Oral delivery of *Bifidobacterium longum* expressing α-melanocyte-stimulating hormone to combat ulcerative colitis

Pijin Wei,† Yan Yang,† Qing Ding, Xiuying Li, Hanxiao Sun, Zhaobing Liu, Junli Huang and Yahui Gong

Institute of Genomic Medicine Research, College of Pharmacy, Jinan University, Guangzhou, 510632, PR China

α-Melanocyte-stimulating hormone (α-MSH) is a tridecapeptide derived from pro-opiomelanocortin that exhibits potent anti-inflammatory properties by regulating the production of inflammatory mediators. This peptide has been well established in several inflammatory models, including inflammatory bowel disease (IBD). However, its extremely short duration in vivo limits its clinical application. To address this limitation, *Bifidobacterium* was used here as a carrier to deliver α-MSH. We utilized α-MSH-engineered *Bifidobacterium* against IBD, which is closely linked to immune and intestinal microbiota dysfunction. First, we constructed a *Bifidobacterium longum* secreting α-MSH (*B. longum*-α-MSH). We then tested the recombinant α-MSH expression and determined its bioactivity in HT-29 cells. To assess its effectiveness, *B. longum*-α-MSH was used against an ulcerative colitis (UC) model in rats induced by dextran sulfate sodium. The data showed that α-MSH expression in *B. longum*-α-MSH was effective, and its biological activity was similar to the synthesized one. This UC model experiment indicated that *B. longum*-α-MSH successfully colonized the intestinal gut, expressed bioactive α-MSH and had a significant anti-inflammatory effect. The results demonstrate the feasibility of preventing IBD by using *B. longum*-α-MSH.

INTRODUCTION

α-Melanocyte-stimulating hormone (α-MSH) is a neuro-peptide derived from pro-opiomelanocortin. In the last few decades, numerous studies focusing on α-MSH have provided substantial evidence that this hormone exhibits potent protection, immunomodulation and anti-inflammatory effects. The immunomodulating and anti-inflammatory effects of α-MSH can be elicited via downregulation of pro-inflammatory cytokines and mediators such as IL-1, IL-2, IL-4, IL-6, IL-8, IL-1β, TNF-α and nitric oxide (NO), as well as upregulation of the anti-inflammatory cytokine IL-10 production (Brzoska et al., 2010). Moreover, an animal model experiment using α-MSH has been demonstrated in various inflammatory diseases, such as irritant and allergic contact dermatitis, vasculitis, inflammatory bowel disease (IBD), allergic airway inflammation, arthritis, acute pancreatitis, and liver, ocular and brain inflammation (Brzoska et al., 2008; Carniglia et al., 2013; Moraes et al., 2014; Tennoune et al., 2014). These diverse physiological and biological capabilities suggest that α-MSH may be a promising therapeutic drug for various inflammatory diseases as mentioned above (Brzoska et al., 2008). Given that the half-life of full-length α-MSH is only a few minutes in vivo (Temp et al., 2013), continuous injections are needed to maintain the therapeutic effects of the hormone. This limitation makes α-MSH unsuitable as a drug. Therefore, an efficient strategy for delivering α-MSH should be developed.

*Bifidobacterium* spp. are non-pathogenic bacteria that represent one of the most dominant groups of intestinal microflora in humans, and these species have a fundamental function from birth to old age (Martinez et al., 2013). Many studies have reported that *Bifidobacterium* spp. confer many health-promoting properties for humans, such as immunomodulatory and gastrointestinal functions; nutrient synthesis; improvement against lactose intolerance, diarrhea, cancer, inflammation, allergies, microbial infection; and serum cholesterol reduction (Medina et al., 2007; Quigley, 2011; Arena et al., 2014; Tsai et al., 2014). Given these beneficial effects, some strains of *Bifidobacterium* have been used as probiotics, which are widely used in food science, medicine and industry.

†These authors contributed equally to this work.

**Abbreviations:** α-MSH, α-melanocyte-stimulating hormone; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase; NO, nitric oxide; RBS, ribosome-binding site; rh, recombinant human; UC, ulcerative colitis.
Furthermore, *Bifidobacterium* has become popular in the field of medicine because it has high security, elicits prominent abilities of fermentation, resists the extreme intestinal environment and adapts to its specific local communities (Meile et al., 2008; Turroni et al., 2011).

IBD, which includes ulcerative colitis (UC) and Crohn’s disease, is an intractable disease. The exact aetiology of IBD is still unknown. A large amount of work in recent decades has made the problem less ambiguous, showing that IBD is related to certain factors like intestinal microflora and immune response (Strober et al., 2008). Considering the complicated nature of IBD, the intestinal microflora must be regulated, and anti-inflammatory action is needed for therapy.

Here, we undertook the construction of a *Bifidobacterium longum* expressing α-MSH, and its oral delivery to combat UC in a rat model induced by dextran sulfate sodium (DSS). We used *B. longum* as a delivery system to make the short half-life α-MSH peptide feasible for oral administration and to combine the regulation of intestinal flora with control of inflammatory reaction for IBD treatment.

**METHODS**

**Construction of Bifidobacterium secreting α-MSH.** The bacterial strains and plasmids used in this research are listed in Table 1. *Escherichia coli* DH5α was used for general cloning purposes and grown at 37 °C in Luria–Bertani broth with vigorous shaking. *Bifidobacterium* strains were grown in MRS (de Man, Rogosa and Sharp) medium supplemented with 0.05 % (w/v) L-cysteine at 37 °C under anaerobic conditions. The *hup* gene promoter and terminator were PCR amplified from *B. longum* HB15. The expression fragment was synthesized and consisted of an optimal ribosome-binding site (RBS), an amylase (*amyB*) signal sequence and the α-MSH gene, and the *hup* gene promoter and terminator were ligated into a shuttle vector, pDG7, to yield a 7.7 kb plasmid named pDGMSH (Fig. 1). Plasmid DNA preparation from *E. coli*, restriction-enzyme digestion, ligation and *E. coli* transformation were performed as described by Sambrook and Russell (2001). The plasmid was transformed into *B. longum* by electroporation, as described previously (Turroni et al., 2014). The transformants were screened via an agar-containing culture medium containing ampicillin (100 mg l⁻¹ final concentration) in anaerobic conditions. Transformed cells were cultivated in MRS agar broth containing 3 μg chloramphenicol ml⁻¹.

**Expression and identification.** Recombinant human α-MSH (rhα-MSH) was separated by sodium bis(2-ethylhexyl) sulfosuccinate PAGE, a small peptides separation system, and was detected by Western blotting. The levels of rhα-MSH in *Bifidobacterium* culture supernatants were measured quantitatively by ELISA. After 20 h, inoculation in buffered DMEM (Dulbecco’s Modified Eagle Medium) containing 3 μg chloramphenicol ml⁻¹ was performed as required under anaerobic conditions. The culture supernatants were collected and filtered through 0.22 μm membranes and 5 kDa ultrafiltration membranes (Millipore Amicon). Then the supernatants were precipitated with trichloroacetic acid (15 % w/v), and the pellets were dissolved in PBS. Sodium bis(2-ethylhexyl) sulfosuccinate PAGE was performed as described previously (Lu et al., 2007). Anti-α-MSH antibodies (LSBio) were used for Western blot analysis according to the manufacturer’s instructions. Synthesized α-MSH (GLS) was used as a standard. Secretion of rhα-MSH in cell culture supernatants was detected on properly diluted samples using an α-MSH ELISA kit (IBL-America) according to the manufacturer’s protocols at 0, 5, 10, 15, 20, 25 and 30 h.

**Bioactivity assay.** HT-29 cells were cultured in RPMI 1640 supplemented with 10 % (v/v) fetal bovine serum containing 10 μg ampicillin ml⁻¹ at 37 °C in humidified 5 % CO₂ until the experiments were performed. Cells were seeded into 24-well plates at a density of 2 × 10⁶ cells per well. Bifidobacterial culture supernatants were sterilized by filtration through 0.22 μm membranes and adjusted to an anticipated concentration (containing 80 pg rhα-MSH ml⁻¹). After removing the growth medium, the cells were incubated with bifidobacterial culture supernatants diluted 1:1 (v/v) with fresh medium. After 1 h of preliminary incubation, cells were exposed to 5 μg LPS (*E. coli; Sigma*) ml⁻¹. After another 4 h, cells were harvested for immunoblotting of NF-kB using an NF-kB p65 kit (Beyotime) as described previously (Fabrikant et al., 2013). The amount of released

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<th>Strain or plasmid</th>
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<td>Bacterial strain</td>
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<td><em>B. longum</em> HB15</td>
<td>Source of <em>hup</em> gene promoter and <em>hup</em> and transformation host</td>
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<td><em>E. coli</em> DH5α</td>
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<td>pDG7</td>
<td><em>Cal</em>, <em>Amp</em>; <em>E. coli–Bifidobacterium</em> shuttle vector</td>
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<td>pDGMSH</td>
<td><em>Cal</em>, <em>Amp</em>; pDG7 derivative containing a functional <em>hup</em> gene promoter, RBS, <em>amyB</em> signal sequence, <em>hup</em> gene terminator from <em>B. longum</em> and an α-MSH peptide gene</td>
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*Restriction endonuclease recognition sites are underlined.

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Histological evaluation. On day 7 of administration, tissue colon samples obtained from the middle colon were weighed and homogenized in 0.5% (w/v) hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer (pH 6.0). The homogenates were then centrifuged for 15 min at 25 000 g at 4 °C. Supernatants were collected for an MPO assay. Fifty microliters of each supernatant sample was added to 200 μl o-dianisidine solution (30 μl 20 mg o-dianisidine dihydrochloride ml⁻¹ and 30 μl 20 mM hydrogen peroxide added into 50 mM potassium phosphate buffer, pH 6.0). After terminating the reaction with sodium azide, the change in absorbance at 460 nm (A₄₆₀) was read using a microplate reader.

Cytokines and α-MSH measurements. Colon samples were weighed and homogenized in PBS at pH 7.2 containing a protease cocktail at 4 °C and centrifuged at 16 000 g for 10 min. The cytokine levels of TNF-α, IL-6 and IL-10 in the supernatants were determined by ELISA kits in accordance with the manufacturer’s instructions (GenStar). Similarly, the level of α-MSH in the colon tissue fluid was measured by an ELISA kit as described above.

Quantification of faecal bifidobacteria. Bifidobacterium quantification in the colon was determined using real-time PCR as described previously (Delroisse et al., 2008). Collected faecal matter (1 g) was serially diluted 10 times with sterile water, followed by centrifugation for 5 min at 110 g to remove large particles. Cells were collected by centrifugation for 10 min at 7100 g DNA was extracted using a DNA extraction kit (Pico Pure). Afterwards, a real-time PCR assay was performed with primers (forward 5’-GGGGCAATGGGTGAAAG-3’, reverse 5’-CCCCACATCCAGCATCCA-3’), a TaqMan minor groove binder probe (5’-AACAGGATTAGATACCC-3’) and purified target DNA. The amplified fragment length was 244 bp. The reaction mixture was held at 50 °C for 2 min and 94 °C for 10 min, followed by 40 cycles of amplification at 94 °C for 20 s, and finally at 60 °C for 1 min. Detection was performed using a sequence detection system (ABI Prism 7000; ABI Applied Biosystems). Log c.f.u. g⁻¹ was used to express the number of bifidobacteria.

NO determination. After weighing, the colon samples were homogenized in PBS at pH 7.2 and centrifuged at 25 000 g for 15 min at 4 °C. Supernatants were deproteinized by ultrafiltration by using an Ultraclar YM-10 membrane (Millipore Amicon). A Nitrite/Nitrate Colorimetric Assay kit was used according to the manufacturer’s instructions (Cayman) to detect NO levels.

Statistical analysis. All data in the figures and text are expressed as arithmetic means ± SEM. The data are representative of three or more independent experiments. The data were evaluated using GraphPad Prism 5.0. Statistical analysis for significant differences was performed using one-way ANOVA, where appropriate. P<0.05 was considered statistically significant.

RESULTS

Vector construction

pDG7 is an E. coli–Bifidobacterium shuttle vector, which was selected as the replication origin. To achieve increased expression, the constitutive and highly expressed hup promoter and terminator (Tena et al., 2014) were introduced into the expression system. To improve translation, we added an optimal RBS (Park et al., 2008) downstream of the hup promoter. The amyB signal peptide (Chenoll et al., 2015) was fused in frame with the α-MSH gene to facilitate the secretion of α-MSH from the cells. An oligonucleotide carrying a fusion of RBS, the amyB signal sequence and the α-MSH gene was synthesized using codon optimization for bifidobacteria (Reyes Escogido et al., 2007). To utilize the signal peptides, we designed two plasmids, pDG7MSH1 and pDG7MSH2, which consisted of signal peptides with different lengths, with the predicted amino acid sequences shown in Table 2.
α-MSH expression

To confirm the transformants of *B. longum* secreting α-MSH, supernatants from WT strains and transformants of *B. longum* were analysed by Western blotting. Western blot analysis showed that both pDGMSH1 and pDGMSH2 could produce α-MSH (Fig. 2). pDGMSH1 was barely expressed and was almost under the level of detection (Fig. 2, lane 2), which could be ascribed to the failure of the *B. longum* signal peptide to cleave the signal peptide. In pDGMSH2, the result showed a prominent band in the Western blot, indicating that rhα-MSH was released from the cells. Then, we determined the level of α-MSH secretion from pDGMSH2 over time. The result is shown in Fig. 3. The concentration of rhα-MSH in the supernatants increased along with cell multiplication. The increase was almost terminated when entering the stationary period (the concentration reached 287.72 ± 14.90 pg ml⁻¹ in 30 h). This result was possibly caused by the *hup* promoter, which is closely associated with cell multiplication. When the cells multiplied rapidly, α-MSH expression similarly increased in quantity. By contrast, α-MSH was expressed less when cells were entering the stationary period.

Anti-inflammatory activity of *B. longum-α-MSH* (*B. longum-pDGMSH2*)

To determine the biological activity of rhα-MSH expressed by *B. longum*-α-MSH, we tested its anti-inflammatory effect with an LPS-induced inflammatory model of HT-29 cells from colon epithelium. Generally, α-MSH executes its anti-inflammatory action via the NF-κB signal pathway (Fabrikant *et al.*, 2013). We measured the expression levels of NF-κB p65 and IL-8 after treatment with HT-29 cells with adjusted bifidobacterial culture supernatants and LPS. *B. longum*-α-MSH supernatants (containing 40 pg rhα-MSH ml⁻¹) could significantly inhibit NF-κB p65 expression compared with that of the LPS (*P* < 0.001) and WT (*P* < 0.01) group (Fig. 4a), indicating that rhα-MSH was bioactive. From the given data, we observed that WT culture supernatant markedly inhibited NF-κB production (*P* < 0.01 compared with the LPS group), suggesting that *B. longum* HB15 can attenuate inflammation (Fig. 4b). With regard to the IL-8 secretion test, the functional result was found to be similar to NF-κB p65 (Fig. 4c).

Effect of orally administering *B. longum-α-MSH* to combat DSS-induced colitis

Colitis is characterized by many factors, such as increased MPO activity, unbalanced inflammatory cytokines, elevated NO and colon histological damage. We analysed these factors to evaluate the effects of *B. longum-α-MSH* on colitis treatment. Rats were orally administered daily with *B. longum* or not for 7 days after colitis induction. The levels of MPO, TNF-α, IL-6, IL-10 and NO were measured at 1, 3, 5 and 7 days, and histological evaluation was performed.

The MPO enzyme is closely related to IBD. In our experiments, the data reflected that MPO activity of colon tissues on day 7 was significantly (*P* < 0.01) reduced in the *B. longum-α-MSH*/DSS group compared with the DSS and WT/DSS groups (10.94 ± 0.84 vs 61.88 ± 7.07 and

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<th>Plasmid</th>
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<tr>
<td>pDGMSH1</td>
<td>MKHRKPAPAWHRGLSIKKVVGITAATAFGGGLAIASTAAQA ↓ SYSMEHFRWGTKPV</td>
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<tr>
<td>pDGMSH2</td>
<td>MKHRKPAPAWHRGLSIKKVVGITAATAFGGGLAIASTAAQA ↓ STSYSMEHFRWGTKPV</td>
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![Table 2. Predicted amino acid sequences encoded by the constructed pDGMSH plasmid](http://jmm.microbiologyresearch.org)

The α-MSH peptide is underlined, arrows indicate cleavage points, and bold shows the predicted signal peptidase recognition sequences.

![Fig. 2. Western blotting detection of rhα-MSH. Lane 1, WT; lane 2, pDGMSH1; lane 3, pDGMSH2; lane 4, synthesized α-MSH standard.](http://jmm.microbiologyresearch.org)

![Fig. 3. Growth (measured as OD₆₀₀) and α-MSH expression of *B. longum-α-MSH* at the indicated time points (0, 5, 10, 15, 20, 25 and 30 h).](http://jmm.microbiologyresearch.org)
43.73 ± 3.63, respectively), suggesting a reduced neutrophil infiltration in colon tissues (Fig. 5).

Cytokines serve a critical role in regulating IBD. The data showed that the levels of TNF-α and IL-6 were significantly attenuated ($P < 0.05$) in the $B. longum$-$\alpha$-MSH/DSS group (13.50 ± 0.57 and 94.67 ± 6.32 pg ml$^{-1}$, respectively) compared with the DSS group (38.09 ± 2.68 and 260.46 ± 8.08 pg ml$^{-1}$, respectively) and the WT/DSS group (18.64 ± 0.97 and 146.55 ± 6.22 pg ml$^{-1}$, respectively) (Fig. 6a, b). We then measured another cytokine, IL-10, which can exert a strong inflammation inhibitory function by inhibiting the expression of inflammatory cytokines such as IL-1α, IL-1β, IL-6, IL-12, IL-18 and TNF-α (Kuehbacher et al., 2008; Potas et al., 2015). This cytokine is regarded as a promising therapeutic treatment for IBD (Braat et al., 2003; Park et al., 2015). On day 7 after oral administration, we observed that IL-10 levels were significantly ($P < 0.01$) higher in the $B. longum$-$\alpha$-MSH/DSS group (380.29 ± 25.37 pg ml$^{-1}$). By contrast, the expression of IL-10 declined in the DSS group (87.51 ± 6.00 pg ml$^{-1}$) and the WT/DSS group (223.77 ± 13.93 pg ml$^{-1}$) (Fig. 6c).

It has been reported that pro-inflammatory cytokines induce the production of inducible nitric oxide synthase (iNOS), which is associated with promoted inflammation, and that abundant iNOS increases the production of free-radical NO (Huang et al., 2014). This tissue-fluid NO can contribute to inflammation, thus exacerbating colitis (Kriegstein et al., 2007; Chokshi et al., 2008). Compared with the DSS and WT/DSS groups, significantly reduced levels of NO were observed in the $B. longum$-$\alpha$-MSH/DSS group (8.47 ± 0.36 vs 24.01 ± 1.52 and 21.60 ± 1.01, respectively) (Fig. 7).
To determine the growing states of Bifidobacterium in the intestines, quantification was performed using real-time PCR. As shown in Fig. 8(b), the number of Bifidobacterium continuously increased in the WT and B. longum-α-MSH groups, whereas in the DSS group, the amount of B. longum decreased. Along with B. longum-α-MSH multiplication, augmentation of rhα-MSH concentration in colon tissue fluid was found, suggesting that B. longum-α-MSH could successfully express rhα-MSH in the colon (Fig. 8a). To further assess the therapeutic efficacy of B. longum-α-MSH, we observed its protective effects in colon faecal matter. ***P<0.001 vs WT/DSS group, **P<0.001 vs WT/DSS group.

**Fig. 6.** TNF-α (a), IL-6 (b) and IL-10 (c) expression levels in the colon at 1, 3, 5 and 7 days after B. longum-α-MSH administration. **P<0.01 vs DSS group; *P<0.05 vs WT/DSS group; **P<0.01 vs WT/DSS group.

**Fig. 7.** Effects on rats with colitis supplemented with B. longum-α-MSH on NO production of colonic tissues. **P<0.01 vs DSS group, **P<0.01 vs WT/DSS group.

**Fig. 8.** Performance of Bifidobacterium in the colon after consumption of B. longum. (a) Increased concentration of rhα-MSH in the colon tissue fluid. (b) Changes in number of Bifidobacterium in colon faecal matter. ***P<0.001 vs WT/DSS group, **P<0.001 vs WT/DSS group.
DSS-induced colitis. The colon histological lesion results are shown in Fig. 9. Reduced inflammation and less submucosal oedema were observed in the \textit{B. longum}-a-MSH-treated group.

**DISCUSSION**

In this study, we successfully constructed an engineered \textit{B. longum} secreting bioactive \textit{a}-MSH. We then utilized this engineered bacterium against DSS-induced colitis of rats. The results showed that colitis induced by DSS was significantly alleviated. These results were based on several crucial aspects of IBD: the MPO enzyme is a good marker of inflammatory status and tissue injury, and is expressed by neutrophils, which have a major function in causing colitis, as well as in the pathogenesis of the disease (Gambero et al., 2007; Hensel et al., 2014). In addition, TNF-\textit{a} and IL-6 serve a critical function in the modulation of intestinal immunity and inflammation. Considerable research has reported that large amounts of TNF-\textit{a} and IL-6 are found in the colonic mucosa of UC patients (Cekic et al., 2014), and that they play a crucial role in the initiation and continuation of mucosal inflammation and immunity (He et al., 2014). Möller and Villiger (2006) suggested that the blockade of TNF-\textit{a} and IL-6 could treat inflammatory diseases. In our work, MPO, TNF-\textit{a}, IL-6 and the pro-inflammatory factor NO were significantly reduced, whereas the anti-inflammatory cytokine IL-10 was significantly increased in the rats fed with \textit{B. longum} secreting \textit{a}-MSH, as well as preventing colonic pathological damage and recovering the number of bifidobacteria in the bowel, indicating that this engineered \textit{B. longum} has a better capacity to control IBD. Our work presents a feasible way to cure IBD that could be of great advantage to those who suffer from this disease.

IBD is a refractory immune-mediated disease. Although much work has been conducted, its exact pathogenesis remains elusive. The conventional treatments for IBD, including corticosteroids, 5-aminosalicylic acid and immunosuppressive drugs, have focused on controlling the inflammation (Triantafyllidi et al., 2015). However, these pharmaceuticals do not meet some needs of the patients because they have serious adverse events. Therefore, effective new agents should be developed. Substantial research has facilitated progress in IBD prevention, using biological agents such as anti-TNF and anti-CD3 antibodies to block one or several inflammatory cytokines, and which have performed well in colitis of rats. Unfortunately, these treatments are inadequate because IBD is characterized not only by immune disorders and various inflammatory factors but also by abnormalities of the intestinal microflora (Bai & Peng, 2010; Kaser et al., 2010; Cohen et al., 2014). A comprehensive treatment should not only control inflammation but also ensure the balance of intestinal

**Fig. 9.** Histological analysis of colonic samples (magnification \times 100) after application of oral \textit{B. longum} for 7 days. (a) Control group, showing a normal colonic histology. (b) DSS group, showing that the normal colonic mucosa was disrupted, with substantial severe infiltration of inflammatory cells. (c) WT/DSS group, showing fractional destruction of the epithelial mucosa. (d) \textit{B. longum-a}-MSH group, showing a relatively normal colonic histology.
microflora. Hence, we introduced \(\alpha\)-MSH and *B. longum* probiotics into an anti-inflammatory function trying to resist this serious disease.

*Bifidobacterium*, a probiotic, modulates gut microbiota balance through the mucosal barrier effect, inhibits the growth of pathogenic bacteria and prevents gastrointestinal diseases. Some studies have indicated that bifidobacteria are well established in controlling IBD, reducing the symptoms of the disease and leading to auxiliary actions in therapy (Kim et al., 2007; Philippe et al., 2011; Meyer et al., 2015). The results of the current study also support these reports. In addition, decreased counts of *Bifidobacterium* have been observed in the colon of patients with UC (Macfarlane et al., 2004), which suggests that these bacteria might have a protective role against UC. The current study agrees with this observation. After *B. longum* administration, the counts of *Bifidobacterium* in the colon of rats in our experiment were restored (Fig. 8b). However, the use of probiotics alone is inadequate and requires further evaluation.

\(\alpha\)-MSH is a potent anti-inflammatory peptide that can regulate a broad range of inflammatory cytokines and mediators. This physiological characteristic makes \(\alpha\)-MSH a potentially valuable drug for the treatment of inflammation diseases, including IBD. However, the application of this peptide is limited because of its extremely short duration *in vivo*. Hence, \(\alpha\)-MSH is an unsuitable drug. On the contrary, given that \(\alpha\)-MSH is a typical peptide, oral delivery of this peptide results in an extremely low bioavailability because of its abundant and unfavourable degradation in the stomach and lack of stability in the luminal environment (Scheuch & Siekmier, 2007; Singh et al., 2008; Fan et al., 2014). Taking these two problems into consideration, a particular strategy must be adopted. In the current study, *B. longum* was chosen as a carrier and we constructed a *B. longum* expressing \(\alpha\)-MSH and used it for UC treatment. Hypothetically, the administration of *B. longum* expressing \(\alpha\)-MSH could continually colonize the enteric canal and express \(\alpha\)-MSH, thereby presenting one way to compensate for the short biological half-life of \(\alpha\)-MSH. The results presented in our experiment showed that the engineered *B. longum* survived in the gut and increased rh\(\alpha\)-MSH expression (Fig. 8). As expected, rh\(\alpha\)-MSH fully functioned to increase anti-inflammation to improve UC conditions by significantly inhibiting inflammatory factors such as MPO, TNF-\(\alpha\), IL-6 and NO, and enhancing the anti-inflammatory cytokine IL-10 expression. Given that *B. longum* survives in the gut, \(\alpha\)-MSH should be immediately produced in the location of IBD. This method may be a more reasonable therapy for the disease.

In summary, in this work, we treated DSS-induced colitis by administering *B. longum* expressing \(\alpha\)-MSH. We aimed to solve the problem of the short half-life of \(\alpha\)-MSH peptide. In this strategy, we delivered \(\alpha\)-MSH peptide via oral administration to circumvent conventional injection, which is uncomfortable for patients. The therapeutic effects could be adjusted based on changes in the condition of the illness, even eliminating the condition by administering suitable antibiotics. In conclusion, our work represents an alternative option to improve experimental IBD by *B. longum* expressing \(\alpha\)-MSH.

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REFERENCES


