REVIEW

Selective estrogen receptor modulators as brain therapeutic agents

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Abstract

Selective estrogen receptor modulators (SERMs), used for the treatment of breast cancer, osteoporosis, and menopausal symptoms, affect the nervous system. Some SERMs trigger neuroprotective mechanisms and reduce neural damage in different experimental models of neural trauma, brain inflammation, neurodegenerative diseases, cognitive impairment, and affective disorders. New SERMs with specific actions on neurons and glial cells may represent promising therapeutic tools for the brain.

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Introduction

The neuroprotective potency of estradiol (E₂) in different animal models of neurodegeneration, cognitive decline, and affective disorders has been extensively characterized in the last decades (García-Segura & Balthazart 2009). However, the application of E₂ as a neuroprotectant in humans presents numerous limitations, mainly due to the endocrine actions of the molecule on peripheral tissues, including estrogen-dependent tumors. The possibility of using selective estrogen receptor modulators (SERMs) to exert E₂-like neuroprotective actions in the brain has emerged as an alternative to E₂ (DonCarlos *et al.* 2009). According to chemical family, SERMs are classified as triphenylethylene, benzothiophene, or benzopyran compounds. Triphenylethylene SERMs, such as tamoxifen and its derivatives, are also known as first-generation SERMs. Benzothiophene SERMs include second-generation SERMs, such as raloxifene, and third-generation SERMs. Fourth-generation SERMs are benzopyran compounds (Dowers *et al.* 2006). SERMs bind to estrogen receptors (ERs) and induce specific changes in their three-dimensional conformation (Brzozowski *et al.* 1997, Paige *et al.* 1999) allowing a tissue-selective recruitment of transcriptional cofactors (Norris *et al.* 1999, Klinge 2000, McKenna & O'Malley 2002, Belandia & Parker 2003). Therefore, SERMs may act as ER

agonists in the brain and as antagonists in others tissues, such as breast tumors. Here, we will briefly review new advances on the research associated with the potential use of SERMs as neuroprotective agents.

Neuroprotective actions of SERMs

The neuroprotective actions of tamoxifen and raloxifene, two SERMs that are currently used in clinical practice for the treatment of breast cancer and osteoporosis, have been assessed in different experimental models of neural dysfunction. These include animal models of traumatic injury of the central nervous system and peripheral nerves, stroke, multiple sclerosis, Parkinson's disease, Alzheimer's disease, cognitive decline, and mood disorders (Fig. 1). This section is a succinct description of the main findings of these experimental studies, including the limited available information from human studies.

Brain, spinal cord, and peripheral nerve injury

Tamoxifen is a protective factor for spinal cord injury. Treatment with tamoxifen, 30 min after spinal cord injury, results in a decrease in blood spinal cord barrier permeability, reduced edema, reduced microglial activation, decreased myelin and neuronal loss, and

Figure 1 Summary of the neuroprotective effects of SERMs reported in animal models of neurodegenerative, cognitive, and affective disorders.

better functional locomotor recovery (Tian *et al.* 2009). In addition, tamoxifen and raloxifene reduce reactive gliosis after a traumatic brain injury (Barreto *et al.* 2009), and raloxifene has been shown to improve functional recovery after bilateral cortical contusion injury (Kokiko *et al.* 2006). Tamoxifen may also be useful to reduce irradiation-induced brain damage after whole brain irradiation therapy (Liu *et al.* 2010). SERMs may also have potential application for the regeneration of peripheral nerves, since the raloxifene analog LY117018 increases the number of regenerating nerve fibers after sciatic nerve crush injury in mice (McMurray *et al.* 2003).

Stroke

Tamoxifen reduces tissue infarction and behavioral deficits in animal experimental models of stroke (Kimelberg *et al.* 2003, Mehta *et al.* 2003, Feng *et al.* 2004, Kimelberg 2008) and attenuates neuronal excitability impairment caused by ischemic conditions in rat hippocampal slices incubated in oxygen-depleted and glucose-deprived medium (Zhang *et al.* 2009). The raloxifene analog LY353381.HCl is also neuroprotective in experimental stroke (Rossberg *et al.* 2000). However, it has been reported that raloxifene may increase the risk of stroke in a subpopulation of women at high stroke risk (Barrett-Connor *et al.* 2009, Mosca *et al.* 2009).

Multiple sclerosis

SERMs may reduce inflammation in the central nervous system (see below) and may therefore represent interesting therapeutic candidates for multiple

sclerosis. Indeed, tamoxifen reduces in mice the degree of demyelination caused by experimental autoimmune encephalomyelitis (Bebo *et al.* 2009).

Parkinson's disease

Tamoxifen prevents the loss of dopaminergic function in the nigrostriatal system caused by methamphetamine in mice (Mickley & Dluzen 2004, Bourque *et al.* 2007). However, the neuroprotective properties of estrogen against Parkinson's disease occurrence may be disrupted by tamoxifen therapy in breast cancer patients (Latourelle *et al.* 2010). Raloxifene has agonistic estrogenic activity on dopamine receptors (Landry *et al.* 2002) and protects dopaminergic neurons in experimental animal models of Parkinson's disease (Grandbois *et al.* 2000, Callier *et al.* 2001, Morissette *et al.* 2008).

Alzheimer's disease

Tamoxifen protects neurons against β -amyloid toxicity (O'Neill *et al.* 2004). Some human studies also suggest that tamoxifen may decrease the risk of Alzheimer's disease (Breuer & Anderson 2000). Raloxifene has been shown to protect PC12 neural cells against β -amyloid-induced neurotoxicity (Du *et al.* 2004).

Cognitive decline

In spite of the neuroprotective actions of tamoxifen in different forms of neural injury, it is unclear whether this molecule may have some benefits for cognition in humans. Indeed, several studies suggest an increased risk of cognitive impairment in women receiving tamoxifen for the treatment of breast cancer, including worse performance in visual memory, word fluency, immediate verbal memory, visuospatial ability, and processing speed tasks (Paganini-Hill & Clark 2000, Shilling *et al.* 2003, Palmer *et al.* 2008, Phillips *et al.* 2010, Schilder *et al.* 2010). However, other studies have not detected a significant effect of tamoxifen on cognition (Debess *et al.* 2010).

In contrast to the potential negative effects of tamoxifen on cognition, the results of the multiple outcomes of raloxifene evaluation randomized trial suggest that raloxifene prevents cognitive decline in postmenopausal women (Yaffe *et al.* 2005). The results of a recent randomized, double-blind, placebo-controlled trial also suggest that raloxifene improves verbal memory in late postmenopausal women (Jacobsen *et al.* 2010). In addition, raloxifene treatment enhances brain activation during performance on a face-encoding paradigm and during recognition of familiar items in healthy elderly men (Goekoop *et al.* 2005, 2006),

suggesting that SERMs may also be used to promote cognition in men. In agreement with this possibility, we have recently observed that both tamoxifen and raloxifene improve hippocampus-dependent memory in androgen-deprived male rats (N Lagunas, I Calmarza-Font, D Grassi & LM Garcia-Segura, unpublished observations).

Affective disorders

Some studies have suggested a potential therapeutic effect of tamoxifen for the treatment of affective disorders. Tamoxifen reduces amphetamine-induced manic-like behavioral alterations in rats (Einat *et al.* 2007) and reduces acute manic episodes in women with bipolar affective disorder (Kulkarni *et al.* 2006, Zarate *et al.* 2007).

Raloxifene reduces anxiety behavior, assessed in the elevated plus maze test, on ovariectomized rats (Walf & Frye 2010) and decreases anxiety (Strickler *et al.* 2000, Florio *et al.* 2001) and depression (Carranza-Lira *et al.* 2004, Grigoriadis *et al.* 2005, Sugiyama *et al.* 2007) in postmenopausal women. Recent clinical studies also suggest the potential application of raloxifene hydrochloride (120 mg/day oral) for the treatment of postmenopausal women with schizophrenia (Kulkarni *et al.* 2010).

SERMs and neuroprotection: a summary of the findings

We can conclude that the studies conducted so far to evaluate the neuroprotective activity of tamoxifen and raloxifene indicate that these SERMs decrease neuronal damage caused by different forms of neural injury in animal models of neurodegenerative diseases. However, animal models of neurodegeneration do not fully reflect the situation in human pathology. Therefore, whether these findings in animals could be translated to human health is still an open question. Nevertheless, the limited information from human studies suggests that SERMs may have a positive impact on mood and cognition, at least under certain circumstances. These findings justify further research on the possible application of SERMs for the treatment of brain disorders.

Molecular mechanisms involved in the neuroprotective actions of SERMs

SERMs signal on neural cells by multiple mechanisms that may contribute to neuroprotection (Dhandapani & Brann 2002). Some neuroprotective actions of SERMs may be due to mechanisms independent of their activity as ER modulators. For instance,

the efficacy of tamoxifen in the treatment of acute mania has been ascribed to its action as a protein kinase C inhibitor (Einat *et al.* 2007, Zarate *et al.* 2007). Other neuroprotective mechanisms of SERMs are mediated by classical ERs, since they are inhibited by ER antagonists, such as ICI 182 780 (Zhang *et al.* 2009). The neuroprotective signaling of SERMs may involve the activation of kinases, such as mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and Akt (Du *et al.* 2004, Lee *et al.* 2009a,b), and the phosphorylation of CREB (Sharma *et al.* 2007) or the inhibition of nuclear factor (NF)- κ B-induced transcription (Cerciat *et al.* 2010). Through these mechanisms, SERMs control synaptic transmission and the expression of molecules involved in the regulation of cell death, oxidative stress, and inflammation.

We will consider here five important interrelated actions of SERMs in the nervous system that may be highly relevant for their neuroprotective activity: i) the modulation of synaptic transmission; ii) the control of oxidative stress; iii) the control of excitotoxic damage; iv) the control of the apoptotic program; and v) the control of inflammation.

Modulation of synaptic transmission

Some SERMs promote axonal growth (Nilsen *et al.* 1998, O'Neill *et al.* 2004) and the expression of synaptic markers (Sharma *et al.* 2007), suggesting possible actions on synaptic plasticity and synaptic regeneration. These actions, which are still not sufficiently investigated, may contribute to the maintenance of functional neuronal circuits and to the repair of damaged connectivity. Actions of SERMs on mood and cognition may also be related with their pre- and post-synaptic modulation of cholinergic, serotonergic, and dopaminergic neurotransmission (Wu *et al.* 1999, Cyr *et al.* 2000, Smith *et al.* 2004, Sánchez *et al.* 2010). Raloxifene may also regulate opiate and GABAergic neurotransmission by the modulation of the levels of β -endorphin and neuroactive steroids respectively. Chronic raloxifene administration to postmenopausal women increases plasma levels of β -endorphin and tetrahydroprogesterone (allopregnanolone), an anxiolytic metabolite of progesterone that modulates GABA_A receptors (Florio *et al.* 2001, Neele *et al.* 2002, Bernardi *et al.* 2003, Genazzani *et al.* 2003). Changes in the levels of β -endorphin and tetrahydroprogesterone in plasma probably parallel similar changes in the nervous system, since in ovariectomized rats, the raloxifene analog LY17018 has been shown to increase β -endorphin and tetrahydroprogesterone levels not only in serum but also in the brain (Genazzani *et al.* 1999, 2000, Bernardi *et al.* 2003). These findings suggest that raloxifene may regulate synaptic function by the modulation of local levels of neuroactive substances within the brain.

Control of oxidative stress

The well-documented antioxidant effects of tamoxifen, hydroxytamoxifen, and raloxifene in the nervous tissue (Moreira *et al.* 2004, 2005, Biewenga *et al.* 2005, Konyalioglu *et al.* 2007, Armagan *et al.* 2009) may be relevant for its neuroprotective actions on Parkinson's disease (Lee *et al.* 2009a,b), cerebral ischemia (Zhang *et al.* 2007, Wakade *et al.* 2008), and other neurodegenerative conditions.

Control of excitotoxic damage

An important neuroprotective action of SERMs is the prevention of excitotoxicity, since this is a common cause of neuronal death in different neurodegenerative disorders. Several SERMs have shown to reduce neuronal loss in the hippocampus after the administration of the excitotoxin kainic acid (Ciriza *et al.* 2004). Figure 2 shows the effect of tamoxifen, raloxifene, lasofoxifene, and bazedoxifene on hilar neurons of ovariectomized rats injected with kainic acid. Tamoxifen, raloxifene, and bazedoxifene prevent the excitotoxic effect of kainic acid in this model.

Control of the apoptotic program

The neuroprotective actions of SERMs on excitotoxicity and against other neural insults may be mediated by the regulation of molecules involved in the control of apoptosis. For instance, raloxifene increases the expression of Bcl-2 in the cerebral cortex of ovariectomized rats treated with kainic acid (Armagan *et al.* 2009), and chronic administration of tamoxifen to ovariectomized rats increases the expression of Bcl-2 and decreases the expression of Bax in the hippocampus (Sharma & Mehra 2008). Furthermore, tamoxifen and raloxifene up-regulate the expression of the antiapoptotic gene seladin-1 (selective Alzheimer's disease indicator-1) in human neuroblasts (Benvenuti *et al.* 2005). SERMs may also prevent neuronal apoptosis by the activation of telomerase activity via Akt and the phosphorylation of telomerase catalytic subunit (TERT; Du *et al.* 2004).

Control of inflammation

SERMs may also exert neuroprotective actions by the control of local brain inflammation, which is mainly regulated by microglia and astroglia. Tamoxifen and raloxifene are able to decrease the inflammatory response caused by lipopolysaccharide (LPS) in mouse and rat microglia cells *in vitro* (Suuronen *et al.* 2005). In addition, these SERMs, at doses within the range used in clinical practice, reduce microglia activation in the central nervous system of male and

Figure 2 SERMs prevent excitotoxic neuronal death *in vivo* after kainic acid administration. High magnification of the hilus of the dentate gyrus of ovariectomized rats stained with toluidine blue after the i.p. injection of vehicle (Control); kainic acid (KA; 7 mg/kg); tamoxifen (1 mg/kg) and kainic acid; raloxifene (1 mg/kg) and kainic acid; lasofoxifene (1 mg/kg) and kainic acid; bazedoxifene (2 mg/kg) and kainic acid. Kainic acid results in the loss of hilar neurons (large cells). Small cell nuclei correspond to glial cells. Some SERMs, such as tamoxifen, raloxifene, and bazedoxifene, prevent the excitotoxic effect of kainic acid. Adapted, with permission, from Ciriza I, Carrero P, Azcoitia I, Lundeen SG & Garcia-Segura LM 2004 Selective estrogen receptor modulators protect hippocampal neurons from kainic acid excitotoxicity: differences with the effect of estradiol. *Journal of Neurobiology* 61 209–221. © 2004 Wiley Periodicals, Inc.

female rats *in vivo* after the peripheral administration of LPS (Tapia-Gonzalez *et al.* 2008). Figure 3 shows the effect of tamoxifen and raloxifene on microglia activation induced by the systemic administration of LPS. Tamoxifen also reduces microglial inflammatory response induced by irradiation (Liu *et al.* 2010). In addition, raloxifene decreases the number of astrocytes and microglia in the brain of aged animals (Lei *et al.* 2003). Furthermore, tamoxifen and raloxifene are able to significantly reduce the number of reactive astrocytes in the hippocampus of young, middle-aged, and older female rats after a stab wound injury (Barreto *et al.* 2009). Some SERMs have also shown to be able to reduce the inflammatory responses of astrocytes treated with LPS by a mechanism involving ERs and the inhibition of NF- κ B-induced transcription of proinflammatory molecules (Cerciat *et al.* 2010).

Figure 3 SERMs reduce microglia activation after the administration of LPS *in vivo*. Representative images of the central white matter of the cerebellum of male rats showing immunoreactivity for MHC-II, a marker of activated microglia, after the i.p. injection of (A) vehicles for SERMs (DMSO) and LPS (phosphate buffer); (B) LPS; (C) 1 mg/kg tamoxifen and LPS; (D) 1 mg/kg raloxifene and LPS. Insets show details of the morphology of MHC-II immunoreactive cells at high magnification. LPS increases the number of MHC-II immunoreactive microglial cells and induces a reactive phenotype in microglia, with thicker and shorter cell processes. SERMs revert the effect of LPS. Scale bar 100 μ m. In the insets, the scale bar represents 25 μ m. Adapted, with permission, from Tapia-Gonzalez S, Carrero P, Pernia O, Garcia-Segura LM & Diz-Chaves Y 2008 Selective oestrogen receptor (ER) modulators reduce microglia reactivity *in vivo* after peripheral inflammation: potential role of microglial ERs. *Journal of Endocrinology* **198** 219–230. © 2008 Society for Endocrinology.

A summary of the molecular mechanisms of neuroprotection by SERMs

Figure 4 summarizes the mechanisms involved in the neuroprotective actions of SERMs that have been discussed in the previous sections. SERMs act on neurons and glial cells and regulate the activity of kinases, such as MAPK, PI3K, and Akt, which in turn regulate the activity of factors that control transcription, such as CREB and NF- κ B. In addition, SERMs regulate transcriptional activity of classical nuclear ERs. Therefore, SERMs exert multiple actions on signaling pathways that are involved in the modulation of synaptic transmission, the regulation of apoptosis, the control of oxidative stress, and the control of inflammation. Furthermore, actions of SERMs in endocrine glands may also contribute to their central actions by the modulation of the levels of neuroactive molecules such as β -endorphin and tetrahydroprogesterone or by the control of peripheral inflammation. All these actions of SERMs, exerted on neurons and glial cells, probably contribute to their effects on neuronal survival, mood, and cognition. However, the multiple mechanisms activated by SERMs may also represent a potential limitation for their use as neuroprotectants.

Perspectives for the future

Most of the studies showing neuroprotective actions of SERMs have focused on tamoxifen and raloxifene. These molecules are already in use in human clinic, which may facilitate their application as brain therapeutic drugs. However, tamoxifen and raloxifene have secondary effects that may limit their use and may block in some cases neuroprotective actions of E_2 . New SERMs, such as arzoxifene, bazedoxifene, lasofoxifene, and ospemifene, among others, need to be explored in experimental models of neurodegeneration. The few available studies with these SERMs indicate that bazedoxifene protects rat hippocampal neurons from excitotoxic cell death *in vivo* (Ciriza *et al.* 2004) and that ospemifene and bazedoxifene reduce the inflammatory response of astrocytes exposed to LPS (Cerciat *et al.* 2010). More studies are also needed to define the neuroprotective potential of natural SERMs (Azcoitia *et al.* 2006, Schreihöfer & Redmond 2009).

SERMs with better permeability of the blood–brain barrier need to be developed, since the access of these molecules to the central nervous system (CNS) may

Figure 4 Summary of the molecular mechanisms involved in the neuroprotective effects of SERMs. SERMs act in the nervous system through classical ERs or by ER-independent mechanisms and activate a variety of signaling molecules, including MAPK, PI3K, Akt, CREB, and NF- κ B. These molecules, in turn, trigger different coordinated mechanisms to promote neuronal survival and regulate mood and cognition.

represent a limitation for their clinical application as neuroprotectants. Furthermore, new SERMs with preferential selectivity for activating estrogen mechanisms in brain and specifically designed to act as neuroprotectants are under development (Brinton 2004, Zhao *et al.* 2005). One of these molecules is 7 α -(4R,8R)-4,8,12-trimethyltridecyl]estra-1,3,5-trien-3,17 β -diol, a hybrid structure of E₂ and vitamin E. This molecule binds to both ER α and ER β and is neuroprotective in rat primary hippocampal neurons (Zhao *et al.* 2007). Other interesting molecules are estrogen non-feminizing analogs with phenol groups (Simpkins *et al.* 2005). Molecules such as 17 α -E₂, ent-E₂, 2-adamantylestrone, and ent-17-desoxyestradiol have been shown to be effective in the protection of neural tissue under different neurodegenerative conditions (Green *et al.* 2001, Liu *et al.* 2002, Yang *et al.* 2003, Jung *et al.* 2006, Wang *et al.* 2006).

In general, there is still poor knowledge of the precise molecular targets of SERMs in the nervous system. Although some key molecules have been identified, such as MAPK, PI3K/Akt, CREB, and NF- κ B, the molecular mechanisms involved in the neuroprotective actions of SERMs should be investigated with more detail in the different cellular populations of the nervous system. Ideally, SERMs with cellular specificity for neurons, astrocytes, oligodendrocytes, and microglia may promote cell-specific responses to decrease neuronal death, increase remyelination, enhance the production of neuroprotective growth factors by astrocytes, and reduce the chronic proinflammatory response of astrocytes and microglia.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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