Dear Mr. David:

I am writing in response to your inquiry regarding a petition by Robert Thomson on behalf of LDN to the Scottish Parliament requesting information. The petition information you provided is included in the paragraph below:

Petition by Robert Thomson on behalf of LDN Now Scotland calling on the Scottish Parliament to urge the Scottish Government to make Low Dose Naltrexone readily available on the NHS to auto-immune disease sufferers as well as other conditions not classified as auto-immune such as HIV/AIDS, cancer and fertility, in each NHS board area thereby reducing the danger of sufferers having to access riskier alternatives and also incurring higher costs by purchasing the drug through private medical providers and to provide guidance to all GPs on LDN protocol and require them to collect LDN clinical data.

You have asked me to respond to specifics in the petition and answer the questions below:

- What is the clinical evidence of the effectiveness of LDN (providing that the clinical trials/tests you carried out were for Naltrexone in 4.5mg or lower dosage)?
- Is there any clinical and scientific evidence that Naltrexone in 4.5mg or lower dosage is unsafe?
- Has any guidance to clinicians in the USA about LDN and its clinical uses been issued?

I am a board certified gastroenterologist at a University hospital (Pennsylvania State University) and I have conducted research in both animals and human subjects using naltrexone for Crohn’s disease. I have not conducted research on any of the other disorders listed in the petition and therefore, I am not eligible to comment on them. We carried out a study in mice where we induced colitis with a chemical and treated the mice with injections of naltrexone. We found that...
the naltrexone decreased inflammatory cytokine expression and improved the histology of the colon lining under the microscope. This work was published in the J Immunotoxicology 15:179-187, 2008 (attached) and was the basis for the dose used in the human trials.

Our first clinical human trial published in the Am J Gastroenterology in 2007 (attached) showed that naltrexone orally at 4.5 mg per day improved active Crohn’s disease in 89% and induced remission in 67% of those treated. We have extended our research and recently completed a clinical double blind placebo controlled trial testing the efficacy of naltrexone versus placebo in 40 adult subjects with active Crohn’s disease. Although the research is complete, it has not yet been published; therefore, I cannot release any of these results yet. We are currently also conducting a clinical trial in children with active Crohn’s disease where we treat with either capsules or liquid at 0.1 mg/kg not to exceed 4.5 mg. I have applied to the Food and Drug Administration (FDA) in the USA for FDA Orphan Drug Status for naltrexone in children.

Currently naltrexone is approved by the US FDA at a dosage of 50mg for alcohol withdrawal syndromes. Our animal studies suggest that the higher dose may not work as effectively at reversing inflammation as the lower dose. Naltrexone is not approved in the USA for Crohn’s disease therapy or at the 4.5 mg dosing. In order for me to treat patients with this medication I had to apply for a license from the FDA and be given an Investigator New Drug number. I am required to keep accurate records and file annual reports to the FDA regarding subjects treated with naltrexone in our clinical trials.

I also have submitted a patent for the use of naltrexone in inflammatory bowel disease using the lower dosing. This application was filed in the USA, UK and many other international countries and is currently under review. We have also applied to the National Institutes of Health for additional research funding to answer your question about dosing; ie., to try both higher and lower doses. There is no scientific data on the use of naltrexone as doses different from our current publications to accurate answer your second question.

Regarding your 3rd question about guidance provided to US physicians prescribing naltrexone, my comment is that most physicians are not prescribing it because it is not yet approved and is considered experimental. Although some physicians may prescribe it ‘off-label’, this practice is not supported by the FDA or the American Medical Association.

Because the current treatments available for Crohn’s patients include biological agents and immunomodulating drugs that suppress the immune system, there is a serious risk of developing infections and even malignancies with these drugs. Because of the increased incidence of leukemias and hepatosplenic lymphomas using infliximab, the UDA FDA has issued a black box warning on the use of biologics in patients. Also the cost of biologics is extreme, and that along with the potential risks is why there is a desperate need for safer drugs to treat patients with Crohn’s disease.

Naltrexone is an inexpensive medication that has the advantage of being given orally. In our human studies the side effects were minimal and the results very good. Although we believe more studies using this compound will improve our understanding and use of it in the future, I cannot encourage its use outside of medically monitored trials at the present time. If the FDA supports our application for approval for this indication and at this dose, that will open the door for new treatments of patients with Inflammatory bowel disease and also spark research in other areas of autoimmune conditions as mentioned in the petition above.
I hope this communication will help you in responding to the petition.

Sincerely

Jill P. Smith, MD
Professor of Medicine
Penn State University
College of Medicine
H-045; 500 University Drive
Hershey, PA 17033
717-531-3694
The Opioid Antagonist Naltrexone Improves Murine Inflammatory Bowel Disease

Gail L. Matters
Departments of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA

John F. Harms and Christopher McGovern
Department of Medicine, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Leo Fitzpatrick
Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA

Anuj Parikh, Nicholas Nilo, and Jill P. Smith
Department of Medicine, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Inflammatory bowel disease (IBD) is a condition of the intestine with significant morbidity. Although hereditary, environmental, immunologic, and bacterial factors have been implicated, the etiology of IBD remains unknown. Since opioid peptides modulate inflammatory cytokine production and opioid antagonists promote tissue growth and repair, we hypothesized the opioid antagonist naltrexone could reduce inflammation of the bowel. Using a chemically-induced mouse model of IBD, C57BL/6J mice received either untreated drinking water or water containing 2% dextran sulfate sodium (DSS) in two parallel regimens modeling moderate and severe colitis. After colitis was established, animals in the moderate colitis study were administered either saline (control) or naltrexone (NTX; 8 or 400 \( \mu \)g/kg) daily, while those in the severe colitis study received 0.1 or 10 mg/kg NTX. DSS-treated animals had significant weight loss (\( p = 0.006 \)) and higher disease activity index (DAI) scores (\( p < 0.001 \)) compared to water controls. However, NTX treatment of mice with moderate colitis resulted in less weight loss, lower DAI scores, and less histologic evidence of inflammation compared to controls. Significantly, elevated levels of colonic RNA for pro-inflammatory cytokines interleukin (IL)-6 and IL-12 were also decreased toward normal with NTX. Similar to patients with severe and unresponsive disease, animals in the severe colitis study did not significantly respond to treatment. Thus, NTX therapy reverses physical symptoms, histologic evidence, and molecular markers of inflammation in moderate colitis. The mechanism by which NTX acts to reverse colitis is related in part to the decreased expression of pro-inflammatory cytokines.

Keywords: Naltrexone, inflammatory bowel disease, opioids, antagonists

INTRODUCTION

Inflammatory bowel disease (IBD) includes two idiopathic conditions termed ulcerative colitis and Crohn’s disease which affect approximately 1 million people in North America (Cominelli, 2004). The pathogenesis of IBD appears to involve the dysregulation of the immune system in the intestine in response to either commensal bacteria or environment in a genetically predisposed host (Cominelli, 2004; Sands, 2007). Cytokines have been implicated in the pathogenesis of Crohn’s disease, in particular the pro-inflammatory/T\( \gamma \)1 cytokines interleukin-1 (IL-1), IL-2, IL-6, IL-12, IL-18, interferon (INF)-\( \gamma \), and tumor necrosis factor-alpha (TNF\( \alpha \)) (Pizarro and Cominelli, 2007).

Medical treatment of this condition has focused on targeting the inflammatory response with immunosuppressive drugs (i.e., corticosteroids, azathioprine) or immune-specific biological drugs, such as monoclonal antibodies against TNF\( \alpha \) (Targan et al., 1997; Sandborn and Hanauer, 1999; Hanauer and Present, 2003; Navarro and Hanauer, 2003; Bell and Kamm, 2000;
Kamm, 2006). Unfortunately, chronic immunosuppression may increase the risk of developing infections such as tuberculosis (Keane et al., 2001) and lymphoma (Kandiel et al., 2005). Since the risks may at times outweigh the benefits when immunomodulating drugs are combined with biologic agents (Hanauer, 2007), novel therapeutic approaches to treat IBD are needed.

Accumulating evidence points to roles for endogenous opioid peptides in the development or perpetuation of inflammation. Immune cells have been shown to express μ, κ, and δ-opioid receptors that bind both opioid agonists and antagonists (McCarthy et al., 2001). In vivo treatments with opioids have been shown to induce the release of pro-inflammatory cytokines, such as IL-12 and TNFα, by mouse peritoneal macrophages (Tomassini et al., 2003). Studies have verified similar roles with endogenous opioids, demonstrating that opioids, including [Met]3- enkephalin, stimulate peritoneal macrophages in rodents systems (Vujic et al., 2004). Opioid receptors are found throughout the gastrointestinal tract in the myenteric and submucosal plexus as well as in epithelial cells (Jimenez et al., 2006).

Furthermore, in these studies, cytokine production was decreased by simultaneous treatment with the opioid receptor agonist, naltrexone, indicating the effects were mediated by opioid receptors expressed by peripheral and intestinal immune cells. Pre-treatment with naltrexone (10 mg/kg) has also been shown to block TNFα synthesis and induction of septic shock in LPS/d-galactosamine-treated mice (Greeneltch et al., 2004). Recently, a clinical study in human subjects with active Crohn’s disease demonstrated that naltrexone therapy improved Crohn’s inflammatory scores and quality of life (Smith et al., 2007). Given the role of endogenous opioids in inflammation and the inhibition of these effects by the opioid receptor antagonist, naltrexone, we hypothesized that naltrexone therapy could improve colitis in mice. In order to test this hypothesis we used the DSS (dextran sulfate sodium) model of experimental colitis in mice to examine symptoms, tissue histology, and RNA profiling of cytokines in response to naltrexone. The addition of DSS to the drinking water induces hematochezia, weight loss, intestinal shortening, and infiltration of neutrophils, and thus serves as a model for human inflammatory bowel diseases, particularly ulcerative colitis (Okayasu et al., 1990). Breakdown of epithelial barrier function in DSS-treated mice leads to an induction of pro-inflammatory cytokines, which are thought to play a central role in disease progression (Strober et al., 2002). Treatments aimed at reducing this excessive inflammatory response have demonstrated therapeutic promise in DSS models; therefore, DSS-treated mice are particularly suitable for proof-of-concept studies of novel IBD therapeutics and treatments that may reduce the cytokine-induced inflammatory state (Pizarro et al., 2003).

METHODS

Animals and Experimental Procedures

Two separate studies were performed to test the effects of naltrexone: chemically induced moderate or severe colitis. The research protocol was approved by the Institutional Animal Care and Usage Committee of the Pennsylvania State University College of Medicine and animals were housed in accordance with the AAACCR guidelines for veterinary medicine.

In the moderate colitis study, 6- to 8-wk-old male C57BL/6J mice (Charles River, Wilmington, MA) were randomly allocated into one of two groups of 24 mice each. Food and water were provided ad libitum. Individual mice were housed in separate cages for accurate measurement of food and water intake to determine whether NTX alone affected animal weight, water intake, and food consumption, each measured daily. The first group (Normal) received untreated drinking water and the second group (DSS) received water containing 2% dextran sulfate sodium (DSS; TDB Consultancy AB, Uppsala, Sweden; molecular weight, 40,000) for six days followed by untreated water for three additional days (See treatment schedule, Figure 1A).

Mice in each group (Normal or DSS) were randomly subdivided into three treatment groups of 8 mice each. Starting on Day 3, mice were treated once daily for six consecutive days with a subcutaneous (SC) injection (0.1 ml) of one of the following: saline (control), 8.0 μg/kg naltrexone (NTX), or 400 μg/kg NTX. These doses were selected to bracket the approximate clinical dosing reported with efficacy in humans with Crohn’s disease (Smith et al. 2007). On Day 9, all animals were necropsied and their colons resected and analyzed.

In the severe colitis study, C57BL/6J mice were placed into one of four groups of 10 mice each. The first group received normal water (control). The remaining groups received water containing 2% DSS (30 mice) for the duration of the study (9 days). Beginning on Day 3, the normal water mice and 10 of the DSS-treated mice were injected with vehicle (water; 0.1 ml SC daily). For NTX treatment in this study, an intermediate dose (100 μg/kg NTX) and an escalated dose (10 mg/kg NTX) were used to test efficacy against the more severe symptoms anticipated with extended DSS treatment. Mice were injected (0.1 mg/kg or 10 mg/kg; 0.1 ml SC daily; n = 10) until the end of the experiment on Day 9 (See treatment schedule, Figure 2B).

The colitis disease activity index (DAI) was calculated for each mouse according to the system established by Murthy and colleagues (1993) using animal weight, stool occult blood, and stool consistency. Overt changes in stool consistency were rarely discerned in the moderate colitis study so a modified DAI was calculated based on percent weight loss and stool hemoccult or presence of gross bleeding.

Histologic Evaluation

At necropsy, the entire colon was excised, measured in length, and bisected into proximal and distal portions. The proximal and distal colons were additionally divided for RNA extraction and histology. Each histology specimen was fixed in 10% neutral buffered formalin, paraffin embedded and sectioned for hematoxylin and eosin (H&E) staining. Specimens were
FIG. 1. Daily weight, food and water consumption. (A) Mean weight (expressed as % starting weight ± SEM) of animals receiving either normal drinking water (upper panel) or 2% DSS in drinking water (lower panel) is shown. DSS-induced weight loss was lessened by treatment with naltrexone (NTX) in moderate colitis by Day 6. (B) Mean daily food intake in grams per mouse and (C) total water intake in ml per mouse over the 9-day course of study is shown. Asterisks indicate significantly different values between corresponding DSS and Normal (No DSS) treatment groups (* p < 0.025; ** p < 0.005). Significant differences in water intake between saline- and 8 µg/kg NTX-treated mice are indicated by a † (p = 0.022).

examined microscopically and scored based upon the criteria established by Williams et al. (2001) by an investigator blinded to the treatment groups. Briefly, a representative longitudinal section from each mouse was scored at six random fields for inflammation severity, extent of inflammation (mucosa, submucosa, transmural) and crypt damage. Each of these scores was weighted to reflect the percent involvement of the overall section and the weighted scores from each of the six fields were averaged to achieve an overall inflammation score for each mouse.

Statistical Analysis

Results were calculated as mean ± SEM. To eliminate undue influence of abnormally sick or resistant mice, statistical outliers from normally distributed data in weight loss and histological sectioning were determined (Minitab 13, State College, PA; below Q1 – 1.5×IQR, above Q3 + 1.5×IQR) and excluded. Pairwise Student t-tests were performed (Minitab 13) using a modified Bonferroni method to correct for multiple comparisons to controls. Statistical comparisons were performed between NTX treatment sub-groups and their corresponding vehicle control, as well as between the Normal and DSS groups, comparing corresponding vehicle or NTX treatments.

Quantitative Real-Time PCR

Total RNA was extracted from the distal colon samples (Trizol; Invitrogen, Carlsbad, CA) and subjected to analysis by Real-Time RT-PCR. RNA (18S and 28S bands) was visualized using the Agilent 2100 Bioanalyzer (Agilent Technologies) and concentrations were adjusted. First strand cDNA was then produced from 1.0 µg of total RNA using random hexamer primers and the SuperScript III Reverse Transcription kit (Invitrogen). The concentration and quality of resulting cDNA was quantified and analyzed using the Agilent 2100 Bioanalyzer or spectrophotometrically with the NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE). Samples were standardized to 30 ng/µl, and 60 ng of cDNA per sample was then utilized as a template.
FIG. 2. Naltrexone treatment reduces Disease Activity Index (DAI) scores. Mice that received normal drinking water (saline or NTX-treated) showed no evidence of colitis by DAI score (A, upper panel; mean ± SEM). DAI scores increased over time in both the moderate (A, lower panel) and the severe colitis (B) studies. Treatment with 400 µg/kg NTX significantly lowered DAI scores in moderate colitis on Day 6 († p = 0.015). In contrast, NTX failed to improve DAI scores of the severe colitis model (B).

for Real-Time RT-PCR using a SYBR Green Master Mix (Qiagen, Valencia, CA). 18S rRNA primers (Eurogentec, San Diego, CA) and the following gene-specific primer sequences obtained from PrimerBank (Wang and Seed, 2003) were utilized: β-actin, 6671509a; IL-5, 6754336a; IL-6, 13642311a; IL-12, 6680395a; STAT3, 13277852a; STAT4, 6755670a; Muc2, 3452503a2; TFF3, 6755773a1; Palladin, 9828173a1; TGF-β BP, 7305243a1; and, TNFα, 202093a3. To exclude the possibility of genomic DNA contamination, control reactions with no cDNA template were also performed for each gene-specific primer set. PCR amplification and analysis were performed with the Applied Biosystems Sequence Detection System 7300 using the Relative Quantification (ddCt) Plate setup. At least six replicates were performed for each target gene set. Amplification data for the target genes were calibrated using the 18S rRNA endogenous control within each sample. The resulting mean ΔC_T values were then normalized to the β-actin expression (corresponding treatment groups) to ensure observed differences were biologically robust. Pairwise Student t-tests were performed on the normalized mean ΔC_T values for each group using a modified Bonferroni method to correct for multiple comparisons.

RESULTS

Effects of Naltrexone on Animal Weight, Food and Water Consumption

Over the 9-day course of the moderate colitis study, Normal control animals given untreated drinking water exhibited steady weight gain (Figure 1A, upper panel) while DSS mice showed weight loss beginning between Days 4 and 6 (Figure 1A, lower panel) and continuing until necropsy (Day 9). Animals treated with naltrexone exhibited less weight loss compared to DSS + saline mice, reaching statistical significance on Day 6 (p = 0.02). NTX-treated mice also had a tendency toward decreased weight loss on Days 7 and 8, although they did not reach statistical significance.

Food consumption was significantly decreased in the animals with DSS-induced colitis compared to animals without colitis (Figure 1B). Naltrexone treatment alone had no effect on food consumption in both normal animals and DSS-treated animals. Animals treated with NTX (8 µg/kg) had slightly decreased water consumption (Figure 1C). DSS-treated mice drank less water that their treatment counterpart, but there was no difference between the DSS groups treated with saline or NTX (Figure 1C).
Disease Activity Index (DAI) Scores are Reduced by Naltrexone Treatment

To monitor disease progression, a disease activity index (DAI) was assessed daily for each mouse including weight and stool hemoccult. DAI scores for all Normal mice (no DSS), regardless of saline or NTX treatment, showed no clinical evidence of colitis (Figure 2A, upper panel), suggesting that NTX alone has no deleterious effects on the colon. All DSS-treated mice developed colitis symptoms (hemoccult-positive stools and increased DAI scores) by Day 4, which continued to increase through the end of each study (moderate colitis, Figure 2A lower panel; severe colitis, Figure 2B). A reduction in the DAI scores was evident with NTX treatment in animals with moderate colitis; on Day 6, DSS + 400 μg/kg NTX animals had significantly lower (55%) DAI scores than DSS + saline mice (p = 0.015). DAI scores for 400 μg/kg NTX mice at Day 7 were also improved, but did not reach significance (p = 0.038). In contrast, neither dose of NTX (0.1 mg/kg or 10 mg/kg) improved the DAI score in severe colitis (Figure 2B).

Colon Length

Reduced colon length, another indicator of colitis, was also evident in all DSS-treated animals. In the moderate colitis study, control mice drinking normal water and injected with saline had colon length 26% longer than DSS-treated animals (9.32 ± 0.39 cm vs. 6.95 ± 0.43 cm, respectively; p = 0.002). DSS-mice treated with naltrexone had more typical colon lengths (7.86 ± 0.31 cm; p = 0.1), although they were still 15% shorter than in the Normal (no DSS) animals.

Histological Evidence for Reduced Colonic Inflammation in Naltrexone-Treated Mice

Histologic inflammation scores and H&E stains of the distal colon in untreated and treated animals are shown in Figure 3. No inflammation was observed in control animals drinking normal water (saline or NTX-treated), indicating that naltrexone alone did not alter the mucosal integrity of the colon (Figure 3A). All DSS animals had increased inflammation scores, and exhibited crypt damage and increased leukocyte infiltration. In moderate colitis (Figures 3A and 3B), the DSS + NTX animals had a significant decrease in inflammation and damage. The DSS + 400 μg/kg NTX treated mice had reduced histology scores (p = 0.018; Figure 3A), with improved crypt architecture and fewer invading leukocytes than were observed in the DSS + saline mice (Figure 3B).

Animals with severe colitis had no improvement in their histology scores with NTX. Although those mice treated with the lower dose of NTX (0.1 mg/kg) had less inflammation than saline-treated mice and high-dose NTX (10 mg/kg) mice, this difference was not statistically significant (Figure 3C and 3D).

No histologic changes consistent with colitis were observed in the proximal colons of the mice (data not shown).

Expression of DSS-Induced, Pro-inflammatory Cytokine Genes is Decreased by Naltrexone Treatment

Because naltrexone reduced the inflammatory histology of DSS-induced colitis, the expression of several genes of interest, including both cytokines and downstream mediators, was examined by Real-Time RT-PCR. Expression of β-actin, cytokines IL-5, IL-6, IL-12, and transcription factors STAT3 and STAT4 were assessed using 18S rRNA as an endogenous control. After normalizing to β-actin, IL-5 levels were not significantly changed by either DSS + saline or by DSS + NTX (Figure 4A). Similarly, levels of TNFα were not significantly changed in DSS-treated animals compared to normal, uninflamed tissue (data not shown). Because TNFα is an early mediator of inflammation, transcript levels may have already subsided during the post-DSS recovery that preceded necropsy.

By contrast, mRNA encoding the cytokines IL-6 and IL-12, known to be up-regulated in IBD, were increased in DSS + saline animals in comparison to Normal controls (Figures 4B and 4C). The increase in IL-6 mRNA was 66-fold, while the increase in IL-12 was more modest (2.7-fold). Upon treatment with naltrexone, levels of IL-6 and IL-12 were reduced and, for IL-12, naltrexone treatment restored mRNA expression to that seen in the colitis-free, Normal mice. The reduction in IL-6 was also significant, although levels were not completely restored to those seen in the colitis-free animals.

The mRNA for cytokine signaling intermediates STAT3, downstream of IL-6, and STAT4, downstream of IL-12 (Mudter et al., 2005), also were increased in DSS + saline animals (2.20- and 8.03-fold, respectively) (Figures 4B and 4C). However, STAT3 and STAT4 mRNA levels were not decreased to as great an extent by naltrexone treatment. This may reflect the fact that STAT 3 and STAT 4 activities are regulated both at the level of transcription and post-transcriptionally by phosphorylation and nuclear relocalization in response to cytokine signaling (Mudter et al., 2005). In the severe colitis study, inflammatory cytokine markers, as measured by Real-Time RT-PCR, were also significantly elevated by DSS treatment. However, in these animals exhibiting severe, rather than moderate colitis, neither NTX dose significantly affected RNA levels (data not shown).

DISCUSSION

This study is the first to report improvement of colitis in a murine model upon treatment with an opioid antagonist. In both the moderate and severe colitis studies, we administered DSS for three days prior to either vehicle or naltrexone injections, emulating a condition of established bowel inflammation preceding treatment. Naltrexone treatment resulted in a rapid mitigation of moderate colitis symptoms in DSS mice, including weight loss and bleeding. Furthermore, the modest differences in histology (DSS ± NTX) may have been even greater if the diseased animals were not allowed potential time for recovery (post-DSS) prior to necropsy.
FIG. 3. Histologic scores are improved in moderate colitis mice treated with naltrexone. At necropsy, longitudinal sections of the distal colon were H&E stained and evaluated using the scoring system of Williams and colleagues (2001). Histology scores are expressed as the mean ± SEM. Normal histology was observed in animals drinking untreated water compared to high inflammatory scores in DSS-treated animals (A). Naltrexone treatment improved the histologic scores in animals with moderate colitis (A). Representative H&E sections of distal colon from animals with moderate colitis are shown in B. Compared to the normal appearance of healthy murine colon (Normal + saline; top), leukocyte infiltration and an absence of normal crypt architecture are evident in the DSS + saline mice (middle). Improved architecture and less inflammation are clearly discernable in DSS mice treated with 400 µg/kg NTX (bottom). Cross indicates significantly different values between DSS + saline and DSS + 400 µg/kg NTX (p = 0.018). In contrast, NTX was unable to reverse the inflammatory response observed in the severe colitis model (C, D).

A robust, impact on pro-inflammatory gene expression by naltrexone was also confirmed. Characteristic of IBD, significant elevations in the gene expression of IL-6 and IL-12 cytokines were induced with DSS. However, naltrexone treatment significantly decreased their expression in DSS mice to normal or near-normal levels. This suggests a possible pathway by which NTX improves colitis. Pro-inflammatory NF-κB signaling is associated with opioid receptor activity in immune cells...
FIG. 4. Gene expression of DSS-induced pro-inflammatory cytokines is decreased by naltrexone. Relative mRNA levels for (A) beta-actin and the cytokine IL-5; (B) cytokine IL-12 and downstream mediator STAT4; and (C) cytokine IL-6 and downstream mediator STAT3, were determined by Real-time RT-PCR using total RNA from the distal colon. Target genes were calibrated to 18S rRNA, and means were normalized to beta-actin expression for each corresponding treatment group. Histogram columns represent the mean relative quantity (RQ = $2^{-\Delta \Delta CT}$) and bars represent a 95% confidence interval (CI; RQ = $2^{-\Delta \Delta CT} \pm CI$). DSS induced significant elevations in the RNA of pro-inflammatory cytokines IL-6 and IL-12. However, mice treated with naltrexone exhibited restoration toward normal levels. Asterisks indicate significantly different values between corresponding Normal + saline and each DSS treatment group (*p < 0.0008). Significant differences between DSS-Saline and DSS-NTX mice are indicated by a † (IL-12, †p < 0.005; STAT4, †p < 0.024; IL-6, †p < 0.0008).

(Chen et al., 2006). When opioid receptor signaling is blocked by naltrexone, the over-stimulation of immune system is moderated, cytokine levels are restored, and a more normal mucosal structure reappears.

There is ample evidence that opioid peptides, particularly delta opioid receptor agonists, can regulate immune responses (House et al., 1996). First, immune cells secrete opioid peptides (Cabot, 2001; McCarthy et al., 2001) and activation of murine T-lymphocytes increases the synthesis and secretion of [Met$^5$]-enkephalin (Zurawski et al., 1986). Second, immune cells express delta, mu, and kappa opioid receptors that respond to endogenous and synthetic opioids (Cabot, 2001; McCarthy et al., 2001). In fact, [Met$^5$]-enkephalin knock-out mice exhibit a defect in T-lymphocyte activation and a reduced ability of T-lymphocytes to proliferate (Hook et al., 2003).

Both endogenous and exogenous opioids have been shown to intensify the production of pro-inflammatory cytokines by immune cells and, in some cases, this opioid-stimulated increase in cytokines has been abrogated by opioid receptor antagonists (Vujic et al., 2004). Third, while opioid peptides do not, in themselves, induce inflammatory responses, they can sensitize T-lymphocytes and macrophages to other pro-inflammatory stimuli (Hucklebridge et al., 1990; Kamphuis et al., 1998). Thus, because opioids can enhance the immune response to pro-inflammatory stimuli and thereby contribute to the escalation of inflammation, it is reasonable to suggest that naltrexone’s action...
on both T-lymphocytes and macrophages could effectively moderate the excessive pro-inflammatory response in IBD. Consistent with this notion, the DSS-induced pro-inflammatory response in this study was down-regulated by opioid receptor blockade.

Although naltrexone is a competitive antagonist at delta, mu and kappa opioid receptors, the effects on immune function reported herein are consistent with delta receptor blockade. In a broad sense, while delta opioid receptor stimulation is pro-inflammatory, signaling through mu opioid receptors has been shown to be immunosuppressive (McCarthy et al., 2001). Indeed, the use of specific mu receptor agonists for treatment of IBD has shown therapeutic benefit both in mouse models and in human mucosal explants (Philippe et al., 2003, 2006). Studies with delta and mu receptor-specific antagonists will further clarify the role of these receptors in colonic inflammation. Conceivably, a delta receptor antagonist and mu receptor agonist may function synergistically with maximal therapeutic benefit.

It has yet to be determined whether naltrexone blocks an overactive opioid peptide system which is aggravating the inflammatory response, or whether it augments the release of endogenous opioids to alter diarrhea and pain through specific receptors in the gut. In addition to inflammatory modulation, naltrexone may elicit other complementary effects, potentially mediated through blockade of a second type of opioid receptor, the opioid growth factor (OGF) receptor. [Met5]-enkephalin binding to the OGF receptor generally inhibits growth and repair. Naltrexone blockade of the receptor, which is expressed throughout the gastrointestinal tract, has been shown to promote cell proliferation and repertithelialization in esophageal epithelium (Zagon et al., 1997). As evidenced by the improved histology scores, healing of the gastrointestinal mucosa observed in our studies may be the result of the simultaneous effects of naltrexone on inflammation and epithelial renewal.

Current therapies for IBD that target pro-inflammatory cytokines (i.e., anti-TNFα monoclonal antibodies) eliminate the cytokines and carry an increased risk of infection due to immune suppression (Sandborn and Targan, 2002; Hanauer, 2007). Humanized monoclonal antibodies also show diminished efficacy over time and have significant secondary complications, decreasing their suitability for long-term use. Because opioid receptor blockade down-regulates, but does not eliminate, pro-inflammatory cytokines, naltrexone therapy may have fewer undesirable side-effects than currently-used agents. Additionally, the versatility of naltrexone for oral administration presents advantages in patient compliance.

Our results also support our previous clinical report of efficacy with low-dose naltrexone. A low-dose of naltrexone (4.5 mg), was shown to decrease inflammation in human subjects with Crohn’s disease (Smith et al., 2007). In the moderate colitis study presented here, naltrexone was effective in microgram per kilogram doses. In the severe colitis portion, the lower dose (0.1 mg/kg) exhibited a trend toward improvement, while high-dose (10 mg/kg) showed no effect, although both treatments were arguably complicated by the induction of more severe colitis than in the moderate study. This reflects current care for patients with severe colitis, in which the inflammation at times exceeds medical management, necessitating surgery. While naltrexone has been approved by the Food and Drug Administration for alcohol withdrawal syndromes (Petrakis et al., 2007) at a dose of 50 mg daily, it is unknown how this higher dose affects inflammatory responses and cytokines.

Precedence for greater efficacy of low-dose therapies already exists. Others have shown that micromolar concentrations of dextromethorphan, a D-isomer of the codeine analog levorphanol, induces a neuroprotective effect in the brain by suppression of proinflammatory factors superoxide, NO and TNFα (Liu et al., 2003, 2005). This inhibitory effect on free radical generation by ultra-low concentrations of dynorphins is shared by another opioid peptide, enkephalin (Zaitsev et al., 1991; Efanov et al., 1994), where femtomolar concentrations of an enkephalin analog inhibited the reactive burst from human neutrophils and mouse macrophages. Thus manipulating the opioid-opioid receptor axis with small doses of these compounds plays a role in modulating inflammatory mechanisms.

These data demonstrate a role of endogenous opioids in the development and progression of IBD and the effectiveness of opioid receptor blockade in reducing of colonic inflammation and damage. On the whole-animal, tissue, and molecular levels, naltrexone treatment alleviated the effects of DSS-induced colitis. Further work will more specifically define the role of endogenous opioids in IBD, the mechanisms linking opioid receptor activation and cytokine production, and the therapeutic potential of naltrexone or other opioid antagonists for the treatment of IBD.

REFERENCES


Low-Dose Naltrexone Therapy Improves Active Crohn’s Disease

Jill P. Smith, M.D., Heather Stock, M.D., Sandra Bingaman, R.N., David Mauger, Ph.D., Moshe Rogosnitzky, and Ian S. Zagon, Ph.D.
Departments of Medicine and Health Evaluation Sciences, Pennsylvania State University College of Medicine, Hershey, Pennsylvania; Consultant, Telz Stone, Israel; and Neural and Behavioral Sciences, Pennsylvania State University, Hershey, Pennsylvania

OBJECTIVES: Endogenous opioids and opioid antagonists have been shown to play a role in healing and repair of tissues. In an open-labeled pilot prospective trial, the safety and efficacy of low-dose naltrexone (LDN), an opioid antagonist, were tested in patients with active Crohn’s disease.

METHODS: Eligible subjects with histologically and endoscopically confirmed active Crohn’s disease activity index (CDAI) score of 220–450 were enrolled in a study using 4.5 mg naltrexone/day. Infliximab was not allowed for a minimum of 8 wk prior to study initiation. Other therapy for Crohn’s disease that was at a stable dose for 4 wk prior to enrollment was continued at the same doses. Patients completed the inflammatory bowel disease questionnaire (IBDQ) and the short-form (SF-36) quality of life surveys and CDAI scores were assessed pretreatment, every 4 wk on therapy and 4 wk after completion of the study drug. Drug was administered by mouth each evening for a 12-wk period.

RESULTS: Seventeen patients with a mean CDAI score of 356 ± 27 were enrolled. CDAI scores decreased significantly (P = 0.01) with LDN, and remained lower than baseline 4 wk after completing therapy. Eighty-nine percent of patients exhibited a response to therapy and 67% achieved a remission (P < 0.001). Improvement was recorded in both quality of life surveys with LDN compared with baseline. No laboratory abnormalities were noted. The most common side effect was sleep disturbances, occurring in seven patients.

CONCLUSIONS: LDN therapy appears effective and safe in subjects with active Crohn’s disease. Further studies are needed to explore the use of this compound.

INTRODUCTION
Chronic relapsing and remitting inflammation of the gastrointestinal tract is the hallmark of ulcerative colitis and Crohn’s disease, conditions termed inflammatory bowel diseases (IBD) (1). The peak age of onset of this disease is between the first and fourth decades of life, with a prevalence of 100–200 per 100,000 in Europe and North America. IBD accounts for significant morbidity and lower quality of life, and is responsible for nearly $2.0 billion in annual medical costs in the United States (2). Crohn’s disease is characterized by transmural, patchy, granulomatous inflammation of any part of the gastrointestinal tract, although it is most common in the ileocecal area (3). The major symptoms of Crohn’s disease include abdominal pain, diarrhea, gastrointestinal bleeding, malabsorption, and weight loss. Although the etiology of Crohn’s disease is unknown, research suggests it involves a complex interplay of environmental, genetic, microbial, immune, and nonimmune factors. Biopsies obtained from the bowel in subjects with Crohn’s disease reveal inflammatory cells suggesting that the bowel is either reacting immunologically to a stimulus or the endogenous immune system of the gastrointestinal tract is off balance (4).

Although there has been progress in defining the pathogenesis of these diseases, their cause remains obscure. The current most comprehensive hypothesis is that IBD is a heterogeneous group of diseases that have a final manifestation, which is mucosal inflammation, and that several genetic and environmental factors are implicated in the pathogenesis of the disease (5–8). The result of these events in some way leads to a disordered immune response to one or more mucosal antigens or bacteria in a genetically determined host (9, 10).

Traditionally, treatment of Crohn’s disease includes compounds designed to reduce the inflammatory response, such as corticosteroids, cyclosporine, and azathioprine, which often lead to serious side effects (11, 12). Major advances in the understanding of the pathogenesis of IBD have led to the development of novel immunotherapies. Such treatments include the administration of chimeric antibodies specific for
molecules such as cytokines known to be central to the pathogenesis of mucosal inflammation (antitumor necrosis factor [TNF], interleukin [IL]-2, IL-10) (13, 14). Although this specific immunotherapy has helped those with Crohn’s disease, still about 20% do not respond to this treatment (14) and many cannot continue this therapy due to untoward side effects (15, 16). Additionally, treatment with the monoclonal antibody infliximab is expensive with each infusion costing thousands of dollars.

Endogenous opioid systems (i.e., opioids and opioid receptors) have been shown to participate in a wide variety of functions including growth and immunity (17). [Met\(^\text{5}\)]-enkephalin is an endogenous pentapeptide that is located throughout the gastrointestinal tract (18). In addition to the growth characteristics of [Met\(^\text{5}\)]-enkephalin, this endogenous opioid has also been shown to influence the immune system with effects on OK10 cells, Leu\(^\text{11}\), and natural killer cells (19). Acetorphan is an oral enkephalinase inhibitor that elevates endogenous enkephalin blood levels and has been used in Europe and elsewhere to treat diarrheal disorders such as cholera (20) and AIDS diarrhea (21). In a clinical study (22), 193 subjects with diarrhea received either acetorphan or placebo for 10 days, and the incidence of diarrhea was reduced by 30%. Additionally, the symptoms of abdominal pain, anorexia, and nausea were also significantly reduced compared with placebo (22).

Zagon and McLaughlin (23) have reported in an animal model that a low dose of naltrexone can produce an intermittent blockade of the opioid receptor. This short-term blockade resulted in a rise in the endogenous tissue levels of [Met\(^\text{5}\)]-enkephalin and endorphins and results in the same effects on growth as exogenous enkephalin (23). It is presumed that too high a dose of receptor antagonist would block the receptor completely and obliterate the effects of the endogenous opioids (24). In fact, naltrexone therapy has been used to aid in the healing of corneal abrasions (25). Naltrexone has also been shown to block TNF-\(\alpha\) synthesis and induction of septic shock in LPS/D-galactosamine-treated mice (26), suggesting that perhaps naltrexone itself may have anti-inflammatory effects.

In this pilot study, we investigated the effects of low-dose naltrexone (LDN) in patients with active Crohn’s disease. It was hypothesized that LDN would improve activity of Crohn’s disease in patients by showing a decline in the Crohn’s disease activity index (CDAI) scores and blood inflammatory markers (C-reactive protein and ESR), and improve quality of life. It is proposed that the mechanism by which LDN will improve Crohn’s disease will be by causing an elevation in endogenous opioid levels.

PATIENTS AND METHODS

Patient Selection

Eligible patients were both male and female, at least 18 yr of age, and with the confirmed diagnosis of Crohn’s disease by either endoscopic or radiographic procedures. Patients had moderate to severely active disease as defined by a CDAI score of >220 and <450 (27). Patients taking stable doses of aminosalicylates, immunomodulators, corticosteroids, or antibiotics were permitted to enter the study, and were continued at the same dosage throughout the trial. Women of childbearing age were permitted to enroll and, if not surgically sterile, were required to use adequate contraception (defined as oral or depot contraceptive, IUD, or barrier plus spermicide) for the duration of the study. These women were required to continue adequate contraception for 3 months after the completion of the study. Exclusion criteria included: women who were pregnant or breastfeeding, subjects with an ileostomy, colostomy, ileorectal anastomosis, or short bowel syndrome from surgery, and patients with abnormal liver function tests. Subjects taking tacrolimus, cyclosporine, mycophenolate, or infliximab within 8 wk of enrollment were excluded.

Approval for the study was granted by the Institutional Review Board of the Human Subjects Protection Office at the Pennsylvania State University Milton S. Hershey Medical Center. The LDN was assigned an Investigational New Drug Number 67,442 by the Food and Drug Administration (FDA).

Study Design

The study was designed as an open-labeled pilot investigation to evaluate response, safety, and toxicity to LDN in subjects with active Crohn’s disease. Eligibility was assessed by telephone, and potential candidates were scheduled for a screening visit in the General Clinical Research Center (GCRC). At the screening visit, patients were subjected to a history and physical examination and laboratory testing (chemistry panel, complete blood count [CBC], urinalysis, and erythrocyte sedimentation rate [ESR]). Patients were dispensed a 7-day diary to record symptoms of frequency of diarrhea, abdominal pain, and general well-being. Within 14 days, patients returned for assessment and calculation of the CDAI score. Qualifying subjects were dispensed medication and given a new diary in order to calculate the subsequent month’s CDAI score at the conclusion of this visit (baseline). Patients returned after 2 wk for an interim visit to evaluate side effects and perform a CBC. Follow-up visits were scheduled for weeks 4, 8, 12, and 16.

Treatment

Naltrexone hydrochloride was compounded into capsules containing 4.5 mg by GMP-approved standards at Williams Apothecary in Lancaster, PA. Because the dosage used in this study was lower than the FDA-approved dose of 50 mg, it will be referred to as “low-dose naltrexone” or LDN. Quality assurance of packaging and purity were confirmed by Analytical Research Laboratories (Oklahoma City, OK). Patients were treated with LDN orally each evening for 3 months. A monthly supply of medication was dispensed to patients by the Investigational Pharmacy of the Pennsylvania State University Milton S. Hershey Medical Center. On the first
visit, an additional 10-day supply of LDN was provided in the event of an appointment delay. Subjects were required to bring the vials to each appointment for counting and drug accountability; extra capsules were returned to the Investigational Pharmacy the day of the visit and another month’s supply dispensed.

**Assessments**

In order to assess LDN’s effect on disease activity, patients kept a Crohn’s symptom diary for the 7 days preceding each visit for calculation of the CDAI score (27). A response to therapy was defined as a 70-point decline in the CDAI score and remission was defined as attaining a CDAI score of 150 or less. To assess quality of life, patients completed two standardized quality of life surveys, the inflammatory bowel disease questionnaire (IBDQ) (28) and SF-36 health survey (29). Appropriate licensure was purchased through contractual agreement for the use of these surveys from each facility.

Routine blood work including CBC, chemistry panel, and ESR were assessed monthly. In addition, urine tests and pregnancy tests were done for monitoring and safety purposes pretreatment and at each monthly visit. C-reactive protein (C-RP) was measured at baseline and at week 12. [Met5]-enkephalin levels were determined by radioimmunoassay (RIA) at baseline and wk 4, 8, 12, and 16 (Peninsula Laboratories, San Carlos, CA).

**Safety Measures**

The study was monitored by the data safety monitoring board (DSMB) at the Pennsylvania State University Milton S. Hershey Medical Center. The safety and toxicity of LDN were assessed by adverse events, laboratory parameters, and vital signs. Nonhematologic and hematologic toxicities were determined by the WHO criteria (30). All adverse events were reported to the Institutional Review Board according to the guidelines established by the Pennsylvania State University Milton S. Hershey Medical Center.

Patients who required rescue medication based on an increase in CDAI score of 100 points were terminated from the study. These subjects were given a tapering regime of LDN, involving dose reduction to every other day for 10 days before discontinuing the medication. Patients necessitating discontinuation from the study were required to return for follow-up visits and analyzed as intent-to-treat subjects.

**Statistical Analysis**

Data were entered into a secure computer in the GCRC by a nurse assigned to this project and analyzed by the Department of Health Evaluation Sciences. An intent-to-treat analysis was performed in which the available data from all evaluable patients were included in the statistical analysis. The parameters of measurement (CDAI scores, laboratory values, and quality of life surveys) were analyzed by SAS statistical software system (version 8.1) computer program by the biostatistician comparing baseline values to those obtained monthly and 4 wk post-therapy. Data from laboratory results and quality of life surveys were entered into an Excel spreadsheet. A longitudinal data analysis, based on the linear mixed-effects model was applied using PROC MIXED program. The Bonferroni statistical method was used to adjust significance, where analysis including multiple comparisons to the baseline were made. P values for binary outcomes of response and remission were calculated using the exact test for binomial proportions.

**RESULTS**

**Patients and Demographics**

Twenty-one subjects were screened for the study and seventeen were eligible to participate. Of the four who were screened that did not participate: one was a screening failure due to elevated liver enzymes, one failed the screen secondary to severe psychiatric illness, and the other two subjects opted for other therapy and declined before receiving drug. Of the seventeen subjects who enrolled in the study, only one subject terminated before wk 12 secondary to a flare-up in Crohn’s disease when she discontinued her concomitant medications. This subject was followed and data included throughout the study as an intent-to-treat subject. The characteristics of the patients at enrollment are shown in Table 1, including age, gender, and body weight. Most patients had both small bowel and colonic disease, and two patients had active perianal fistulas. Eight patients had prior surgical resection performed for their Crohn’s disease. Seventy-six percent of patients had prior treatment with anti-TNF-α therapy, and were either allergic, intolerant, or unresponsive to this medication. Concomitant medications for Crohn’s disease taken by patients throughout the study are also shown in Table 1.

**General**

Statistical analysis showed that there was no significant change in body weight from screening visit through wk 16 of

<table>
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<tr>
<th>Table 1. Patient Demographics</th>
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<tr>
<td>Mean age ± SEM (yr)</td>
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<td>Gender, N (% of patients)</td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
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<td>Mean body weight ± SEM (kg)</td>
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<td>Colon</td>
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<tr>
<td>Past resection performed, N (% of patients)</td>
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<tr>
<td>Prior anti-TNF-α therapy, N (% of patients)</td>
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<tr>
<td>Concomitant meds for Crohn’s, N (% of patients)</td>
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<tr>
<td>Aminosalicylates</td>
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<td>Immunosuppressors</td>
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the study (data not shown). Two patients elected to discon-
tinue taking routine medications for Crohn’s disease prior to
wk 12 and symptoms of Crohn’s disease recurred in one of
them. Data from both patients were analyzed with an intent-
to-treat paradigm. The two subjects with entercutaneous and
rectovaginal fistulas had closure of the fistulas with LDN ther-
apy. Unexpectedly, one study subject with Crohn’s disease
and multiple sclerosis was found also to have improvement
in her neurological symptoms and manifestations of multiple
sclerosis with LDN.

**Inflammatory Response (CDAI Scores)**

CDAI scores were used to measure the patient’s disease activ-
ity and inflammatory response to LDN therapy. Mean CDAI
scores (Fig. 1) at wk 4, 8, and 12 following the initiation of
LDN therapy were 41, 55, and 49%, respectively, decreased
from baseline. Four weeks after discontinuation of therapy
(wk 16), the mean CDAI score was 45% less than baseline
and not statistically different from the mean scores measured
during the therapy. Figure 2 shows the percentage of patients
responding to therapy (Fig. 2A), as well as the percentage
of subjects achieving a remission of disease (Fig. 2B). At 1
month after treatment, 76% had achieved a response to ther-
apy (a decrease in the CDAI score by 70 points), and at 8
and 12 wk, 88% showed a response. Four weeks after discon-
tinuation of LDN, 73% continued to show a response. At 1
month after starting LDN therapy, 29% of the patients had
achieved a remission (a CDAI score of 150 points or less),
and at wk 8 and 12 of LDN therapy, 53 and 47%, respectively,
had achieved remission (Fig. 2B). Four weeks after discontin-
tuation of LDN therapy, 33% of the subjects were in clinical
remission. Therefore, at some point during the 16-wk trial,
89% of patients exhibited a response ($P < 0.001$), and 67%
achieved a remission ($P = 0.07$) with LDN.

When the components of the CDAI scores were evaluated
separately, the number of bowel movements and pain assess-
ment both independently improved significantly ($P < 0.01$)
from baseline at each 4-wk interval on LDN and 4 wk after
discontinuing LDN. In addition, the CDAI score minus the
number of bowel movements and pain was also statistically
improved with LDN therapy ($P < 0.01$). These results indi-
cate that both pain and number of bowel movements are im-
portant markers in the CDAI score; however, they were not
the only parameters contributing to the improved response
found.

**Quality of Life**

Two standardized quality of life surveys, the IBDQ (Fig. 3)
and the SF-36 health survey (Fig. 4), were administered to pa-
tients receiving LDN treatment. By both measures, patients
experienced a significant improvement in their quality of life
on LDN therapy. With regard to the IBDQ survey, signifi-

![Figure 1](image1.png)

**Figure 1.** Mean Crohn’s disease activity index (CDAI) scores ± SEM are shown at baseline (wk 0), wk 4, 8, and 12 after initiation of LDN therapy and 4 wk after discontinuation of LDN therapy (wk 16). ****Significantly different from baseline at $P < 0.0001$.

![Figure 2](image2.png)

**Figure 2.** The percent of patients responding with a decline in CDAI score of at least 70 points (A), and the percent of patients achieving remission by a CDAI score of 150 or less (B), to LDN therapy are shown at wk 4, 8, and 12 and 4 wk after discontinuation of LDN therapy (wk 16).
Patients experienced a significant improvement in quality of life was noted compared with baseline at wk 4, 8, and 12 on LDN, as well as 1 month after completion of treatment. Figures 3.

**Side Effects**

The most frequently reported side effect with LDN therapy was sleep disturbances, and this was noted in seven patients; one reported unusual dreams. Five subjects changed the timing of LDN from the evening to morning due to insomnia. In no instance was a dose reduction necessary for sleep disturbances. Other rare reported events included nausea (N = 1), hair thinning (N = 1), blurred vision (N = 1), irritability (N = 1), mood swings (N = 1), and mild disorientation (N = 1).

**DISCUSSION**

The results of this pilot study are the first to show that LDN therapy significantly decreases symptoms and improves quality of life in patients with active Crohn’s disease. In fact, two-thirds of enrolled patients achieved remission at some point during LDN treatment. It is known in a condition such as Crohn’s disease that remissions of activity occur spontaneously (31); therefore, it is possible the remission occurred by chance. In a recent large randomized placebo-controlled trial for Crohn’s disease, the remission rate with a placebo was recorded at 23% at wk 12 with even lower placebo remission rates earlier in the study (32). Therefore, in the present study, with 67% achieving remission, it would appear that LDN is effective; however, a randomized placebo-controlled trial is warranted.

Another finding in this trial was the fairly rapid onset of effect from LDN in that by 4 wk there was significant improvement. Corticosteroids may be effective in decreasing symptoms of Crohn’s patients in 7–10 days, but other medications such as the immunomodulators (azathioprine and 6-mercaptopurine) may take 3–4 months to demonstrate improvement in symptoms (33). Often symptoms recur within 1 month after discontinuing corticosteroids or aminosalicylates (31, 33). However, in the present study, continued improvement in CDAI scores and quality of life was reported even 4 wk after discontinuing LDN. Longer studies are needed to evaluate the long-term effects of LDN and whether it can be used for maintenance therapy as well as induction therapy.

Another finding in this pilot study was that LDN improved the quality of life of subjects with active Crohn’s disease. The baseline value on the IBDQ was similar to that reported in other clinical trials (32), indicating that our subject group did not differ from those used in other studies. Statistical analysis indicated that for two separate quality of life surveys, a significant difference from baseline occurred in those individuals on LDN. Moreover, even 1 month after discontinuation of LDN therapy, the quality of life remained better in almost
all parameters measured for these patients. It is unknown at this time, how long the quality of life benefit of LDN persists after discontinuing therapy, but this observation merits further investigation for duration of response.

Treatment with LDN may provide some advantages over other standard therapy for Crohn’s disease. Although the long-term safety profile of LDN in Crohn’s patients is unknown, the safety profile of LDN appears to be excellent in
this short-term study, with infrequent and minor side effects and no known suppression of immunity or greater risk of secondary infections. Corticosteroids have short-term side effects of weight gain, emotional lability, glucose intolerance, and risk of secondary infections, especially fungal (34). Acute complications with some immunomodulators (azathioprine, 6-mercaptopurine) include idiopathic pancreatitis and neutopenia (35). Acute allergic reactions have been reported primarily, 6-mercaptopurine) include idiopathic pancreatitis and neutopenia (35). Acute allergic reactions have been reported with the new anti-TNF-α compounds; these drugs can also increase the risk of reactivation of tuberculosis (36) and induce a lupus-like reaction, serum sickness syndrome, and/or anaphylaxis (37). Higher doses of naltrexone (i.e., 50 mg) used for alcohol and opioid abuse have been reported to elevate liver transaminases (38). In contrast, the use of LDN herein at 4.5 mg daily did not change liver transaminases during treatment.

Infliximab has become the standard medical therapy for patients with fistulizing disease associated with Crohn’s disease (39). It is of interest that the two subjects in our study with enterocutaneous fistulas noted closure with LDN when they had not previously responded to infliximab. Perhaps closures of the fistulas may be related to lower intestinal secretions or mucosal healing. Naltrexone has been reported to promote healing of corneal abrasions and epithelial wound healing by stimulating DNA synthesis (25); therefore, this compound may promote healing. Perhaps, the fistulas closed as a result of a lower number of bowel movements and improved mucosal fluid absorption as reported in diarrheal disorders of other etiologies that respond to enkephalins (22).

It was of interest that one study subject with multiple sclerosis and Crohn's disease in our study also had improvement of her neurologic symptoms with LDN. Although the etiology of both disease processes is unknown, another monoclonal antibody, natalizumab, has been useful in treating both of these conditions (40), suggesting perhaps a similar underlying defect. If so, perhaps evaluation of LDN in other inflammatory conditions such as multiple sclerosis would be warranted.

Medical care for IBD is costly (41, 42). Aminosalicylate therapy can cost several hundred dollars per month, and an infliximab infusion generally exceeds several thousand dollars (not to mention the time away from the workplace for IV administration) (43). Naltrexone is a generic medication and the cost is therefore inexpensive. Moreover, effective mesalazine therapy (Pentasa) may require up to 8–16 tablets per day. Another advantage of LDN is the once-a-day dosing, which may improve patient compliance.

The mechanism by which LDN improves symptoms and reduces inflammation of those individuals with active IBD is unknown. Opioid receptors for µ, κ, and δ have been identified on immune cells (44) and morphine has been shown to induce the release of proinflammatory cytokines from mouse peritoneal macrophages (45). [Met5]-enkephalin has similarly been shown to stimulate peritoneal macrophages in rodents (46). In contrast, Philippe and coworkers have shown that stimulation of the µ opioid receptor with elective agonists reduces inflammation in the TNBS (2,4,6-trinitrobenzene sul-

fonic acid) murine model of colitis (47). Plasma enkephalin levels were not altered in this study; however, the enkephalin levels were only obtained monthly at the time of the AM clinic appointment. Because enkephalin levels have been shown to increase in animals administered LDN through transient receptor blockade, it is possible that the plasma enkephalin levels were increased by the compound, but the time of the blood sampling was inappropriate. Peptide levels usually are short-lived in the peripheral blood and frequent sampling postgestion would be necessary to perform accurate pharmacokinetic assays. Another possible explanation for the unchanged enkephalin levels in this study may be that perhaps the dose used in this study was too low and did not effect a sufficient blockade to stimulate upregulation of [Met5]-enkephalin.

LDN may also be acting by another mechanism unrelated to changes in enkephalin levels such as through a reduction in cytokine activity or promotion of direct growth and mucosal repair. In fact, opioids have been shown to increase release of peritoneal cytokines (45) and naltrexone has been shown to block TNF-α production in a murine model (26). Another possibility is that therapy with the low-dose opioid antagonist may have affected a different endogenous opioid substance, such as endorphins, which were not measured.

Naltrexone may be playing a role in direct mucosal healing unrelated to its effects on cytokines. Opioids have been shown to decrease cell growth (48) through the interaction with the nuclear opioid growth factor receptor (49), and indeed blockade of the opioid receptor with naltrexone has been shown to promote DNA synthesis and healing of corneal ulcers (25). Lastly, there are opioid receptors throughout the gastrointestinal tract that are involved in analgesia, fluid, and water absorption. Because the CDAI scores are partially calculated with the patient’s number of liquid bowel movements and perceived pain, naltrexone may have improved the CDAI scores in these individuals through another opioid-mediated mechanism.

The results of this feasibility study support the need for further investigation with a randomized controlled Phase 2 trial of LDN therapy and comparison to a placebo group. Because the present study found that subjects were still unchanged and improved 4 wk after stopping naltrexone therapy, a longer follow-up period should be observed to determine the durability of response. Extended treatment periods in future studies would further define optimal naltrexone treatment parameters. It is unknown whether naltrexone may be beneficial in reducing the amount of additional Crohn’s medications required, i.e., steroid sparing. Future studies are needed to further explore these interesting results and perhaps provide hope for a common frequently debilitating disease.

**ACKNOWLEDGMENTS**

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**STUDY HIGHLIGHTS**

**What Is Current Knowledge**
- The current medical therapy of Crohn’s disease includes medications that target the immune system or inflammatory modulators.
- Opioid systems (peptides and receptors) play an integral role in gastrointestinal fluid regulation, pain perception, and inflammation.
- Many of the current drugs for treatment of Crohn’s disease carry a greater risk of infection from immunosuppression or allergic reactions, and some must be administered parenterally.

**What Is New Here**
- An opioid antagonist, naltrexone 4.5 mg, administered by mouth once daily significantly improved Crohn’s disease activity index (CDAI) scores and symptoms in subjects with active Crohn’s disease.
- Quality of life significantly improved with low-dose naltrexone therapy and remained improved after discontinuation of the drug.
- Naltrexone therapy was well tolerated in Crohn’s disease with minimal side effects.

**REFERENCES**


**CONFLICT OF INTEREST**

The authors declared no conflicts of interest.