Anti-melanin-concentrating hormone treatment attenuates chronic experimental colitis and fibrosis

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Ziogas DC, Gras-Miralles B, Mustafa S, Geiger BM, Najarian RM, Nagel JM, Flier SN, Popov Y, Tseng YH, Kokkotou E. Anti-melanin-concentrating hormone treatment attenuates chronic experimental colitis and fibrosis. Am J Physiol Gastrointest Liver Physiol 304: G876–G884, 2013. First published March 28, 2013; doi:10.1152/ajpgi.00305.2012.—Fibrosis represents a major complication of several chronic diseases, including inflammatory bowel disease (IBD). Treatment of IBD remains a clinical challenge despite several recent therapeutic advances. Melanin-concentrating hormone (MCH) is a hypothalamic neuropeptide shown to regulate appetite and energy balance. However, accumulating evidence suggests that MCH has additional biological effects, including modulation of inflammation. In the present study, we examined the efficacy of an MCH-blocking antibody in treating established, dextran sodium sulfate-induced experimental colitis. Histological and molecular analysis of mouse tissues revealed that mice receiving anti-MCH had accelerated mucosal restitution and lower colonic expression of several proinflammatory cytokines, as well as fibrogenic genes, including COL1A1. In parallel, they spared collagen deposits seen in the untreated mice, suggesting attenuated fibrosis. These findings raised the possibility of perhaps direct effects of MCH on myofibroblasts. Indeed, in biopsies from patients with IBD, we demonstrated expression of the MCH receptor MCHR1 in α-smooth muscle actin (+) subepithelial cells. CCD-18Co cells, a primary human colonic myofibroblast cell line, were also positive for MCHR1. In these cells, MCH acted as a profibrotic modulator by potentiating the effects of IGF-1 and TGF-β on proliferation and collagen production. Thus, by virtue of combined anti-inflammatory and anti-fibrotic effects, blocking MCH might represent a compelling approach for treating IBD.

IN MANY ORGANS, including the intestine, chronic inflammation results in impaired healing and fibrosis, a process characterized by excessive synthesis and deposition of extracellular matrix components, including collagen. In parallel, there is an inhibition of degradation of extracellular matrix, resulting from an imbalance between the various matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) (38, 47). In inflammatory bowel disease (IBD), the manifestation of fibrosis reflects the distinct pathological characteristics of each subtype; however, in both Crohn’s disease (CD) and ulcerative colitis, fibrosis represents a serious clinical problem, even after the remission of acute inflammation. In CD, transmural granulomatous inflammation results in the formation of strictures, which can require surgical intervention. Fibrosis in ulcerative colitis is less pronounced and limited to the submucosa, leading to severe disruption of tissue architecture and failure of epithelial regeneration (37). TGF-β, a profibrogenic factor, is upregulated in the intestine of patients with CD, specifically in the mucosa overlying strictures, along with TIMP1 and MMPs, in particular MMP-3 and MMP-12 (3, 13). TGF-β signaling results in phosphorylation of Smad2 and Smad3, which in turn form a complex with Smad4 and translocate to the nucleus, where they regulate transcription of several genes, including procollagen and fibronectin, extracellular matrix-degrading enzymes (MMPs), and their inhibitors (TIMP1, TIMP6), as well as regulators of mesenchymal cell proliferation and apoptosis (17). Beside TGF-β, IGF-1 and TNF-α have also been shown to stimulate type I collagen synthesis, and their expression is increased in collagen deposition areas in the intestine of patients with CD (36, 41).

Myofibroblasts are α-smooth muscle actin (α-SMA) (+), vimnetin (+), and desmin (−) mesenchymal cells in the lamina propria of the gut, which secrete extracellular matrix components, along with cytokines and growth factors (35, 37). Although transient appearance of myofibroblasts contributes to mucosal healing, their sustained proliferation and activation in response to chronic inflammation leads to intestinal fibrosis. Indeed, increased numbers of myofibroblasts were found in the affected mucosa of patients with CD and in mouse models of IBD (36).

It is estimated that 1.4 million patients suffer from IBD in the US alone, a chronic condition characterized by periods of remission and relapses. The clinical management of IBD, despite the revolutionizing use of biological therapies, remains problematic (30). For instance, treatment with anti-TNF-α, the prototype of this class of therapeutics, significantly improves the disease free time and the quality of life of patients with IBD. Meta-analyses of large clinical trials, however, indicate that less than 40% of patients respond to anti-TNF-α monotherapy (9). Therefore, the need to identify novel molecular pathways modulating the inflammatory and fibrogenic processes in IBD and develop more effective therapies and with fewer side effects is greater than ever. One such promising candidate is melanin-concentrating hormone (MCH).

MCH is a 17–19-amino-acid peptide highly conserved in evolution. It was originally identified in fish as a hormone secreted by the pituitary, causing, as its name indicates, skin paleness in response to an environmental threat (22). MCH is also expressed in the mammalian hypothalamus, where its primary role is in the regulation of appetite and energy balance (34). In addition to brain, the gut represents a significant source of neuropeptides (18). Studies in the rat revealed numerous MCH-containing cells, some of them enterochromaffin cells, in gastric antrum and corpus, duodenum, and colon (19). Our own group has demonstrated MCH and MCHR1 immunoreactivity in the rat myenteric and submucosal plexus (24), whereas...
others have shown that vagal afferent neurons in both human and rat express MCH and its G protein-coupled seven-transmembrane receptor (MCHR1) (6). Moreover, when tested in an in situ ligated loop experiment, MCH stimulated Na\(^+\) and K\(^+\) fluxes in proximal colon; water, as well as Na\(^+\) and K\(^+\) fluxes in duodenum; and bicarbonate absorption in the jejunum (20).

Beside the still elusive roles of MCH in gastrointestinal physiology, we have found a significant upregulation of MCH expression in the affected mucosa of patients with IBD, suggesting involvement of this molecule in the pathogenesis of IBD (24). Indeed, MCH-deficient mice appear to be less susceptible to 4,6-dinitrobenzene sulfonic acid (TNBS)-induced acute colitis, pointing to proinflammatory effects of MCH. However, the significance of MCH in chronic experimental colitis and its associated fibrosis, which better recapitulate the human condition, have not yet been investigated.

In the present study, we evaluated the efficacy of an anti-MCH antibody in treating established dextran sodium sulfate (DSS)-induced experimental colitis. Among the available models, mice treated with multiple cycles of DSS develop chronic colitis characterized by transmural inflammation, muscularis overgrowth, and collagen deposition, which starts in the lamina propria and can extend up to muscularis propria (37). It has been claimed that this type of experimental colitis shares features with CD based on T- and B-cell involvement and histological hallmarks although it lacks the development of strictures and intestinal obstruction (37). In our experiments, treatment of mice with anti-MCH resulted in accelerated mucosal healing associated with reduced markers of inflammation and fibrosis. The effects of MCH on fibrosis were found to be, at least in part, inflammation independent. Indeed, we describe that human myofibroblasts express MCHR1, the activation of which, in combination with additional factors, promotes cell proliferation and collagen production.

**MATERIALS AND METHODS**

**Mice.** Protocols of studies involving animal research were submitted to and approved by the Institutional Animal Care and Use Committee (IACUC) of Beth Israel Deaconess Medical Center.

Eight-week-old CD1 male mice were purchased from Charles River Laboratories and used for the experiment 4 wk later (body wt 30–35 g). Chronic colitis was induced by exposure to 3% (wt/vol) DSS (molecular weight 36,000–50,000, kDa, MP Biomedicals) dissolved in drinking water as previously described (31). DSS was given ad libitum for 5 days per cycle, followed by 5 days of normal drinking water (Fig. 1A). Beginning on the day after the third DSS cycle, mice were treated i.p. with 1 mg/kg per day anti-MCH antibody (kindly provided by Dr. Eleftheria Maratos-Flier) (n = 12) or control IgG (n = 11) for a total of 7 days (Fig. 1A). No mouse death occurred during any of the treatments. Control mice received no DSS and no antibody treatment (n = 7). MCH has identical sequence in mouse, human, and rat, and the anti-MCH antibody was raised in rabbits against the whole peptide. The IgG fraction of the anti-MCH serum was used in our studies, along with control IgG, isolated likewise from preimmune rabbit serum. The specificity of this antibody has been previously confirmed in neuronal mapping studies of human and rat brain slices in combination with MCH mRNA detection by in situ hybridization (16), as well as in hypothalamic sections of transgenic mice overexpressing MCH (28). In an in vitro functional assay, the anti-MCH antibody could block the MCH-mediated inhibition of cAMP upregulation (23). In vivo treatments of mice with the same antibody resulted in prevention of TNBS-induced acute experimental colitis (24) and of *Clostridium difficile* toxin A-mediated enteritis (23).

**Assessment of colonic inflammation and fibrosis.** Tissue segments taken from the distal, middle, and proximal parts of the colon were fixed in 10% buffered formalin and paraffin embedded. Colitis grading was performed in a blinded fashion by a pathologist specializing in gastroenterology (R. Najarian). Hematoxylin and eosin (H&E)-stained transverse colonic sections were evaluated (3 areas per section, 3 sections per mouse), and an average was calculated for each mouse. Histology score (maximum score 9) is the sum of three partial scores (0–3) for epithelial damage (E), inflammation (I), and crypt shortening and distortion (D), where 0 represents normal histology, 1 mild, 2 moderate, and 3 severe histological abnormality.

Fibrosis was evaluated in Sirius red-stained sections and severity scored as follows: 0 = no fibrosis, 1 = mild fibrosis limited to the mucosal layer, 2 = mucosal and submucosal fibrosis, and 3 = fibrosis involving mural (muscularis propria) and/or serosal layers. The fibrosis score assigned to each mouse (0–3) is based on the average of three areas per section/three sections per mouse as in the colitis scoring.

**Cell culture experiments.** CCD-18Co cells (ATCC, passages 8–15) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 20% fetal bovine serum and 1% antibiotic/antimycotic (Invitrogen). These cells share morphological and functional characteristics of human subepithelial myofibroblasts (45). CCD-18Co cells at 80% confluence were stimulated with 3% DSS for 1 h. Cells were then washed and subsequently treated with 10\(^{-6}\) M MCH (Bachem), or vehicle for 24 h (Fig. 6). Collagen production by CCD-18Co cells was determined using the Sircol collagen assay (Biocolor). To measure cell proliferation, cells were incubated with bromodeoxyuridine (BrdU) labeling media for 18 h followed by BrdU detection using a colorimetric immunoassay (Roche Applied Science). Assays for each
condition were performed in six replicas, and the experiment was repeated twice. Results are shown relative to no-peptide-treated cells (control = 100).

In other experiments (Fig. 7), CCD-18Co cells, at 50–70% confluence, were incubated overnight (16 h) with reduced serum media (2% FBS) and subsequently treated with MCH (10^{-6} M), IGF-1 (10 ng/ml), their combination, or vehicle in the same media for 4 h. Cell proliferation was assessed as above. CCD-18Co cells were cultured in MEM media (10% FBS) and treated with MCH, TGF-β, their combination, or vehicle for 36 h. At the end of the experiment, collagen was measured using the Sircol assay.

For the wound-scratch assay, after removal of the DSS, a linear wound was generated in the CCD-18Co monolayer by scraping a sterile 200-μl pipette tip across the bottom of the well. Following three washes, cells were incubated with growth media containing MCH (10^{-6} M) or vehicle. Results represent the average of six replicas per condition.

Quantitative RT-PCR. From each mouse, tissue samples corresponding to the distal, middle, and proximal parts of the colon were pulled for gene expression analysis. Total RNA was extracted using the RNeasy mini-kit (Qiagen), according to manufacturer’s instructions. One microgram of RNA was reverse-transcribed into cDNA using the Advantage RT for PCR reagents with oligo (dT) (Clontech). Quantitative gene expression was assessed using gene-specific primers purchased from Applied Biosystems and TaqMan Universal PCR Master Mix in an ABI PRISM 7700 Sequence Detection System. Results have been normalized to expression of the TATA-binding protein housekeeping gene and are expressed as arbitrary mRNA units (AU) relative to control group (control = 100). The following groups of mice were included into the gene expression analysis: control, no DSS (n = 5), DSS + IgG (n = 9), and DSS + anti-MCH (n = 10).

Immunostaining. Human colonic tissue samples derived from surgical resection specimens were obtained as frozen sections from the Ardais/Beth Israel Deaconess Medical Center Biomatlerals and Information for Genomic Research Tissue Library (Boston, MA). The panel included areas of active disease from patients with IBD (5 with CD and 4 with ulcerative colitis) as well as histologically normal tissue from patients undergoing surgery for noninflammatory conditions (n = 3).

Slides were fixed in 4% paraformaldehyde and incubated with anti-α-SMA mouse anti-human monoclonal antibody (clone1A4, dilution 1:50, Dako) and with a rabbit polyclonal antibody against human/rat/mouse MCHR1 (24) (dilution 1:200) for 2 h at room temperature followed by incubation with FITC- and Texas Red-labeled secondary antibodies, respectively. As negative controls, either or both of the primary antibodies were omitted from the staining procedure. Sections were treated with Prolong Gold antifade plus DAPI (Invitrogen) mounting media and viewed under a Zeiss LSM510 META confocal microscope.

Statistical analysis. Results are expressed as group means ± SE. Data were analyzed in STATView using the nonparametric Mann-Whitney U-test or repeated-measures ANOVA as appropriate. A P value <0.05 was considered statistically significant.

RESULTS

Treatment with an anti-MCH antibody attenuates chronic intestinal inflammation and fibrosis. To evaluate the therapeutic potential of targeting MCH in chronic experimental colitis, mice were exposed to three cycles of DSS treatment, followed by daily injections of anti-MCH or control antibody for 7 days (Fig. 1A). Body weight curves were indistinguishable between the two treatment groups throughout the study (Fig. 1B). Histological analysis of H&E-stained mouse colonic sections revealed more severe epithelial damage, based on areas of necrosis, crypt abscesses, and mucosal ulcers in mice treated with control antibody compared with mice that received anti-

![Fig. 2. Disease attenuation in mice with DSS colitis treated with anti-MCH. Representative hematoxylin and eosin (H&E)-stained (top) and Sirius red-stained (bottom) colonic sections of mice with chronic DSS colitis treated with anti-MCH or control IgG. Mice with no colitis are included for comparison (×200 magnification).](http://ajpgi.physiology.org/10.1152/ajpgi.00305.2012)
MCH treatments (Fig. 2). Architectural distortion, another marker of chronic epithelial injury, was also less pronounced in mice treated with anti-MCH. Mice treated with control antibody, in contrast to anti-MCH-treated mice, also had significant ongoing inflammation. Furthermore, the study of mouse colonic sections stained with Sirius red (Fig. 2) demonstrated extensive collagen deposits in mice with DSS colitis treated with control IgG, in contrast to mice treated with anti-MCH. Overall, the histology score was lower in mice receiving anti-MCH (1.75 ± 0.69 vs. 4.54 ± 1.13; anti-MCH vs. IgG; \( P = 0.043 \); Fig. 3A), as well as their fibrosis score (0.58 ± 0.19 vs. 1.64 ± 0.39; anti-MCH vs. IgG; \( P = 0.024 \); Fig. 3B). Despite the chronic inflammation and injury, none of the mice developed colonic tumors.

Corroborating the histology findings, mice treated with anti-MCH had significantly lower colonic levels of several proinflammatory cytokines (TNF-\( \alpha \); 274.7 ± 65.4 vs. 127.7 ± 31.5; \( P = 0.014 \); IL-1\( \beta \); 4.104.8 ± 1,811.1 vs. 588.6 ± 239.5, \( P = 0.036 \); keratinocyte chemokine: 2,174.2 ± 1,397.4 vs. 481.8 ± 176.9, \( P = 0.037 \); expressed as arbitrary mRNA units, control IgG vs. anti-MCH, respectively, Fig. 3C). These findings further support the role of MCH as a modulator of intestinal inflammation, as previously described by our group (23, 24).

Molecular analysis of fibrosis markers in mice with chronic colitis treated with anti-MCH. We did not find any differences in TGF-\( \beta \) mRNA expression, a major drive of fibrotic processes, among the two treatment groups (Fig. 4A). In addition to TGF-\( \beta \), IGF-1 and TNF-\( \alpha \) have also been shown to regulate type I collagen synthesis by myofibroblasts (14, 36, 42). Along with lower TNF-\( \alpha \) mRNA expression in mice treated with anti-MCH (Fig. 3C), we found a similar trend for IGF-1 expression although it did not reach statistical significance (344.7 ± 86.5 vs. 227.1 ± 29.1 IGF-1 AU, control IgG vs. anti-MCH; \( P = 0.110 \); Fig. 4A). Because TGF-\( \beta \) mRNA levels might not accurately reflect its activity, we subsequently measured expression of SMAD2, SMAD3, and SMAD4, the primary downstream effectors of TGF-\( \beta \) receptor I signaling (17). Indeed, SMAD3 was upregulated almost twofold in mice with colitis treated with control antibody, compared with mice with no DSS treatment, whereas it was similar to baseline levels in mice with colitis treated with anti-MCH antibody (171.9 ± 13.3 vs. 101.5 ± 9.8 AU, control IgG vs. anti-MCH, \( P = 0.0005 \); Fig. 4B). There was a similar trend for SMAD2 expression (177.2 ± 36.7 vs. 111.9 ± 18.4 AU, control IgG vs. anti-MCH, \( P = 0.0661 \)), whereas changes in SMAD4, although significant, were of a lesser mag-

Fig. 3. Treatment with anti-MCH attenuates inflammation and fibrosis in mice with chronic DSS colitis. A: histological scoring of H&E-stained colonic biopsies of mice with colitis treated with anti-MCH or control IgG. B: fibrosis scoring of Sirius red-stained colonic biopsies from the same mice as above. C: colonic mRNA expression of cytokines in mice with chronic DSS colitis treated with anti-MCH or control IgG. Mice that were not treated with DSS were included as a baseline control (control = 100). Results as expressed as arbitrary units. \( ^* P < 0.05 \), anti-MCH vs. IgG control treatment. KC, keratinocyte chemokine.
Colonic mRNA levels of COL1A1 were significantly reduced in DSS-exposed mice that received treatment with anti-MCH antibody (334.2 ± 76.5 vs. 118.6 ± 19.9 arbitrary mRNA units, control IgG vs. anti-MCH; \( P = 0.007 \); Fig. 4C), consistent with the findings on histology (Sirius red-stained sections).

Collagen deposition and extracellular matrix remodeling are parts of a dynamic process regulated by various MMPs and TIMPs. Mice with DSS-induced chronic colitis had increased mRNA expression of MMP-3, MMP-9, and MMP-12. Among them, MMP-3 upregulation was significantly attenuated in mice treated with anti-MCH antibody (723.1 ± 220.2 vs. 396.3 ± 151.5, control IgG vs. anti-MCH, \( P = 0.033 \); Fig. 4D), whereas MMP-9 levels followed a similar trend. In parallel to the induction of MMPs, mice with chronic DSS colitis also had increased Timp-1 and Timp-6 mRNA expression, whereas there was a trend for lower Timp-1 levels in mice treated with anti-MCH (685.0 ± 215.9 vs. 347.1 ± 79.2, control IgG vs. anti-MCH, \( P = 0.106 \), Fig. 4E).

Upregulation of MCHR1 expressing myofibroblasts in patients with IBD. Areas of collagen deposition in patients with IBD were identified by Masson's Trichrome staining (Fig. 5A). Sequential sections were stained for \( \alpha \)-SMA, a marker for myofibroblasts and MCHR1. Confocal analysis revealed that the majority of myofibroblasts from the inflamed mucosa of patients with IBD were MCHR1 positive. Pictures of representative patients from each group are shown in Fig. 5B.

Direct effects of MCH on myofibroblasts. CCD18-Co primary human intestinal subepithelial myofibroblasts were also found to express MCHR1 (Fig. 6A). In these cells, we examined the effect of MCH treatment on cell proliferation, collagen production, and cell migration at baseline conditions and after exposure to DSS-induced cell injury (15). Following DSS treatment, we observed a moderate increase in cell proliferation in response to MCH, as measured by a BrdU incorporation assay (135 ± 11 vs. 100 ± 5, relative units, MCH vs. vehicle, \( P = 0.004 \); Fig. 6B). Furthermore, under similar conditions, MCH promoted collagen production, although to a lesser extent, as measured by a biochemical assay (107.3 ± 1.4 vs. 100 ± 0.6 relative units, MCH vs. vehicle, \( P = 0.008 \), Fig. 6C). However, under the conditions tested, no effects of MCH were apparent in the absence of DSS treatments. This suggested that perhaps MCH modulates the effects of factors secreted in response to myofibroblast activation (2). To further explore this possibility,
CCD18-Co cells were treated with IGF-1, alone or in combination with MCH. Indeed, we observed a potentiation of the IGF-1 effects on cell proliferation in the presence of MCH (97.05 ± 3.0 vs. 110.0 ± 2.9 relative BrdU units, IGF-1 vs. IGF-1 plus MCH, P = 0.025; Fig. 7A). Likewise, MCH enhances TGF-β1-induced collagen production in the same cells (118.5 ± 7.3 vs. 160.3 ± 8.3 relative units, TGF-β1 vs. TGF-β1 plus MCH, P = 0.01; Fig. 7B).

**Effects of MCH on wound healing.** To determine the role of MCH in wound healing, a scratch was made into a confluent monolayer of CCD-18Co myofibroblasts (100%), and closure of the wound was monitored at different time points. No differences in migration were observed between MCH- and vehicle-treated cultures without prior stimulation with DSS. In cells that had been pretreated with DSS, differences between MCH and vehicle-treated myofibroblasts became

**Fig. 5.** MCHR1-positive myofibroblasts in the colon of patients with inflammatory bowel disease (IBD). A: Masson’s trichrome staining of colonic biopsies from controls and patients with IBD, which included fibrotic areas (blue deposits). B: confocal analysis of staining with α-smooth muscle actin (SMA) (green), a marker of myofibroblasts and MCHR1 (red), revealed a large number of myofibroblasts stained positive for MCHR1 (yellow) in the subepithelium of patients with IBD but not in normal colonic tissue.

**Fig. 6.** In vitro effects of MCH on myofibroblasts in response to cell injury. CCD-18Co cells were stimulated with 3% DSS or media alone for 1 h. Cells were then washed and subsequently treated with MCH (10⁻⁶ M) or vehicle for 24 h. A: immunofluorescence analysis confirmed the presence of MCHR1 (green) in CCD-18Co human myofibroblasts. B: bromodeoxyuridine (BrdU) incorporation (Roche Applied Science) was used to access proliferation of CCD-18Co human myofibroblasts in response to MCH treatment. C: collagen production by CCD-18Co cells in response to MCH treatment was evaluated using the Sircol collagen assay (Biocolor). **P < 0.01, MCH vs. vehicle treatment.
Fig. 7. MCH modulates the effects of IGF-1 and TGF-β in vitro. A: CCD-18Co human myo- 
fi broblasts were treated with MCH (1 μM), IGF-1 (10 ng/ml), their combination, or vehicle 
for 4 h, and cell proliferation was measured by 
BrdU incorporation the following 16 h, using a 
colorimetric assay. *P < 0.05; IGF-1 vs. IGF-1 
plus MCH. B: CCD-18Co cells were treated 
with MCH (1 μM), TGF-β1 (2 ng/ml), their combination, or vehicle for 36 h, and collagen 
production was evaluated using the Sircol col-
lagen assay. Results are expressed are relative 
units (vehicle treated = 100). **P < 0.01; 
TGF-β1 vs. TGF-β1 plus MCH.

apparent at 24 h postwounding. At 48 h, 74.1 ± 3.5% of the 
wound area had been closed in MCH-treated cells vs. 62.2 ± 
1.7% in vehicle-treated cells (P = 0.0117; Fig. 8).

DISCUSSION

In the present study, we demonstrate attenuation not only of 
chronic experimental colitis by blocking MCH but also of 
testinal fibrotic processes associated with ongoing inflamma-
tion. Intestinal fibrosis represents a recurring clinical problem 
in IBD that leads to bowel dysmotility, narrowing, and obstruc-
tion, complications that often require surgical intervention 
(38). Although limited fibrosis in mice with chronic DSS 
colitis treated with an anti-MCH antibody could be secondary to 
reduced inflammation, our in vitro studies suggest that 
additional direct effects of MCH on intestinal myofibroblasts are 
possible. It would be of interest to test whether MCH 
modulates collagen secretion from myofibroblasts in other 
organs, including liver, lung, and kidney (47).

Earlier studies from our group using MCH-deficient mice 
pointed to MCH as a proinflammatory mediator in experimen-
tal colitis (24). Investigating potential underlying mechanisms 
of such effects, we uncovered that MCHR1 expression on 
intestinal subepithelial α-SMA (+) cells, presumably 
myofibroblasts. Furthermore, we provide evidence for 
direct effects of MCH on myofibroblast proliferation, collagen 
production, and migration under proinflammatory conditions. 
These findings are in agreement with a previous report showing 
that MCH promotes migration of 3T3-preadipocytes via actin 
cytoskeleton rearrangements (10). Additionally, the functional 
antagonist of MCH, α-MSH (44), suppresses TGF-β1-induced 
collagen synthesis in human dermal fibroblasts and has antifi-
brogenic activity in vivo (5). However, in our experimental 
setting, the effect of MCH on myofibroblasts was moderate and 
required the presence of cofactors (42). This is not totally 
unexpected, given that, in other studies, substance P induces 
collagen production by CCD18-Co only in the presence of
TGF-β and IGF-1 (25), bradykinin synergizes with TNF-α to induce expression of COX-2 in the same cells (48), and vasoactive intestinal peptide increases proliferation of primary colonic myofibroblasts only in the presence of platelet-derived growth factor (21). At the molecular level, this MCH requirement for costimulators could perhaps be explained by the fact that G protein-coupled receptors, like MCHR1, lack intrinsic kinase activity, and thus they need to transactivate growth factor receptors to convey mitogenic and growth signals (12). For instance, it has been previously shown that neurotensin promotes the growth of colonic epithelial cells via IGFR transactivation (49) and substance P promotes the proliferation of U-373MG astrogloma cells via EGFR transactivation (7).

In an in vitro assay, we have shown that MCH, under certain conditions (DSS treatment), can accelerate wound healing. Although this effect is highly desirable as a response to acute tissue injury, in cases of persistent inflammation, as in IBD, it might become deleterious. In patients with CD, chronic or excessive wound healing results in fibrostenotic lesions, a major indication for surgery, whereas, in patients with ulcerative colitis, it may cause colon shortening and dysmotility (39). As such, from a therapeutic perspective, blocking MCH might prevent such complications.

The molecular analysis of tissues from mice with colitis treated with anti-MCH provides insights into potential downstream mechanisms by which MCH modulates fibrotic processes in the context of inflammation, as for example regulation of SMAD3 expression. This finding might be of significance in IBD, given that SMAD3 is not only upregulated in the strictured areas of patients with CD (13), but also its downregulation or genetic ablation inhibits intestinal fibrosis while accelerating reepithelialization and mucosa healing in mouse models of experimental colitis (27, 43) and in mouse skin wound healing (1). Furthermore, mouse fibroblasts null for Smad3 showed decreased production of collagen in vitro (33).

In the present and our previous studies, lower TNF-α expression as a result of MCH ablation or inhibition is a common finding in mice with intestinal inflammation (23, 24). Notably, TNF-α has been implicated in inflammatory and fibrotic processes by directly regulating collagen production (36). Indeed, myofibroblasts isolated from patients with CD expressed high levels of TNF-α, and treatment of these cells with an anti-TNF-α antibody (infliximab) decreased collagen production (14). Hence, blocking MCH might be valuable as a combination, “add-on” treatment to current therapies for IBD, including blocking TNF-α.

In conclusion, our findings suggest that targeting MCH might have dual benefit in the treatment of IBD by reducing the severity of inflammation itself as well as preventing fibrosis. Interestingly, the field of IBD therapeutics can move forward by repurposing several existing MCHR1 antagonists initially developed for the treatment of obesity and the metabolic syndrome (8, 29). These candidate drugs have already been tested in early clinical trials and were found to be relatively safe and well tolerated. However, they have been abandoned by pharmaceutical companies primarily because of lack of efficacy in obese individuals, and some of them are readily available for new therapeutic uses (http://www.ncats.nih.gov/research/reengineering/rescue-repurpose/therapeutic-uses/directory.html).

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DISCLOSURES
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AUTHOR CONTRIBUTIONS

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