Tripterygium wilfordii polycoride regulate UC inflammatory ferroptosis process through S100A8/TLR4/NF-κB signaling pathway

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Aim of the study:
Ulcerative colitis is a condition characterized by chronic and progressive inflammation of the intestinal tract. It has been observed that ferroptosis, a form of cell death, can stimulate an immune response and contribute to intestinal inflammation. *Tripterygium wilfordii* polycoride (TWP) have shown promising therapeutic effects in treating UC, although the exact mechanism of action remains uncertain.
Materials and methods:
This study is divided into three parts. Firstly, to investigate the relationship between ferroptosis and inflammation, THP-1 cells were subjected to in vitro experiments using LPS and the ferroptosis inhibitor (Fer-1). In addition, UC mouse models were constructed using DSS for in vivo experiments, and various indicators including S100A8, TLR4, NF-κB, inflammatory factors, and ferroptosis-related markers were examined. Secondly, the involvement of the S100A8/TLR4/NF-κB signaling pathway in the process of intestinal ferroptosis in UC was explored. This was achieved by constructing a S100A8 knockdown cell model in vitro, inducing THP-1 cell ferroptosis with LPS, and creating a S100A8-KO mouse model in vivo. In these models, colonic inflammation was induced by DSS, and the TLR4/NF-κB pathway, inflammatory factors, and ferroptosis-related indicators were assessed. Finally, the mechanism of TWP was studied by conducting in vivo and in vitro experiments, evaluating the TLR4/NF-κB pathway, inflammatory factors, and indicators related to iron death.
Results:
In vitro experiments demonstrated that LPS stimulation resulted in an increased expression of S100A8, TLR4, NF-κB, TNF-α, and IL-1β in THP-1 cells. This stimulation also led to a decrease in cell viability, an increase in intracellular ferrous ions, lipid peroxidation (MDA), and ROS levels. Additionally, the expression of PTGS2, FTH, FTL, and P53 increased, while the expression of GPX4 and SLC7A11 decreased. However, treatment with TWP significantly improved these indicators. Knockdown of S100A8, TLR4, NF-κB, TNF-α, and IL-1β resulted in decreased intracellular iron ion content, reduced ROS fluorescence, decreased MDA levels, and decreased expression of PTGS2, FTH, FTL, and P53. On the other hand, the expression of GPX4 and SLC7A11 increased. In vivo experiments demonstrated that TWP attenuated total weight loss, shortened colon length, disease activity index (DAI)

score, and histological damage in DSS-induced mice. Furthermore, TWP decreased the expressions of S100A8, TLR4, NF-κB, TNF-α, IL-1β, PTGS2, FTH, FTL, and P53, while increasing the expressions of GPX4 and SLC7A11. After S100A8 knockout, the expressions of various inflammatory factors, as well as PTGS2, FTH, FTL, and P53, decreased, while the expressions of GPX4 and SLC7A11 increased. **Conclusion:** our study demonstrates that S100A8 plays a crucial role in regulating intestinal ferroptosis in patients with ulcerative colitis (UC). We have identified the S100A8/TLR4/NF-κB pathway as the main mechanism underlying UC ferroptosis. Furthermore, our findings indicate that treatment with TWP effectively inhibits ferroptosis by suppressing the S100A8/TLR4/NF-κB pathway, thereby alleviating intestinal inflammation associated with UC.