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Research Report

Dose-dependent inhibition of GCPII to prevent and treat cognitive impairment in the EAE model of multiple sclerosis

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ABSTRACT

There are no treatments for cognitive impairment in multiple sclerosis (MS). Novel treatments can be evaluated in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS that displays both physical and cognitive impairments. Inhibition of the neuropeptidase glutamate carboxypeptidase II (GCPII) has previously been shown to ameliorate cognitive impairment in EAE, but dosing has not yet been optimized and only a prevention treatment paradigm has been explored. In the study described herein, the dose response of the GCPII inhibitor 2-(phosphonomethyl)pentanedioic acid (2-PMPA) was evaluated for preventing cognitive impairment in EAE mice. Mice were immunized and received daily injections of vehicle or 2-PMPA (10, 30, 100, or 300 mg/kg) from the time of immunization (i.e. day 0). Although no doses of the drug altered physical disease severity, the 100 mg/kg dose was most efficacious at preventing cognitive impairments in Barnes maze performance. Dose-related increases in brain NAAG levels were observed in post-mortem analysis, confirming target engagement. Using the 100 mg/kg dose, we subsequently evaluated 2-PMPA's ability to treat EAE-induced symptoms by commencing treatment after the onset of physical signs of EAE (i.e. day 14). Mice were immunized for EAE and received daily injections of vehicle or 100 mg/kg 2-PMPA starting two weeks post-immunization. Significant improvements in both cognitive performance and increases in brain NAAG levels were observed. GCPII inhibition is a promising treatment for cognitive impairment in MS, and doses providing equivalent exposures to 100 mg/kg 2-PMPA in mice should be evaluated in clinical studies for the prevention and/or treatment of MS-related cognitive impairment.

Abbreviations: 2-PMPA, 2-(phosphonomethyl)pentanedioic acid; DMTs, disease-modifying therapies; EAE, experimental autoimmune encephalomyelitis; GCPII, glutamate carboxypeptidase II; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; MS, multiple sclerosis; TGF, transforming growth factor; TBI, traumatic brain injury

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1. Introduction

Cognitive impairment is present in approximately half of the patients with multiple sclerosis (MS), but none of the thirteen FDA-approved disease-modifying therapies (DMTs) for MS target cognition. Recent clinical data collected in MS patients revealed a positive correlation between hippocampal concentrations of the neuropeptide N-acetylaspartylglutamate (NAAG) and patients' performances on a standard battery of cognitive assessments (Rahn et al., 2012). Notably, MS patients with low hippocampal NAAG levels showed cognitive impairment, while those with higher levels of hippocampal NAAG exhibited normal cognition. Similar to humans with MS, cognitive impairment and correspondingly low levels of brain NAAG are present in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS (Rahn et al., 2012; Ziehn et al., 2010). Pharmacological upregulation of brain NAAG levels is possible via inhibition of Glutamate CarboxyPeptidase II (GCPII), the zinc metalloenzyme responsible for cleavage of NAAG into N-acetylaspartate (NAA) and glutamate following its synaptic release. In EAE mice, the potent and selective GCPII inhibitor 2-(phosphonomethyl) pentanedioic acid (2-PMPA) elevates brain NAAG concentrations and improves learning and memory performance to levels observed in healthy, non-EAE control mice (Rahn et al., 2012).

GCPII inhibition's cognitive effects are not limited to MS, as GCPII inhibitor-mediated improvements in cognition have been reported in preclinical models of schizophrenia (Janczura et al., 2013; Olszewski et al., 2012b) and traumatic brain injury (TBI) (Gurkoff et al., 2013). Doses tested in these models were broad, ranging from 0.2 to 150 mg/kg. The only dose of 2-PMPA utilized in EAE studies to date has been 100 mg/kg, which was chosen because it afforded neuroprotection in numerous animal models of disease (Harada et al., 2000; Slusher et al., 1999; Thomas et al., 1999). It is possible, however, that lower and/or higher doses of 2-PMPA might show additional benefit in EAE. Therefore, the first purpose of the present study was to determine the optimal dose of 2-PMPA's therapeutic effect.

Given that MS-related cognitive impairment is often not detected until a MS diagnosis has been made (Glanz et al., 2007), it is important to evaluate potential treatments for cognitive impairment in MS using a treatment paradigm (i.e. drug administration begins after disease onset). Therefore, the second purpose of the present study was to determine if 2-PMPA can treat EAE-related cognitive impairment when administered after EAE disease onset.

2. Results

2.1. *Dose response prevention study*

2.1.1. *EAE disease score*

EAE mice were treated daily, beginning on the day of immunization (day 0), with vehicle or 2-PMPA at a dose of 10 mg/kg, 30 mg/kg, 100 mg/kg, or 300 mg/kg to determine the optimal dose of the drug for preventing cognitive impairment in EAE. No significant differences in EAE disease score were noted between groups (Fig. 1A). Average day of disease onset, approximately 12 days post-immunization, also did not differ between groups. Although the peak and mean daily EAE scores tended to be lower in 2-PMPA versus vehicle treated mice, especially at the highest dose (i.e. 300 mg/kg), no statistically significant treatment effects were observed. Consistent with these disease score trends, the 2-PMPA daily treatment at all four doses resulted in a body weight that trended higher with increasing doses of drug compared to placebo, although statistically significant differences in body weight were only observed between EAE+vehicle and EAE+2-PMPA 300 mg/kg on day 27 ($P<0.01$, Fig. 1B). As expected, all mice lost weight between days 10 and 17, but regained weight during the chronic stage of EAE, when behavior tests were conducted (28–33 days post-immunization).

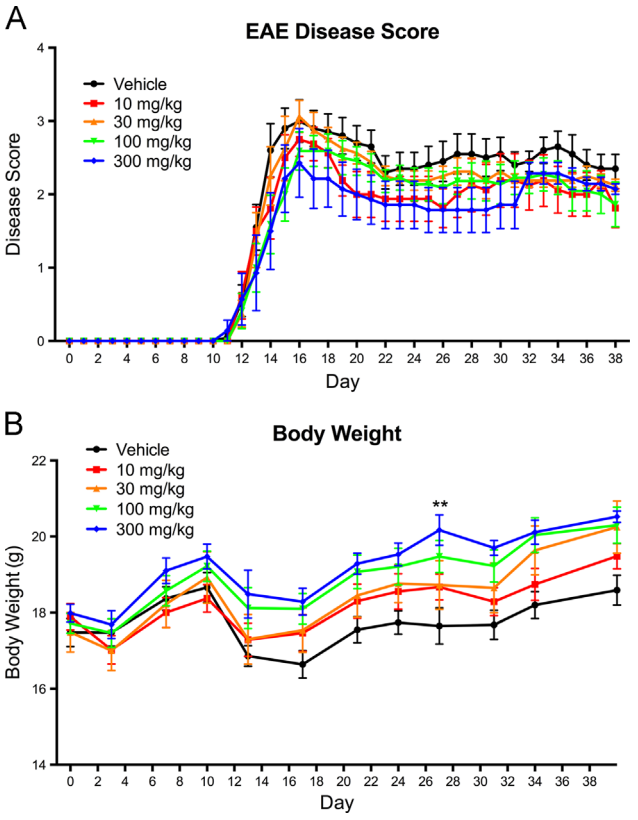

Fig. 1 – 2-PMPA does not significantly alter EAE disease score. Physical disability as measured by the EAE disease score scale was evaluated daily (A). While there was a trend for a slight reduction in EAE severity in mice treated with 2-PMPA, particularly in the high dose 300 mg/kg 2-PMPA group, no significant differences were observed between groups ($n=7$–11). Starting body weight did not differ between groups (B). Body weight declined at the time of acute EAE disease onset, approximately 10–17 days post-immunization. Mice treated with all doses of 2-PMPA had higher average body weight than EAE+vehicle mice, with 300 mg/kg 2-PMPA EAE mice reaching statistical significance on day 27 ($P<0.01$).