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Maintenance of [Met^5]-enkephalin Blood Levels Corresponds with a Positive Outcome in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic immunological disorder of multifactorial etiology. Genetics,geographical location, age, and gender are risk factors, but the underlying causes are not fully defined. Research has provided substantial evidence that pro- and anti-inflammatory cytokines, as well as glial cell proliferation are involved in the progression of the disease. However, there is still a need to define noninvasive biomarkers to track the onset and course of MS. New treatmentsare effective at reducing signs and symptoms of the disease, but little change has been made in altering the overall course of MS.

Clinical reports from individuals using a low dose of the opioid receptor antagonist naltrexone prompted research using animal models of chronic experimental autoimmune encephalomyelitis (EAE) or relapsing-remitting EAE (RR-EAE). These studies reported that serum levels of the endogenous peptide Opioid Growth Factor (OGF), chemically termed [Met^5]-enkephalin, declined during the course of disease and could be restored to normal following therapeutic intervention. β-endorphin, another endogenous neuropeptide, was less responsive to treatment, suggesting that OGF may be a selective biomarker for MS that can be obtained non-invasively in order to monitor the course of disease.

There is a rapid decrease in OGF serum levels within days of EAE induction, as well as reduced levels of OGF in individuals with MS. This reduction in the negative growth factor may contribute to the uncontrollable lymphocytic proliferation and cytokine storm observed in early stages of MS that correspond to the physical and mental decline measured in humans, and to the behavioral deficits and central nervous system pathology recorded in animal models. Therapies that increaseOGF serum values and result in improved clinical signs and perceived good health are warranted.

Keywords: Opioid Growth Factor, [Met5]-enkephalin, ELISA tests, MS, Experimental autoimmune encephalomyelitis

Introduction

Multiple sclerosis (MS) is a debilitating disease with a multifactorial etiology that remains to be fully defined. In the United States alone it is estimated that there are one million individuals livingwith MS [1,2]. Development of MS involves inflammatory reactions against self-antigens and progressive demyelination in the brain and spinal cord. Epidemiological data on MS indicate a 2:1 ratio of women to men with a worsening disease prognosis for men than for women [1]. In addition to a genetic predisposition [3], geography [4] and sun exposure increasing melatonin [5] and vitamin D levels [6] are risk factors for the development of MS. The disorder is prevalent in younger individuals of Northern European ancestry who may have an accumulation of genetic and biological risk factors, one or more of which may be involved in pathways that have become dysregulated.

Clinical reports first described a relationship between enkephalins, endorphins, and MS in individuals receiving low doses of naltrexone (LDN) who reported that they "felt" better followingthis non-traditional treatment [7-9]. A retrospective chart review of more than 50 MS patients, 23 receiving LDN alone, revealed that in comparison to the disease-modifying therapy (DMT) Copaxone, LDN maintained stable physiological signs, with no change in MRIs, and health in the RR-MS individuals [9]. Evaluation of MRIs, blood laboratory data (e.g., white cell counts, platelet count, hemoglobin

levels, liver enzymes), and behavior as assessed by a timed 25-foot walk revealed no differences in values for patients on Copaxone and those on LDN only [9].

Because of the variability in patient profiles, as well as the broad array of DMTs that are now available, it is difficult to perform rigorous and reproducible studies on humans. Given our understanding of the Opioid Growth Factor (OGF) - OGF receptor (OGFr) axis, and the role that naltrexone plays in modulating this pathway, we investigated the role of OGF and LDN utilizing the experimental autoimmune encephalomyelitis (EAE) mouse model [10-18]. Different antigens and strains of mice can be used to develop chronic progressive EAE (Ch-EAE) or relapsing-remitting EAE (RR-EAE). Accumulated evidence from our research supports the hypothesis that down-regulation of the OGF-OGFr pathway resulting in decreased blood levels of OGF are associated with a poor prognosis for MS [10,11,19] or EAE [19,20]. Although animal models of MS are imperfect, they provide a reliable platform to assess independent variables in a controlled setting. Manipulation of the OGF-OGFr axis in Ch-EAE mice by injecting OGF or intermittent blockade of the pathway with LDN provides a reproducible animal model enabling rigorous investigations. Data demonstrated that increased blood levels of OGF resulted in positive outcomes and reduction in clinical disease and central nervous system pathology [12-20].

Behavioral and Pathological Response to OGF Therapy in Ch-EAE

Initial preclinical work utilized the EAE model that was established by immunization with myelin oligodendrocytic glycoprotein (MOG) of female C57Bl/6J mice. The regimen produced behavioral patterns that resembled primary progressive MS or a chronic course of behavioral decline until full paralysis was observed and animals were humanely euthanized. This Ch-EAE model presents with a caudal to rostral pathology detected by demyelination of the spinal cord and glial cell activation [e.g., 12,13]. Daily treatment with OGF beginning at the time of disease induction delayed, and even prevented in some mice, the onset of EAE which was otherwise present in all saline-treated mice inoculated with MOG within 3 weeks of immunization [13]. Behavioral scores of limp tail, wobbly gait, and hind limb paralysis were markedly reduced in OGF-treated EAE mice relative to saline-treated EAE mice.

Recognizing that clinical therapy routinely begins after symptoms and signs of MS have appeared for a number of weeks or months, another mouse paradigm was established to study a more clinically relevant model. Mice were immunized with MOG and treatment began 2 days afterthe first appearance of disease (i.e., limp tail or wobbly gait). OGF therapy reversed the disease progression; disease scores of the OGF-treated EAE mice declined significantly from those of saline-treated EAE mice. Moreover, spinal cord pathology and astrocyte activation were substantially reduced in the EAE mice receiving OGF [14].

RR-MS represents more than 85% of all individuals with MS [1], warranting preclinical investigations in a mouse model of RR-EAE. Female SJL mice were immunized with proteolipid proteins to establish such a model. Several studies conducted on RR-EAE have documented that induction of this form of disease is variable, and lends itself to having incomplete responses [15,16]. Some animals are responders to therapy and others are non-responders. While this phenomenon is not unique to EAE, and is particularly evident in addiction studies, it presents somedifficulty in the interpretation of data. Several studies that established RR-EAE showed repeatedly that mice injected daily with OGF had lower behavioral scores and reduced disease severity [15]. The length of remissions as measured by a return to a disease score of 0.5 or less was significantly greater in terms of days, as was the length of time mice had mild disease for mice receiving OGF.

Investigations on the treatment of OGF using established RR-EAE mouse models were completed with daily injections of OGF beginning 2 days after behavioral signs of disease were observed [16] and continued once daily for 40 days. OGF treated RR-EAE mice had markedly reduced clinical signs of disease, more periods of remission with each one being longer that those recorded for saline-injected RR-EAE mice. OGF inhibited proliferation of T lymphocytes, reduced the number of Iba-1+ cells and CD3+ cells, and decreased proliferating astrocytes. Exploration of the mechanism of OGF and LDN therapy in Ch-EAE mice revealed that these treatments delayed CNS infiltration of CD4+ T lymphocytes, another sign of inflammation [17]. Thus, multiple studies demonstrated that daily injections of OGF could reduce the severity of both relapsing remitting and chronic forms of established EAE.

OGF Levels in Experimental Autoimmune Encephalomyelitis

Once we established that manipulation of the OGF-OGFr axis was involved in EAE progression, we focused on determining the serum levels of endogenous peptides, OGF and β-endorphin, in Ch-EAE mice. Within 5 days of immunization of MOG to 6 week old C57BL/6J female mice, OGF levels were significantly reduced (~70 pg/ml) from normal levels of approximately 150 pg/ml [18]. The deficits in OGF preceded the appearance of any clinical behavioral signs by at least 4 days. Treatment of EAE mice with injections of 10 mg/kg OGF daily increased the blood levels of OGF above those of saline-treated EAE mice, but still below those in normal mice. However, the serum OGF levels correlated with reduced open field movement and von Frey sensitivity, and were inversely correlated with disease severity. Thus, the progression of disease corresponded with declining OGF blood levels.

Investigations examined whether the timing of OGF administration to mice altered their immune response. In a well-controlled set of experiments, female mice were immunized with MOG and received OGF prophylactically

beginning at the time of disease induction or as traditional therapy once clinical behavior was observed for 2 days [20]. Prophylactic OGF delayed the onset of disease behavior and suppressed lymphocyte and neutrophils replication. Traditional treatment regimens of OGF resulted in a reversal of the clinical behavior, restored OGF serum levels, and inhibited microglial activation within an 8 day period of time. Longer studies were aborted due to the pandemic. This work suggests that treatments to increase serum OGF levels should begin as early as possible following diagnosis of MS, and that maintenance of blood levels of OGF is a positive biomarker of disease progression.

OGF Levels in Multiple Sclerosis

Clinical studies on MS are complicated by a number of factors including length of MS disease, age of patient at the onset of disease, and the patient's self-reported symptoms. Moreover, OGF is not FDA approved for clinical use in humans. An alternative therapy is low dosages of an opioid receptor antagonist such as naltrexone that invokes a biofeedback mechanism to increase production and/or release of endogenous opioids including β-endorphin and [Met^5]-enkephalin. In clinical studies, elevated levels of enkephalins, specifically OGF, as well as β-endorphin, were measured following either disease modifying therapy or LDN leading to self-reported higher scores on the MS-QoL survey [9,10]. A clinical study (IRB protocol 9784) conducted with consented volunteer patients at the Penn State Hershey Neurology Clinic revealed that serum [Met^5]-enkephalin (i.e., OGF) levels were lower in MS patients relative to values from non-MS control humans [10]. LDN therapy restored OGF levels to near normal, but small sample size and demographic variability in length of disease and treatment were too large for reliable comparisons.

In a follow-up study, a larger cohort of individuals with diagnosed RR-MS was consented and blood samples assayed for OGF levels, IL-17A and TNFα cytokines; comparisons were made to blood samples for age and sex matched non-MS individuals [10]. Serum samples were analyzed using commercial ELISA kits for OGF, β-endorphin, IL17A, 1L17, and TNFα. Data revealed that MS diagnosis resulted in lower OGF serum values, even in patients on disease modifying therapy relative to OGF levels in non-MS individuals; a small cohort of RR-MS individuals on LDN had OGF comparable to those of non-MS individuals, but sample size was too small for statistical reliability. Serum β-endorphin levels ranged between 1 and 4 ng/ml, and were elevated in RR-MS individuals receiving glatiramer acetate therapy. A direct correlation was found between OGF levels and IL- 17A cytokine values, the pro-inflammatory T-helper cell marker, but no correlation was noted between OGF and TNFα values. Sample sizes and treatment variability prevented additional reliable comparisons. Additional research is needed to expand on the observations that OGF inhibits activated T and B cells in EAE with a focus on whether this mechanism can be modulated by LDN [21].

Conclusions

Preclinical and clinical data reveal that OGF serum levels are significantly decreased in mouse models of EAE and in RR-MS. Treatment with OGF for mice, or LDN for humans, appears to increase serum OGF leading to improved disease outcomes. In Ch-EAE and RR-EAE mice models, OGF treatment reduced behavioral signs, decreased spinal cord pathology, and correlated with a cytokine profile supporting immunological repair. In humans, restored serum enkephalin is associated with a reduced inflammatory cytokine profile. Data distinguishing whether different disease modifying therapies are more likely to alter OGF serum levels are warranted. Collectively, higher serum OGF levels are predictive of better patient outcome. The addition of OGF serum values to the diagnostic panel for MS may be warranted. This would enable physicians to begin early treatment with LDN or another disease modifying therapy.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. National Multiple Sclerosis Society. MS Prevalence. 2021. https://www.nationalmssociety.org

2. Wallin MT, Culpepper WJ, Campbell JD, Nelson LM, Langer-Gould A, Marrie RA, et al. The prevalence of MS in the United States: a population-based estimate using health claims data. Neurology. 2019 Mar 5;92(10):e1029-40.

3. Baranzini SE, Oksenberg JR. The genetics of multiple sclerosis: from 0 to 200 in 50 years. Trends in Genetics. 2017 Dec 1;33(12):960-70.

4. Ebers GC, Sadovnick AD. The geographic distribution of multiple

sclerosis: a review. Neuroepidemiology. 1993;12(1):1-5.

5. Hawkes CH, Baker MD, Pohl D, Lechner-Scott J, Levy M, Giovannoni G. Melatonin and multiple sclerosis. Mult Scler Relat Disord. 2021 Jun;51:103032.

6. Scazzone C, Agnello L, Bivona G, Lo Sasso B, Ciaccio M. Vitamin D and genetic susceptibility to multiple sclerosis. Biochemical Genetics. 2021 Feb;59(1):1-30.

7. Gironi M, Martinelli-Boneschi F, Sacerdote P, Solaro C, Zaffaroni M, Cavarretta R, et al. A pilot trial of low-dose naltrexone in primary progressive multiple sclerosis. Multiple Sclerosis Journal. 2008 Sep;14(8):1076-83.

8. Cree BA, Kornyeyeva E, Goodin DS. Pilot trial of low-dose naltrexone and quality of life in multiple sclerosis. Annals of Neurology. 2010 Aug;68(2):145-50.

9. Ludwig MD, Turel AP, Zagon IS, McLaughlin PJ. Long-term treatment with low dose naltrexone maintains stable health in patients with multiple sclerosis. Multiple Sclerosis Journal–Experimental, Translational and Clinical. 2016 Sep;2:2055217316672242.

10. Patel CL, Zagon IS, Thomas GA, McLaughlin PJ. Enkephalin therapy improves relapsing-remitting multiple sclerosis. In: An Overview and Management of Multiple Chronic Conditions. 2020 Jan 28. IntechOpen.

11. Patel C, Thomas G, Zomorodi N, Zagon IS, McLaughlin PJ. β-endorphin and opioid growth factor as biomarkers of physical ability in multiple sclerosis. Multiple Sclerosis and Related Disorders. 2021 May 1;50:102868.

12. Zagon IS, Rahn KA, Turel AP, McLaughlin PJ. Endogenous opioids regulate expression of experimental autoimmune encephalomyelitis: a new paradigm for the treatment of multiple sclerosis. Experimental Biology and Medicine. 2009 Nov;234(11):1383-92.

13. Zagon IS, Rahn KA, Bonneau RH, Turel AP, McLaughlin PJ. Opioid growth factor suppresses expression of experimental autoimmune encephalomyelitis. Brain Research. 2010 Jan 15;1310:154-61.

14. Campbell AM, Zagon IS, McLaughlin PJ. Opioid growth factor arrests the progression of clinical disease and spinal cord pathology in established experimental autoimmune encephalomyelitis. Brain Research. 2012 Sep 7;1472:138-48.

15. Hammer LA, Zagon IS, McLaughlin PJ. Treatment of a relapse-remitting model of multiple sclerosis with opioid growth factor. Brain Research Bulletin. 2013 Sep 1;98:122-31.

16. Hammer LA, Zagon IS, 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 Research Bulletin. 2015 Mar 1;112:42-51.

17. Hammer LA, Waldner H, Zagon IS, McLaughlin PJ. Opioid growth factor and low-dose naltrexone impair central nervous system infiltration by CD4+ T lymphocytes in established experimental autoimmune encephalomyelitis, a model of multiple sclerosis. Experimental Biology and Medicine. 2016 Jan;241(1):71-8.

18. Ludwig MD, Zagon IS, McLaughlin PJ. Elevated serum [Met5]-enkephalin levels correlate with improved clinical and behavioral outcomes in experimental autoimmune encephalomyelitis. Brain Research Bulletin. 2017 Sep 1;134:1-9.

19. Ludwig MD, Zagon IS, McLaughlin PJ. Featured Article: Serum [Met5]-enkephalin levels are reduced in multiple sclerosis and restored by low-dose naltrexone. Experimental Biology and Medicine. 2017 Sep;242(15):1524-33.

20. Patel C, Zagon IS, Pearce-Clawson M, McLaughlin PJ. Timing of treatment with an endogenous opioid alters immune response and spinal cord pathology in female mice with experimental autoimmune encephalomyelitis. Journal of Neuroscience Research. 2022 Feb;100(2):551-563.

21. McLaughlin PJ, McHugh DP, Magister MJ, Zagon IS. Endogenous opioid inhibition of proliferation of T and B cell subpopulations in response to immunization for experimental autoimmune encephalomyelitis. BMC Immunology. 2015 Dec;16(1):24.