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ORIGINAL ARTICLE

Therapy with the Opioid Antagonist Naltrexone Promotes Mucosal Healing in Active Crohn's Disease: A Randomized Placebo-Controlled Trial

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Abstract
Background Endogenous opioid peptides have been shown to play a role in the development and/or perpetuation of inflammation. We hypothesize that the endogenous opioid system is involved in inflammatory bowel disease, and antagonism of the opioid–opioid receptor will lead to reversal of inflammation.
Aims A randomized double-blind placebo-controlled study was designed to test the efficacy and safety of an opioid antagonist for 12 weeks in adults with active Crohn's disease.
Methods Forty subjects with active Crohn's disease were enrolled in the study. Randomized patients received daily oral administration of 4.5-mg naltrexone or placebo. Providers and patients were masked to treatment assignment. The primary outcome was the proportion of subjects in each arm with a 70-point decline in Crohn's Disease Activity Index score (CDAI). The secondary outcome included mucosal healing based upon colonoscopy appearance and histology.
Results Eighty-eight percent of those treated with naltrexone had at least a 70-point decline in CDAI scores compared to 40% of placebo-treated patients ($p = 0.009$). After 12 weeks, 78% of subjects treated with naltrexone exhibited an endoscopic response as indicated by a 5-point decline in the Crohn's disease endoscopy index severity score (CDEIS) from baseline compared to 28% response in placebo-treated controls ($p = 0.008$), and 33% achieved remission with a CDEIS score <6, whereas only 8% of those on placebo showed the same change. Fatigue was the only side effect reported that was significantly greater in subjects receiving placebo.
Conclusions Naltrexone improves clinical and inflammatory activity of subjects with moderate to severe Crohn's disease compared to placebo-treated controls. Strategies to alter the endogenous opioid system provide promise for the treatment of Crohn's disease.

Keywords Opioids · Naltrexone · IBD · Mucosal healing

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Introduction

Crohn's disease is a chronic inflammatory condition of the gastrointestinal tract causing abdominal pain, diarrhea, gastrointestinal bleeding, malabsorption, and weight loss [1]. Although the etiology of Crohn's disease is unknown, research suggests it involves a complex interplay of environmental, genetic, microbial, immune, and nonimmune factors [2–4]. Biopsies obtained from the bowel in subjects with Crohn's disease reveal an inflammatory cellular infiltrate with crypt abscesses, architectural distortion, and occasional granulomas [5].

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Traditionally, treatment of Crohn's disease includes compounds designed to reduce the inflammatory response, and the mainstay of therapy has included the 5-aminosalicylate compounds, corticosteroids, immunomodulators (e.g., 6-mercaptopurine, methotrexate, and azathioprine) and, more recently, anti-TNFα biologic agents [6]. Unfortunately, treatment with many of these compounds may be associated with rare yet serious side effects including opportunistic infections and lymphoma [7–10].

Accumulating evidence supports a role for endogenous opioid peptides (enkephalins and endorphins) in the development and/or perpetuation of inflammation [11, 12]. One factor that has clearly been shown over years is that chronic use of narcotic analgesics significantly reduces immune cell function [13] and opiates inhibit chemokine induced chemotaxis [14]. Immune cells have been shown to express μ, κ, and δ-opioid receptors that bind both agonists and antagonists [15, 16]. There is increasing evidence that opioid peptides can, both directly and indirectly, regulate immune responses. First, immune cells secrete opioid peptides such as [Met^5]-enkephalin, and synthesize opioid receptors [15, 16]. Second, while opioid peptides do not, by themselves, induce inflammatory responses, they can sensitize T-cells and macrophages to other pro-inflammatory stimuli [17]. For example, [Met^5]-enkephalin stimulates the production of hydrogen peroxide and nitrous oxide in rodent peritoneal macrophages [18]. In vivo treatments with some opioids have been shown to induce the release of pro-inflammatory cytokines such as IL-12 and TNFα by mouse peritoneal macrophages [19], which were reversed by simultaneous exposure to the opioid receptor antagonist naltrexone. Additional evidence for the role of opioids in moderating immune cell function comes from [Met^5]-enkephalin knock-out mice, that show a defect in T-cell activation and a decreased ability of T-cells to proliferate [20]. Thus, signaling through opioid receptors expressed by peripheral and intestinal immune cells has a significant impact on cytokine production and intestinal inflammation. In addition to such pro-inflammatory roles, endogenous opioid peptides may also adversely impact normal healing. Opioids can decrease cell growth in vitro [21], and sustained receptor blockade of opioid receptors promotes re-epithelialization, cell migration, and repair in rodent cornea [22].

Our research team has been studying the role of endogenous opioid systems and their role in carcinogenesis, wound healing, development, angiogenesis, and inflammation. We have also demonstrated in a mouse model with chemically induced colitis that intermittent opioid receptor blockade by naltrexone, significantly decreased inflammation of the bowel and improved the inflammatory index using a low dose of naltrexone [23]. In a pilot clinical study, we found that naltrexone treatment at 4.5 mg daily to patients with active Crohn's disease exhibited significant improvement in Crohn's Disease Activity Index (CDAI) scores, plasma inflammatory markers, and quality of life [24]. The purpose of the current investigation was to evaluate the ability of naltrexone to reverse the inflammatory activity and promote mucosal healing in patients with moderate to severe Crohn's disease compared to placebo treated controls.

Methods

Study Design

This prospective, double blind, randomized placebo-controlled trial, undertaken between September 2006 and September 2009 at the Pennsylvania State University College of Medicine, was an investigator-initiated translational research study based upon a pilot clinical trial in subjects with active Crohn's disease [24] and animal dosing study [23]. The protocol was approved by the Institutional Review Board of the Pennsylvania State University on August 28, 2006. The study was designed to test the efficacy of naltrexone (4.5 mg) in subjects with moderate to severe Crohn's disease compared to a placebo at a University hospital. The primary outcome was the proportion of patients achieving a clinical response based upon a 70-point decline in CDAI scores from baseline values at 12 weeks. The secondary outcome was that of endoscopic healing with colonoscopy and biopsies. Written informed consent approved by the Institutional Review Board committee was obtained prior to screening subjects for inclusion. Eligible patients were required to have confirmed Crohn's disease by endoscopic or radiographic methods and moderate to severe clinical disease activity with a CDAI of $\geq$220 [25]. Patients were randomized using a block randomization schedule designed by the biostatistician with a block size of four which was stratified based upon location of disease, and C-reactive protein (CRP) greater than 2.5 mg/dl or less than 2.5 mg/dl (normal $<$ 0.8 mg/dl). Assignments were made by the investigational pharmacy department. The treatment randomization sequence was not concealed to the investigational pharmacists; however, all care providers, endoscopists, pathologist, and patients were masked to the treatment until completion of the entire study for all subjects.

A colonoscopy with mucosal biopsies was performed before initiation of therapy and at 12 weeks. Subjects received either naltrexone or placebo orally each day and were evaluated clinically every 4 weeks with CDAI scores, laboratory tests, and physical examination in the General Clinical Research Center at the Pennsylvania State University College of Medicine. An independent data safety

monitor and statistician reviewed accrual, adverse events, and dose-limiting toxicities, and submitted an interval report to the data safety monitoring board that evaluated interim safety and efficacy outcomes at 6-month intervals. An investigational new drug number was assigned to the Principal Investigator by the Food and Drug Administration for the clinical use of naltrexone for this indication at the dose prescribed. This study was registered with http://www.ClinicalTrials.gov website (NCT00663117) and on CRISP of the NIH website at http://www.report.nih.gov.

Patient Population

Eligible subjects were both male and female, age 18 or older, who were able to read and sign an informed consent and had confirmed Crohn's disease by radiographic, endoscopic, and/or histologic criteria. Patients taking stable doses of medications for Crohn's disease were allowed to enroll with the following stipulations: 4 weeks of stable medications prior to screening for aminosalicylates and steroids (prednisone at 10 mg or less daily and budesonide at 3 mg/day), and 12 weeks for thiopurines (azathioprine or 6-mercaptopurine). These medications were maintained at the same dose throughout the duration of the study. Anti-TNFα biologic agents were not allowed during the study, and subjects were required to discontinue these agents at least 8 weeks prior to enrollment. Lomotil© (diphenoxylate hydrochloride and atropine sulfate) also was not allowed since this medication may interfere with binding to opioid receptors. Women of childbearing age were allowed to enroll in the study, but were required to use two adequate means of contraception until 3 months after completing the study if they were not surgically sterile. Patients were excluded who were pregnant or breastfeeding, had an ileostomy, colostomy, or ileoanal anastomosis, short bowel syndrome, or abnormal liver enzymes.

Study Intervention

Patients were treated with naltrexone or placebo orally at bedtime for 12 weeks. The drug was compounded at Williams Apothecary in Lancaster, PA according to GMP-approved standards, with 4.5 mg of active drug per capsule. Identical placebo capsules were packaged with the filler, Avacil PH105. Packaging and purity were tested for quality control and approved by Analytical Research Laboratories (Oklahoma City, OK).

Study Outcome Measures

The primary parameter of measurement was the proportion of subjects with a clinical response as indicated by a 70-point decline in the CDAI score from baseline [25]. The score was tabulated from a patient diary which included a record of frequency of diarrhea, abdominal pain, general well-being, temperature, antidiarrheals consumed, physical exam, and laboratory tests. The information gathered on the diary corresponded to 7 days prior to each clinic visit. Comparisons were made between the naltrexone-treated and placebo-treated groups at baseline and week 12. Remission was defined as a CDAI score of 150 or less.

The second major outcome evaluated in this study was the endoscopic and histological inflammatory scores obtained during colonoscopy with biopsies comparing naltrexone therapy to placebo controls. Mucosal healing was judged using endoscopic appearance on colonoscopy performed by an endoscopist who was blinded to the treatment regimen according to the established Crohn's Disease Endoscopic Index of Severity (CDEIS) score described by Mary et al. [26]. Histological inflammation was assessed by a pathologist masked to the treatment using microscopic hematoxylin and eosin stained tissue biopsies from five segments of each patient's gastrointestinal tract (i.e., ileum, right colon, transverse colon, left colon, and rectum), and graded according to Dieleman et al. [27] criteria for inflammation.

CRP, erythrocyte sedimentation rate (ESR), hemoglobin, white blood count, albumin and potassium values were compared to baseline levels, as well as between naltrexone and placebo treated groups. Liver enzymes, electrolytes, glucose and renal profiles were monitored for safety and toxicity.

Two standardized quality of life (QOL) surveys were evaluated monthly. One survey was the inflammatory bowel disease questionnaire (IBDQ), a survey that scores the quality of life giving a range from poor (i.e., 32) or very well (i.e., 224) [28]. The IBDQ survey also assessed patients in the domains of bowel, systemic, emotional, and social function. A second survey administered, the Short-Form General Health Survey (SF-36), contained 36 items that measured eight domains of health: physical functioning, role limitations due to physical health, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems, and mental health [29]. Permission to use these questionnaires was granted from the licensing agencies through an agreement with Pennsylvania State University.

Extended Open-Labeled Study

After completing the 12-week double-blinded study, all subjects were continued in an open-labeled extension protocol for an additional 12 weeks, and were treated daily with 4.5 mg of naltrexone. Patients and providers remained blinded to the first part of the study during this

open-labeled extension. The purpose of this open-labeled part of the study was twofold: to improve patient recruitment since all subjects would eventually receive the study drug, and to determine whether longer treatment duration (i.e., 12 weeks vs. 24 weeks) with naltrexone was safe and efficacious. Subjects underwent a colonoscopy after the additional 12 weeks of treatment; therefore, all together these patients had three colonoscopies (baseline, week 12, and week 24).

Statistical Analysis

The information used to calculate sample size was from the results of a prior open-labeled trial [24]. In this previous trial, 15 of 17 subjects (88%) responded favorably to daily administration of 4.5 mg of naltrexone for 12 weeks. Thus, the sample size for this study was calculated under the assumption that at least 60% of the naltrexone-treated patients, and no more than 10% of the placebo-treated patients, would respond with at least a 70-point decline in CDAI scores. Allowing for a 10% withdrawal rate, a sample size of forty subjects yielded an 86% power using a two-sided, 0.05-significance level Fisher's exact test for comparison of treatment response.

Descriptive statistics (mean, median, standard deviation, minimum and maximum) were calculated for each of the CDAI outcome measurements at the baseline visit and at weeks 4, 8, 12, and 24, and for colonoscopy scores at baseline, week 12, and week 24. Comparisons between treatment groups and changes from baseline values were calculated and tested for statistical significance with two-sample and paired t tests, respectively. The proportion of those achieving a response with a 70-point decline in CDAI score was compared between naltrexone and placebo-treated subjects using the Fisher's exact test. Analysis was performed with the intent-to-treat criteria. Clinical significance was accepted if the difference met 95% confidence ($p < 0.05$). Outcomes exhibiting a skewed distribution were analyzed with Wilcoxon Signed-Rank and Mann–Whitney tests. All analyses were carried out using SAS software version 9.1 (SAS Inc, Cary, NC). No interim analyses were performed during the course of the study.

Results

Patient Demographics, Disease Characteristics, and Disposition

The demographics and disease characteristics of the subjects enrolled in the study are shown in Table 1. The percentage of subjects taking concomitant medications at stable dosages during trial included is shown in Table 1. There were no statistical differences in concomitant medications utilized between placebo and naltrexone randomized subjects. Twenty-seven percent of the patients were taking more than one of the concomitant medications for maintenance therapy. The location of disease is also shown with few subjects having colonic disease alone. Figure 1 outlines the disposition of the 40 subjects enrolled in the trial and screened for eligibility. Six subjects that did not meet the inclusion criteria were not randomized or treated due to screening CDAI scores less than 220.

CDAI Scores

In spite of maintenance medications, all subjects had significantly elevated CDAI scores upon enrollment to the study implying their concomitant medication was insufficient to induce or maintain a remission. Since subjects were not randomized based upon CDAI scores, the baseline CDAI score of the placebo group was lower (327 ± 19) than that of the naltrexone-treated subjects (365 ± 16), but this difference was not statistically significant ($p = 0.13$).

Eighty-eight percent of those treated with naltrexone achieved the primary outcome of a 70-point decline in CDAI scores compared to 40% of those on placebo at week 12. The difference between the groups was significant ($p = 0.009$) indicating that naltrexone improved the clinical activity of Crohn's disease. No statistical difference was observed between the treatment groups at week 4 or 8 (data not shown) suggesting that determination of response requires at least 12 weeks of naltrexone therapy. Using an even more stringent criteria of a 100-point decline in the CDAI score [30], 63% of the subjects receiving naltrexone and 33% of those taking placebo achieved this response. Despite an almost twofold greater response in naltrexone-treated individuals compared to placebo controls, this difference was not statistically significant ($p = 0.16$). Perhaps a larger sample size would clarify whether this decline is valid. Although, only 30% of those treated for 12 weeks with naltrexone achieved remission with a CDAI score of <150; this may be due to the fact that they started with very high CDAI scores at baseline.

Colonoscopy Evaluation

Gastrointestinal inflammation was evaluated by both the endoscopic appearance and histological inflammatory scores in four areas of the colon and the ileum. Baseline endoscopic mucosal appearance was slightly higher in those randomized to the placebo arm (16 ± 2.5) compared to those randomized to receive naltrexone (15.4 ± 2.7); however, this difference was not significant ($p = 0.45$). There was no endoscopic improvement in the mucosa of those patients treated with placebo for 12 weeks compared

Table 1 Patient demographics and disease characteristics

Treatment group parameter	Placebo	Naltrexone	*p* value
Age (years); mean ± SEM	44.8 ± 2.8	40.5 ± 2.4	1.0
(range)	(26–67)	(21–60)	
Gender			
% of males	37.5	35.3	1.0
% females	62.5	64.7	1.0
Prior anti-TNFα treatment %	56	61	1.0
Concomitant medications for Crohn's (% of patients)			
Aminosalicylates	44	56	0.73
Immunomodulators	31	6	0.08
Corticosteroids	19	28	0.70
Antibiotics	6	6	1.0
None	38	17	0.25
Location of disease (%)			
Small bowel only	38	34	1.0
Ileocolic	44	55	1.0
Colon	13	6	0.59
Baseline CDAI (mean ± SEM)	327 ± 19	365 ± 16	0.13
Baseline IBDQ (mean ± SEM)	136 ± 5.8	121 ± 6.1	0.08
Baseline SF36 (mean ± SEM)	44.5 ± 3.9	35.9 ± 4.6	0.16
Baseline CRP mg/dl (mean ± SEM)	1.19 ± 0.3	1.55 ± 0.3	0.41
Baseline ESR mm/h (mean ± SEM)	33.5 ± 6.3	26.6 ± 5.7	0.45

CDAI Crohn's disease activity index, *IBDQ* Inflammatory bowel disease questionnaire, *SF36* short form general health survey, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *SEM* standard error of mean

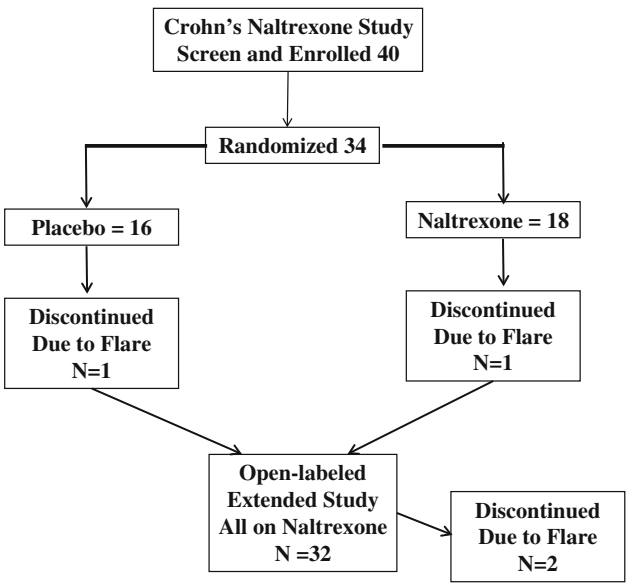

Fig. 1 Patient disposition according to CONSORT (consolidated standards of reporting trials). The numbers of subjects who enrolled in the trial and were randomized to either placebo or naltrexone therapy is shown. The outcome of those in each arm is demonstrated and the number of subjects rolling over into the extended open labeled study

to baseline values ($p = 0.4$). In contrast, endoscopic scores improved by 48% ($p = 0.018$) in naltrexone-treated subjects compared to baseline values and by 36% ($p = 0.052$) compared to placebo-treated controls. Seventy-eight percent of those on naltrexone experienced an endoscopic response with a 5-point drop in CDEIS scores from baseline whereas only 28% of those on placebo demonstrated the same decrease (Fig. 2a). Thirty-three percent of those treated with naltrexone had CDEIS scores of less than 6 suggesting endoscopic remission whereas 8% of the placebo treated subjects had scores less than six at week 12 (Fig. 2a), although this fourfold difference did not reach statistical significance. Complete endoscopic remission (CDEIS score < 3) was found at week 12 in 22% of those on naltrexone and none of the placebo-treated subjects.

Inflammatory scores determined by mucosal biopsies exhibited no change in placebo-treated controls ($p = 0.6$) over the 12-week period. On the contrary, microscopic inflammatory scores decreased significantly with naltrexone therapy compared to both baseline values ($p = 0.016$) and those subjects treated with placebo ($p = 0.048$; Fig. 2b). Photographs obtained of the colonic mucosa from representative patients at baseline, and in the same patients after 12 weeks of either placebo or naltrexone therapy, are shown in Fig. 3. Endoscopic appearance revealed a loss of normal vascularity, with increased mucosal edema, erythema, and ulceration at the baseline colonoscopy (Fig. 3a, c). No change in mucosal appearance was observed endoscopically in subjects treated with placebo (Fig. 3b). However,

in patients treated with naltrexone for 12 weeks, the endoscopic inflammatory features were not found and mucosal healing was noted (Fig. 3d). The corresponding histological specimens at baseline (Fig. 3a1, c1) revealed a marked inflammatory infiltration with ulceration at baseline in all subjects. Persistent inflammation, crypt distortion and ulceration remained present in patients receiving placebo (Fig. 3b1). However, the histological profile of subjects treated with naltrexone revealed decreased inflammation and a restoration of crypt architecture (Fig. 3d1).

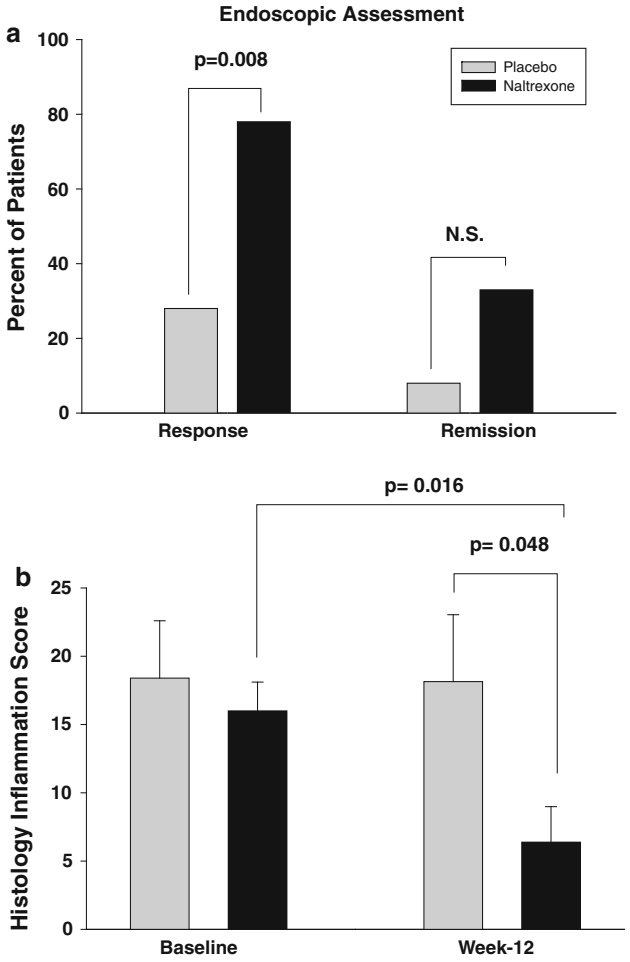

Fig. 2 Endoscopic and histological scores improve with naltrexone therapy. **a** After 12 weeks, 78% of subjects treated with naltrexone exhibited an endoscopic response as indicated by a 5-point decline in the CDEIS score from baseline compared to 28% response in placebo-treated controls ($p = 0.008$). Thirty-three percent of naltrexone treated subjects showed endoscopic remission with CDEIS scores of less than six compared to 8% of placebo treated subjects. **b** Histology scores to assess microscopic inflammation and structural architecture were determined at baseline and after 12 weeks of either naltrexone therapy or placebo by mucosal biopsy samples obtained during colonoscopies. No difference in histology scores was noted at baseline between those randomized to placebo or naltrexone ($p = 0.8$). Histology inflammation scores significantly improved in those treated with naltrexone compared to baseline values ($p = 0.016$) and compared to placebo-treated controls ($p = 0.048$). No improvement was noted histologically in placebo-treated patients at 12 weeks compared to baseline ($p = 0.06$). Columns represent means ± SEM. *N.S.* not statistically significant

Quality of Life Surveys

The results of both quality of life surveys (IBDQ and SF-36) indicated an improvement over the course of the study in both the naltrexone and placebo treated groups from baseline (Fig. 4). Although the total IBDQ and total SF36 scores improved to a greater extent in those on naltrexone compared to placebo over the 12 week study, this difference was not significant ($p = 0.3$).

Side Effects and Toxicity

The incidence of side effects reported during the study is shown in Table 2. Although some side effects were frequently reported (insomnia, diarrhea, pain) there was no difference in the incidence of these complaints between patients receiving naltrexone compared to placebo. The only side effect reported with increased frequency in placebo-treated subjects compared to naltrexone-treated patients was fatigue ($p = 0.04$). Two patients exhibited a flare of Crohn's disease with worsening of gastrointestinal symptoms during the first month of the study. One patient had been randomized to naltrexone and was rescued with steroids and withdrawn from the study. The other patient was in the placebo arm and was crossed over prematurely to naltrexone therapy; his symptoms responded to naltrexone, and he successfully completed the study with significant improvement. One patient with reflux sympathetic dystrophy experienced aggravation of her neurogenic pain on naltrexone suggesting an interaction with the opioid receptors on nerve tissue. Two patients had transient elevation in liver transaminases on naltrexone which resolved the following month. In one of these patients the liver enzyme increase coincided with a respiratory infection.

Laboratory Values

No significant changes were noted in any laboratory tests compared to baseline or between treatment groups. Specifically, the blood counts, electrolytes, liver function panels remained stable. No significant differences were noted in the CRP or ESR values between the groups. Patients who exhibited a 70-point drop in CDAI scores with naltrexone had higher CRP values at baseline (2.0 ± 0.5 mg/dl) compared to those subjects on naltrexone who did not exhibit a response (0.8 ± 0.3 mg/dl); however, this difference was not statistically significant. One reason for the lack of difference may be that all values

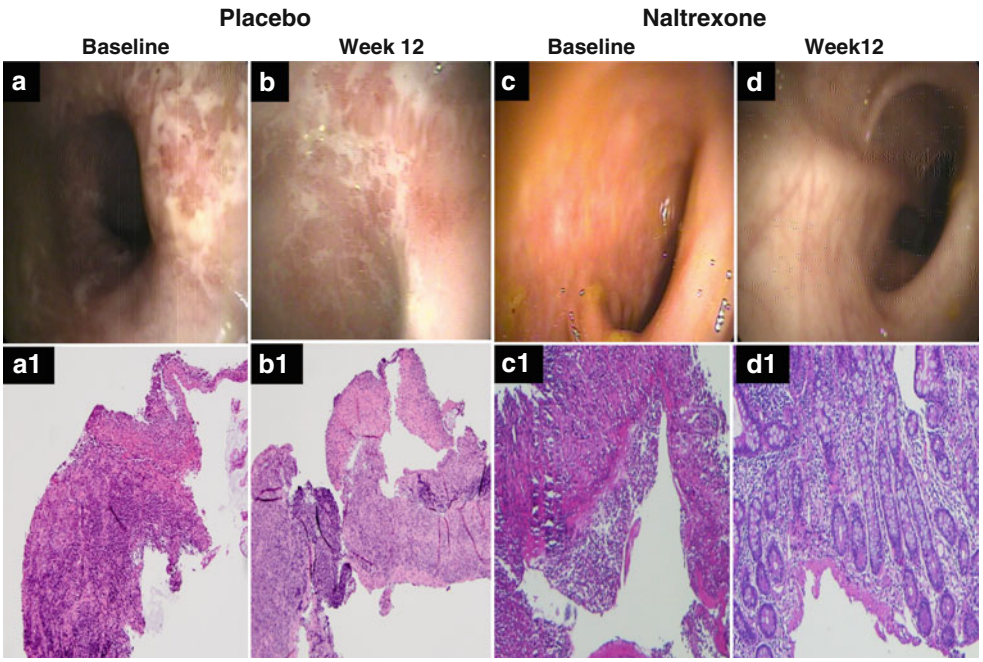

Fig. 3 Naltrexone promotes mucosal healing in Crohn's disease. The endoscopic appearance of representative patients' colonic mucosa is shown at baseline (**a**, **c**) demonstrating erythema, edema, ulceration, and loss of vascularity. Corresponding H & E histologic sections obtained from the same areas demonstrate marked inflammation and ulceration with crypt distortion at baseline (**a1** and **c1**). No change was found in the endoscopic appearance (**b**) or the histological score (**b1**) in subjects randomized to placebo for 12 weeks. In contrast, the subjects treated with naltrexone for 12 weeks exhibited endoscopic mucosal healing (**d**) and histologic examination showed decreased inflammatory cells with restoration of crypt architecture (**d1**)

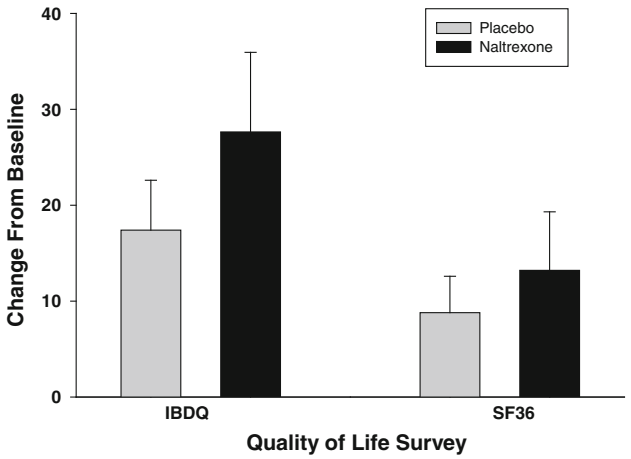

Fig. 4 Quality of life surveys. Changes from baseline scores for the IBDQ (*left*) and the SF36 (*right*) quality of life surveys are shown. Although the quality of life indices improved to a greater extent in subjects treated with naltrexone compared to placebo, this difference was not significant

were included in the analysis as proposed in the study design for intent-to-treat; hence, the subjects who flared during the study due to an increase in Crohn's symptoms were included in these analyses.

Table 2 Number of patients reporting side effects

Side effect/symptom	Placebo	Naltrexone	*p* value
Insomnia	5	5	0.3
Unusual dreams	3	2	0.3
Headache	2	4	1.0
Flatulence	5	6	0.5
Loss of appetite	0	2	0.6
Vomiting	1	3	1.0
Diarrhea	5	7	0.7
Abdominal pain	5	5	0.3
Nausea	4	4	0.5
Hair loss	1	0	1.0
Fatigue	3	0	0.04*
Constipation	0	2	0.6
Hair growth	0	1	1.0

* Statistically significant ($p < 0.05$)

Extended Open-Labeled Study

Seventy percent of those patients who had received placebo for the first 12 weeks and then subsequently were placed on naltrexone experienced at least a 70-point decline in the CDAI score. The mean CDAI scores of placebo-treated controls over the 3-month period of time were compared to

the CDAI score of these same subjects after 12 weeks on open-labeled naltrexone therapy. A significant response ($p < 0.005$) with a decline in CDAI score was recorded in these patients indicating that naltrexone was capable of 'rescuing' their active Crohn's disease (Fig. 5a). In the open labeled part of the study, patients who continued on naltrexone therapy for an additional 12 weeks (24 total weeks of therapy) had a further 75-point decline in CDAI scores ($p < 0.01$) (Fig. 5a). This 24-week score was also significant compared to baseline ($p < 0.0001$). The percentage of subjects on achieving remission with a CDAI score of less than 150 was 50% with 24 weeks of naltrexone therapy which was greater than the 30% of subjects achieving remission with only 12 weeks of therapy.

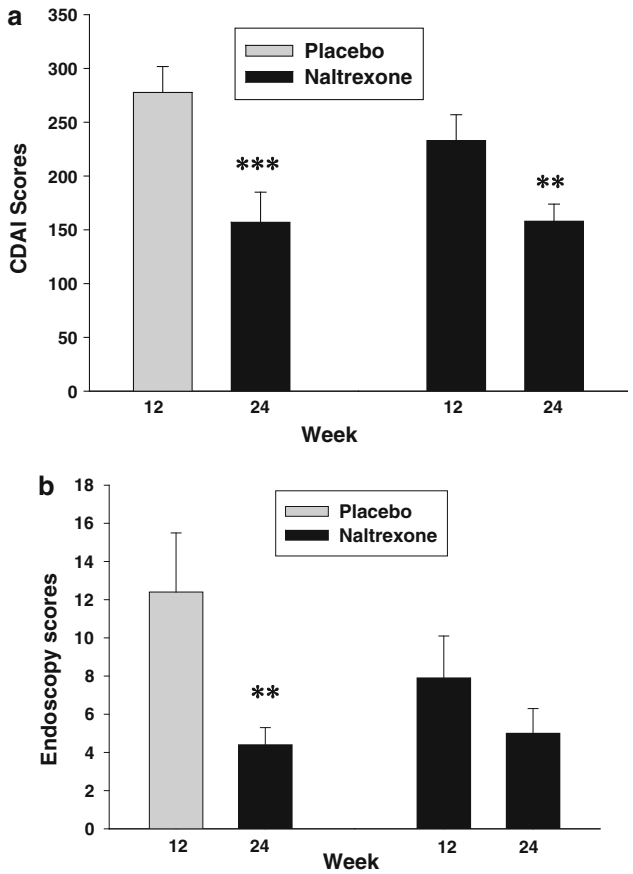

Fig. 5 Extended open-labeled study. **a** With the extended naltrexone therapy (24-weeks), CDAI scores demonstrated further significant reduction compared to the 12-week naltrexone treatment values. When subjects who had been on placebo for 12 weeks were then treated with naltrexone, a significant decline in CDAI scores also occurred. Columns represent means ± SEM. (Significantly different from corresponding week 12 at $^{**}p < 0.01$ and $^{***}p < 0.005$). **b** Mucosal healing as determined by endoscopic appearance was maintained when naltrexone was extended beyond the 12-week time point to 24 weeks. Although a decrease by 36% in endoscopic scores was reported in those continued on naltrexone compared to week 12, this difference was not significant. However, the week-24 values were significantly improved ($p = 0.005$) compared to baseline. Mucosal healing was evident in those who had taken placebo for 12 weeks and then subsequently were treated with naltrexone. **Significantly different from corresponding week 12 level at $p < 0.01$

Endoscopic scores significantly decreased by 65% from the scores recorded at the week-12 colonoscopy when naltrexone was subsequently introduced and administered for 12 weeks to those who had been initially randomized to placebo (Fig. 5b). These data further support the role of naltrexone in mucosal healing. Colonoscopies performed at week 24 showed a 36% decline in endoscopy inflammation scores compared to the 12-week scores in subjects initially randomized to the naltrexone arm (Fig. 5b). Although this value was significantly lower than baseline ($p = 0.005$), there was no statistical difference from the week-12 scores ($p = 0.26$). No further improvement in histological scores was observed in those treated for 24 weeks of naltrexone compared to 12 weeks of therapy; however, significant improvement in histology was also observed when former placebo-treated patients were subsequently administered naltrexone ($p = 0.006$) (data not shown). Two subjects experienced a flare in their Crohn's symptoms during the extended protocol. One of these subjects had been on naltrexone during the blinded study and one had been randomized to placebo. Both subjects were withdrawn and rescued with steroids.

Discussion

The present study involved a novel approach for treating inflammation of the bowel in subjects with Crohn's disease using an opioid receptor antagonist. This clinical trial is the first randomized double-blind placebo controlled study demonstrating that naltrexone improves clinical activity index scores and improves gastrointestinal mucosal inflammation in subjects with moderate to severe Crohn's disease. Over a 12-week period of time, subjects were treated with either placebo or naltrexone in a blinded fashion, and mucosal healing was evaluated by endoscopic appearance and histology from biopsies obtained during colonoscopies. Only those patients treated with naltrexone showed significant reversal of gastrointestinal inflammation by histology, whereas, placebo-treated subjects had no improvement. Thus, using a medication that alters the interaction of endogenous opioids at their receptors to decrease inflammation provides an innovative approach that utilizes native biological pathways.

Evidence for efficacy of naltrexone for Crohn's disease is further supported by the extension study which followed the double blind protocol. Those subjects that were in the placebo arm of the study that subsequently were treated with naltrexone in an open-labeled fashion showed significant improvement both in clinical activity and in

colonoscopy scores. Patients who had been randomized to active drug in the first 12-week period remained on naltrexone for a total duration of 24 weeks, and the medication did not lose its effectiveness nor did any untoward side effects develop. Over the extended duration of therapy for 24 weeks, laboratory values and quality of life parameters remained stable.

Our study employed the CDAI score as a major outcome, a standard method for evaluating activity of Crohn's disease and response to therapy for the past four decades [25]. Compared with other published clinical studies utilizing the CDAI scoring system [31, 32], the subjects included in our trial had higher baseline activity scores thereby indicating the presence of moderate to severe disease in our patient population. In addition, over half of our patient volunteers had previously failed or were intolerant of anti-TNFα biologic agents, suggesting a more resistant disease expression. Based upon the CDAI score response rates, our subjects showed a significant improvement with naltrexone therapy, with 88% meeting the primary end point of the study. The clinical response to naltrexone in this double-blind placebo controlled study was similar to that which we previously reported using naltrexone in an open-labeled pilot trial [24]. These findings thus support the efficacy of an opioid receptor antagonist in the treatment of inflammatory bowel disease.

Although the CDAI score is beneficial for assessing response in clinical trials, there are also some drawbacks to utilizing this measure of analysis. Because part of the assessment involves subjective information gathered from a 7-day patient diary, placebo response rates using this parameter are often reported in the 23–40% range [33, 34]. Indeed we also found a placebo response rate using this parameter. While most clinical trials involved in the testing of new therapies for Crohn's disease have utilized the CDAI score as an indicator of response, it has become evident that the CDAI score does not correlate with the endoscopic lesions of Crohn's disease [35]. In a large Scandinavian clinical trial in subjects with IBD, Froslie et al. [36] showed that mucosal healing was a valuable indicator of long-term efficacy. Moreover, in a review by Devlin and Panaccione [37], it was suggested that healing of mucosal ulceration should be a superior outcome over measurement of disease activity (i.e., CDAI score) for achieving clinical remission. D'Haens et al. [31] showed that infliximab therapy improved the endoscopic appearance of the bowel mucosa and decreased the inflammatory infiltrate compared to placebo treated controls. In the present study, we demonstrated that naltrexone improves the inflammatory response and also reverses the distorted histological architecture. Enough evidence now exists to support the rationale for determining 'response to therapy' based upon endoscopic changes rather than solely on CDAI scores.

One of the drawbacks to this report is that this trial was a single center study and all the subjects were aware they would be receiving the active drug at some point. Since all the patients were included in an intent-to-treat analysis, it is likely that the inclusion of the data from the subjects who flared resulted in the lack of significance in CRP levels between the groups. Since we demonstrated that those patients with high CRP levels were more likely to demonstrate a clinical response, perhaps a larger study would demonstrate correlation between CRP and treatment. Likewise, subjects receiving naltrexone had greater improvement in the quality of life scores compared to placebo treated controls; however, due to the smaller sample size and intent-to-treat analysis this was not significant in this study.

The side effects in our trial using naltrexone were minimal. In fact the only significant difference in reported side effects between naltrexone-treated and placebo-treated controls was that those taking placebo had significantly more fatigue. There was no difference in the frequency of Crohn's disease flares between the groups. Naltrexone is approved as a generic medication by the Food and Drug Administration for the management of addiction disorders at a dose more than tenfold higher than that administered in this study. Because of its long-term use in the United States for addictive disorders, the occurrence of serious side effects at higher doses has been monitored and few have been reported. Indeed, the only infrequent reported toxicity has been elevated liver transaminases occurring at naltrexone doses of 300 mg daily (almost 100 times the dose used in this study). We recorded two subjects with transient asymptomatic elevation of liver transaminases that resolved within a month without interruption of naltrexone therapy. It is unclear whether there was any relationship to naltrexone use or if the mild elevation in transaminase levels was coincidental, but the risk of hepatotoxicity should continue to be monitored in future clinical trials.

An advantage of the naltrexone therapy includes its oral route of administration and its once-a-day dosing regimen facilitating patient compliance. With the improved endoscopic appearance, clinical status, and histology with naltrexone, further investigation and larger studies using opioid receptor antagonists in Crohn's disease are warranted.

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Conflict of interest Drs. Smith and Zagon have intellectual property rights and have a patent for the use of naltrexone in IBD. This disclosure was provided to all study participants. The statistical analysis of the entire data sets pertaining to efficacy (specifically

primary and major secondary efficacy endpoints) and safety (specifically, serious adverse events as defined in federal guidelines) have been independently confirmed by a biostatistician who has no conflict of interest.

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