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Research Report

Opioid growth factor arrests the progression of clinical disease and spinal cord pathology in established experimental autoimmune encephalomyelitis

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ABSTRACT

An endogenous neuropeptide, opioid growth factor (OGF), chemically termed [Met^5]-enkephalin, arrested the progression of established disease in a mouse model of multiple sclerosis (MS) called experimental autoimmune encephalomyelitis (EAE). This study treated mice who demonstrated 2 consecutive days of behavioral decline following injections of myelin oligodendrocyte glycoprotein (MOG) with daily injections of OGF (10 mg/kg) or saline (0.1 ml) for 40 days. Within 6 days of OGF treatment, mice initially demonstrating clinical signs of EAE had significant reductions (45% reduction) in their behavioral scores relative to EAE mice receiving saline. Behavior was attenuated for the entire 40-day period with mice receiving OGF showing only limp tails and wobbly gait in comparison to saline-treated EAE mice who displayed paralysis of one or more limbs. Neuropathological studies revealed that OGF treatment initiated after the appearance of disease reduced the number of activated astrocytes and damaged neurons, decreased demyelination, and inhibited T cell proliferation. These results demonstrate that OGF can halt the progression of established EAE, return aberrant pain sensitivity to normal levels, inhibit proliferation of T cells and astrocytes, and prevent further spinal cord pathology. The data extend our observations that OGF given at the time of disease induction prevented disease onset, reduced the severity of clinical signs of disease, and reversed neurological deficits in a non-toxic manner. Our data substantiate the role of the OGF–OGFr axis in EAE and support the use of OGF as a biotherapy for MS.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease associated with the central nervous system (CNS) that impacts ~400,000 people in the United States and 2 million individuals worldwide (Forte et al., 2007; Rizvi and Agius, 2004) to cause lifelong loss of quality of life. MS manifestation involves inflammation, demyelination, and axonal damage in the CNS (Bennett and Stuve, 2009; Van der Walt et al., 2010; Weiner, 2009). The onset of disease is associated with the activation of astrocytes (Axelsson et al., 2011), proliferation of T and B cells (Ercolini and Miller, 2006), and subsequent production of cytokines and myelin-specific antibodies at the site of active CNS lesions. Current therapies for MS are

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expensive, associated with toxic side-effects, and often not well tolerated as long-term therapy by patients.

The opioid growth factor (OGF)–OGF receptor (OGFr) axis is a physiological pathway that is effective in the amelioration of experimental autoimmune encephalomyelitis (EAE), the mouse model of MS. Exogenous injections of the neuropeptide OGF, as well as production of endogenous opioid peptides following daily, short-term blockade of receptors by naltrexone (low dose naltrexone, LDN) (Zagon and McLaughlin, 1984), have been shown to diminish behavioral signs of disease as well as neuropathology (Rahn et al., 2011; Zagon et al., 2009, 2010). Studies (Zagon and McLaughlin, 1989, 1991) revealed that one particular endogenous opioid acting to tonically regulate cell proliferation through an inhibitory pathway was the pentapeptide [Met^5]-enkephalin, termed opioid growth factor (OGF). OGF activity is mediated by the non-classical opioid receptor, OGFr (Zagon et al., 1992, 2002), and the mechanism of OGF–OGFr action involves the upregulation of cyclin-dependent inhibitory kinase pathways (p16, p21) which in turn delay transition from the G_1 phase to S phase of the cell cycle (Cheng et al., 2009a).

Chronic treatment with OGF beginning at the time of disease induction with MOG resulted in a neuroprotective effect on encephalitogenic processes in mice with experimental autoimmune encephalomyelitis (EAE). Signs of behavioral deficits such as limp tail and limb paralysis were delayed in onset, prevented, reduced, or reversed in mice receiving 10 mg/kg OGF or 0.1 mg/kg naltrexone (LDN) from day 0. Neuropathological evaluation of lumbar spinal cord on days 20, 30 and 60 following induction of disease revealed significant reductions in the number of activated astrocytes, and the area of demyelination in mice with EAE receiving OGF or LDN. However, this paradigm represents a clinically isolated event whereby patients with the first signs of MS (e.g., tremor, weakness in limb, blurred vision) may present to a physician and begin treatment. The vast majority of patients have established disease (confirmed brain lesion, behavioral episode) at the time of presenting to a physician. In this study we examined the efficacy of daily injections of 10 mg/kg OGF to mice with established EAE. Mice were observed daily for changes in behavior over a 40-day period of treatment. Lumbar spinal cord tissue was collected 5, 10, 20, and 40 days after the establishment of disease and initiation of treatment to assess the expression and proliferation of T lymphocytes, astrocytes, and oligodendrocyte precursor cells, as well as demyelination and neuronal damage.

2. Results

2.1. Behavioral results

2.1.1. General observations

Normal mice did not develop neurological abnormalities over the entire experimental period, and no mouse in the Normal group died. Following MOG inoculations, a few mice displayed redness and/or minor lesions, but these healed within a few days of injection. Body weights of mice receiving MOG were comparable for the first week, but declined as disease became evident. MOG injected mice receiving saline had body weight loss that reached 16% below that of Normal mice, whereas MOG-injected mice receiving OGF weighed only 7% less than Normals at the time of peak disease (~day 18). One mouse in each MOG injected group died over the course of the 40 days experiment.

2.1.2. Behavioral assessments

MOG peptide immunization induced the first appearance of clinical signs of EAE on day 9, with an average day of disease onset occurring at 9.5 ± 0.1 days following the first injection of MOG. After two consecutive days of disease, EAE mice were randomly assigned to EAE+Vehicle (saline) or EAE+OGF treatment groups. 100% disease incidence occurred by day 11 of the study. At disease onset, behavioral scores were comparable in EAE+Vehicle and EAE+OGF mice. However, after 5 treatment days, the average behavioral scores were significantly lower in OGF treated EAE mice, being reduced 45% from EAE mice receiving saline. Behavioral scores continued to be significantly lower in the EAE+OGF group compared to the EAE+Vehicle group for the duration of the 40-treatment-day study (Fig. 1A, $P<0.0001$ by 2-way ANOVA). To assess overall disease burden, cumulative disease scores were determined for mice collected after 5, 10, 20 and 40 treatment days. Cumulative disease scores were 48% and 36% lower in EAE+OGF mice after 20 and 40 treatment days, respectively, compared to EAE+Vehicle mice (Fig. 1B). The effects of OGF treatment on the behavior of mice with established EAE were comparable in 3 independent studies.

2.1.3. Pain sensitivity

The effect of OGF on pain sensitivity was tested by subjecting mice to hotplate testing and recording latency to a response (Fig. 1C). At disease onset (treatment day 0), EAE mice had a mean response time (11.7 ± 0.6 s) to the thermal stimulus that was 19% faster than response times of Normal mice. After 5 and 18 treatment days, EAE+Vehicle mice continued to exhibit increased pain sensitivity with 44% and 31% faster mean response times compared to Normal controls who had 16.2 ± 2.1 and 14.3 ± 0.7 s, respectively, latencies on the hotplate. However, at both 5 and 18 treatment days, OGF treated mice with EAE had response times that were comparable to Normals.

2.2. Histopathology results

2.2.1. Microglia/macrophages

To evaluate microglia/macrophages, lumbar spinal cord sections collected after 5, 10, 20 and 40 days of treatment were stained with Iba1 antibody (Fig. 2) and positive staining recorded. After 5 treatment days, the number of Iba1$^+$ cells were upregulated 3.1-fold and 3.2-fold in EAE+Vehicle and EAE+OGF mice, respectively, compared to Normal controls. After 10 treatment days, the number of Iba1$^+$ cells was increased 3.8- and 3.3-fold in both groups of EAE mice relative to Normals, however, the number of Iba1$^+$ cells was reduced 30% and 24% after 20 and 40 days of OGF treatment, respectively, in comparison to values in EAE+Vehicle treated mice.