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Estrogen receptors alpha mediates postischemic inflammation in chronically estrogen-deprived mice

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ABSTRACT

Estrogens are known to exert neuroprotective and immuneomodulatory effects after stroke. However, at present, little is known about the role of estrogens and its receptors in postischemic inflammation after menopause. Here, we provide important in vivo evidence of a distinct shift in microglial phenotypes in the model of postmenopause brain. Using a model-system for live imaging of microglial activation in the context of chronic estrogen- and ERα-deficiency associated with aging, we observed a marked deregulation of the TLR2 signals and/or microglial activation in ovariectomized and/or ERα knockout mice. Further analysis revealed a 5.7-fold increase in IL-6, a 4.7-fold increase in phospho-Stat3 levels suggesting an overactivation of JAK/STAT3 pathway and significantly larger infarction in ERα knockouts chronically deprived of estrogen. Taken together, our results suggest that in the experimental model of menopause and/or aging, ERα mediates innate immune responses and/or microglial activation, and ischemia-induced production of IL-6. Based on our results, we propose that the loss of functional ERα may lead to deregulation of postischemic inflammatory responses and increased vulnerability to ischemic injury in aging female brains.

1. Introduction

To date, studies have clearly demonstrated difference on stroke outcome between genders (Alkayed et al., 1998; Cordeau et al., 2008; Gibson, 2013; Suzuki et al., 2007). In fact, women are less vulnerable to stroke when their level of estrogen is elevated. Interestingly however, this protective gender effect is completely reverted after menopause as the incidence of stroke increases substantially after menopause with women having worst outcome for stroke than men. In addition, recent evidence suggests that menopause and aging have been associated with proportionally higher activation of immune-related genes in female brain (Berchtold et al., 2008).

Although incidence of stroke increases with aging, at present, little is known about the role of estrogens and its receptors in the brain response to ischemic injury in aged female brain. The effects of estrogen are mediated through estrogen receptors (ERs), ERα and ERβ, both widely distributed through various tissues and organs including brain. As previously reported, ERs are expressed in many cell types including microglial cells, and it has been clearly demonstrated that in different experimental models (in in vitro and in vivo conditions) inflammatory response and activation of microglial cells can be modulated via ERα and ERβ (Baker, 2004; Ghisletti et al., 2005; Vegeto et al., 2003; Wu et al., 2013). At present, the immuneomodulatory role of ERs after menopause remains unclear. Evidence suggests that ERα expression and/or function is decreased with aging. Namely, Ishunina and Swaab (2008b, 2009) reported that elderly women show increased expression of the ERα splice variants that act as dominant negative regulators of the ERα—mediated transcriptional activation and function, thus suggesting a loss of receptor function with advanced age. Therefore, we hypothesized that the described progressive decline and/or loss of function of ERα in concert with increased expression of proinflammatory genes detected in aged female brains may represent an additional risk factor for increased vulnerability following stroke. In the present study, we investigated how ischemia-induced microglial activation and innate immune responses are regulated in the mouse model of menopause and female aging (ovariectomized [OVX] mice derived in ERα-deficient background).

Cerebral ischemia is associated with strong inflammatory and glial responses that may contribute to neuronal damage and/or provide a trophic support (Iadecola and Anrather, 2011; Kriz and Lalancette-Hebert, 2009). Using live imaging approach, we

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previously showed the effects of estrogens and gender on neuroinflammation after stroke (Cordeau et al., 2008). In the present study, we took advantage of transgenic mouse model, a Toll-like receptor 2 (TLR2)–luc-green fluorescent protein (GFP) reporter model developed in our laboratory (Lalancette-Hebert et al., 2009). In this model, microglial activation and innate immune response can be visualized in real time from the brains of live animals using in vivo bioluminescence and/or biophotonic imaging and high resolution charge-coupled devices camera. Using this model system, we investigated the effects of estrogen deficiency alone or in the context of ERα or ERβ deficiency on microglial activation after cerebral ischemia. We report here that chronic estrogen deprivation markedly increases microglial activation profile and the innate immune responses (TLR2 signals) before and after stroke. To our surprise, the absence of ERα in chronically OVX females blunted innate immune responses, whereas the absence of ERβ did not exert significant effects on stroke-induced TLR2 signal induction. Importantly, the observed attenuation of the TLR2 responses in chronically estrogen- and ERα-deprived females was associated with markedly altered profiles of activated microglial cells, selective overexpression of IL-6, and over-activation of JAK/STAT3 pathway leading to significantly larger ischemic lesions.

2. Material and methods

2.1. Generation of transgenic mice and genotyping

The transgenic TLR2-luc-GFP mice derived in C57BL/6 background were used as described (Lalancette-Hebert et al., 2009). Transgenic animals were identified by polymerase chain reaction detection of luciferase. The genotyping was performed as previously described (Lalancette-Hebert et al., 2009). To derive double transgenic colonies, as a first step, the TLR2-luc-GFP mice were then crossed with an estrogen receptor alpha heterozygous mouse, and in the second step of breeding, we obtained the TLR2-luc-GFP mice in ERαKO background (Jackson Laboratory, strain name: B6.129P2-Esr1^{tm1Ksk}/J).

2.2. Surgical procedures

2.2.1. Ovariectomy

As previously described (Cordeau et al., 2008), 2-month-old transgenic female mice were anesthetized with 2% isoflurane in 100% oxygen at a flow rate of 2 L/min. Under anesthesia, both flanks of the animal were shaved before incision. A 5-mm, dorsal and/or ventral incision was made through the skin, the ovarian fat pad was located, and the ovaries were removed bilaterally.

2.2.2. Middle cerebral artery occlusion

As previously described, the unilateral transient focal cerebral ischemia was induced by intraluminal filament occlusion of the left middle cerebral artery (MCAO) during 1 hour followed by a reperfusion period of 48 hours or 7 days (Cordeau et al., 2008; Lalancette-Hebert et al., 2007, 2011, 2012). The surgery was carried out on adult, 4- to–5-month-old females (25–30 g), TLR2-luc-GFP; Erα^{KO} and their wild-type (C57BL/6) littermates. The animals were anesthetized with 2% isoflurane in 100% oxygen at a flow rate of 2 L/min. To avoid cooling, the body temperature was regularly checked and maintained at 37 °C with a heating pad. The correct placement of the filament was confirmed by the Laser Doppler measurements (PF5001, Perimed, Sweden). As previously described, to additionally confirm successful MCAO, at 6 and 24 hours after surgery, the animals were examined for early neurological deficits (Lalancette-Hebert et al., 2007). All experimental procedures were approved by the Laval University animal care ethics committee and are in accordance with The Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

2.3. Vaginal smears and hormone detection

To avoid the effects of physiological fluctuation of estrogen levels on inflammatory response, we regularly controlled the vaginal smears (Cordeau et al., 2008). The vaginal epithelial cells were obtained from mice and smeared onto cleaned slides. Slides were then evaluated for the presence of white blood cells and the morphology of epithelial cells to determine the stage of the estrus cycle. For the female control group, they were cycled to perform the MCAO at the end of the diestrus period.

2.4. In vivo bioluminescence imaging

The images were gathered using IVIS 200 Imaging System (Xenogen, Alameda, CA, USA). Before imaging session, the mice received intraperitoneal injection of D-luciferine, a luciferase substrate (150 mg/kg, Xenogen, Alameda, CA, USA) dissolved in 0.9% saline. The mice were then anesthetized with 2% isoflurane in 100% oxygen at a flow rate of 2 L/min and placed in the heated, light-tight imaging chamber. Images were collected using high-sensitivity charge-coupled devices camera with wavelengths ranging from 300–600 nm. Exposition time for imaging was 2 minutes using the smallest field of views and f/1 lens aperture. As previously described, bioluminescence emission was normalized and displayed in physical units of surface radiance, photons per second per centimeter squared per steradian (photons/sec/cm2/sr; Cordeau and Kriz, 2012; Cordeau et al., 2008; Lalancette-Hebert et al., 2009). The data were presented as pseudocolor images indicating light intensity (red and yellow, most intense), which were superimposed over grayscale reference photographs.

2.5. Tissue collection

The animals were anesthetized via an intraperitoneal injection of 0.1 mL/10 g of ketamine (10 mg/mL)/xylazine(1 mg/mL) and transcardially perfused with 30 mL of 0.9% NaCl, followed by phosphate-buffered saline (PBS) 1X buffered 4% paraformaldehyde at pH 7.4. Tissue sample were then postfixed overnight in 4% paraformaldehyde and equilibrated in phosphate-buffered 30% sucrose for 48 hours. Brains were embedded into Tissue-Tek (O.C.T. compound, Sakura, USA) and freeze at −20 °C and cut into coronal section with a cryostat (25 μm thick) and stored at −20 °C.

2.6. Size of infarct

For measurements of the lesion size, wild type littermate and transgenic mice were anesthetized 48 hours after the tMCAO. The brains were then quickly removed from the skull, chilled at −80 °C for few minutes, placed in a mouse brain mold (Stoelting) and cut in 1-mm thick slices. The slices were then immersed in 2% solution of 2,3,5-phenyltetrazolium chloride (Sigma, Oakville, ON, USA) for 20 minutes at 37 °C in the dark (Lalancette-Hebert et al., 2007, 2012). The relative size of infarction was quantified by using the Scion Image processing and analysis program (Scion Corporation), calculated in arbitrary units (pixels), and expressed as a percentage of the control, nonstroked area of the nonischemic hemisphere. The total size of infarction was obtained by numeric integration of area of marked pallor measured in 6 consecutive 1-mm coronal sections affected by MCAO, with appropriate correction for brain edema (Lalancette-Hebert et al., 2007). Five to 8 mice were used for each experimental group.