UNIVERSITI TEKNOLOGI MARA

INTERACTIONS OF SELECTED FLAVONOIDS WITH MESALAZINE AND BUDESONIDE IN CCD18-Co CELLS AND DSS-INDUCED COLITIS MODEL

OMAR ABDULHAFIZ ALHARASSTANI

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ABSTRACT

Background and Objective: Although some phytochemicals demonstrate a wide range of health benefits, thought to be by directly targeting the underlying cascades responsible for the production of key pro-inflammatory mediators involved in inflammatory bowel disease (IBD), no study has compared the efficacy of current IBD treatments with such phytochemicals and their combinations or the possible drug-drug synergism that would be applicable to IBD treatment. This study therefore aimed to compare the interactions of selected promising natural phytochemicals which have been assessed in recent years for their therapeutic potentials, namely gallic acid (trihydroxybenzoic acid), thymoquinone, and bryonolic acid and their combinations, with current IBD treatments such as aminosalicylates (mesalazine) and corticosteroids (budesonide) in *in vitro* model and in an acute DSS-induced colitis animal model utilizing modern isobolographic analysis. Recent research employed *in vitro* screening of anti-inflammatory activities of phytochemicals using CCD18-Co human colon fibroblast cell line which was employed as the *in vitro* model in this study. Cells were treated with the selected phytochemicals either individually or in combination with current IBD treatments (mesalazine and budesonide) and co-stimulated with LPS for selected cytokines assays to detect synergistic response of the following cytokines: IL-2, IL-4, IL-6, IL-10, IL-17A, IL-23, TNF-α, IFN-γ and PGE2. For the *in vivo* study, induction of colitis in mice was achieved by daily challenge with 5% DSS in drinking water for 7 days. Mice groups were orally treated with different drugs and/or combinations on a daily basis. Disease scores (bleeding, diarrhoea and stool consistency) was observed, systemic effects (total body weight loss, serum cytokine levels and anti-neutrophil cytoplasm antibodies (ANCAs) levels were quantified by flow cytometry and ELISA assays, colonic tissue myeloperoxidase (MPO) activity was measured by standard colourimetric assay, histopathology, inflammatory parameters such as erythrocyte sedimentation rate, C-reactive protein, and faecal calprotectin were measured using conventional ELISA method and flow cytometry. Results showed that all flavonoids had efficacy *in vitro* & *in vivo*, of the three flavonoids tested, thymoquinone is the most efficacious compared to gallic acid while bryonolic acid was the least efficacious. Current treatment of mesalazine and budesonide are efficacious in both *in vitro* & *in vivo* models. The three flavonoids showed synergistic effect with mesalazine or budesonide on multiple parameters, however, the most significant synergism was observed between thymoquinone and mesalazine or thymoquinone and budesonide. The two standard treatments mesalazine and budesonide synergize with each other. All flavonoids (thymoquinone, gallic acid and bryonolic acid) showed inhibition of pro-inflammatory cytokines *in vitro* & *in vivo* but, the most potent is thymoquinone, accounting for its significant synergism with mesalazine and budesonide, resulting in clinical improvement of DSS-challenged acute colitis mice with the underlying shift of the immune paradigm towards Th2 mediated response while enhancing the expression of Th2 anti-inflammatory cytokines. Thus, nominating thymoquinone as a promising adjunctive therapy to mesalazine or budesonide for IBD and UC.

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