REGULAR ARTICLE

Synergistic effect of probiotics, butyrate and L-Carnitine in treatment of IBD

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Abstract Genetic, environmental factors, dysregulation of immune system, intestinal microbes and oxidative stress are the most important factors that play the role in the pathogenesis of inflammatory bowel disease (IBD). Current treatments do not always result in complete remission and usually accompanied with several adverse effects. Recent studies showed that nuclear factor-kappa B (NF-κB), tumor necrosis factor-α (TNF-α) and oxidative stress play the pivotal role in the induction of inflammation. Butyrate, L-Carnitine, and probiotics have the potential to control inflammation by reduction of main inflammatory cytokines, including NF-κB and TNF-α. They also stimulate antioxidant enzymes and inhibit IxB kinase (IKK). Regarding the beneficial effects of these three compounds in inflammation via several mechanisms, we hypothesize that the mixture of these compounds would be synergistically effective in reduction of inflammation and alleviation of IBD. Further experimental investigations are needed, to evaluate the hypothesis.

Introduction

Inflammatory bowel disease (IBD), representing as ulcerative colitis (UC) or Crohn’s disease (CD), with an increasing incidence, can be debilitating in affected patients. It associates with colonic mucosal inflammation that causes bloody diarrhea and abdominal pain. Although, activation of inflammatory process and oxidative stress are believed to be the main causes to the disease, but the exact pathophysiology of IBD is under debate yet [1–4]. The most of currently used medicines can only reduce the severity of IBD symptoms and cannot cure the disease or do not result in complete remission [5]. Conventional treatments are accompanied with several adverse effects resulting in less tolerability and compliance in patients [2,6,7]. Therefore, in the recent years, many investigations have been done to develop new natural, non-synthetic
compounds to be safer with lesser adverse effects besides more efficacies [4,8,9]. Previous studies have shown the efficacy of butyrate, l-Carnitine, and probiotics in IBD when used alone or in combination with conventional therapies [10–13]. These compounds have the potential to control inflammation by reduction of main inflammatory cytokines, the nuclear factor-kappa B (NF-κB) and tumor necrosis factor-α (TNF-α), which play the pivotal role in induction and progress of IBD [14–17]. The protective effect of butyrate, l-Carnitine, and probiotics against oxidative stress, is well documented based upon the results from previous studies.

Butyrate is produced naturally by bacterial fermentation in colonocytes. It affects IκB kinase (IKK) by suppressing phosphorylation of IκB-α or IκB-β. This results in reducing the stimulation of NF-xB pathway and thus decreases the level of reactive oxygen species (ROS) [2,16,18–23]. In addition, butyrate prevents elevation of TNF-α which occurs during the process of inflammation. Then, silencer of death domain (SODD) dissociates from adaptor protein so called TNF receptor type 1-associated death domain protein (TRADD), which finally activates the ribosome inactivating protein (RIP) and TNF receptor associated factor 2 (TRAF2). These activated proteins stimulate IKK and NF-κB [14,24].

On the other hand, probiotics have preventive effects on the induction of NF-xB, either directly or indirectly. They can delay degradation of IκB-α, so they inhibit the activation of NF-xB, expression of TNF-α and other pro-inflammatory cytokines. In addition, by producing anti-oxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), probiotics act against ROS, which induces IKK [18,25].

The third compound l-Carnitine (β-hydroxy-γ trimethylamino butyrate) has anti-oxidant activity as mainly reduces myeloperoxidase (MPO) and malondialdehyde (MDA), two active elements of oxidative stress. l-Carnitine up-regulates SOD and prevents reduction of glutathione (GSH). It also inhibits accumulation of lipid peroxides. Therefore, it suppresses the formation and the activation of ROS, resulting in inhibition of NF-κB pathway [15,20,23,26] (Fig. 1).

The hypothesis

It is revealed that suppression of NF-κB through inhibition of IKK or via stimulating of antioxidant enzymes may suppress oxidative stress. Based on the beneficial effects of butyrate, l-Carnitine and probiotics in inflammation, via mentioned mechanisms, we hypothesize that combination of these compounds would be synergistically effective in reduction of inflammation.

Some studies support the present idea. For instance, probiotics cause an over expression in sodium-coupled monocarboxylate transporter 1 (SMCT1) gene, which is responsible for butyrate’s absorptions. This gene is down regulated by TNF-α. In addition, over expression of l-Carnitine’s transporter (OCTN2) by Saccharomyces boulardii (a type of probiotics), and enhancement in the metabolism of butyrate (β oxidation) by l-Carnitine have been demonstrated. Therefore, by considering these interactions, the combination effect might be a more advantageous in IBD treatment [22].

Evaluation of the hypothesis

To evaluate the efficacy of combination treatment, first, an appropriate experimental model of IBD should be set up.

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**Figure 1** The effects of butyrate, l-Carnitine, and probiotics on IKK. SOD: Superoxide Dismutase; CAT: Catalase; IKK: IκB Kinase; iNOS: inducible Nitric Oxide Synthase; NO: Nitric Oxide; NF-κB: Nuclear Factor-kappa B; RIP: Ribosome Inactivating Protein; ROS: Reactive Oxygen Species; TNF: Tumor Necrosis Factor; TNF-R: Tumor Necrosis Factor Receptor; TRADD: Tumor Necrosis Factor Receptor Type 1-Associated Death Domain Protein; TRAF2: Tumor Necrosis Factor Receptor Associated Factor 2; SODD: Silencer of Death Domain.
Among several models, dextran or trinitrobenzene sulfonic acid (TNBS) induced colitis, are similar models to human IBD with the most reproducibility. To set up the study, seven groups, including, sham, negative, positive control, and four treatment groups (each compound alone or in combination) should be assigned. To prepare the mixture, containing these three compounds, a proper formulation should be provided. The effective doses of each compound and duration of treatment should be adjusted based on literature and previous studies [27]. After the treatment period, macroscopic, microscopic and biochemical bio markers of colon should be evaluated. The main factors of bowel inflammation such as oxidative stress markers and inflammatory cytokines, including TNF-$\alpha$ and NF-$\kappa$B, should be measured and compared among treatment groups [14,16,21,23,28,29]. Given that dextran and TNBS-induced colitis models are safe used in IBD patients as a novel and desirable combination. By adding this combination to chemical therapy of patients, then the doses of chemicals and thus chance of adverse effects might be reduced.

Discussion

Current treatments of IBD such as corticosteroids, immunosuppressive agents and 5-aminosalicylic acid (5-ASA) are not completely curative and satisfying. Recent studies have focused upon the role of NF-$\kappa$B, TNF-$\alpha$ and oxidative stress in the induction of inflammation. Regarding the positive effects of butyrate, L-Carnitine and probiotics via regulation of main inflammatory cytokines and anti-oxidant enzymes, we hypothesize that the combination of these compounds would be synergistically effective in reducing inflammation and alleviation of IBD.

These compounds have protective effects against oxidative stress through several mechanisms. Butyrate inhibits degradation of IkBx bound NF-$\kappa$B (inhibitory protein) by suppression of IKK. In colonic tropism, butyrate stimulates the proliferation of immature epithelial cells at the base of colonic crypts and improves electrolyte absorption that leads to colonic repair stimulation [30].

Several investigations have reported that probiotics such as Lactobacillus casei, Bifidobacterium bifidum, and Saccharomyces boulardii recover immune colitis and reduce pro-inflammatory mediators inside the lamina propria of inflamed mucosa [31,32]. They activate T-cells via Th1 or Th2 immune responses. Some studies have shown that probiotics decrease the production of TNF-$\alpha$, and the activation of NF-$\kappa$B cascade. They probably affect the innate immunity cells [33–37].

It has been clearly demonstrated that L-Carnitine can act as an anti-inflammatory and anti-oxidant. It protects tissues from ROS-induced damages. Several mechanisms of action of L-Carnitine on T-cell activation remain hard to define. Several investigations have reported that probiotics such as Lactobacillus casei, Bifidobacterium bifidum, and Saccharomyces boulardii recover immune colitis and reduce pro-inflammatory mediators inside the lamina propria of inflamed mucosa [31,32]. They activate T-cells via Th1 or Th2 immune responses. Some studies have shown that probiotics decrease the production of TNF-$\alpha$, and the activation of NF-$\kappa$B cascade. They probably affect the innate immunity cells [33–37].

Considering the demonstrated beneficial interactions between probiotics, L-Carnitine and butyrate, when used in combination, such as effects on the metabolism or expression of each other, it is suggested that the mixture of these three compounds may be effective in treatment of IBD by via the common pathways.

Conclusion

In conclusion, mixture of butyrate, L-Carnitine and probiotics may have great anti-inflammatory and antioxidant properties via down regulation of TNF-$\alpha$, inhibition of NF-$\kappa$B, or suppression of ROS [19,20,23,25,27,28]. These benefits would be considered in therapy of IBD.

Conflict of interest statement

The authors have no conflict of interest. Authors try to test the idea in animal models. Authors acknowledge assistance of INSF and TUMS. Since Editor-in-Chief of the journal is one of authors, all review process was conducted by one of section editors.

Overview Box

First question: What do we already know about subject?
There are some information about pathogenesis of IBD, therapeutic methods and alternative therapies. Recent studies have shown positive effects of butyrate, L-Carnitine and probiotics in IBD when used with conventional therapies.

Second question: What does your proposed theory add to the current available knowledge and what benefits does it have?
Mixture of butyrate, L-Carnitine and probiotics can be safely used in IBD patients as a novel and desirable combination. By adding this combination to chemical therapy of patients, then the doses of chemicals and thus chance of adverse effects might be reduced.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?
Evaluation of inflammatory cytokines such as TNF-$\alpha$, NF-$\kappa$B, oxidative stress markers and, macroscopic and biochemical factors by setting up an appropriate colitis model is proposed.

References

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