Original Article

Long-term treatment with low dose naltrexone maintains stable health in patients with multiple sclerosis

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Abstract

Introduction: A retrospective study was conducted on patients at Penn State Hershey Medical Center diagnosed with relapsing—remitting multiple sclerosis between 2006 and 2015.

Methodology: Laboratory and clinical data collected over this 10-year period were reviewed. Two cohorts of patients were established based on their relapsing—remitting multiple sclerosis therapy at the time of their first visit to Penn State. One group of patients ($n = 23$) was initially prescribed low dose naltrexone at the time first seen at Hershey. This group was offered low dose naltrexone because of symptoms of fatigue or refusal to take an available disease-modifying therapy. The second group of patients ($n = 31$) was treated with the glatiramer acetate (Copaxone) and offered low dose naltrexone as an adjunct therapy to their disease-modifying therapy.

Results: Patient data from visits after 1—50 months post-diagnosis were evaluated in a retrospective manner. Data obtained from patient charts included clinical laboratory values from standard blood tests, timed 25-foot walking trials, and changes in magnetic resonance imaging reports. Statistical analyses between the groups and for each patient over time indicated no significant differences in clinical laboratory values, timed walking, or changes in magnetic resonance imaging.

Conclusion: These data suggest that the apparently non-toxic, inexpensive, biotherapeutic is safe and if taken alone did not result in an exacerbation of disease symptoms.

Keywords: Disease-modifying therapy, low dose naltrexone, magnetic resonance imaging, Copaxone, behavior, walking

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Introduction

Multiple sclerosis and current therapies

Multiple sclerosis (MS) is a chronic and debilitating autoimmune disease of the central nervous system (CNS) that affects approximately 400,000 individuals in the United States and 2 million individuals worldwide.[1] MS occurs in two forms — progressive (primary or secondary) and relapse—remitting, and many patients with relapse—remitting forms often develop a more progressive, non-remitting disorder later in life. Although the etiology of MS is unknown, women and individuals of countries in northern latitudes have a greater incidence of MS. Deficiencies in vitamin D levels and some genetic factors are associated with the disorder.[1,2] MS is a triphasic disease involving astrocyte activation that leads to inflammation and recruitment of activated T cells to the CNS, and subsequent demyelination, axonal damage, and neurodegeneration.[1—3]

The US Food and Drug Administration (FDA) has approved seven disease-modifying therapies (DMTs) to reduce T-cell infiltration, including β-interferon products marketed as Betaferon, Avonex or Rebif, glatiramer acetate (Copaxone), natalizumb (Tysabri), fingolimod (Gilenya) and mitozantrone (Novantrone).[3—11] Two of the most widely used therapies are the oral compound fingolimod and the injectable drug Copaxone. Despite the mechanism of action being unknown, Copaxone is the only DMT with a category B rating for use in pregnancy.

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All current treatment regimens are expensive and have adverse side effects that reduce compliance. There remains a need to identify inexpensive and non-toxic therapies that target the underlying patho-physiology of autoimmune disorders. Blockade of the opioid growth factor (OGF)–OGF receptor (OGFr) pathway with low dose naltrexone (LDN) has been explored as one such therapy.[12,13] The OGF–OGFr axis becomes dysregulated in auto-immune disorders and the intermittent opioid receptor blockade from LDN leads to an increase of endogenous opioids that appears to be effective in both clinical and preclinical studies.[13]

Preclinical studies on LDN therapy

The widely used animal model for MS is experimental autoimmune encephalomyelitis (EAE).[14–21] Chronic progressive EAE is induced by immunization with myelin oligodendrocytic glycoprotein ($MOG_{35–55}$), whereas a relapsing–remitting form of EAE can be induced by immunization with proteolipid protein ($PLP_{139–151}$).[14–18] Although the animal models do not correspond completely to the etiology of MS, the pro-inflammatory diseases are similar, as the levels of IFN-γ, IL-1β, and TNF-α are upregulated in EAE and MS, and both disorders are characterized by CNS demyelination and neurodegeneration.

Endogenous opioids such as OGF or the endogenous secretion of OGF by upregulation of the OGF–OGFr axis following systemic exposure to LDN reverse the progression of EAE, prevent neuronal damage in the CNS, and reduce the frequency and severity of relapses in chronic progressive EAE as well as relapsing–remitting models of EAE.[14–21] Studies utilizing both relapsing–remitting and chronic progressive models of EAE showed that when OGF or LDN was administered at the time of disease induction, or when treatment was started after clinical signs of aberrant behavior were noted, both treatment regimens were effective at reversing the course of the disease. In some cases, onset was delayed.[14–16] Pathological assessments revealed that OGF and LDN reduced activated astrocyte proliferation, demyelination, and neuronal damage; in no instance did treatment of mice with EAE result in deleterious long-term repercussions or exacerbate EAE.[17,18]

Preclinical studies suggest that there is a dysregulated OGF–OGFr axis in EAE,[21] with endorphin and enkephalin levels in MS patients being reduced during flares[22–25] and elevated during periods of clinical inactivity.[24] Furthermore, proteases such as neprilysin/CD10 that degrade enkephalins appear to be elevated in animal models of EAE,[26,27] thus inferring an important role in the etiology of MS. Neprilysin/CD10 (neutral endopeptidase-NEP; EC 3.4.24.11) and CD13 (aminopeptidase N; AP-N, EC 3.4.11.2) that break down OGF are increased in patients with active MS, and reduced in patients undergoing remission,[26,27] suggesting that an aberrant OGF–OGFr pathway contributes to the underlying pathophysiology of EAE/MS, and establishes a central target for treatment.

Endogenous opioids and the treatment of MS

Confirmation of the efficacy of the biotherapeutic OGF, as well as understanding the underlying mechanistic pathways in MS, is particularly attractive because OGF was demonstrated to be safe, non-toxic, and efficacious in phase I and phase II studies of human cancer therapy.[28,29] Similarly, LDN has been reported to be non-toxic and effective in clinical trials for the treatment of other autoimmune disorders including Crohn's disease[30] and fibromyalgia.[31] At this time OGF is not available by prescription, whereas LDN can be obtained as an off-label prescription therapy under a physician's guidance. At least three clinical trials have been published in which LDN was found to increase the quality of life of MS patients with relapsing–remitting multiple sclerosis (RRMS) or secondary progressive MS, and significantly improve mental health.[32–34] In a single center, double-masked, placebo-controlled, crossover study, patients were given 4.5 mg naltrexone (i.e. LDN) nightly, with no serious adverse events reported.[33] The longest treatment regimen (6 months) of LDN was in a study by Gironi et al. in a phase II multicenter trial in which LDN was found to be safe and well tolerated.[34] Thus, the reports on controlled clinical trials, as well as numerous websites (http://www.ldnnow.co.uk/, http://www.ldnresearchtrust.org/), show that LDN is a safe, non-toxic and apparently effective therapy.

Evaluation regarding perceived levels of fatigue of patients with clinically defined MS and treated with LDN for sustained periods of time revealed that LDN was well tolerated and safe.[35] No serious adverse effects were recorded and patients reported that fatigue levels were stable or decreased, and perceived quality of life was stable. Moreover, patients identified with clinically isolated syndrome (one of the first indications of MS) and treated with LDN had no adverse reaction to the biotherapy.[36] However, there are no in-depth studies of patients who have received LDN for a sustained period of time. No data are available on physiological parameters (clinical data) following long-term LDN

treatment in excess of 2 or 3 years. In this study, data on clinical blood samples, walking timed tests, and magnetic resonance imaging (MRI) scans collected retrospectively from charts of patients prescribed LDN alone were compared with data from patients taking LDN in combination with other DMTs. At Penn State Hershey, a majority of patients diagnosed with RRMS are offered Copaxone; thus, our sample cohorts were LDN only and LDN–Copaxone; in some cases, patients were followed for more than 4 years. The purpose of the study is not a prospective efficacy trial, but rather a retrospective study to assess whether those patients on only LDN experienced an exacerbation of MS or had any deleterious events relative to patients prescribed LDN and the DMT.

Materials and methods

Chart review and patient inclusion

This chart review was conducted on data obtained from patients seen at the Penn State Hershey Medical Center Neurology Clinic between January 2006 and April 2016. All patients were diagnosed with clinically defined MS, and only patients with RRMS were included in the database (~430). Physician-collected data at each visit were retrospectively entered in the Redcap database allowing for de-identified patient analysis. Data for two cohorts of RRMS patients were established based on inclusion/exclusion criteria. Patients 18 years or older, and prescribed daily use of LDN as an oral medication (3, 3.5 or 4 mg) for at least 3 months as confirmed by clinical charts were included. One cohort consisted of patients with no other DMT when LDN therapy was initiated. This group was offered LDN (oral tablet) because of symptoms of fatigue or refusal to take an available DMT. The second cohort included patients receiving Copaxone as a DMT and offered LDN as an adjunct therapy; these patients continued on both medications. The cohort of patients is limited by patient preferences for treatment after discussion of all available DMT therapies. Thus, the DMT Copaxone provided the largest cohort of patients. The nature of a retrospective study does not allow for random assignment of treatment.

Evaluation parameters

Three research questions formed the basis of evaluation:[37] (1) Was disease progression for patients on LDN alone as measured by MRI different from that observed in patients on both Copaxone and LDN? (2) Did long-term LDN treatment change the overall health status of patients as measured by blood counts and liver enzymology? (3) Did long-term LDN treatment alone result in behavioral deficits as measured by the time required to walk a 25-foot course unassisted?

Disease progression was monitored by evaluating reports from an initial MRI collected during early stages of disease onset, and the last MRI taken during our observation period ending April 2016. MRIs were obtained at the recommendation of physicians and based on clinical need and were not obtained at prospectively determined time points. The MRI data were collected from the radiologist's interpretation of the image and placed into the following categories: (1) improved, (2) stable, (3) slightly worse, and (4) active enhancing lesions.

Overall physical wellness was assessed by analysis of clinical laboratory data. The blood laboratory data were collected periodically at the request of either the primary care physician or the neurologist at Penn State. The data were collected from the following panels: complete blood counts (CBCs), blood chemistry, nutrition, liver and gastrointestinal function, immunology, rheumatology, cardiac and lipids, and coagulation. In addition, cerebrospinal fluid data were collected if available. The data consistently available for most patients were CBCs, blood urea nitrogen (BUN) and creatinine, and liver function parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and total bilirubin.

Behavior was assessed by timed 25-foot walks when obtained by the clinical nurse or examining neurologist. It was noted if the patient performed the 25-foot timed walk with or without the assistance of a cane, walker, or a Rollator. In most cases, patients were asked to repeat the walk at 6-month or yearly visits.

Statistical analyses

RRMS patients meeting the inclusion criteria were assigned a number, and de-identified data were collected through Redcap, entered into Excel spreadsheets, and subsequently analyzed by the public health sciences department at Penn State Hershey. Parametric data (clinical values, walking) were analyzed using two-tailed *t*-tests or analysis of variance with subsequent comparisons made using Newman–Keuls procedures. Ambulation data were organized into baseline measurements followed by measurements at 6-month intervals. Data were analyzed by the Wilcox rank sum test and expressed as median (range). The proportion of MRIs in each category (e.g. stable) were evaluated by Chi-square tests.

Statistical reliability was set at a *P* value less than 0.05.

Results

Patient demographics

Most patients were diagnosed with RRMS prior to being seen at Penn State Hershey. These patients were continued on their FDA approved therapies. In the retrospective chart review, the largest cohort of patients received Copaxone and thus constituted our study population. At the start of the present study, all patients in the LDN–Copaxone cohort were receiving Copaxone for a period of time prior to beginning LDN therapy. The LDN-only cohort was receiving no other treatment at the onset of their LDN therapy. Patient decision not to start an available FDA-approved DMT was based on concerns regarding side effects, requirement of injections, and personal opinions. The LDN-only cohort was composed of 10 men and 17 women with an age range of 34–66 years for men and 38–77 years for women at the termination of the study (April 2016). The LDN–Copaxone cohort was composed of 11 men and 21 women, with an age range of 37–72 years for men and 32–65 years for women by April 2016 (Table 1).

Medications and incidence of flares

The average length of disease for those individuals in the LDN-only cohort was approximately 14 years, with a range of 4–29 years reported for men and 3–31 years for women. The LDN-only cohort was supplied with tablets of 3 or 4 mg naltrexone to be taken orally once daily. This cohort of patients reported LDN use alone for an average of 1095 days (~3 years), with an individual range of LDN use being 30–2169 days.

The average length of disease for the LDN–Copaxone cohort was approximately 13.7 years; men had MS for 4–29 years and women had MS for 3–31 years. The LDN–Copaxone cohort received LDN therapy (daily oral tablets of 3 or 4 mg naltrexone) for an average of 1418 days (approximately 47 months). Importantly, all patients in both cohorts have remained on LDN therapy throughout the duration of the study (to April 2016). A small portion (less than 25%) of the total patient population changed DMT or added a DMT during the course of the study; LDN dosage remained constant.

Regarding the incidence of flares, or attacks requiring additional physician visits, there was only one patient in the LDN-only cohort with multiple reported flares, having five flares during the course of the study. The LDN–Copaxone cohort had six patients with multiple flares during the course of the study. The remaining patients all had a singular reported flare during the course of the study.

MRI reports

MRI data for each patient were collected from the radiologist interpretations of brain MRI, cervical spinal MRI, and thoracic spinal MRI (Figure 1). The number and frequency of spinal MRIs made their analysis non-contributory. Data were organized into one of the following categories: normal stable, improved, worse, new lesions, and active lesions. Data from the most recent brain MRI prior to the study revealed no statistically significant difference between the LDN-only and LDN–Copaxone cohorts ($N = 32$ and $N = 27$, respectively). At this time the majority of the patients in both cohorts had non-active MS lesions suggestive of a diagnosis of MS.

**Table 1.** Patient demographics, number and duration of visitation, and walking.

	LDN–Copaxone	LDN only
Number	32	27
Mean age, years	52.5 (M)	50.3 (M)
	46.7 (M)	55.7 (F)
Sex, % female	66	63
Number of visits (range)	6.4 ± 0.5 (2–10)	4.6 ± 0.5 (1–10)*
Mean length of LDN, days	1095 ± 113	1418 ± 97*
Range of LDN treatment, months	5–52	1–72

Values represent means ± SEM.
Significantly different from values in the Copaxone + LDN cohort at $P < 0.05$ (*).
LDN: low dose naltrexone.
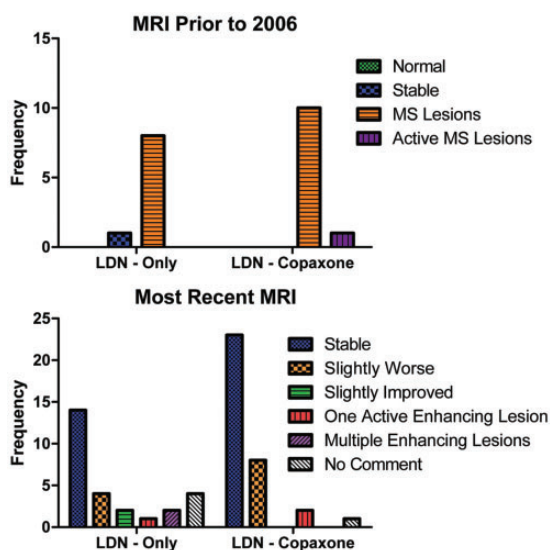
Figure 1. Categorization of magnetic resonance imaging (MRI) scans from patients treated with either low dose naltrexone (LDN) (LDN-only) or LDN and Copaxone for relapsing–remitting multiple sclerosis. MRIs were taken (a) at the time of initial diagnosis (prior to 2006) and (b) in 2016 (most recent). Groups in (a) are based on eventual treatment cohorts. MRIs were scored based on radiologists' reports. The proportion of MRIs in each category were evaluated by chi-square analyses. No differences were noted.

Intermediate MRIs were shown to have no significance between the cohorts. Following the first intermediate MRI, the majority of the patients in both cohorts were considered as stable. The first intermediate MRI, or the first MRI after 2006, had shown both cohorts having mainly non-active MS lesions. Again, suggesting that many of these MRIs were the initial MRIs for the diagnosis of MS. Some fluctuation was noted in all of the intermediate MRIs, in which some patients in both cohorts had active lesions or new lesions, or were considered improved. The majority of the patients whose MRIs that were classified as improved were seen in the LDN–Copaxone cohort, but were not exclusive to that cohort.

Data from the most recent brain MRI revealed no significant differences between the cohorts. The last MRI showed that more than 50% of LDN-only patients, and a comparable number of LDN–Copaxone patients, were considered to have stable disease. For patients considered in the 'slightly worse' category, there were twice as many in the LDN–Copaxone cohort than in the LDN-only

group (Figure 1). None of the LDN–Copaxone patients fell into the slightly improved category, while two of the patients in the LDN-only cohort were in this category. Two patients in the LDN–Copaxone cohort had active lesions, whereas only one patient in the LDN-only group had an MRI with an active lesion. Two patients in the LDN-only group had MRIs categorized with multiple enhancing lesions.

Given that MRIs are a common method to determine disease progression, the lack of differences in the status of MRI readings between LDN and LDN–Copaxone cohorts demonstrates that LDN alone did not result in detectable inflammatory disease progression.

Blood laboratory data

Data from the blood laboratoriess were collected throughout the course of the 6-year study. However, because the patient visits were not evenly distributed and blood collected every 6 months, as well as the fact that blood was not tested at each visit, only data from the last patient visit at which blood was collected and tested were analyzed. In general, blood values and liver enzymes did not fluctuate between treatment cohorts, and did not differ from the standard values accepted as 'normal' by the Penn State Hershey Medical Center as analyzed by the Wilcox rank sum test (Figures 2, 3, and 4).

The CBC panel, when compared between the cohorts, revealed statistical significance in the baseline absolute basophils and consequently in the percentage basophils ($P = 0.008$ and 0.007, respectively). The LDN-only cohort had a higher median value of 0.1 K/µl, whereas the LDN–Copaxone group had a median value of 0.0 K/µl. Other values in the CBC panel including white cell counts, red cell counts, hematocrit, hemoglobin, etc., showed no statistical significance (Figures 2 and 3). In addition to this general lack of statistical significance, overall the laboratory values remained within normal levels. Elevations were seen in the white blood cell, hemoglobin, hematocrit, red blood cell distribution width (RDW) platelet count, absolute numbers of neutrophils, lymphocytes, basophils, and eosinophils. These fluctuations were observed infrequently (three samples at one time point) and only in the blood counts of patients in the LDN-alone cohort. All other values and time points were within the normal range, showing that LDN therapy does not cause elevation of the measurements of the CBC panel. RDW and the absolute number of
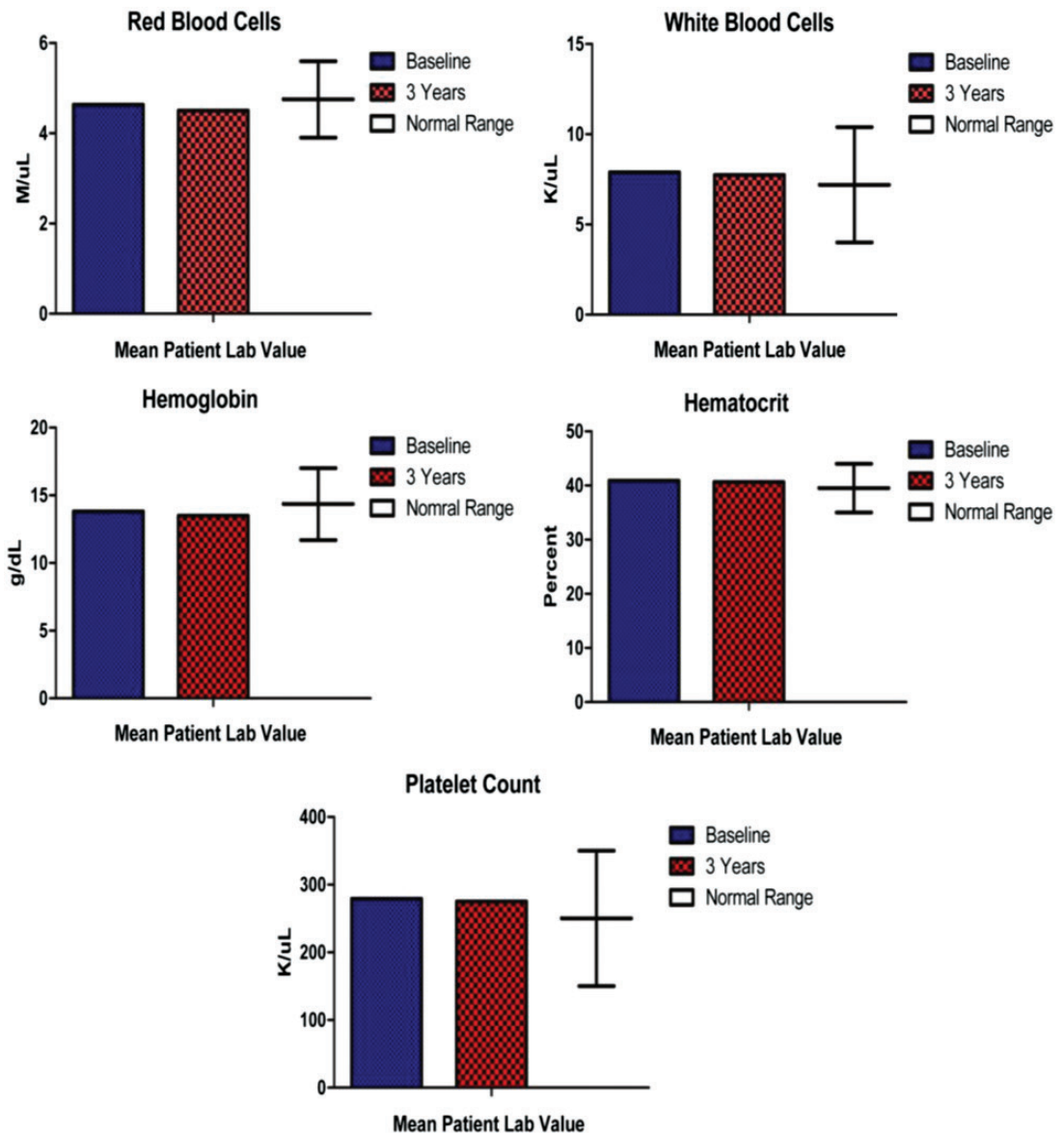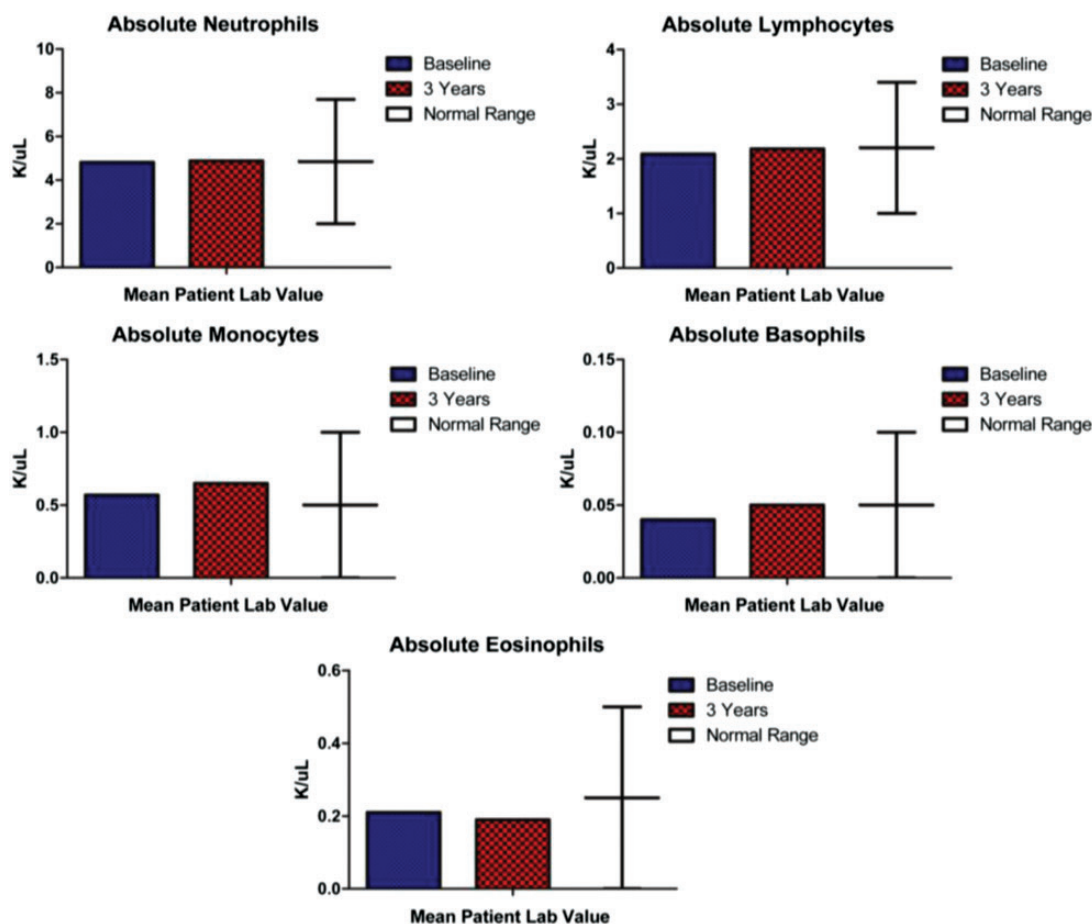
Figure 2. Mean laboratory values for all patients at their initial diagnosis of multiple sclerosis (baseline) and at the 3-year time point of treatment with low dose naltrexone (LDN) only or LDN + Copaxone. Bars represent mean values for all patients as they did not differ between treatments for total red blood cells (M/μL), total white blood cells (K/μL), hemoglobin (g/dL), hematocrit (%), and platelet count (K/μL). The whisker plot indicates the normal range of values for each measurement.

neutrophils were also elevated for at least one blood test in the LDN–Copaxone cohort. Based on evaluation of the Redcap data, abnormal blood values were transient and did not cause discontinuation of treatment. All patients were on LDN at the time of sampling.

Liver enzymes
The liver panel assessed levels of AST, ALT, total bilirubin and alkaline phosphatase (Figure 4).

There were no statistical differences between the LDN-only cohort and the LDN–Copaxone cohort. A few values from individual screenings were outside the normal range; these fluctuations occurred in patients within both treatment cohorts. With regard to ALT, median values ranged from 19 to 43.5, with one patient in the LDN–Copaxone group and two subjects in the LDN group expressing values in excess of 40; the range of ALT median values excluding those patients was 19–37. Total bilirubin

Figure 3. Absolute numbers (K/μL) of neutrophils, lymphocytes, monocytes, basophils and esoinophils for all patients at their initial diagnosis of multiple sclerosis (baseline) and at the 3-year time point of treatment with low dose naltrexone (LDN) only or LDN + Copaxone. Bars represent mean values for all patients as they did not differ between treatments. The whisker plot indicates the normal range of values for each measurement.

data were very consistent for all subjects, ranging from 0.1 to 0.9 across 10 visits. The median levels of alkaline phosphate over 10 visits ranged between 59 and 85; one value was reported to be 30 for one patient in the LDN-alone group. Finally, values for AST were less than 50 for all patients at all times in both groups, with the exception of one patient on LDN plus Copaxone with AST values of 64 and one LDN-alone subject with a value of 54 reported in the charts. Based on Redcap data, these abnormal values were transient and did not cause discontinuation of treatment.

BUN and creatinine values did not differ between patients across treatment cohorts (Figure 4). BUN values ranged between 11 and 20.5, with Q1 readings of 7–14 for the LDN–Copaxone group, and values ranged between 10 and 27 with Q1 readings

of 10–27. One patient in each group expressed these fluctuations. With regard to creatinine values, one patient in the LDN–Copaxone group had a reported elevated creatinine score; LDN-only patients had an average median creatinine value of 0.79 with no outliers. Data for CBCs and liver function studies show that LDN therapy is safe and not detrimental to the patient physiology.

Ambulation
Ambulation data were collected at each visit and compared for LDN-only and LDN–Copaxone cohorts at 1-year intervals; in some cases patients participated in eight timed walks (Table 2). No mean differences were reported for a single patient across time or for the group at 6-month intervals. The time of the walking test was based on the initiation of LDN treatment; no confounding interactions
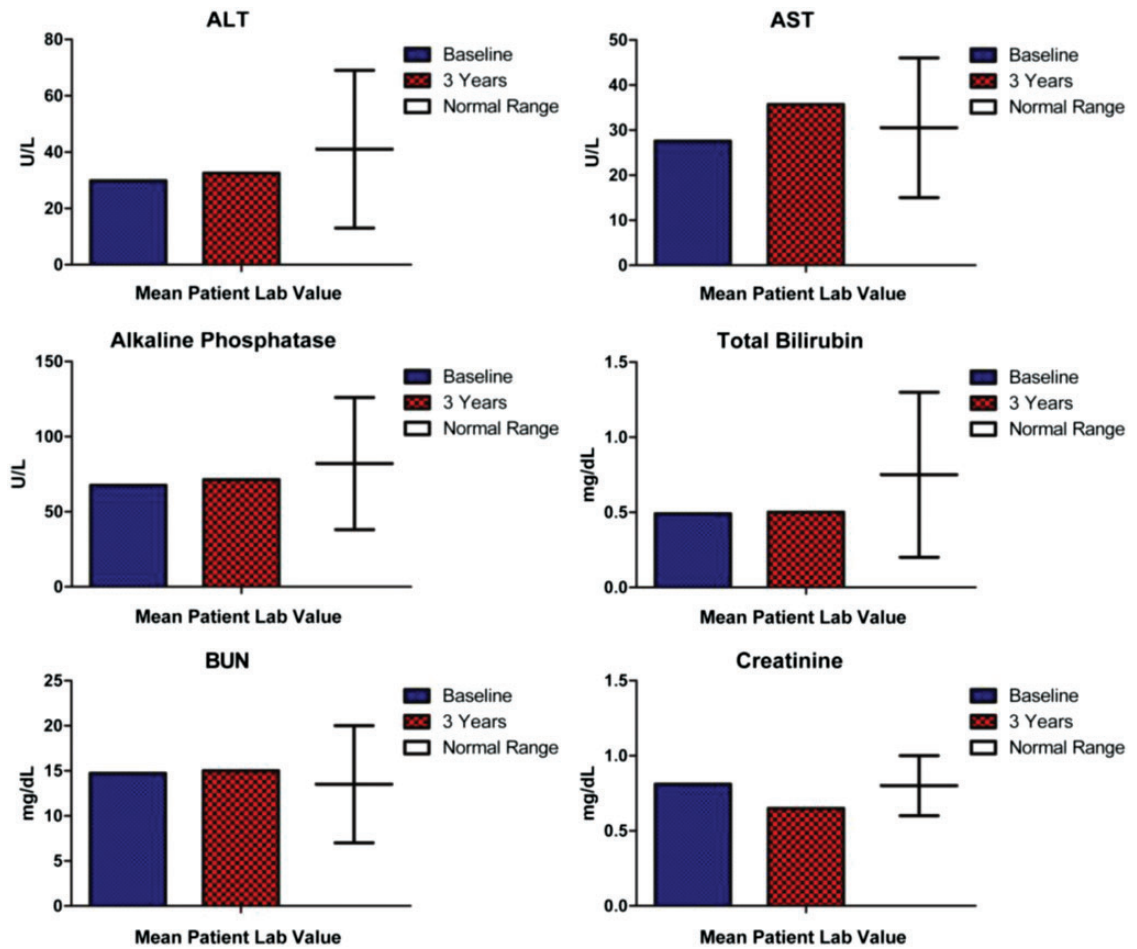
Figure 4. Mean liver values for alanine aminotransferase (ALT) (U/L), aspartate aminotransferase (AST) (U/L), alkaline phosphatase (U/L), bilirubin (mg/dl), blood urea nitrogen (BUN) (mg/dL), and creatinine (mg/dlL) for all patients at their initial diagnosis of multiple sclerosis (baseline) and at the 3-year time point of treatment with low dose naltrexone (LDN) only or LDN-Copaxone. Bars represent mean values for all patients as they did not differ between treatments. The whisker plot indicates the normal range of values for each measurement.

Table 2. Profile of behavior: timed 25-foot walking times.

	LDN−Copaxone	LDN only
Unassisted walking time (s)		
Visit 1	6.3 ± 0.5 ($n = 15$)	6.2 ± 0.5 ($n = 12$)
Visit 2	5.3 ± 0.3 ($n = 15$)	5.4 ± 0.3 ($n = 12$)
Visit 3	5.8 ± 0.6 ($n = 15$)	6.3 ± 0.9 ($n = 12$)
Visit 4	5.1 ± 0.3 ($n = 15$)[a]	5.9 ± 0.4 ($n = 11$)

Values represent means $\pm$ SEM (number of participants).
Walking times were calculated for patients who completed testing on at least four consecutive visits without any assistance at each visit. The first visit was the time when low dose naltrexone (LDN) was prescribed, and each visit thereafter represents approximately 6 months later.
Data did not differ between treatment groups.
[a]Significantly different between visit 1 and visit 4.

were noted. Data collected at the initial visit to Penn State Hershey, which may not be the onset of treatment or diagnosis of MS, showed no significant difference in the time required to walk 25 feet between persons assigned to the LDN-only and the LDN–Copaxone groups. The mean time for LDN-only patients was 6.2 seconds in comparison to a mean of 6.3 seconds for patients in the LDN–Copaxone group. Both of these measures were significantly longer than that measured for healthy individuals (4–5 seconds). However, in both treatment cohorts, some patients ambulated near the 5 second range and were considered normal.

Throughout the course of visits, ambulation times fluctuated slightly for most patients. In both groups, no differences were noted when comparisons were made between cohorts at a given time. However, there was a significant decrease in the timed walk in patients in the LDN–Copaxone cohort when analyzing baseline and final measurements (6.3 and 5.1 seconds, respectively). No differences were noted in baseline to final measurements in the LDN-only group (6.2 and 5.9 seconds, respectively).

Discussion

Evaluation of behavioral data, clinical laboratory blood values, as well as interpretation of MRIs over a period of 10 years revealed that treatment of patients with clinically definite RRMS and receiving only LDN had no significant adverse effects. Disease status did not progress with only LDN in comparison to data obtained from patients prescribed Copaxone and LDN. Subjects prescribed only LDN had timed 25-foot walking tests comparable to those in the LDN–Copaxone group at the start of the study, and after more than 2 years of treatment. Laboratory data on standard blood counts, as well as liver enzymes, were comparable across time within a treatment, and in comparison to a treatment group at a specific time such as the start of treatment (visit 1), at 6 months (visit 2), or 24 months (visit 4). Analyses of data for a given patient across a period of treatment lasting up to 10 years revealed no significant changes in blood counts or liver enzyme panels for patients in either the LDN-only or LDN–Copaxone groups.

Interpretation of MRIs was difficult because not all patients had their original diagnostic MRI performed at Penn State Hershey, leading to variable readings. However, based on the radiologist's report, there were no substantial changes in disease progression based on the number of lesions seen in the MRIs.

As with any retrospective study, the limitations are related to the data available in each patient chart. The accuracy and completeness of the data are uncontrollable. In this study, the patients were seen by one of four physicians beginning in 2006 through to 2016. In most cases, the patients were seen by one of two physicians who are still active members of the multiple sclerosis clinic at Penn State Hershey, thus reducing variability in terminology and MRI interpretation.

However, in this retrospective study, many of the endpoints/measurements were quantitative rather than patient feedback, or 'perceived' data. These data were subjected to parametric analyses allowing for more rigorous and reliable comparisons. The absence of significant physiological changes in patients on LDN support its tolerability over an extended period for clinically defined MS.

Clinical implications and conclusions

This study illustrates that LDN is safe for people with MS, particularly RRMS as it does not appear to increase MRI activity or alter regular blood tests of liver, kidney and hematopoietic function. The efficacy of LDN needs to be evaluated in prospective clinical studies of MS because of its interesting mechanism of action and preclinical data. The safety findings of our study indicate that prospective studies of up to 3 years could be safely performed. Evaluation of clinical values, behavior, and MRIs revealed that patients on a long-term LDN treatment regimen did not show that LDN alone increased inflammatory disease progression or impaired clinical blood values. These data could assist physicians in their decision to prescribe LDN as a safe, inexpensive therapy for MS patients who are reluctant to take other, more costly, or more cumbersome DMTs. Moreover, these data support and warrant prospective clinical studies of MS, examining treatment outcome in patients receiving LDN only.

References

1. National Multiple Sclerosis Society. *What is MS?* www.nationalmssociety.org/about-multiple-sclerosis/index.aspx (accessed 5 August 2016).

2. UpToDate. *Clinical features of multiple sclerosis in adults*. www.uptodate.com/contents/epidemiology-and-clinical-features-of-multiple-sclerosis-in-adults?detectedLanguage=en&source=search_result&search=multiple+sclerosis&selectedTitle=2~150&provider=noProvider (accessed 5 August 2016).

3. Katsavos S and Anagnostouli M. Biomarkers in multiple sclerosis: an Up-to-Date overview. *Mult Scler Int* 2013; 2013: 20. doi: 10.1155/2013/340508.

4. Fox FJ and Rhoades RW. New treatments and treatment goals for patients with relapsing–remitting multiple sclerosis. *Curr Opin Neurol* 2012; 25(Suppl): S11–S19.

5. Stoll SS, Nieves C, Tabby DS, et al. Use of therapies other than disease-modifying agents, including complementary and alternative medicine, by patients with multiple sclerosis: a survey study. *J Am Osteopath Assoc* 2012; 112: 22–28.

6. Hadjigeorgiou GM, Doxani C, Miligkos M, et al. A network meta-analysis of randomized controlled trials for comparing the effectiveness and safety profile of treatments with marketing authorization for relapsing multiple sclerosis. *J Clin Pharm Ther* 2013; 38: 433–439.

7. Cree B. Update on reproductive safety of current and emerging disease-modifying therapies for multiple sclerosis. *Mult Scler* 2013; 19: 835–843.

8. Devonshire V, Havrdova E, Radue EW, et al. Relapse and disability outcomes in patients with multiple sclerosis treated with fingolimod: subgroup analyses of the double-blind, randomised, placebo-controlled FREEDOMS study. *Lancet Neurol* 2012; 11: 386–388.

9. Chiba K, Kataoka H, Seki N, et al. Fingolimod (FTY720), sphingosine-1-phosphate receptor modulator, shows superior efficacy as compared with interferon-β in mouse experimental autoimmune encephalomyelitis. *Int Immunopharmacol* 2011; 11: 366–372.

10. Killestein J, Rudick RA and Polman CH. Oral treatment for multiple sclerosis. *Lancet Neurol* 2011; 10: 1026–1034.

11. Horga A and Tintore M. Natalizumab for relapsing-remitting multiple sclerosis. *Neurologia* 2011; 26: 357–368.

12. Zagon IS and McLaughlin PJ. Endogenous opioids as treatment for multiple sclerosis. *J Neurol Neurophysiol* 2014; S12: S12–011. doi: 10.4172/2155-9562.S12-011

13. McLaughlin PJ and Zagon IS. Duration of opioid receptor blockade determines clinical response. *Biochem Pharmacol* 2015; 97: 236–246.

14. Zagon IS, Rahn KA, Turel AP, et al. Endogenous opioids regulate expression of experimental autoimmune encephalomyelitis: A new paradigm for the treatment of multiple sclerosis. *Exp Biol Med* 2009; 234: 1383–1392.

15. Zagon IS, Rahn KA, Bonneau RH, et al. Opioid growth factor suppresses expression of experimental autoimmune encephalomyelitis. *Brain Res* 2010; 1310: 154–161.

16. Rahn KA, McLaughlin PJ and Zagon IS. Prevention and diminished expression of experimental autoimmune encephalomyelitis by low dose naltrexone (LDN) or opioid growth factor (OGF) for an extended period: therapeutic implications for multiple sclerosis. *Brain Res* 2011; 1381: 243–253.

17. Campbell AM, Zagon IS and McLaughlin PJ. Opioid growth factor arrests the progression of clinical disease and spinal cord pathology in established experimental autoimmune encephalomyelitis. *Brain Res* 2012; 1472: 138–148. PMID: 22820301

18. Campbell AM, Zagon IS and McLaughlin PJ. Astrocyte proliferation is regulated by the OGF–OGFr axis in vitro and in experimental autoimmune encephalomyelitis. *Brain Res Bull* 2013; 90: 43–51.

19. Hammer LA, Zagon IS and McLaughlin PJ. Treatment of a relapse–remitting model of multiple sclerosis with opioid growth factor. *Brain Res Bull* 2013; 98: 122–131. PMID: 23973432

20. Hammer LA, Zagon IS and McLaughlin PJ. Improved clinical behavior of established relapsing–remitting experimental autoimmune encephalomyelitis following treatment with endogenous opioids: implications for the treatment of multiple sclerosis. *Brain Res Bull* 2015; 112: 42–51. PMID: 25647234

21. Hammer LA, Zagon IS and McLaughlin PJ. Low dose naltrexone treatment of established relapsing–remitting experimental autoimmune encephalomyelitis. *J Mult Scler (Foster City)* 2015; 2(2): 1000136.

22. McLaughlin PJ and Zagon IS. The opioid growth factor – opioid growth factor receptor axis: homeostatic regulation of cell proliferation and its implications for health and disease. *Biochem Pharmacol* 2012; 84: 746–755. PMID: 22687282

23. Gironi M, Martinelli V, Brambilla E, et al. β-Endorphin concentrations in peripheral blood mononuclear cells of patients with multiple sclerosis. *Arch Neurol* 2000; 57: 1178–1181.

24. Csontos K, Rust M, Hollt V, et al. Elevated plasma β-endorphin levels in pregnant women and their neonates. *Life Sci* 1979; 25: 835–844.

25. McLaughlin PJ. Proenkephalin-derived peptides. In: Kastin A (ed) *Handbook of Biologically Active Peptides*. Amsterdam: Elsevier, 2013, vol. 219, pp. 1602–1609.

26. Ziaber J, Baj Z, Pasnik J, et al. Increased expression of neutral endopeptidase (NEP) and aminopeptidase N (APN) on peripheral blood mononuclear cells in patients with multiple sclerosis. *Immunol Lett* 2000; 71: 127–129.

27. Halonen T, Kilpelainen H, Pitkanen A, et al. Lysosomal hydrolases in cerebrospinal fluid of multiple sclerosis patients. A follow-up study. *J Neurol Sci* 1987; 79: 267–274.

28. Smith JP, Field D, Bingaman SI, et al. Safety and tolerability of low-dose naltrexone therapy in children with moderate to severe Crohn's disease: a pilot study. *J Clin Gastroenterol* 2013; 47: 339–345. PMID: 23188075

29. Smith JP, Ahmad M, Conter R, et al. Treatment of advanced pancreatic cancer with opioid growth factor: phase I. *Anticancer Drugs* 2004; 15: 203–209. PMID: 15014352

30. Smith JP, Bingaman SI, Ruggiero F, et al. Therapy with the opioid antagonist naltrexone promotes mucosal healing in active Crohn's disease: a randomized placebo-controlled trial. *Dig Dis Sci* 2011; 56: 2088–2097. PMID: 21380937

31. Younger J, Noor N, McCue R, et al. Low-dose naltrexone for the treatment of fibromyalgia. *Arthritis Rheum* 2013; 65: 529–538.

32. Sharafaddinzadeh N, Moghtaderi A, Kashipazha D, et al. The effect of low-dose naltrexone on quality of life of patients with multiple sclerosis: a randomized placebo-controlled trial. *Mult Scler* 2010; 16: 964–969.

33. Cree BA, Kornyeyeva E and Goodin DS. Pilot trial of low-dose naltrexone and quality of life in multiple sclerosis. *Ann Neurol* 2010; 68: 145–150.

34. Gironi M, Martinello-Boneschi F, Sacerdote P, et al. A pilot trial of low-dose naltrexone in primary progressive multiple sclerosis. *Mult Scler* 2008; 14: 1076–1083.

35. Turel AP, Oh KH, Zagon IS, et al. Low dose naltrexone (LDN) for treatment of multiple sclerosis: a retrospective chart review of safety and tolerability. *J Clin Psychopharmacol* 2015; 35: 609–611. PMID: 26203498

36. Ludwig MD, Turel AP, Zagon IS, et al. Treatment of clinically isolated syndrome and low dose naltrexone. *Clin Case Reports Rev* 2015; 1: 182–184.

37. Vassar M and Holzmann M. The retrospective chart review: important methodological considerations. *J Educ Eval Health Prof* 2013; 10: 12.