● **PERSPECTIVE**

Outlook on the neuroprotective effect of estrogen

Epidemiologic studies often consider gender differences in a particular pathology, and constantly observe variations between men and women. Indeed, a remarkable sexual dimorphism exists in the epidemiology of neurological conditions and brain diseases. Physiologically, males and females differ by their levels of circulating hormones that drive sexual behavioral, as well as endocrine functions. Estrogen is the primary female sex hormonal group that enwraps estradiol, estrone and estriol, and which are the major naturally occurring hormones prevalent in women. Their role in the reproductive function has long been established, although the ubiquitous expression of its receptors (alpha, beta and G protein-coupled, GPR30) presumes a broader spectrum of action. This short review will summarize the current knowledge in estrogen therapy with particular focus on some of the recent work that might lead to new neuroprotective treatments.

Neuroprotective properties of estrogen in brain injury and neurodegenerative diseases: At first glance, estrogen has positive impact on general cognitive function, and the experimental and clinical cases that link estrogen concentration to brain protection are plentiful.

Steroid hormones have by some way been presented as potentially beneficial in rescuing the injured brain as in cerebrovascular accidents. Prevalence, and age of stroke, indicate that male patients present a higher risk, although strokes are more severe in women (Appelros et al., 2009). Animal studies also show that 17-β-estradiol (E2) mostly protects the brain against ischemic stroke. Anti-inflammatory and immunomodulatory mechanisms are also involved in the estrogen-mediated neuroprotection. Notably, E2 inhibits the production of neutrophil chemoattractants in the ischemic region, thus limiting excessive and inappropriate inflammation. Experimental treatment by E2 and other selective estrogen receptor modulators (SERMs), such as Raloxifen, enhances neurogenesis in the lateral ventricle neurogenic niche in the post-ischemic area, and reverses the ischemia-induced spine density loss after medial cerebral artery occlusion in rats (Khan et al., 2015).

Estrogenic compounds may provide relative neuroprotection after mechanical injury, thus limiting the cerebral damage. A striking example is the effect of acute administration of estrone 30 minutes after experimental traumatic brain injury (TBI) in rats, which dramatically decreased cell death that normally occurs after TBI (Gatson et al., 2012). Post-trauma treatment with estrogen also proved beneficial on spinal cord injury (SCI). Indeed, low physiological doses of E2 had multi-active effects (protection of cells, preservation of the axon-myelin tract and increase in angiogenesis) that contributed to improving locomotor function in chronic SCI rats (Samantaray et al., 2016).

A growing body of evidence from basic science and clinical studies supports the therapeutic potential of estrogen in neurodegenerative diseases. Clinical observations generally relate memory loss, cognitive decline and certain forms of dementia with the circulating hormones' depletion. In the most common form of dementia, i.e., Alzheimer's disease (AD), the prevalence is higher in women. This is mainly due to female longevity, but men suffer a more aggressive form of AD as men progress faster to death. Animal models mislead the actual role of estrogen as a shield to AD in humans. While E2 exhibits protective properties on AD-related toxicity, including in the context of decreasing cell death, reducing Aβ accumulation and tau hyper phosphorylation, clinical trials involving estrogen replacement showed no benefit (Snyder et al., 2016).

Parkinson's disease (PD) is the second most frequent neurodegenerative disease after AD. It is a progressive movement disorder characterized by the selective depletion of dopamine (DA) neurons in the midbrain. The risk of PD increases with lower circulating sex steroids; such as men have a greater incidence and prevalence of PD. There is extensive evidence that estrogen prevents the loss of DA neuron induced by neurotoxins in PD animal models. However, the picture of estrogenic protection on DA neuron is complex. Growing evidence suggests that estrogen enhances the functionality of the surviving DA neurons rather than simply preventing the cell death (Gillies and McArthur, 2010).

Beneficial effects of estrogen are also met in multiple sclerosis (MS), a demyelinating disease. A recent phase 2 clinical trial showed that anti-inflammatory and neuroprotective properties of estriol (one of the three main endogenous estrogen that is principally produced during pregnancy) decrease MS relapse in patients (Voskuhl et al., 2016).

Mechanisms involved in estrogen neuroprotection: The understanding of the mechanism by which estrogen and SERMs protects the brain is complex because of the multiplicity of responses elicited in the brain.

Activation of the classical nuclear receptors α and β mainly leads to gene transcription. Along with specific co-regulators recruitment, this triggers activation of target promoters that can differ from one cell to another. On the other hand, the cell surface G protein-coupled receptor (GPER) stimulates intracellular signaling pathways such as Ras-Raf, MEK, ERK1/2, p38-MAP kinase, PI3K-Akt/PKB, and PKA, resulting in a variety of biological responses that is dependent on the cell type. Therefore, estrogen properties in the brain range from anti-inflammatory, to prosurvival, and antioxidant effect. As such, estrogen prevents neutrophils recruitment in the damaged region, limits microglia and astrocyte activation and secretion of pro-inflammatory cytokines such as interferon and tumor necrosis factor α (Villa et al., 2016). Beyond this, estrogen may promote apoptotic cells clearance by microglia to help end the inflammation process.

Additionally, stimulation of estrogen receptors prevents the activation of apoptotic signaling cascades and promotes pro-survival signaling. The indirect participation of estrogen in mitochondrial function is also supported, which may reduce ATP decline in cellular stress conditions. Furthermore, in ovariectomized animals neurotrophin synthesis may be involved in the estrogen-elicited survival clues. Estrogen elicits neurotrophin production such as nerve growth factor, insulin-like growth factor and brain-derived neurotrophic factor (BDNF), as well as expression of their specific receptors. The post-TBI estrone-mediated neuroprotection, observed in rats, prevents apoptosis via activation of the ERK1/2-CREB intracellular pathway, and the expression of BDNF. Thus, experimental data indicates that the protective action of E2 partly requires neurotrophin system activation.

The own chemical nature of estrogen also contributes to its neuroprotective properties. Biochemical evidence showed that estrogen provides a "chemical shield" to neurons from the increased level of reactive oxygen species occurring in damaged cells in an experimental stroke reperfusion model (Prokai et al., 2003). Therefore, the A-ring phenolic hydroxyl group confers an intrinsic free-radical scavenging capacity that is believed to protect neurons from oxidative stress. This notably prevents lipid peroxidation and protects cell membrane integrity. Chemically analogous drugs that have antioxidant properties, such as quinol-based compounds, may be more acceptable and alternatively used as estrogenic molecules for neuroprotective therapy.

Recent data from neuroblastoma cell lines showed that E2-activated ERα induce epigenetic modifications in the genomic region, in concert with promoter activation of the *NGB* gene coding for neuroglobin, a protein involved in cellular oxygen homeostasis in the central and peripheral nervous system (Guglielmotto et al., 2016). This represents an exciting new modulatory function of neuroprotection by estrogen.

Pertinence of estrogen as a neuroprotective therapy? To assess the applicability of estrogen as a neuroprotective agent in the clinic, it is essential to consider several aspects.

1. The assumed translational validity of animal models results, later, to failure in clinical trial because models do basically not accurately replicate the human disease. For instance, mouse models based on ovariectomy hardly recapitulate the clinical manifestations of menopause in women, and caution is needed when using preclinical data to predict therapeutic outcome.

2. The rationale for using estrogen as a treatment in brain injury

and neurodegenerative diseases is based on remarkable correlations observed between hormonal levels and case severity in clinical studies. Hormone replacement therapy (HRT) certainly showed positive outcomes in a number of brain pathologies. Although HRT provides neuroprotection, it remains challenging to use as a therapy since the expression of ERs is very broad in the body. This makes an estrogen-based treatment certainly unspecific. To the contrary, the discovery of neuroprotection by estrogen must be employed to innovate and open new research lines.

3. In population studies, for instance women *versus* men, the influence of other sex steroids, *e.g.*, progesterone, hinders the discrimination of the sole action of estrogen. In addition, complex behavior differences and general state of health also misled researchers. Indeed, in a multifactorial disease such as AD, the protecting or worsening role of sex steroids remains undefined. Interestingly, gender differences observed in neuroprotection may not solely lie in the circulating sex steroid level. In fact, the genomic information held by the sex chromosomes is not trivial as in *e.g.* neuronal sensitivity to toxic insults. In other words, inherent cell response to injury can be sex-specific XX or XY. For instance, elegant *in vitro* experiments showed that neurons cultured from male or female embryonic brain respond differently to certain cytotoxic challenge (Du et al., 2004). XY neurons are more susceptible to peroxynitrite and glutamate excitotoxicity, while XX neurons are more vulnerable to staurosporine and etoposide. Thus, gender proclivity does not necessarily imply sex steroids.

New treatments on call: An important issue of estrogen usage relies on the lack of specificity as a consequence of the widespread expression and limited number of different ERs. Clearly identified ER subtypes are the long-known nuclear receptors α and β and the membrane bound GPR30 (also named GPER). A better knowledge of the ER subtypes (α , β, or GPR30) involved in the neuroprotective mechanism allows selection of the appropriate agonist, therefore favoring genomic or non-genomic action as well as cell types. Indeed, recent studies highlighted the use of the specific GPR30 agonist G-1 as a potent neuroprotective nonsteroidal agent. Pretreatment of mice with G-1 presented striking effects on tissue recovery after spinal cord injury. A shift into the BAX-2/Bcl-2 balance prevented apoptosis, while neurotrophic factor such as BDNF expression increased, resulting in improved tissue repair and motor function recovery. GPR30 stimulation also confers central neuroprotection to rat hippocampal neurons following TBI (Wang et al., 2017). The promising debut of the GPER agonist G-1 calls for further evaluation in order to confirm its therapeutic significance. Likewise, since ERα possesses ten different potential phosphorylation sites that, as suggested by Villa et al. (2016), cell-specific ER phosphorylation may be required for "unliganded" receptor activation. Therefore the possibility of specific ERα activation by non-estrogenic molecules should be explored. Other sex steroids like progesterone are also suggested as substantial neuroprotective agents in a variety of CNS models. Estriol is considered as well a safer form of estrogen and might be of a potential alternative to estradiol use. Alternatively, non-feminizing estrogen could be employed, such as 17-α-estradiol that has lower affinity to ERs but nevertheless showed some interesting neuroprotective properties in several experiments, sometimes superior to its β stereoisomer. Other chemical compounds carrying estrogenic characteristics such as the phenolic structure, are potential neuroprotective agents and efforts must be made to develop and test such molecules. At last, the involvement of endogenous estrogen within the neural parenchyma should not be disregarded, and approaches that might potentially enhance its production are worthwhile.

Conclusion: The neuroprotective effect of estrogen is as tangible as it is naïve. On one hand, converging evidence from clinical observations and experimental studies support the use of estrogenic compounds to prevent or moderate the brain tissue damage. On the other hand, the complexity of estrogenic actions throughout the body makes estrogen therapy a rather unspecific and challenging treatment for the injured brain. Consequently, finding the specific intracellular mechanisms promoted by estrogen stimulation may help to optimally tune the cell

homeostasis and raise protective shields to prevent the CNS and PNS damage occurring after nerve injury, or in a disease. Thus, a neuroprotective strategy based on estrogen treatment is conceivable, although much effort has to be made before this can be achieved.

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