

DOCTORAL THESIS

Therapeutic effects and the underlying mechanisms of qing-dai powder against experimental colitis in mice

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Date of Award:
2015

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ABSTRACT

Ulcerative colitis (UC), a subset of inflammatory bowel disease (IBD), is a chronic uncontrolled inflammatory condition of the intestinal mucosa. As its etiology remains unclear, no specific effective treatment is available. Therefore, development of novel strategies for IBD treatment remains a major medical need. Qing-dai Powder (QDP), an ancient herbal medicinal formula, exerted potent therapeutic effect on intractable UC patients; however, evidence-based support is needed. The aims of this study are: i) to delineate the anti-colitis effect of QDP and its underlying mechanisms in murine colitis; 2) to explore the rationality of QDP formula; 3) to investigate the anti-colitis effects of major component(s) or/and active ingredient(s) of QDP and their underlying mechanisms in murine colitis.

In the present study, the therapeutic effect of QDP on UC was investigated on dextran sulfate sodium (DSS)-induced acute murine colitis. Results showed that i) QDP dose-dependently attenuated disease activity index (DAI), colon shortening, histological damage and colonic myeloperoxidase (MPO) activity of DSS-treated mice; ii) QDP significantly decreased the infiltration of immune cells, particularly macrophages and CD4⁺ T cells, colonic levels of pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6, and plasma level of chemokine MCP-1. In RAW 264.7 cells, QDP significantly suppressed lipopolysaccharide (LPS)-induced the production of TNF- α and IL-6, and the expression levels of COX-2 and iNOS via inhibiting I κ B- α degradation and p65 nuclear translocation; Also, in primary CD4⁺ T cells, QDP significantly suppressed the differentiation of Th1 and Th17 cells. These findings indicate that the anti-colitis effects of QDP might be associated with inhibition of inflammatory responses of colonic macrophages and CD4⁺ T cells.

QDP is composed of Qing-dai and Ku-fan. The comparative study of anti-colitis of QDP, Qing-dai and Ku-fan revealed that QDP is a reasonable TCM formula, and Qing-dai is mainly responsible for the anti-colitis effect of QDP and Ku-fan exhibits a weak beneficial effect. Mechanistically, it was found that Qing-dai significantly suppressed Th1 and Th17 responses, characterized as i) suppressing mRNA expression of Th1 cytokine IFN- γ and Th17 cytokine IL-17A, inhibiting the production of Th1 and Th17-related cytokines IFN- γ , IL-17A/F and TNF- α in the colon of DSS-treated mice; ii) restraining the proportions of Th1 and Th17 cells in mesenteric lymph nodes of DSS-treated mice; iii) suppressing the differentiation of Th1 and Th17 cells *in vitro*.

Indirubin is the principle active component of Qing-dai. It was found that indirubin significantly suppressed the generation of Th17 cells in DSS-treated mice, evidenced by i) suppressing the mRNA expression of IFN- γ , IL-17A, and ROR γ t, and inhibiting the production of IL-17A/F, TNF- α , IL-1 β and IL-6 in the colon of DSS-treated mice; ii) reducing Th17 cells in mesenteric lymph nodes of DSS-treated mice through reducing GSK-3 β activity and p-STAT3 expression; iii) suppressing the differentiation of Th17 cells through down-regulating the expression of GSK-3 β and p-STAT3 *in vitro*.

In summary, the present study provides evidence-based support for the clinical use of QDP in the management of UC, and indicates that indirubin is the main active compound of QDP responsible for its anti-colitis effect.

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