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Pioglitazone is superior to quetiapine, clozapine and tamoxifen at alleviating experimental autoimmune encephalomyelitis in mice

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ABSTRACT

Recent evidence suggests that clozapine and quetiapine (atypical antipsychotics), tamoxifen (selective-estrogen receptor modulator) and pioglitazone (PPARγ agonist) may improve functional recovery in multiple sclerosis (MS). We have compared the effectiveness of oral administration of these drugs, beginning at peak disease, at reducing ascending paralysis, motor deficits and demyelination in mice subjected to experimental autoimmune encephalomyelitis (EAE). Mice were immunized with an immunogenic peptide corresponding to amino acids 35–55 of the myelin oligodendrocyte glycoprotein ($MOG_{35–55}$) in complete Freund's adjuvant and injected with pertussis toxin to induce EAE. Unlike clozapine, quetiapine and tamoxifen, administration of pioglitazone beginning at peak disease decreased both clinical scores and lumbar white matter loss in EAE mice. Using kinematic gait analysis, we found that pioglitazone also maintained normal movement of the hip, knee and ankle joints for at least 44 days after $MOG_{35–55}$ immunization. This long-lasting preservation of hindleg joint movements was accompanied by reduced white matter loss, microglial and macrophage activation and the expression of pro-inflammatory genes in the lumbar spinal cords of EAE mice. These results support clinical findings that suggest pioglitazone may reduce the progressive loss of motor function in MS by decreasing inflammation and myelin damage.

1. Introduction

Multiple sclerosis (MS) is characterized by the autoimmune-mediated destruction of myelin in the central nervous system (CNS) (Baecher-Allan et al., 2018; Compston and Coles, 2008). Axonal damage caused initially by activated immune cells (Nikic et al., 2011) and subsequently by the metabolic collapse of denuded axons (Trapp and Stys, 2009) is thought to promote the irreversible loss of neurological function in MS (Mahad et al., 2015; Trapp et al., 1998). Experimental autoimmune encephalomyelitis (EAE) in mice, induced by immunization with $MOG_{35–55}$, models inflammatory processes, demyelination, axonal damage and paralysis seen in MS (Constantinescu et al., 2011; Steinman and Zamvil, 2006). This EAE model has proven to be useful in the identification of immunological disease mechanisms and treatments for MS (Constantinescu et al., 2011; Steinman and Zamvil, 2006). More recent experimentation with the EAE model suggests that drugs approved for the treatment of other conditions might be repositioned to protect the CNS from damage in MS.

Among the classes of Food and Drug Administration (FDA)-approved drugs that have been shown to reduce inflammation,

Abbreviations: 4-HT, 4-hydroxytamoxifen; CNS, central nervous system; CFA, complete Freund's adjuvant; DPI, Days post-immunization; EAE, Experimental autoimmune encephalomyelitis; FDA, Food and Drug Administration; MS, Multiple sclerosis; Hour, hr; MOG, Myelin oligodendrocyte glycoprotein; PBS, Phosphate buffered saline; PLP, Proteolipid protein; PPAR, Peroxisome proliferator activator receptor; qRT-PCR, Quantitative real time-polymerase chain reaction; RMS, Root mean squared; SERM, Selective estrogen receptor modulator; TZD, Thiazolidinedione

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demyelination and clinical disease signs in EAE mice are the atypical antipsychotics (Mei et al., 2012; O'Sullivan et al., 2014), selective estrogen receptor modulators (SERMs) (Bebo Jr et al., 2009) and thiazolidinediones (TZDs) (Feinstein et al., 2002; Niino et al., 2001). Oral administration of the atypical antipsychotic clozapine to mice *via* the drinking water, beginning one day before immunization with $MOG_{35–55}$, produced dose-dependant reductions in EAE disease severity (O'Sullivan et al., 2014). Clinical signs of EAE were also reduced by the oral administration of quetiapine (10 mg/kg/day, po) at the onset of clinical signs (Mei et al., 2012). Unlike quetiapine (Mei et al., 2012), clozapine did not appear to reduce clinical disease signs in the EAE model by suppressing effector T cell function or enhancing T regulatory cell activity (Zareie et al., 2017). Alternatively, the ability of clozapine to improve myelin integrity by increasing oligodendrocyte energy production and lipid synthesis (Ferno et al., 2005; Steiner et al., 2014) may contribute to the beneficial effects of this antipsychotic in models of autoimmune- and cuprizone-induced demyelination (Xu et al., 2010; Xu et al., 2014; Zhang et al., 2008). Quetiapine is structurally similar to clozapine and shares these positive effects on oligodendrocyte function and survival in cell-based and animal models of demyelination (Xu et al., 2010; Xu et al., 2014; Zhang et al., 2008; Zhang et al., 2012; Zhornitsky et al., 2013). Tamoxifen is a SERM that suppresses relapsing-EAE induced by immunization of SJL mice with a synthetic peptide corresponding to amino acids 139–151 of proteolipid protein ($PLP_{139–151}$) (Bebo Jr et al., 2009). In this study, tamoxifen was delivered by a slow-release implant beginning 7 days before $PLP_{139–151}$ immunization. T cell proliferation assays and cytokine measurements suggested that tamoxifen supressed the development of EAE by inducing a Th2 immune bias that suppressed autoimmune-mediated demyelination (Bebo Jr et al., 2009). TZDs such as pioglitazone and rosiglitazone have also been shown to reduce EAE disease severity in association with decreased inflammation and demyelination (Feinstein et al., 2002). Although troglitazone also alleviates $MOG_{35–55}$-induced EAE, this TZD appears to be less effective than pioglitazone (Niino et al., 2001). Lastly, clozapine, quetiapine, tamoxifen and pioglitazone may also reduce the destruction of vulnerable neural elements such oligodendrocyte progenitor cells (De Nuccio et al., 2015; Xu et al., 2014), oligodendrocyte, axons and neurons in EAE mice by improving mitochondrial function.

Based on these findings, we compared the abilities of clozapine, quetiapine, tamoxifen and pioglitazone to alleviate $MOG_{35–55}$-induced EAE for the following reasons. First, the potential for oral efficacy is an important therapeutic consideration for drug repositioning in MS (Vesterinen et al., 2015). In keeping with this criterion, all four of these FDA-approved drugs are typically given by the oral route for their primary indications. Second, autoimmune and inflammatory processes contribute to the neurodegenerative events that drive disease MS progression (Frischer et al., 2009; Mahad et al., 2015; Mandolesi et al., 2015). Atypical antipsychotics, SERMs and PPARγ agonists suppress these autoimmune and inflammatory events in mouse EAE models of MS. Third, remyelination is essential for functional recovery in MS (Franklin and Ffrench-Constant, 2008). Cell-based and animal studies suggest that clozapine, quetiapine, tamoxifen and pioglitazone may promote remyelination in MS. Lastly, mitochondrial dysfunction in MS has been implicated in the destruction of oligodendrocyte progenitor cells (Cui et al., 2013), oligodendrocytes (Mahad et al., 2015), axons (Dutta et al., 2006) and neurons (Friese et al., 2014). *In vitro* and *in vivo* experimental findings suggest that atypical antipsychotics, SERMs and PPARγ agonists may protect these neural elements from damage in MS by improving mitochondrial function. Although these drug classes interact with distinct receptors, they all inhibit common pathological mechanisms considered pivotal in MS.

A major obstacle in the repurposing of clozapine, quetiapine, tamoxifen and pioglitazone for MS is the considerable resources required to test their clinical effectiveness. Preclinical studies are therefore required to identify the most promising of these therapeutic candidates for clinical testing. It is also important in these studies that drug administration to EAE mice is delayed until after disease onset to gauge their therapeutic potential for reducing inflammation, demyelination and neurological deficits in established MS. We have therefore compared the effects of oral administration of either clozapine or quetiapine or tamoxifen or pioglitazone, beginning at peak disease, on these measures in mice with $MOG_{35–55}$-induced EAE. Lastly, kinematic gait analysis (Akay, 2014) was performed in the sagittal plane to determine if the most effective of these drugs also prevented impaired hindleg hip, knee and ankle joint movements for EAE mice that resemble those seen in MS patients (Fiander et al., 2017a; Fiander et al., 2017b).

2. Methods

2.1. Animal care

Each experiment was done in accordance with the Canadian Council on Animal Care guidelines and received ethical approval from the Dalhousie University Committee on Laboratory Animals. All mice were housed in the Life Science Research Institute Animal Care Facility at Dalhousie University and maintained on a 12-hourh (hr) light/dark cycle (7:00 am/7:00 pm) with food and water provided *ad libitum*. Experimentation was performed during the light cycle using 10-week old female C57BL/6 mice (18–20 g; Charles River Canada, St. Constant, QC, CAN). Mice were given one week to habituate to the facility prior to experimentation.

2.2. EAE induction

A peptide corresponding to amino acids 35–55 (MEVGWYRSPFSRWHLYRNGK; Gen Script, Piscataway, NJ, USA) of myelin oligodendrocyte glycoprotein ($MOG_{35–55}$) was dissolved in phosphate buffered saline (PBS; pH = 7.4) and emulsified in complete Freund's adjuvant (CFA; 10 mg/ml) at a 1:1 ratio. This CFA solution included incomplete Freund's adjuvant mixed with *Myobacterium tuberculosis* H37RA (Difco Laboratories, Detroit, MI, USA). Two bilateral subcutaneous (sc) injections (100 µl) of the $MOG_{35–55}$/CFA mixture (300 µg/mouse) were administered near the base of the tail to mice on day 0 (EAE group). Pertussis toxin (300 ng; Sigma Aldrich, St. Louis, MO, USA) was administered by intraperitoneal (ip) injection in a volume of 200 µl on day 0 and 2 post-immunization (DPI) to each mouse. Immunization controls received injections of CFA plus pertussis toxin without $MOG_{35–55}$ (CFA group).

2.3. Behavioural assessment of clinical scores

Starting on DPI 7, mice were weighed and assessed for disease severity each day using a clinical rating scale. The following 11-point ordinal scale was used to assess motor deficits: 0, no motor deficits; 0.5, hooked tail; 1.0, fully flaccid tail; 1.5, bilateral hindlimb splay; 2.0, minor walking deficits; 2.5, major walking deficits; 3.0, dropped pelvis; 3.5, unilateral hindlimb paralysis; 4.0, bilateral hindlimb paralysis; 4.5, forelimb paralysis; and 5.0, moribund. Two experienced individuals, blind to the experimental conditions, performed the clinical scoring.

2.4. EAE animal husbandry

All mice were given access to mashed kibble and Dietgel Recovery (Clear H_2O, Westbrook, ME, USA) at disease onset. If body weight loss excessed 10% of pre-immunization valves, mice were handfed with DietGel Boost (Clear H_2O, Westbrook, ME, USA) and injected with 0.9% sodium chloride solution (25 ml/kg; sc). Humane end-points were one of the following: 1) weight loss exceeded 20% of the pre-immunization values; 2) clinical score of 5; 3) loss of righting reflex or 4) inability to access food or water for 24 hr. A humane end-point was reached in < 5% of the EAE mice.