● INVITED REVIEW

Effects of estrogen receptor modulators on cytoskeletal proteins in the central nervous system

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Abstract

Estrogen receptor modulators are compounds of interest because of their estrogenic agonistic/antagonistic effects and tissue specificity. These compounds have many clinical applications, particularly for breast cancer treatment and osteoporosis in postmenopausal women, as well as for the treatment of climacteric symptoms. Similar to estrogens, neuroprotective effects of estrogen receptor modulators have been described in different models. However, the mechanisms of action of these compounds in the central nervous system have not been fully described. We conducted a systematic search to investigate the effects of estrogen receptor modulators in the central nervous system, focusing on the modulation of cytoskeletal proteins. We found that raloxifene, tamoxifen, and tibolone modulate some cytoskeletal proteins such as tau, microtuble-associated protein 1 (MAP1), MAP2, neurofilament 38 (NF38) by different mechanisms of action and at different levels: neuronal microfilaments, intermediate filaments, and microtubule-associated proteins. Finally, we emphasize the importance of the study of these compounds in the treatment of neurodegenerative diseases since they present the benefits of estrogens without their side effects.

Key Words: estrogen receptor modulators; selective estrogen receptor modulators; microtubules; neurofilaments; tibolone; tamoxifen; raloxifene

Introduction

In mammals, endogenous estrogens are involved in the regulation of many processes ranging from tissue growth maintenance to reproduction. Their action is mediated by estrogen receptors (ERs): estrogen receptor alpha (ERα) and estrogen receptor β (ERβ), which are located in the cell nucleus where they act as nuclear transcription factors. Both subtypes of estrogenic receptors are markedly expressed in the central nervous system (CNS). The activation of these nuclear receptors is responsible for the well-known genomic events produced by estrogens since they regulate the transcription of target genes through binding to specific DNA sequences. The tissue-specific and pleiotropic actions of estrogens are influenced by ER subtypes differential expression and their coregulatory proteins (Farooq, 2015; Farzaneh and Zarghi, 2016).

Estrogen receptor modulators (ERMs) constitute a group of compounds with a chemical structure that gives them an affinity to bind to estrogen receptors depending on the target tissue where this binding is performed (Pérez-Edo, 2004). Within the ERMs, selective estrogen receptor modulators (SERMs) (Pérez-Edo, 2004) and selective tissue estrogenic activity regulators (STEARs) (Reed and Kloosterboer, 2004) are found. SERMs induce estrogen agonistic (bone tissue, cardiovascular system, liver, brain) and antagonistic (breast, endometrium) effects in contrast to the purely agonistic effects of estrogens (Pérez-Edo, 2004). The term STEAR focuses on the estrogenic activity, which is particularly expressed in a tissue selective manner; moreover, steroid metabolism plays an essential role in establishing the availability of the ligand for the receptor (Reed and Kloosterboer, 2004).

A neuroprotective effect of SERMs and STEARs has been described in different models of damage, such as epilepsy (Velisek et al., 2013), focal cerebral ischemia (Zhang et al., 2005), traumatic brain injury (Kokiko et al., 2006), ozone exposure (Farfán-García et al., 2014; Pinto-Almazán et al., 2014), aging (Neri-Gomez et al., 2017) and Parkinson's

disease (Morissette et al., 2008). One of the mechanisms by which SERMs and STEARs exert this neuroprotective effect is through the modulation of the expression of cytoskeletal proteins (Barreto et al., 2009; Pinto-Almazán et al., 2014).

In this work, a systematic review of the effects of SERMs and STEARs on the modulation of cytoskeletal proteins in the CNS was performed. This manuscript included the search of controlled clinical trials from MEDLINE (*via* PubMed), LILACS (*via* BIREME), Ovid Global Health, SCOPUS, Scielo and Web of Science. Language restriction was applied to English. All searches were performed from 1990 to January 2017 and included the controlled vocabulary indexed on databases as well as keywords. Terms used on Medical Subject Heading (MeSH) were "tamoxifen", "clomiphene", "toremifene", "GW 5638", "raloxifene", "arzoxifene", "lasofoxifene", "basedoxifene", "tibolone", "tubulin", "actin", "MAP", "MAP1", "MAP2", "tau", "GFAP", "ketarin", "nestin", "neurofilament", "vimentin", "brain" and "central nervous system". The Boolean operator "AND" was used to perform a broad range of combinations in databases and find all relevant studies.

An Overview of ERMs

As mentioned before, ERMs are compounds that lack the steroid structure of estrogens but selectively bind to ERs, depending on their chemical structure as well as their tissue specificity. They exhibit diverse agonist and antagonist characteristics (biocharacter) in a given tissue. Furthermore, *in vitro* experiments have shown that individual SERMs can exhibit distinct activities in the same cell type (Dutertre and Smith, 2000). Due to their structure, ERMs also present antioxidant activity (Yu et al., 2007; Farfán-García et al., 2014).

Classification of ERMs

According to their chemical structure, SERMs have been classified into five groups: triphenylethylenes, benzothiophenes, tetrahydronaphtylenes, indoles and benzopyrans (Diez-Perez, 2006). In contrast, only one STEAR has been described so far: tibolone (Reed and Kloosterboer, 2004) (**Figure 1**).

Mechanism of action of ERMs

The mechanism of action of ERMs relies on their tissue-selective ER agonist or antagonist activities. The ER has two subunits (α and β chains), and SERMs interact with either of these subunits. From this interaction, there is a certain level of target-site specificity and tissue specificity for SERMs action. This differential behavior of SERMs depends on eliciting varying signaling properties from the ER that is tissue specific (An, 2016). SERMs bind to ERα and ERβ selectively. Moreover, the ligand nature and ER subtype determine the conformation of the ER-ligand complex. Hence, this structure determines its ability to interact with other molecules (coactivators, corepressors, coregulators). The molecular interactions involving the ER

determine its activity on a target promoter because some of these molecules, as well as the ER subtypes themselves, are differentially expressed or accessible to the SERM-ER complex in the different cell types (Dutertre and Smith, 2000). Therefore, the total activity (agonist/antagonist) of ER ligands depends on the conformational changes of the receptor isoforms induced by specific ligands and the particular ensemble of other proteins (coregulators) and promoters that provide functional specificity of the receptor at a gene level (Osborne et al., 2000).

Estrogens and SERMs also activate other pathways that are non-genomic or rapid acting, such as those dependent upon nitric oxide (NO): vasodilatation, ischemic myocardial damage, response to endothelial damage and coronary artery relaxation, among others (Navarro-Despaigne, 2001; Diez-Perez, 2006).

Tissue-selective effects of STEARs result from metabolism, enzyme regulation and receptor activation, which are different depending on the type of tissue. Tibolone is rapidly converted in the organism into three metabolites: 3α- and 3β-hydroxy-tibolone, with estrogenic effects, and Δ(4)-isomer, with progestogenic and androgenic effects (Kloosterboer, 2004).

Clinical use of ERMs

As these drugs can act as estrogen agonists and antagonists depending on the target tissue, they are used as a treatment for different conditions. For example, clomiphene has been used in the management of infertility (Goldstein et al., 2000), whereas tamoxifen, toremifene and droloxifene have been used as adjuvant therapy in breast cancer (Osborne et al., 2000). Raloxifene has been used to prevent osteoporosis in postmenopausal women (Kulak et al., 2010; Maximov et al., 2013) and it has been suggested that it may exert cardioprotective effects (Goldstein et al., 2000). Ospemifene has been used to treat postmenopausal dyspareunia associated with vaginal atrophy (Pinkerton and Thomas, 2014). Tibolone has been used to diminish climacteric symptoms in menopausal women (Pinto-Almazán et al., 2017).

One key difference among the effects of ERMs is breast and endometrial cancer risk safety (Pinkerton and Thomas, 2014). Many clinical trials examining various SERMs preparations in postmenopausal osteoporotic women showed that SERMs can maintain bone mineral density (BMD) and reduce the incidence of vertebral fractures but did not reduce non-vertebral fracture risk, indicating that their benefit for fractures is anatomically limited (An, 2016). The study of the mechanisms of action of SERMs has increased the understanding of hormone-receptor regulatory processes. Their development has allowed a certain efficacy profile to avoid some of the side effects of hormone therapy. Their clinical utility relies today mostly on the effects on breast cancer and BMD (Diez-Perez, 2006).

Many other SERMs such as GW 5638, arzoxifene and pipendoxifene are on preclinical stage trials, and other SERMs like MDL 101,986, SR16234, tetrahydroisoquinoline derivatives and 2-phenylspiroindenes are being developed to

prevent and treat osteoporosis, breast cancer and cardiovascular diseases (Terán and Teppa, 2005).

Neuroprotection by ERM

Extensive work has reported the neuroprotective activity of estrogens (Green et al., 1997) as well as ERMs. In a neuronal cell culture, raloxifene was found to be protective against a variety of toxic insults including glutamate, $A\beta_{25-35}$, and H_2O_2. The neuroprotective activity of raloxifene in an oxygen-glucose deprivation model was observed to be G protein-coupled receptor 30 (GPR30)-dependent and ER-independent and not mediated by antioxidant effects (Abdelhamid et al., 2011). Raloxifene and tamoxifen reduced microglial activation induced by neuroinflammatory stimuli in young and aged rats, which suggests the neuroprotective effects of these SERMs in brain trauma (Barreto et al., 2014). They also reversed spine density loss observed in a cerebral ischemia model in ovariectomized adult female rats (Khan et al. 2015). After the administration of kainic acid in adult ovariectomized rats, tamoxifen, raloxifene and bazedoxifene prevented hippocampal neuronal loss (Ciriza et al., 2004). In a male mouse model of Parkinson's disease, raloxifene showed distinct neuroprotective actions similar to those of estradiol and progesterone following a cytotoxic brain insult (Littleton-Kearney et al., 2002). In an ovariectomized rat focal stroke model, azoxifene significantly reduced ischemic infarction volume in the caudoputamen providing some degree of neuroprotection (Littleton-Kearney et al., 2002).

In the brain, SERMs may exert therapeutic potential either by modulating brain neurotransmitter transmission or through neuroprotective activity. The clinical potential for raloxifene in neurodegeneration and cognitive decline is shown by studies in elderly males and postmenopausal women (Cyr et al., 2000). Recent studies hint that raloxifene and arzoxifene are neuroprotective and may preserve some elements of cognitive function. Raloxifene mimics estrogen in the cholinergic system and increases brain-derived neurotrophic factor (BDNF) and nerve growth factor receptors, and may influence cognitive status. In postmenopausal women, raloxifene produced a small but significant improvement in verbal memory scores. Improvements in cognitive function were also observed in postmenopausal women with and without Alzheimer's disease (AD) (Littleton-Kearney et al., 2002).

Tibolone has been described to present neuroprotective effects and to modulate neuroplasticity in some animal models of oxidative stress and aging (Pinto-Almazán et al., 2014; Neri-Gómez et al., 2017). In postmenopausal women, tibolone improves well-being, cognition, and mood (Genazzani et al., 2006).

Importance of Cytoskeletal Proteins in Neuronal Physiology

The major intracellular structure that dictates morphology, polarization and motility of all cell types, known as the cytoskeleton, is a complex network of interlinking filaments and tubules. This structure is of main importance for neurons, which develop an uttermost differentiation in axonal and somatodendritic compartments (Menon and Gupton, 2016; Brandt and Bakota, 2017).

The neuronal cytoskeleton is composed of three interconnected types of long chains of protein subunits, each with specific properties. Neurofilaments are formed by intermediate filament (IF) proteins; microtubules (MT) are cytoskeleton heterodimers comprising protofilaments of α/β-tubulin; and actin microfilaments (MF), which contain both filamentous actin (F-actin) and polymerized globular actin (G-actin) (Hansberg-Pastor et al., 2015; Menon and Gupton, 2016).

The diameter of IF is about 10 nm, intermediate between microfilaments (6 nm) and myosin filaments (15 nm). Neurofilaments (NF) are heteropolymers composed of four subunits: heavy (NFH), medium (NFM) and light polypeptides (NFL). They are the major IF present in adult neurons, and their expression is restricted to neuronal cell types. NF are particularly abundant in axons; however, they are also present in perikarya and dendrites (Perrot et al., 2008; Yuan et al., 2012).

Outstanding properties of NF include a long half-life and elastic fibrous nature that allows upholding the distinctively asymmetrical shape of neurons. Whereas the main role of NF is to increase the axonal caliber of myelinated axons, and consequently the velocity of transmission of electrical impulses, they also contribute to the dynamic properties of the axonal cytoskeleton during neuronal differentiation, axon outgrowth (where NFL and NFM subunits are especially important), regeneration and guidance (Perrot et al., 2008; Yuan et al., 2012).

Before the arrival of the electronic microscope, MT could only be described as part of the mitotic spindle and cytoplasm. At present, it has been found that MT are hollow tubes with an approximate diameter of 25 nm and characteristically assembled from 13 laterally associating protofilaments of αβ-tubulin heterodimers. They constantly alternate between rapid phases of dynamic instability: growth (known as "rescue") and shrinkage (named "catastrophe"). As a result, MT cytoskeleton remains suitable for swiftly remodeling according to intracellular cues (Menon and Gupton, 2016; van de Willige et al., 2016).

Evidence in different eukaryotic cells has reported that MT cytoskeleton serves as a primary spatial regulator of cell shape. Its high dynamic properties compel to interact with actin in areas of cellular growth or reorganization during cell division, polarization and migration. With no exception, neurons depend on MT to determine their development for their distinctive morphology (Kaech et al., 2001; Jaworski et al., 2008).

Since early migration stages from the ventricular zone into more remote regions, MT are indispensable to provide the track path. However, ever since mature neurons must remain plastic as a response to their continuous rewiring connections, this development also depends on both the MT and MF, their crosstalk and accessory proteins (van de Willige et al., 2016).

This large number of proteins known as MAPs (for microtubule-associated proteins) can influence MT or relay signals from the MT cytoskeleton to other parts of the cell. MT behavior, such as stability, assembly, bundling and targeting, is regulated by MAPs. MAP2 and tau, two well-known examples of neuronal MAPs, keep a polarized, mutually exclusive distribution and decorate MT bundles in dendrites and axons, respectively. Abnormal phosphorylation of tau produces its dissociation from MT and originate tau aggregation into neurofibrillary tangles, which are potentially toxic tau deposits found in the brains of patients with AD and other tauopathies (van de Willige et al., 2016).

A mature neuron comprises several structures, including the axon, which transmits and propagates electric stimuli, and its dendrites, the receivers of the input from other neurons. From the very beginning, upon the stage of differentiation, neurite formation (protrusions which later become axons and dendrites) is yet powered by MT sliding. As soon as the axon is newly formed, it also relies on stable MT tracks for the transport of proteins, vesicles and organelles necessary for the formation of new axonal segments (van de Willige et al., 2016).

MTs also play a key role when participating in DNA segregation during mitosis, cell migration and maintenance of cell polarity; therefore, they are involved in neuronal plasticity (van de Willige et al., 2016; Brandt and Bakota, 2017).

In combination, MF and MT work to guide and support the growth and differentiation of axons and dendrites. Though dynamic actin filaments, they lead the course of growth cones and MT stabilize the structure of the new process. The axon outgrowth direction is defined by the growth cone, a specialized cytoskeletal-based motile structure, which responds to extracellular cues to guide the axon toward postsynaptic partners. Despite the fact that dynamic MT play only a minor role in neurite outgrowth, their role is crucial for axon polarization, pathfinding and branching (Kaech et al., 2001; Menon and Gupton, 2016; van de Willige et al., 2016).

The ultimate elements of the cellular cytoskeleton to be endorsed are MF. It should be reminded that actin is one of the most prominent proteins in neurons as well as in muscle in cells. The main components of the actin skeleton are bundles and networks of filamentous actin (F-actin). Moreover, actin is also present as a monomer (G-actin; globular actin) in living cells. In vivo, G-actin-binding proteins (e.g., thymosin β4) are responsible for assembly/disassembly and higher-order organization of actin filaments, isolate G-actin and prevent the assembly of G-actin into F-actin. Additionally, G-actin/F-actin equilibrium in dendritic spines also depends on the presence of a sequestered G-actin pool, which allows site-directed F-actin polymerization in response to synaptic activity (Sekino et al., 2007).

The integrity of the actin cytoskeleton is responsible for forming and maintainingthe shape and structure of the cell. In non-neuronal cells, actin plays the main role in the production of motile force; in fact, a relationship between actin and the motility and morphology in the dendritic spines of neurons has been observed. Rapid morphological changes in the peripheral region of spines resemble lamellipodial motion (Pollard and Borisy, 2003; Sekino et al., 2007).

An actin filament is a double helix of actin protomers decorated with binding proteins. A cohort of actin-binding proteins determines the particular organization of F-actin, either in bundles or networks, providing each F-actin to have unique physical and biochemical properties according to its binding proteins. In neurons, F-actin networks are found in spine heads whereas straight bundles are present in spine necks. However, the subcellular localization of actin-binding proteins can be changed by extracellular stimulation. Many actin-binding proteins identified in dendritic spines, such as Arp2/3, cortactin, ADF/cofilin, profilin, gelsolin, drebrin and neurabin (Sekino et al., 2007; Shirao and González-Billault, 2013) have been described.

Actin filaments possess polarity. Also, actin filaments keep treadmilling reaction when their ends are not covered by actin-capping proteins, which perform as fine regulators of actin polymerization. According to the treadmilling reaction, actin protomers are continuously polymerized at the barbed end and depolymerized at the pointed end. As some studies suggest, treadmilling reaction of actin filament occurs in dendritic spines; however, it does not generate the force to change spine morphology (Shirao and González-Billault, 2013).

A comprehensive approach to cytoskeletal proteins allows deepening knowledge beyond form and structure of cells. Particularly in neurons, these proteins have associated and specific functions merging on the cytoskeleton, which enable a model for both physiological and pathological cellular conditions from which pharmacological tests might develop.

Effect of ERMs on Neuronal Microfilaments

NFs or intermediate filaments are the most resistant elements of the cytoskeleton. NFs are heteropolymers, which are abundant in neuronal axons, with extremely elastic fibrous properties that help to maintain the asymmetrical shape of the neuronal cell and regulate the axon diameter and growth (Yuan et al., 2012). NFs are composed of three distinct polypeptides with a molecular weight of 200, 160 and 68 kDa.

They represent a class of intermediate filament proteins highly specific for neurons (Julien and Grosveld, 1991; Fracy et al., 1993). Some authors have described a subset of anterior pituitary cells that express immunoreactivity for neuronal markers, including the 68 kDa neurofilament NF68. It has been observed that the expression of NF68 is sexually dimorphic. Moreover, a drastic decrease in NF68 expression in anterior pituitary cells was observed when intact female rats were treated with tamoxifen, which can be an agonist or antagonist of ERs (Dutertre and Smith, 2000). Evidence that pituitary cells expressing neuronal traits correspond to subsets of lactotrophs, somatotrophs, thyrotrophs and gonadotrophs has been provided with double-immunolabelling experiments (Fiordelisio and

Hernández-Cruz, 2000).

This discovery suggests a different physiological role for a subset of pituitary NF68-positive cells in the organism. For this reason, it is important to be cautious in the use of neuronal markers like NF38 or other elements of the cytoskeleton and consider that the expression of such proteins, including NF38, can be influenced by steroid hormones or by SERMs.

The structure of astrocytes presents intermediate filaments constituted by glial fibrillary acidic protein (GFAP). This protein has been implicated in cell motility (Elobeid et al., 2000), astrocyte proliferation (Toda et al., 1999), integrity of the blood-brain barrier, myelination (Liedtke et al., 1996), neuroprotection and brain plasticity (Eddleston and Mucke, 1993; Otani et al., 2006). Some factors such as neuronal damage, stress, age or hormones can modify GFAP expression (Day et al., 1993).

Brain injury produces reactive gliosis (Williams et al., 2006), causing a glial scar to avoid the propagation of inflammation and damage. Astrocytes and NG2 cells participate in glial scar formation (Alonso, 2005).

A reactive phenotype characterized by a series of morphological and molecular modifications, including the expression of the cytoskeletal protein vimentin, is acquired by astrocytes.

In the rat brain, tamoxifen exhibited an antagonist action on ER (Zhao et al., 2005). Furthermore, it had a significant effect on reducing reactive astrocytes after brain injury (Barreto et al., 2009) and reduced the increased number of astrocytes, perhaps as a consequence of emigration from the injury zone or death (Arevalo et al., 2012). Tamoxifen could favor brain repair by promoting neuron survival, adjusting glial cell number and recover adequate neural communication (Franco-Rodríguez et al., 2013).

Other SERMs, like bazedoxifene, tamoxifen and raloxifene, present different dose-dependent neuroprotective effects. In the hippocampus of adult ovariectomized rats, the administration of kainic acid induced the expression of vimentin in reactive astroglia and a significant neuronal loss in the hilus. At different optimal doses, bazedoxifene, tamoxifen and raloxifene prevented neuronal loss. These SERMs may act through different neuroprotective mechanisms. Despite the fact that they were unable to reduce reactive gliosis, these molecules prevented neuronal loss in the hippocampus after kainic acid excitotoxicity (Ciriza et al., 2004).

In another study, young and aged ovariectomized rats received a stab wound brain injury before the treatment with estradiol, raloxifene or tamoxifen. The results showed that reactive astrogliosis was reduced in all animal groups, including controls. These findings indicate that SERMs are potential candidates for the control of astrogliosis in individuals and after a prolonged depletion of ovarian hormones (Barreto et al., 2014). The effects on astrogliosis could be attributed to the different doses administered, the model used to induce the injury or the age of the animals used in the study.

Tight junction proteins (TJs) are connected to the cortical actin cytoskeleton *via* multi-domain scaffolding proteins of the peripheral membrane-associated guanylate kinase (MAGUK) family. ZO-1 is not a transmembrane protein but a cytoplasmic TJ-associated protein, which can determine whether and where claudins are polymerized in an independent manner (Umeda et al., 2006). Claudins are members of a family of transmembrane proteins, which establish the structural and functional features of TJs with tissue-specific expression. ZO-1 deficiency disrupts TJs, and reduced ZO-1 levels are associated with barrier breakdown in many neurological disorders (Katsuno et al., 2008). The immunostaining for ZO-1 in the brain cortex and hippocampus of ovariectomized rats administered with vehicle, tibolone or 17β-estradiol (E2) revealed similar staining patterns. In this study, the authors also evaluated GFAP expression. The staining of GFAP was more intense in tibolone and E2 groups than in the control group (Ceylan et al., 2012). These results showed that some cytoskeleton-associated proteins are regulated by ERMs, and other are less responsive.

Effect of ERMs on Neuronal Intermediate Filaments

MF are formed by actin filaments. Their polymerization dynamics are associated with the activity of actin-binding proteins like drebrin and the ADF/cofilin. Drebrin is a protein located in the dendritic spines of the neuron that plays a role in the synaptic plasticity together with actin filaments. Drebrin binds to and organizes filamentous actin (F-actin) in dendritic spines, the receptive regions of most excitatory synapses that play a crucial role in higher brain functions (Kojima and Shirao, 2007). Moreover, the ADF/cofilin family comprises small actin-binding proteins that enhance actin dynamics in three ways: by depolymerization (accelerating monomer loss at the pointed end), by severing filaments into shorter protomers and by directly or indirectly facilitating actin filament growth (Bernstein and Bamburg, 2010). ADF and cofilin-1 are both expressed in the mammalian brain. The genetic deletion of cofilin in the nervous system reduces neuronal cell proliferation and migration but not neurite formation (Bellenchi et al., 2007). Moreover, the genetic ablation of ADF affects neither the development of the nervous system nor the formation of neurites in particular (Bellenchi et al., 2007). Therefore, MF regulate the function of synapses, axonal cone growth and protein trafficking (Disanza et al., 2005).

The cytoskeletal rearrangements are controlled by the Rho family of GTPases, which regulate the activity of diverse cytoskeleton-associated proteins such as actin-binding proteins (Gonzalez-Billault et al., 2012). The process of cytoskeleton remodeling including the formation of new MF and their interaction with the plasma membrane depends on the participation of diverse actin-binding proteins (Kim et al., 2006). MF can be modulated by hormones (Arevalo et al., 2010; Ferri et al., 2014). However, to the extent of our knowledge, the effect of MF regulation by SERMs has not been described.

Effect of ERMs on Neuronal Microtubules and Microtubule-Associated Proteins

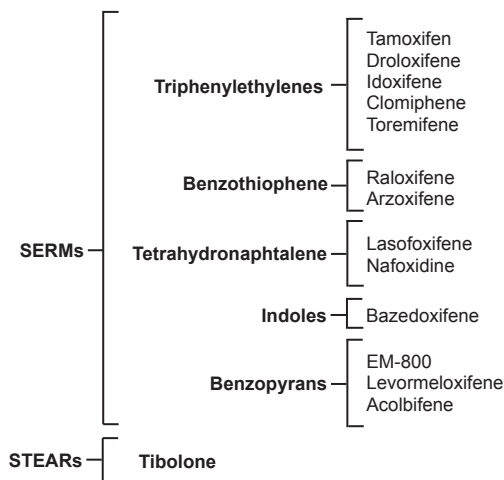Microtubules (MT) are highly dynamic polymers of α and

Figure 1 Classification of ERMs.
ERMs: Estrogen receptor modulators; SERMs: selective estrogen receptor modulators; STEARs: selective tissue estrogenic activity regulators.

Figure 2 Chemical structure of some estrogen receptor modulators (ERMs) and effects on cytoskeletal proteins.
The similarities between ERMs and estradiol structures can be observed. Their structure allows them to bind to estradiol receptors with different affinities.

β tubulin essential for the growth and maintenance of the shape, movement, signaling and reproduction of cells. Polymerization is indispensable for MT functions, allowing their reorganization depending on the cell necessities (Jordan, 2002). MT nucleation is the process by which soluble αβ-tubulin subunits are arranged parallel to a cylindrical axis and are converted into a growing MT that may be as long as millimeters (Jordan, 2002; Wieczorek et al., 2015). MT arrays in axons and dendrites are necessary for both assembly and transport properties of these neurites (Conde and Cáceres, 2009).

Kinetics of MT assembly and disassembly dynamics can be influenced by non-enzymatic proteins called microtubule-associated proteins (MAPs), being MAP1B and tau the first proteins implicated (Conde and Cáceres, 2009; Wieczorek et al., 2015).

Structural MAPs belong to four different families: MAP1, MAP2, MAP4 and tau proteins that are different in type and structure. MAP1, MAP2 and tau are the most important in neurons. The MAP1 family is formed by MAP1A, MAP1B and MAP1S; MAP1A and MAP1B have a key role in the stabilization, guidance and function of axons. MAP1S is important for the regulation of cell division; its expression in neurons is lower when compared with MAP1A and B. MAP1B is essential for the development and maturing of dendritic spines (Conde and Cáceres, 2009; Mohan and John, 2015).

MAP2a, MAP2b, MAP2c and MAP2d, members of the MAP2 family, are formed by alternate splicing. In neurons, the most abundant proteins are those from MAP2 family. They are part of axons and dendrites in initial stages of neurodevelopment but limited only to dendrites in adults. In addition, MAP2 have been associated with actin in the development of axons and the inside of dendritic spines (Conde and Cáceres, 2009; Mohan and John, 2015).

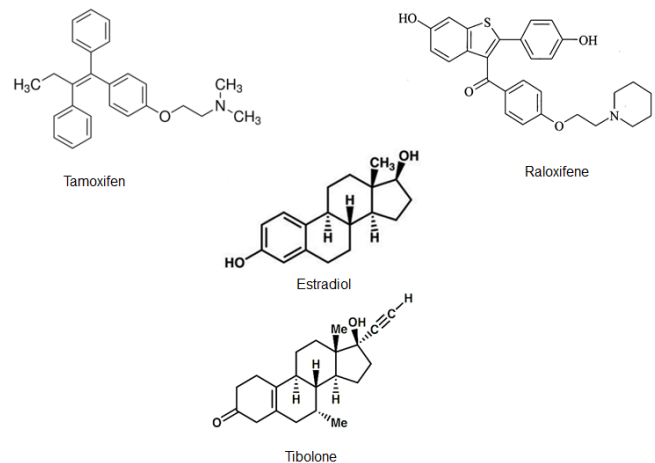Tau family is formed of six different isoforms produced by alternative splicing and post-translational modifications. The length of each isoform depends on the number of repeats (3 or 4) of the microtubule-binding domain at their C-terminus and the number of N-terminal inserts (0-2). Tau proteins are associated with establishing neuronal polarity and axon elongation by controlling the assembly and stabilization of neuronal MT allowing the regulation of intracellular transport, which in turn plays a critical role during myelin formation (Conde and Cáceres, 2009; LoPresti, 2015; Mohan and John, 2015).

The expression of MAPs is differentiated in neurons. MAP1A expression is localized in dendrites and mainly expressed in adult neurons. MAP1B, MAP2c and tau could be differentially found in axons. MAP1B and MAP2c are predominantly expressed in embryonic and neonatal brains. MAP2a, MAP2b and MAP2d are expressed only in adult neuronal cells specifically in the soma and dendrites (Mohan and John, 2015). Therefore, MAP2 antibodies are excellent markers in neurons (Zhang et al., 2001; Conde and Cáceres, 2009).

The effects of SERMs on neuronal MAPs have been studied indirectly (Zhang et al., 2001; Haynes et al., 2003; Wang et al., 2015). For example, tamoxifen (TMX) has been used for the understanding of E2 neuroprotective effects *via* an estrogen receptor-dependent process in different models (Zhang et al., 2001; Haynes et al., 2003).

Zhang et al. (2001) studied the mechanisms underlying the neuroprotective effects of estrogens in a neurotoxic β-amyloid peptide model. In this model, $A\beta_{31-35}$ significantly decreased the number of neurons. Furthermore, it was demonstrated that the total number of MAP2 positive cells (MAP2$^+$) decreased. TMX was used for its estrogen receptor antagonist characteristics, abolishing the neuroprotective effects of E2. However, the total number of MAP2$^+$ cells did not change with TMX treatment. Therefore, according to these results, TMX had no effects on the expression or con-
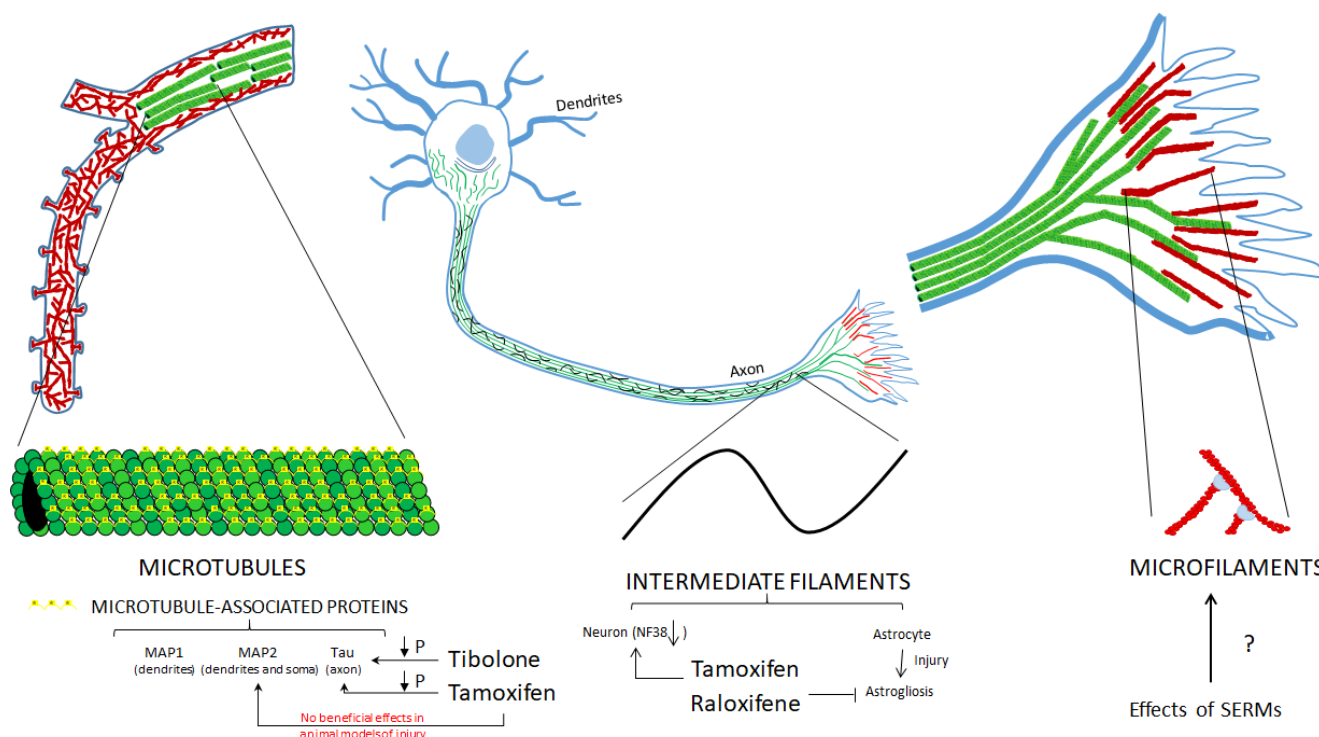
Figure 3 Effect of estrogen receptor modulators (ERMs) on cytoskeletal proteins at different levels of organization.
The effect of ERMs on microtubules, mainly on microtubule-associated proteins (MAPs), is shown. Their main effect is to decrease the phosphorylation of these proteins (↓ P), which can be considered as a marker of neuronal damage as well as pathogenicity in some neurodegenerative diseases such as Alzheimer's disease. On the intermediate filaments, ERMs can regulate the astrogliosis by diminishing the expression of proteins like the neurofilament of 68 kilodaltons (NF68). Finally, although it is known that steroid hormones, such as estradiol and progesterone, can have effects on microfilaments, the effect that ERMs may have on these structures is unknown.

tent of MAP2 (Zhang et al., 2001).

Haynes et al. (2003) studied the effects of E2 in the overall neuronal damage produced by dexamethasone. The authors evaluated the levels of MAP2$^+$ in the striatum and hippocampus. They reported that TMX pretreatment prevented estrogen neuroprotection given that they observed similar results in the MAP-2 scores between both vehicle/vehicle/dexamethasone and TMX/estrogen/dexamethasone groups. Interestingly, the same damage produced by dexamethasone alone (reduction of MAP2$^+$ neurons) in the hippocampus was observed for the TMX/vehicle/dexamethasone group; moreover, the damage was increased in the striatum (Haynes et al., 2003).

At the present day, only the effects of TMX treatment on MAPs have been studied. The results on animal models have shown that TMX does not exert neuroprotective effects and even increases the damage produced by dexamethasone.

Modulation of Cytoskeletal Proteins by ERMs in Neurodegenerative Diseases

MT can display multiple functions due to their flexibility. Tubulin assembly can accomplish several functions depending on their binding partners, generating different physiological or pathological microtubule structures (Oláh et al., 2013).

Some neurodegenerative diseases known as tauopathies present a pathological aggregation of tau called neurofibrillary tangles. Abnormal phosphorylation of tau decreases its capability for stabilizing microtubules, generating cytoskeleton destabilization and perturbation of axonal transport. In affected neurons, hyperphosphorylation of tau followed by neurofibrillary tangles, which aggregate into paired helical filaments (PHFs), have been reported (Alvarez-de-la-Rosa et al., 2005; Pinto-Almazán et al., 2012; Corbel et al., 2015).

Several studies have been designed with the idea of using sex hormones and analogs as therapeutic strategies for tauopathies and dementia. Neuroprotective effects of ERMs have been evaluated on tau phosphorylation, and also their role as ER antagonists (Alvarez-de-la-Rosa et al., 2005; Pinto-Almazán et al., 2012; Corbel et al., 2015; Lo-Presti, 2015).

Corbel et al. (2015) screened over 1,760 compounds that could inhibit the activity of CDK5, one of the major tau kinases, from which they identified TMX as a prospect. They performed BRET-based screening assays, cellular and western blots studies and determined that TMX inhibits CDK5/p25 protein-protein interaction by preventing the increase of CDK5 kinase activity and the increase of tau phosphorylation produced by glutamate.

In concordance with these findings, Alvarez-de-la-Rosa et al. (2005) treated neuroblastoma and female neuronal cells for 24 hours with E2 or E2 + TMX to study the effects of E2 on tau phosphorylation. Western blot analyses were performed to analyze tau dephosphorylation (Tau-1 epitope), tau hyper-

phosphorylation (12E8Site) and total tau. The treatments with E2 alone decreased okadaic acid-induced tau hyperphosphorylation (12E8Site) and increased tau dephosphorylation (Tau-1 epitope) and total tau expression. These E2 effects were blocked with E2 + TMX treatment. Therefore, TMX alone demonstrated to increase Tau-1 and total tau expression without an effect on 12E8Site epitope.

As the studies performed with TMX, the effects of tibolone on tau phosphorylation also have been evaluated. Pinto-Almazán et al. (2012) reported that chronic treatment with 0.5 mg/kg of tibolone decreased tau hyperphosphorylation, increased tau dephosphorylation and correlated with an increased phosphorylation of GSK3 in Ser9, which is the inactive form of this kinase in the hippocampus and cerebellum of ovariectomized adult rats.

Interestingly, other pharmacological properties of TMX have helped in the understanding of the pathophysiological process of tauopathies and dementia (LoPresti, 2015; Wang et al., 2015). Wang et al. (2015) produced Akt cTKO mice to understand the pathological process of tauopathies and the importance of Akt contribution. First, they created viable Akt1[f/f]; Akt2^{−/−}; Akt3^{−/−}; CAG-CreER mice that became Akt cTKO after the treatment with TMX. In this novel Akt cTKO mouse model, tau hyperphosphorylation was reported without significant changes in the total number of TUNEL^{+} cells or NeuN^{+} cells and also unchanged levels of GSK3β, CDK5, ERK and p38 in their active forms.

Furthermore, LoPresti designed a truncated tau (ΔTau) inducible expression model in oligodendrocytes (OLGs) for studying the effects of this non-microtubule-associated tau in neurodegenerative diseases. She generated a Floxed LacZ-STOP/EGFP–ΔTau founder transgenic mice, which expressed EGFP-ΔTau and Cre transgenes. The Cre recombinases system activated by administration of TMX has been an important tool for inducing *in vivo* gene activity in space- and time-dependent manner. In this study, she injected TMX to the 12-day-old offspring mice for three days and observed gait abnormalities, such as stumbles and loss of balance, in the p18 mice, which were correlated with myelin decrease.

Unlike other studies on MAPs, not only the effects of TMX but also the effects of TIB on tau protein have been evaluated. Both treatments demonstrated their neuroprotective effects by increasing the dephosphorylated form of tau (Tau-1). Moreover, other pharmacological properties of TMX have been used to produce different models of neurodegeneration, useful for a better understanding of the mechanisms of tauopathies.

Perspectives

Some ERMs have an effect on the modulation of cytoskeletal proteins (**Figure 2**) through several mechanisms, mainly on filaments and MAPs (**Figure 3**). Because of the diverse functions of these proteins, ERMs can modulate cell motility, astrocyte proliferation, the integrity of the blood-brain barrier, myelination and neuronal plasticity. Hence, they can exert a neuroprotective effect in different models of neuronal

damage, as well as in neurodegenerative diseases such as AD in which these proteins participate.

The study and development of more ERMs are important. In addition, the study of the interaction mechanisms between ERMs and ERs is necessary for the better understanding of the interactions with cytoskeletal biomolecules, which in turn are involved in some neurodegenerative disorders.

An important aspect to be considered in the observed effects of ERMs is the route of administration. Throughout the review, it was observed that several routes of administration of the ERMs were used in the different studies, which included intraperitoneal, esophageal, ICV and the addition to the means of culture in the case of cell lines. It was also observed that depending on the route of administration the administered dose varied because through both intraperitoneal and esophageal routes ERMs can be metabolized in the liver. Also, a higher concentration was required to cross the blood-brain barrier and observe an effect in the CNS. In contrast, ICV administration and drug addition to cell cultures had a more direct and powerful action. For this reason, a smaller dose was required to observe an effect. Hence, it is important to carry out more studies in which the effects of different doses of ERMs are evaluated, as well as the routes of administration used.

Finally, it is necessary to continue the search for an ideal ERM that has all the benefits of estrogens, even the neuroprotective effects, but without the risk of side effects, such as breast or endometrial cancer and cardiovascular risks and stroke.

Open peer review reports:
Reviewer 1: Sarah L. Ferri, University of Iowa, USA.
Comments to authors: This is a thorough and interesting review of literature pertaining to estrogen receptor modulators (ERMs) in the central nervous system. Several mechanistic levels are described for a number of ERMs, which have important health implications. I think this overview of what is known is a valuable resource.
Reviewer 2: Arash Abdolmaleki, Ferdowsi University of Mashhad, Iran.

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