The immune-stimulating peptide WKYMVm has therapeutic effects against ulcerative colitis

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In this study, we examined the therapeutic effects of an immune-stimulating peptide, WKYMVm, in ulcerative colitis. The administration of WKYMVm to dextran sodium sulfate (DSS)-treated mice reversed decreases in body weight, bleeding score and stool score in addition to reversing DSS-induced mucosa destruction and shortened colon. The WKYMVm-induced therapeutic effect against ulcerative colitis was strongly inhibited by a formyl peptide receptor (FPR) 2 antagonist, WRWWWW, indicating the crucial role of FPR2 in this effect. Mechanistically, WKYMVm effectively decreases intestinal permeability by stimulating colon epithelial cell proliferation. WKYMVm also strongly decreases interleukin-23 and transforming growth factor-β production in the colon of DSS-treated mice. We suggest that the potent immune-modulating peptide WKYMVm and its receptor FPR2 may be useful in the development of efficient therapeutic agents against chronic intestinal inflammatory diseases.

Experimental & Molecular Medicine (2013) 45, e40; doi:10.1038/emm.2013.77; published online 13 September 2013

Keywords: therapeutics; ulcerative colitis; WKYMVm

INTRODUCTION

Inflammatory bowel diseases (IBDs) such as Crohn’s disease or ulcerative colitis are chronic diseases that cause inflammation of the intestine.1,2 Although the incidence of IBD varies in different countries, a gradual increase has been noted recently, and the disease is a major health problem worldwide.2,3 Although the search for the cause of IBD has been a hot issue for several decades, it is still not clear what causes IBD. Currently, various factors, including environment, diet and genetic makeup, have been suggested to be associated with IBD pathogenesis.1–3 Among these, a genetic defect that affects the response of the human immune system to offending agents, such as bacteria, viruses or proteins in food, has been associated with IBD.1–3

The host immune system is closely associated with the pathogenesis and progress of IBD.4 Both innate and adaptive immune systems are critically involved in the response to intestinal microbiota.4,5 Pattern recognition receptors, such as the toll-like receptor, have roles in sensing conserved microbial molecules in the intestinal environment.5 Despite the need for efficient therapeutic molecules to treat human IBD, no cure has yet been developed. Clinically, some immunosuppressive agents that target tumor necrosis factor (TNF) are currently used to treat IBD.6

WKYMVm is a synthetic peptide that was identified by screening a peptide library.7,8 The peptide binds to at least three formyl peptide receptors (FPRs): FPR1, FPR2 and FPR3.9–11 WKYMVm stimulates the chemotactic migration of leukocytes such as neutrophils, monocytes, dendritic cells and natural killer cells.12–15 It also stimulates superoxide anion production in phagocytes including neutrophils and monocytes.12,16 Recently, we demonstrated that WKYMVm administered to a polymicrobial sepsis model had potent therapeutic activity in cecal ligation and puncture mice.17 The peptide was shown to inhibit the production of inflammatory cytokines, such as TNF-α and interleukin (IL)-1β, and augment the production of Th1 cytokines (IFN-γ (interferon-γ) and IL-12) to achieve this therapeutic effect against sepsis.17 Here, we investigate the effects of WKYMVm on dextran sodium sulfate (DSS)-induced ulcerative colitis, including its effects on cytokine production.
MATERIALS AND METHODS

Animals and DSS-treated ulcerative colitis model

Six-week-old C57BL/6 mice were obtained from Orient Bio Inc. (Seongnam, Korea). After adapting for 1 week following arrival, the animals were given drinking water containing 3% DSS (m.w. 36 000–50 000; Sigma-Aldrich, St Louis, MO, USA) w/v for 5 days followed by fresh water until the end of the experiment on the 8th day. WKYMVm (Anygen, Gwangju, Korea) or vehicle (phosphate-buffered saline) was subcutaneously injected into DSS mice six times (at 0, 12, 24, 36, 48 and 60 h after DSS treatment). Body weight, rectal bleeding and stool score (stool consistency or diarrhea) were measured daily according to a previous report.18

Histology

The mice were subjected to DSS treatment and were administered with phosphate-buffered saline or WKYMVm at a dose of 8 mg kg⁻¹. The mice were euthanized 8 days after DSS treatment, and the intestines were fixed, sectioned and stained with hematoxylin and eosin for morphological analysis.

Measurement of intestine permeability

Food was withdrawn from the mice for 6 h, and the animals were gavaged with fluorescein isothiocyanate–dextran (10 mg per head; Sigma-Aldrich). Serum was collected retro-orbitally 4 h after the gavage. Fluorescein isothiocyanate–fluorescence was measured using the Gemini XPS fluorescence microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Cell proliferation assay

Caco-2 human epithelial colorectal adenocarcinoma cells were cultured with Dulbecco’s modified Eagle’s medium containing 20% fetal bovine serum (Life Technologies, Grand Island, NY, USA). Healthy cultured cells were seeded at 4 × 10⁴ cells per well. After 24 h, WKYMVm was added at several concentrations (0, 10, 100 and 1000 μM) in the absence or presence of cyclosporine H (CsH, 1 μM) or WRW4 (WRW4) (10 μM) for 24 h. Quantification of cell proliferation was performed using the Cell Counting Kit-8 (Dojindo Molecular Technologies Inc., Rockville, MD, USA).

Wound healing assay

Caco-2 human epithelial colorectal adenocarcinoma cells were cultured with Dulbecco’s modified Eagle’s medium containing 20% fetal bovine serum. Vehicle or WKYMVm (1 μM) was added into a scratched Caco-2 cell layer for 0, 12 or 24 h. Images were obtained using a digital camera attached to a light microscope.

Measurement of colon cytokines

To measure DSS treatment-induced cytokine production, mice were administered with WKYMVm at 0, 12, 24, 36, 48 and 60 h after DSS treatment. Intestines were collected at day 7 after DSS treatment and sliced into 1-cm segments. Each segment was incubated in Roswell Park Memorial Institute 1640 medium for 24 h, and cytokines secreted into the supernatant were measured using enzyme-linked immunosorbent assay (BD Biosciences, San Jose, CA, USA or eBioscience Inc., San Diego, CA, USA) according to the manufacturer’s instructions.

Statistical analysis

All data were evaluated using the t-test, and statistical significance was set at P < 0.05.

RESULTS

Administration of WKYMVm ameliorates DSS-induced ulcerative colitis

To examine whether WKYMVm has a therapeutic effect on ulcerative colitis, we used the DSS model. DSS-treated C57BL/6 mice showed a decreased body weight over to time compared with control mice (Figure 1a). WKYMVm administration to DSS-treated mice reversed this decrease in body weight (Figure 1a). Bleeding and stool scores were also monitored after DSS treatment. WKYMVm administration effectively attenuated the DSS-induced increase in the bleeding score and the stool score (Figures 1b and c). As WKYMVm binds to both FPR1 and FPR29–11, we also examined the effect of an FPR1 antagonist (CsH)19 and an FPR2 antagonist (WRW4)20 on the WKYMVm-induced therapeutic effect on ulcerative colitis. The WKYMVm-induced therapeutic effect on ulcerative colitis was strongly inhibited by WRW4, but not by CsH, indicating that WKYMVm exerts its effects via FPR2 (Figures 1d–f).

Colon shortening is associated with the progression of ulcerative colitis. As shown in Figure 2a, DSS treatment strongly elicited colon shortening, which was efficiently reversed by WKYMVm administration. The WKYMVm-induced reversal of colon shortening was inhibited by WRW4, but not by CsH, supporting the hypothesis that the peptide exerts its effects via FPR2 but not FPR1 (Figure 2a). The histological data indicate that DSS treatment caused destruction of the mucosa structure, which was dramatically inhibited by WKYMVm administration (Figure 2b).

WKYMVm administration protects intestinal permeability

Intestinal permeability is increased during the pathogenesis of ulcerative colitis induced by DSS treatment owing to the destruction of the intestinal epithelial barrier.21 We also observed that DSS treatment increased intestinal permeability in mice, whereas WKYMVm administration effectively decreased the intestinal permeability in DSS-treated mice (Figure 3a). Changes in intestinal permeability are associated with the proliferation and migration of intestinal epithelial cells.22 As we observed that WKYMVm inhibits intestinal permeability, we hypothesized that WKYMVm may affect the proliferation of intestinal epithelial cells. Stimulation of Caco-2 cells (the intestinal epithelial cells) with WKYMVm induced cell proliferation (Figure 3b). To examine whether FPR1 and FPR2 have roles in WKYMVm-induced Caco-2 cell proliferation, the cells were pretreated with CsH (an FPR1 antagonist)19 or WRW4 (an FPR2 antagonist)20 before WKYMVm treatment. Inhibition of FPR2, but not FPR1, caused inhibition of WKYMVm-induced cell proliferation (Figure 3c). We also examined the effects of WKYMVm treatment on Caco-2 cell migration. WKYMVm addition into a scratched Caco-2 cell layer strongly induced wound healing (Figure 3d).
WKYMVm administration affects cytokine profiles in the DSS colitis model

We measured cytokine profiles in the DSS model and found that DSS treatment increased IL-17, IFN-γ, IL-6, IL-1β and TNF-α levels (Figure 4a–e). WKYMVm administration augmented the production of these cytokines in the intestine of DSS-treated mice (Figure 4a–e). However, IL-12, IL-23 and transforming growth factor (TGF)-β were significantly decreased by WKYMVm administration (Figure 4f–h). No significant changes in IL-22 levels were observed in the intestine of DSS-treated mice that were administered with WKYMVm (data not shown).

DISCUSSION

As the intestine has a unique microbial environment, the intestinal innate immune system has an important role in
Figure 3 Protective effects of WKYMVm on the intestinal barrier in colitis. C57BL/6 mice were treated with vehicle, 3% dextran sodium sulfate (DSS) or 3% DSS plus WKYMVm (8 mg kg⁻¹, six subcutaneous administrations at 12-h intervals) for 5 days, and fresh water was provided for an additional 2 days. On day 7, fluorescein isothiocyanate (FITC)–dextran was administered by gavage and serum was collected. The amount of FITC–dextran in the serum was measured (a). Caco-2 cells were stimulated with several concentrations (0, 10, 100 and 1000 nM) of WKYMVm (b), or with 1 µM of WKYMVm in the absence or presence of cyclosporine H (CsH; 1 µM) or WRWWWW (WRW²) (10 µM; c) for 24 h. Cell proliferation was measured using the Cell Counting Kit-8 (b, c). The data are expressed as the mean ± s.e.m. (n = 7 mice per group). ***P < 0.001 compared with control. *P < 0.05 compared with DSS alone (a). **P < 0.05 compared with vehicle. #P < 0.05 compared with WKYMVm alone (b, c). Vehicle or WKYMVm (1 µM) was added to a scratched Caco-2 cell layer for 0, 12 or 24 h. Images were obtained with a digital camera attached to a light microscope. The data are representative of four independent experiments (d).

In this study, we demonstrated that WKYMVm has therapeutic activity against colitis in DSS-treated mice. The therapeutic effect of WKYMVm in this colitis model was markedly inhibited by an FPR2 antagonist (WRW¹), but not by an FPR1 antagonist (CsH), indicating that FPR2 is critically involved in this process (Figures 1 and 2). Very recently, Wang and colleagues also demonstrated that FPR2-deficient mice showed defects in commensal bacterium-dependent homeostasis in response to DSS challenge and exhibited delayed mucosal restoration after injury, suggesting that FPR2 is important in mediating homeostasis, inflammation and the epithelial repair process in the colon. These results support our hypothesis that the receptor for WKYMVm, FPR2, can be regarded as a target molecule for treatment of ulcerative colitis.

The WKYMVm peptide was shown to stimulate the bactericidal activity of phagocytic cells by enhancing the production of reactive oxygen species such as superoxide anions. In DSS-treated mice, the WKYMVm peptide increased the proliferation and migration of intestinal epithelial cells, resulting in the inhibition of intestinal permeability. Maintenance of intestinal permeability is important for maintaining the intestinal homeostasis by inhibiting harmful actions of microorganisms in the intestinal microenvironment. As intestinal epithelial cells express three FPRs (FPR1, FPR2 and FPR3) and WKYMVm stimulated Caco-2 cell proliferation and migration, which were inhibited by WRW² but not by CsH, it is reasonable to assume that WKYMVm stimulates FPR2, resulting in the proliferation and migration of intestinal epithelial cells and the subsequent recovery of DSS-induced intestinal permeability in mice.
Figure 4 Effect of WKYMVm on cytokine production in the colon of dextran sodium sulfate (DSS)-treated mice. Mice were administered with WKYMVm or phosphate-buffered saline (PBS) at 0, 12, 24, 36, 48 and 60 h after DSS treatment. The intestine was collected on day 7 from untreated, DSS-treated and DSS + WKYMVm-treated mice, and was sliced into 1-cm segments. Each segment was incubated in Roswell Park Memorial Institute (RPMI) 1640 medium for 24 h, and cytokines secreted into the supernatant were measured using enzyme-linked immunosorbent assay (ELISA). (a) Interleukin (IL)-17; (b) interferon-γ (IFN-γ); (c) IL-6; (d) IL-1β; (e) tumor necrosis factor (TNF)-α; (f) IL-12; (g) IL-23; (h) transforming growth factor (TGF)-β. The data are expressed as the mean ± s.e.m. (n = 7 for a–h). *P<0.05, **P<0.01, ***P<0.001 compared with DSS alone.

We previously demonstrated that the administration of WKYMVm in a cecal ligation and puncture sepsis model markedly decreased the production of TNF-α and IL-1β.17 However, WKYMVm administration in a DSS-induced ulcerative colitis model significantly augmented the levels of these two proinflammatory cytokines in the intestine (Figure 4d,e). It is not clear why WKYMVm exerted differential effects on TNF-α and IL-1β production in a sepsis model versus an ulcerative colitis model. As the same dose was used in both studies (8 mg kg⁻¹), the in vivo effects of WKYMVm may therefore depend on the sampling time (∼7 days versus ∼72 h) or the sampled tissue (the intestine slice versus the peritoneal fluid). In ulcerative colitis, WKYMVm likely exerts its effects by dampening local levels of IL-23, TGF-β or IL-17.

In conclusion, the potent immune-modulating peptide WKYMVm had a therapeutic effect on ulcerative colitis by inhibiting the epithelial permeability and modulating the cytokine profile. We suggest that WKYMVm is a potent agonist of FPRs that may be a potential therapeutic agent in the management of chronic intestinal inflammatory diseases.
CONFLICT OF INTEREST

SK and YB have pending patent applications. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This research was supported by National Research Foundation of Korea (NRF) grants funded by the Korean government (MEST; No. 35B-2011-1-E00012, 2013 041811, 2012R1A2A2A01007751) and by a grant from Next generation of BioGreen 21 project (PJ008196) from Rural Development Administration, Republic of Korea.

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