Neurobiology of Aging 48 (2016) 61–71

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Neuroprotective and immunomodulatory effects of raloxifene in the myenteric plexus of a mouse model of Parkinson's disease

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ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form 29 June 2016
Accepted 6 August 2016
Available online 16 August 2016

Keywords:
Enteric nervous system
GPER1
Gut
Hormonotherapy
Inflammation
MPTP

ABSTRACT

Motor symptoms in Parkinson's disease (PD) are often preceded by nonmotor symptoms related to dysfunctions of the autonomic nervous system such as constipation, defecatory problems, and delayed gastric emptying. These gastrointestinal impairments are associated with the alteration of dopaminergic (DAergic) neurons in the myenteric plexus of the gut. Recently, we demonstrated the anti-inflammatory properties of estrogens to treat intestinal neurodegeneration in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. The present study aimed to investigate the neuroprotective and anti-inflammatory roles of raloxifene, a selective estrogen receptor modulator (SERM) already commercialized for osteoporosis treatment. In MPTP-treated mice, we found that raloxifene decreased the loss of DAergic neurons and prevented the increase in proinflammatory macrophage density in the myenteric plexus. Interestingly, raloxifene activity was prevented by the G protein–coupled estrogen receptor 1 (GPER1) antagonist G15, suggesting that raloxifene effects were mainly mediated by GPER1. Moreover, monocytic cell proinflammatory polarization, nuclear factor-kappa B (NF-κB) response, nitric oxide (NO), and proinflammatory cytokines production following 1-methyl-4-phenylpyridinium (MPP^+) treatment were also prevented by raloxifene in vitro. Overall, the present results suggest that raloxifene may help preventing the loss of DAergic neurons in the myenteric plexus in an MPTP mouse model of PD, at least in part through its anti-inflammatory effects. This suggests that drug repurposing of raloxifene might represent a promising therapeutic avenue to prevent systemic inflammation and peripheral neuronal dysfunction at early PD stages.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder affecting more than 6 million people worldwide (Dorsey et al., 2007). The degeneration of the dopaminergic (DAergic) nigrostriatal system as well as motor dysfunctions, including muscular rigidity, bradykinesia, tremors at rest, and postural instability, represent only one aspect of this multifaceted and complex disorder (Jankovic, 2008). In addition to parkinsonism, nonmotor symptoms also significantly reduce the quality of life and are a key component of PD. Dysautonomia, which may include constipation, defecation problems, and delayed gastric emptying, is present from the "premotor" phase until the final palliative stage (Savica et al., 2009). Over the last decade, several studies and models have provided strong evidence for the occurrence of gastrointestinal dysfunctions years before the onset of motor symptoms, suggesting that the intestine could be one of the first organs affected in the pathogenesis of PD (Braak et al., 2004, 2003; Phillips et al., 2008). Considering the intensive exposure of the enteric nervous system (ENS) to environmental toxins and the vulnerability of DAergic neurons to various physicochemical aggressions, understanding the mechanisms involved in neuronal alteration of the gut has become crucial.

Peripheral immunological challenges and chronic inflammatory diseases influence the pathogenesis and progression of PD. Communication between the immune and nervous systems is active and bidirectional (Collins et al., 2012). Proinflammatory cytokines (IL-1β, IL-6, and TNFα) and innate immune cell activation are the main mediators of inflammation in both brain (Nagatsu and Sawada, 2005; Sawada et al., 2006) and intestine (Devos et al., 2013; Herrera et al., 2015). Using the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD, we previously demonstrated the role of

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http://dx.doi.org/10.1016/j.neurobiolaging.2016.08.004

the innate immune system in the alteration of DAergic neurons in the brain and the gut (Cote et al., 2011, 2015b). Since partial depletion of the proinflammatory monocyte population protects DAergic neurons of the myenteric plexus from the toxic effects of MPTP (Cote et al., 2015b), an immunomodulatory-based therapy may likely be beneficial against PD development.

As PD is widely known to affect men more frequently than women (ratio of 3:2; Wong et al., 2014), several studies have assessed possible factors to account for the differential incidence of the disease according to sex and evaluated the possible benefit conferred by female sex steroids (Bourque et al., 2011; Gillies et al., 2014; Kotagal et al., 2013; Lubomski et al., 2014; Miller and Cronin-Golomb, 2010). Studies have demonstrated that estrogens may be used as neuroprotective agents (Liu and Dluzen, 2007), but also as modulators of the immune system (Kovats, 2015). However, hormonal therapies are known to induce various undesirable side effects via the activation of estrogenic receptors ERα and ERβ, such as increased risk of breast cancer, venous thrombosis, and stroke (Taylor and Manson, 2011). Taking these limitations into account, alternative targets with similar neuroprotective and immunomodulatory effects have been attracting interest, such as the G protein–coupled estrogen receptor 1 (GPER1). GPER1 is known for its non-feminizing effect, and studies have supported the major contribution of this receptor in the neuroprotective effect of estrogens in PD (Bessa et al., 2015; Bourque et al., 2013). Recently, we investigated the role of GPER1 in the myenteric plexus of the MPTP mouse model of PD and reported that the estrogen-mediated activation of this receptor had neuroprotective and immunomodulatory effects similar to the activation of ERα and ERβ (Cote et al., 2015a). Therefore, GPER1 could constitute a promising therapeutic target to treat premotor symptoms of PD in both men and women.

Raloxifene, commercially available for estrogen-based therapy, is commonly used for the treatment and prevention of postmenopausal osteoporosis. This drug is a part of the second generation of selective estrogen receptor modulators (SERMs), which are known to induce selective estrogenic effects in tissues. Depending on the tissue studied, raloxifene can act as either as an agonist or antagonist of the ERs and GPER1 (Dutertre and Smith, 2000; Sato et al., 1996; Yaffe et al., 2005). The antagonistic effect of raloxifene in mammary and uterine tissues decreases the risk of cancer in reproductive organs (Dutertre and Smith, 2000; Heringa, 2003; Shelly et al., 2008). The beneficial effects of this drug were also reported in the brain of both women and men in the absence of known side effects (Goekoop et al., 2005; Jacobsen et al., 2010; Yaffe et al., 2005). Many studies have already demonstrated the potential neuroprotective effect of raloxifene in animal models of PD (Baraka et al., 2011; Bourque et al., 2014; Callier et al., 2001; Grandbois et al., 2000). In both men and women, raloxifene is a very interesting option in view of its non-feminizing action and considering its neuroprotection via GPER1 already demonstrated in the central nervous system (CNS) of MPTP mice (Bourque et al., 2014).

In this study, we have investigated the therapeutic properties of raloxifene in early gastrointestinal inflammation and on DAergic neuron integrity in the MPTP mouse model of PD. We thus evaluated (1) whether raloxifene induces a neuroprotective effect on DAergic neurons in the myenteric plexus and whether this effect involves; (2) the activation of GPER1; and/or (3) the inhibition of the innate immune response.

2. Materials and methods

2.1. Animals

Ten-week-old C57BL/6 male mice were purchased from Charles River Canada (Montreal, Quebec, Canada). Mice were housed in cages under standard laboratory conditions, had access to food and water, and were acclimatized to a controlled-temperature environment maintained under a 12-hour light/dark cycle. Mice were handled in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, using a protocol approved by the Laval University Animal Care Committee. All efforts were made to minimize animal suffering and to reduce the number of mice used.

2.2. Animal treatments

Mice were divided into 6 groups comprised of 8–9 mice per group and respectively treated with vehicle (0.9% saline containing 0.3% gelatin, twice per day, s.c.), raloxifene (62.5 μg, twice per day, s.c.; Tocris, Ellisville, MO, USA) or a combination of raloxifene and the GPER1 antagonist G15 (10 or 50 μg, twice per day, s.c.; Tocris) for 10 days. On day 5, mice received 4 injections of MPTP (4.75 mg/kg i.p.; Sigma-Aldrich, St. Louis, MO, USA) at 2-hour intervals, whereas the control groups received only saline. On day 11, mice were killed and the gut was collected. The drug doses chosen were based on those used in our previous studies in MPTP mice (Bourque et al., 2014).

2.3. Tissue processing

Guts were collected and prepared as described (Cote et al., 2011; 2015a, 2015b). Briefly, mice were anesthetized with isoflurane and decapitated. Guts were removed and placed overnight in phosphate buffer saline containing 4% paraformaldehyde, pH 7.4. For each animal, the ileum was excised and microdissected to isolate the myenteric plexus.

2.4. Immunohistochemistry

DAergic neurons and macrophages were identified following an immunohistochemistry as reported (Cote et al., 2011; 2015a, 2015b). Briefly, myenteric neurons were stained with the Cuprolinic blue coloration (Holst and Powley, 1995), DAergic neurons and macrophages were identified with a polyclonal tyrosine hydroxylase antibody (TH; 1:1000; Cedarlane, ON, Canada) and the pan-macrophage/microglia marker–ionized calcium binding adapter molecule 1 (Iba-1; 1:1000; Cedarlane), respectively. Biotinylated goat antirabbit IgG (Cedarlane) was used as a secondary antibody, and the signal was revealed with the ABC Vectastain Elite kit (Vector Laboratories Inc, Ontario, Canada) and 3,3′-diaminobenzidine (DAB, Vector Laboratories Inc) as chromagen. All pictures were acquired with a Nikon C80i microscope equipped with a MicroFire digital camera (MBF Bioscience, Williston, VT, USA). Figures were assembled using Adobe Illustrator CS3.

2.5. Immunofluorescence

Proinflammatory macrophage density was measured using an immunofluorescence-based approach. Myenteric plexi were incubated overnight with a monoclonal rat antibody for the major histocompatibility complex (MHC) class II molecule (MHCII; 1:500; BD Biosciences Pharmingen). The next day, myenteric plexi were stained with a donkey antirat secondary antibody conjugated to Alexa Fluor 488 (1:1000; Invitrogen Corporation, CA, USA). Nuclei were counterstained with 0.022% DAPI (Invitrogen). Images were taken using a confocal laser scanning microscope (Olympus IX81-FV1000; Ontario, Canada) and were acquired by sequential scanning using a 2-frame Kalman filter and optimal *z*-separation.