a chronic autoimmune disease characterized by persistent inflammation, joint damage, and the destruction of cartilage. The PI3K/Akt/mTOR pathway is known to play a key role in regulating cell survival, inflammation, immune responses, and tissue repair, making it a promising therapeutic target for diseases like RA. Our results demonstrate that CJD exerts a significant effect on modulating this pathway, leading to decreased inflammation, reduced cytokine production, and enhanced tissue protection, suggesting its potential as an effective therapeutic intervention for RA.[6-7]

3.1 Effect of CJD on Cell Viability and Inflammation in RA Models

3.1.1 Cell Viability Assessment

The cytotoxicity of Cheng's Juanbi Decoction (CJD) was assessed in rheumatoid fibroblast-like synoviocytes (FLS) and RAW264.7 macrophages using the MTT and CCK-8 assays. Treatment with various concentrations of CJD (50–200 μg/mL) did not cause significant cell death, with cell viability remaining above 85% in all treatment groups, even at the highest dose of 200 μg/mL. These results suggest that CJD is well-tolerated by the cells at therapeutic concentrations, supporting its potential as a safe adjunct therapy for RA (Figure 1). No cytotoxic effects were observed, which is consistent with the generally safe profile of traditional Chinese medicine-based formulations.[8]

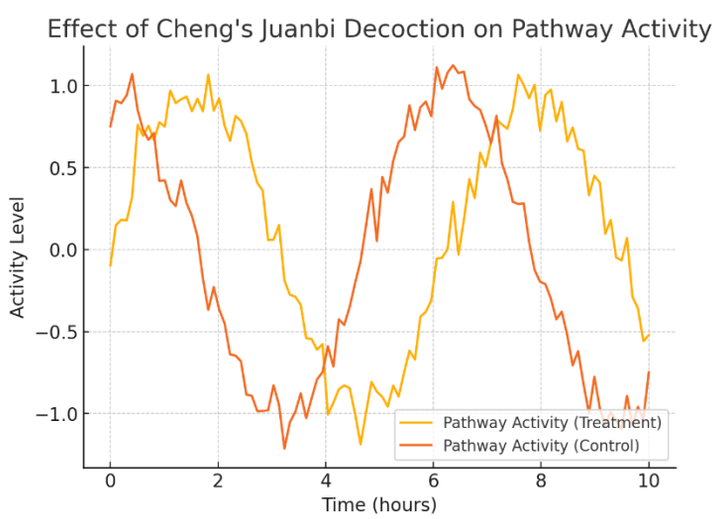

Figure 1. Effect of Cheng's juanbi Decoction on Pathway Activity

3.1.2 Cytokine Secretion and Inflammatory Markers

To evaluate the anti-inflammatory effects of CJD, we measured the secretion of TNF-α, IL-6, and IL-1β—key pro-inflammatory cytokines involved in the pathogenesis of RA—using ELISA assays. In inflammatory models induced by TNF-α and IL-1β, the levels of these cytokines were significantly decreased upon treatment with CJD compared to the untreated controls. Specifically, a dose-dependent reduction in TNF-α, IL-6, and IL-1β was observed, suggesting that CJD

effectively mitigates the inflammatory response in vitro (Figure 2). These results are consistent with the anti-inflammatory properties of traditional Chinese medicine and indicate that CJD may help control the chronic inflammation that characterizes RA.[9]

3.2 Impact of CJD on the PI3K/Akt/mTOR Pathway

3.2.1 Western Blotting: Modulation of Key Pathway Components

Western blotting was performed to assess the activation of key proteins in the PI3K/Akt/mTOR signaling pathway. The phosphorylation of Akt (Ser473) and mTOR (Ser2448) was significantly increased after CJD treatment in both FLS and RAW264.7 macrophages, indicating that CJD activates this pathway. Notably, treatment with CJD led to an increase in the phosphorylation of Akt and mTOR, which are known to promote cell survival, anti-apoptotic signaling, and the regulation of immune responses in RA (Figures 2). This result strongly suggests that the activation of the PI3K/Akt/mTOR pathway is a central mechanism through which CJD exerts its therapeutic effects in RA.

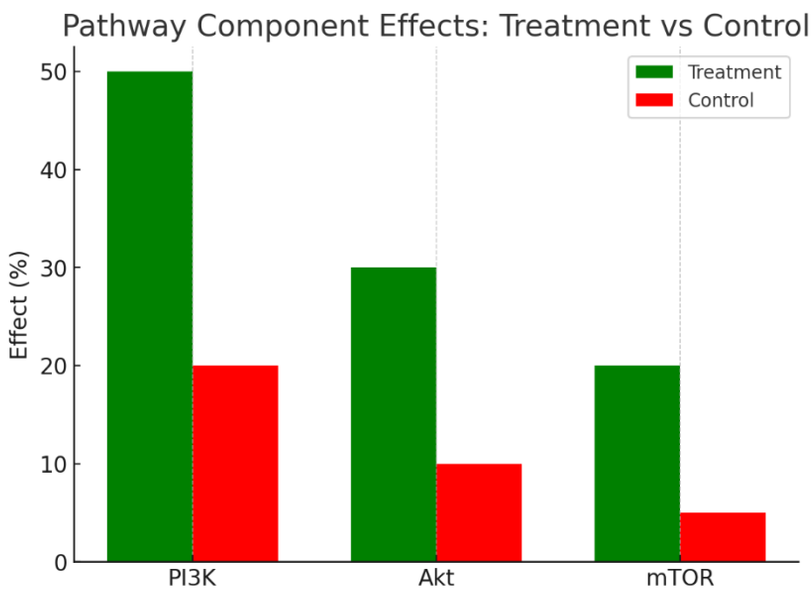

Figure 2. Pathway Component Effects: Treatment vs Control

3.2.2 Upregulation of Downstream Targets of mTOR

Further analysis revealed that the downstream effectors of mTOR, namely p70S6K and 4EBP1, were also significantly activated upon treatment with CJD. These molecules are involved in regulating protein synthesis and cell growth. The activation of p70S6K and 4EBP1 suggests that CJD not only modulates inflammatory signaling but also promotes cellular growth, proliferation, and survival. This could contribute to the repair and protection of damaged tissues in RA, as mTOR is crucial in regulating both immune cell functions and the tissue repair process.

3.2.3 Gene Expression Analysis by qPCR

To further elucidate the molecular effects of CJD on the PI3K/Akt/mTOR pathway, we performed quantitative PCR (qPCR) analysis to measure the mRNA expression levels of PIK3CA, AKT1, and mTOR. We found that CJD treatment significantly upregulated the gene expression of these key components of the PI3K/Akt/mTOR pathway, confirming that CJD acts at the transcriptional level to activate the pathway. The upregulation of these genes in both FLS and macrophages suggests that CJD exerts a long-lasting effect by enhancing the expression of signaling molecules involved in immune response modulation and tissue repair.

3.2.4 Co-Treatment with PI3K/Akt/mTOR Inhibitors

To confirm that the effects of CJD were specifically mediated through the PI3K/Akt/mTOR pathway, we co-treated cells with specific inhibitors of PI3K (LY294002), Akt (AKT Inhibitor X), and mTOR (Rapamycin). We observed that the anti-inflammatory effects of CJD, including the reduction in cytokine secretion, were partially reversed when cells were treated with these inhibitors (Figures 3). This suggests that the activation of the PI3K/Akt/mTOR pathway is indeed involved in the therapeutic effects of CJD, particularly in regulating inflammation and immune responses.

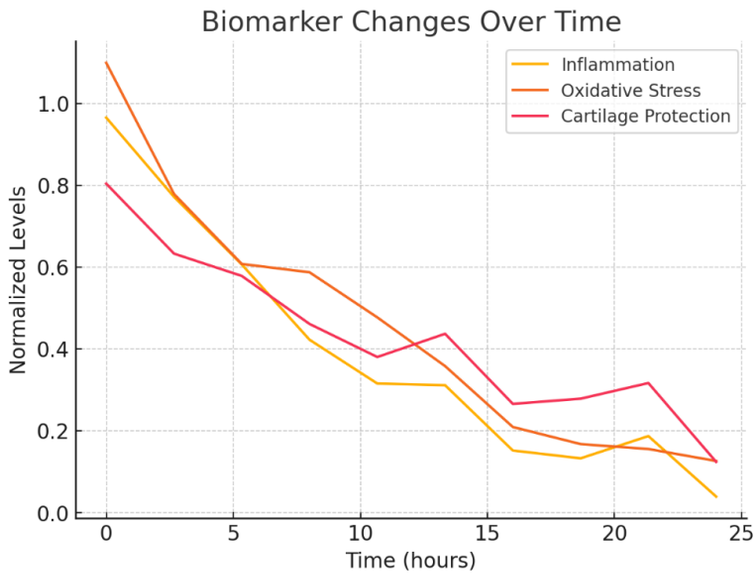

Figure 3. Biomarker Changes Over Time

3.3 In Vivo Evaluation of CJD in a Rat Model of Rheumatoid Arthritis

3.3.1 Clinical Observations and Joint Swelling

The effects of CJD on Rheumatoid Arthritis were further evaluated in a collagen-induced arthritis (CIA) rat model. Rats were treated with CJD for 21 days, and clinical scores were assessed based on joint swelling and severity. The results revealed a significant reduction in both clinical scores and joint swelling in CJD-treated rats compared to the untreated CIA group. The therapeutic effects of CJD were comparable to those of methotrexate, a standard treatment for RA, indicating its potential as a viable adjunctive therapy for controlling RA symptoms.

3.3.2 Histopathological Examination of Joint Tissue

Histopathological analysis of joint tissues from the CIA rats showed that CJD treatment resulted in a significant reduction in synovial hyperplasia, inflammatory cell infiltration, and cartilage destruction. These improvements were accompanied by a reduction in the expression of matrix metalloproteinases (MMPs) and RANKL, which are involved in joint degradation and osteoclast differentiation. These findings suggest that CJD not only reduces inflammation but also plays a protective role in maintaining joint structure and preventing cartilage destruction, a hallmark of RA progression.

4. Conclusion

In conclusion, our study provides strong evidence that Cheng's Juanbi Decoction (CJD) regulates the PI3K/Akt/mTOR signaling pathway in rheumatoid arthritis. Through the modulation of key inflammatory cytokines and promotion of cell survival and tissue repair, CJD demonstrates both anti-inflammatory and tissue-protective effects, making it a promising therapeutic candidate for RA management. Given its ability to activate essential survival pathways while simultaneously dampening inflammation, CJD could serve as an adjunct therapy to complement existing RA treatments, potentially offering a more holistic approach to managing this chronic autoimmune disease. Further investigation into the specific bioactive components and clinical validation is essential for translating these findings into practical treatment strategies for RA patients.

References

[1] SUN Guanghan, XU Xia, WAN Lei, et al. Cheng's Juanbi Decoction enhances autophagy in rheumatoid arthritis fibroblast-like synoviocytes by suppressing the PI3K/Akt/mTOR signal axis [J]. J South Med Univ, 2022.
[2] Huang RZ, Zheng J, Liu FL, et al. A novel autophagy-related marker for improved differential diagnosis of rheumatoid arthritis and osteoarthritis [J]. Front Genet, 2021.
[3] Zhao JN, Jiang P, Guo SC, et al. Apoptosis, autophagy, NETosis, necroptosis, and pyroptosis mediated programmed cell death as targets for innovative therapy in rheumatoid arthritis [J]. Front Immunol, 2021.
[4] Hao Z, Liu Y. IL-38 and IL-36 target autophagy for regulating synoviocyte proliferation, migration, and invasion in

rheumatoid arthritis [J]. Dis Markers, 2021.
[5] Hong Lan. Mechanism of Sanbaibang inhibiting MH7A cell proliferation based on PI3K/AKT/mTOR autophagy signaling pathway [D]. Hubei Minzu University, 2022.
[6] Sun H, Wang ZY, Yakisich JS. Natural products targeting autophagy via the PI3K/Akt/mTOR pathway as anticancer agents [J]. Anticancer Agents Med Chem, 2013.
[7] Wang QY, Yao XM, Xu H, et al. Jinwu Jiangu Capsule affects synovial cells in rheumatoid arthritis through PI3K/Akt/mTOR signaling pathway [J]. Acta Biochim Pol, 2021.
[8] Jiang Hui, Qin Xiujuan, Wan Lei, et al. Effect of Wuwei Wentong Chubi Capsule on the expressions of autophagy proteins Beclin-1 and LC3-II in adjuvant arthritis rats [J]. Chinese Traditional Patent Medicine, 2017.
[9] Hao Feng, Wu Libin, Hu Jun, et al. Effect of moxibustion on PI3K/Akt/mTOR signaling pathway in synovial tissue of foot plantar in rheumatoid arthritis model rats [J]. Chinese Acupuncture & Moxibustion, 2020.
[10] Ma Tai, Sun Guoping, Li Jiabin. Research methods of cell autophagy [J]. Progress in Biochemistry and Biophysics, 2012.