Astrocytes Mediate Protective Actions of Estrogenic Compounds after Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) is a serious public health problem. It may result in severe neurological disabilities and in a variety of cellular metabolic alterations for which available therapeutic strategies are limited. In the last decade, the use of estrogenic compounds, which activate protective mechanisms in astrocytes, has been explored as a potential experimental therapeutic approach. Previous works have suggested estradiol (E2) as a neuroprotective hormone that acts in the brain by binding to estrogen receptors (ERs). Several steroidal and nonsteroidal estrogenic compounds can imitate the effects of estradiol on ERs. These include hormonal estrogens, phytoestrogens and synthetic estrogens, such as se-

lective ER modulators or tibolone. Current evidence of the role of astrocytes in mediating protective actions of estrogenic compounds after TBI is reviewed in this paper. We conclude that the use of estrogenic compounds to modulate astrocytic properties is a promising therapeutic approach for the treatment of TBI.

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Introduction

Traumatic brain injury (TBI) is generated by a traumatic event, which causes a strong impact on the brain and results in acute or chronic impairments in the func-

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tion of neural cells [1]. TBI is a severe public health problem, particularly among male adolescents, young adults, and older people of both sexes [2]. Indeed, TBI is a familiar source of morbidity and mortality all over the world, whereby 69 million people are estimated to be affected by TBI each year [3]. Besides, it is the main cause of mortality and impairment among young people in the low- and middle-income countries [4]. Currently, brain injury has a higher frequency than other highly spread diseases such as cancer, acquired immune deficiency syndrome, Parkinson's disease and multiple sclerosis [1]. TBI has heterogeneous aetiology, type, severity and outcomes. There are 2 main types of TBI: penetrating and non-penetrating. Penetrating TBI is an open lesion in the head causing intracranial hemorrhage. Non-penetrating TBI causes brain damage because of indirect impact without penetration of any objects into the brain [5]. As a result of TBI, one or more brain regions are damaged and become swollen [3, 6].

Despite the availability of standardized treatment guidelines and improvements in understanding the mechanisms of cellular damage, therapeutic strategies for patients with TBI remain unsatisfactory because of their reduced efficacy in preventing the metabolic changes and brain dysfunctions caused by the injury. Brain responses to damage and disease involve several cell types that interact with each other in an attempt to preserve cell viability and homeostatic physiological function [7]. In the last few years, protective strategies have been focused on modulating the responses of different brain cells to injury. In this regard, astrocytes may represent relevant candidates, since they play critical roles in the central nervous system (CNS) both under healthy and pathological conditions. In the healthy CNS, astrocytes accomplish a significant number of homeostatic functions, including the control of neuronal metabolism, production of antioxidants and regulation of synaptic transmission and plasticity. In addition, astrocytes respond to brain injury with a series of morphological, metabolic and cellular changes involved in the protection of the damaged neural tissue [8]. Studies with intravital time-lapse imaging have shown that ATP and other metabolites released by astrocytes play a pivotal role in stimulating beneficial cellular responses after brain injury [9]. Therefore, the identification of molecules/compounds that activate protective mechanisms in astrocytes may lead to the development of potential therapeutic strategies for TBI [10–12].

Estrogenic compounds include a wide variety of natural and synthetic steroid and non-steroid molecules that bind to ERs, thereby mimicking the activity of estradiol

in different tissues. Estrogenic compounds can affect astrocytes, either directly or indirectly, and exert numerous actions in the CNS under physiological and pathological conditions [13]. For instance, it has been shown that estrogenic compounds regulate oedema, extracellular glutamate levels, reactive astrogliosis and inflammatory response after brain trauma [14, 15]. Therefore, they may be useful molecules for the management of TBI [11]. In the next sections, we review the participation of astrocytes in the response of neural tissue to injury and their role in mediating neuroprotective actions of estrogenic compounds to improve neuronal survival and functional outcomes after TBI.

Cellular and Molecular Alterations after TBI

Depending on the outcome of damage to brain parenchyma, the tissue injury produced by TBI is classified into 2 substantially different stages associated with different mechanisms of neural damage: primary and secondary [16]. The primary injury occurs as a consequence of the initial physical insult [17]. This stage often requires surgical attention and happens at the moment of trauma, when tissues and blood vessels are extended, compacted, and damaged [6]. The secondary stage appears hours to days later as a consequence of the activation of signalling pathways that provoke oedema, inflammation and brain cell death [16]. The secondary injury is characterized by high levels of lactate [18], oxygen-free radicals [1], interleukins [19] and intracellular free Ca^{2+} in response to the primary injury [20]. In addition, impairment of the blood-brain barrier (BBB), excessive release of the neurotransmitter glutamate and dysfunction of mitochondria in the brain [21] contribute to the secondary injury. Other alterations that can be detected in secondary brain injury are decreased brain blood flow, ischemia, increased reactive oxygen species (ROS) and brain cell death [22].

In comparison with primary injuries, secondary ones are progressive, a fact that can be vital for patient recovery [23]. TBI may be associated with different injuries in other body regions (limb fractures, thoracic, or abdominal injuries) [24], which increase the risk of secondary brain injury due to hypotension, hypoxia, pyrexia and coagulopathy. In addition to brain cell death, TBI is associated with neuroinflammation, axonal damage and vascular abnormalities [25]. TBI includes anomalous ranges of oxygenation, upregulation of inflammatory pathways and irregular endocrine secretion [26]. Interestingly, there is evidence suggesting that neuroendocrine dysfunction

might contribute negatively to the severity and the TBI outcome [27]. Recent studies using controlled cortical impacts in mice have demonstrated that the alterations in growth hormone levels after brain injury are associated with a disruption in barrier organization of tanycytes [28, 29]. It has been suggested that these cells play a crucial role in the maintenance of BBB, the control of the pituitary function and the secretion of active compounds. All these functions, which are critical to the protection of the homeostasis of the brain, have highlighted the important role of tanycytes during TBI [30].

The early secondary stages of cerebral injury after TBI are characterized by the accumulation of lactic acid due to an increase in anaerobic glycolysis, excess membrane permeability and oedema [22, 31]. The latest pathological stages are characterized by excessive production of excitatory neurotransmitters (glutamate, aspartate), and activation of α -amino-3-hydroxy-5-methyl-4-isoxazolpropionate, N-Methyl-D-aspartic acid and voltage-dependent calcium and sodium channels, which consequently generate terminal membrane depolarization and mitochondrial dysfunction [32, 33]. The imbalance of calcium and sodium intake could trigger autophagy mechanisms [34], activation of caspases, translocases and endonucleases, and inhibition of DNA repair, which may induce structural modifications of biological membranes and DNA damage [35, 36]. Consequently, all these events lead to membrane decoupling and finally cell death by necrosis and apoptosis [37]. Furthermore, the excessive production of excitatory neurotransmitters causes excitotoxicity. In this regard, excitotoxicity could lead to excessive stimulation of glutamate receptors, occasioning damage and death of brain cells. Neurons are especially vulnerable to excitotoxicity [38]. Furthermore, astrocytes are less vulnerable and reuptake glutamate from synapses, thanks to their fast conversion from glutamate into glutamine by the glutamine synthetase, thus preventing the excessive and toxic extracellular glutamate accumulation [39].

Excitotoxicity induces harmful effects on the mitochondrial electron transport chain that generates the oxidation of cellular structures and molecules by the newly generated oxidative species [40]. Indeed, another cell death mechanism elicited by TBI is oxidative stress caused by increased levels of ROS and reactive nitrogen species (superoxide, hydrogen peroxide and nitric oxide) [41]. This mechanism is fundamental due to the fact that the brain is highly susceptible to free radical damage because of its oxidative metabolism and its high levels of polyunsaturated lipids [42]. Some studies have demonstrated that superoxide radicals and nitric oxide impair cerebral

Table 1. Astrocyte functions under physiological conditions

Function	References
Control of cerebral blood flow by the regulation of vascular tone	[229]
Uptake of glutamate and GABA	[230]
Adjustment of synaptic transmission	[231]
Antioxidant functions related with key proteins involved in neutralizing damaging free radicals in the form of reactive oxygen species	[232]
Organization of the structural architecture of the brain	[233]
Preservation of neural tissue and restriction of inflammation after moderate focal brain injury	[234]
Regulation of brain homeostasis	[235]

vascular function after TBI due to their strong oxidative effects [43, 44].

Increased levels of ROS can be triggered not only by an increase in their generation, but also due to a decreased capacity of the enzymatic antioxidant system (superoxide dismutase, glutathione peroxidase and catalase). In this regard, it is relevant to note that astrocytes maintain high expression of intracellular antioxidant enzymes. This antioxidant activity of astrocytes helps to protect the neuron against the oxidative stress preventing the damage of DNA, RNA, and proteins [45, 46]. In this regard, previous works indicated that astrocytes are pivotal mediators in TBI due to their ability to provide neurons (and other cells) metabolic support [47–49].

Role of Astrocytes after TBI

Function of Astrocytes under Physiological and Pathological Conditions

Astrocytes are the most abundant group of glial cell, representing approximately around 20–40% of the total brain cell population [48, 50, 51]. Their function depends on their shape, position, subtype, developmental stage and metabolic state [48]. Astrocytes are recognized for being multifunctional housekeeping cells involved in a permanent cross-talk with neurons and other neighbouring glial cells [52]. They regulate the concentration of potassium, sodium and glutamate at the synaptic cleft [47–49], provide lactate and glutamine to neurons and participate in neuron signalling modulation [53–57].

Table 2. Molecules with the ability to activate astrocytes

Processes	Activating molecules	References
Growth factor and interleukins	TGFβ-1, TNFα, INFγ, IL-1β, IL6, LIF, CNTF, IL1, IL10, IL-6, and EGF	[236–239]
Inflammatory modulators	LPS, TLRs, DAMPs, PAMPs	[74, 94, 240, 241]
Cytotoxicity	Glutamate, ammonia	[242, 243]
Oxidative stress	ROS, H2O2, NO, O2– and OH	[84, 113, 244]
Neurodegenerative diseases	Amyloid-beta, alpha-synuclein and presenilin	[84, 245, 246]
Brain damage	Hypoxia, glucose deprivation and TBI	[113]

Fig. 1. Schematic representations of different phenotypic changes in reactive astrogliosis. **a** Healthy astrocytes have many physiological functions aimed to maintain CNS homeostasis. **b** Under pathological conditions, astrocytes undergo a variety of phenotypic and functional changes, known as reactive astrogliosis. Reactive astrocytes show a variety of changes in the activation of specific signaling cascades, such as STAT3, SOCS3 or NF-κB, cellular morphol-

ogy and alterations in the expression of intermediate filament proteins such as nestin, glial fibrillary acidic protein (GFAP) and vimentin. **c** Astrogliosis progress over the time and this can become maladaptive, whereby scar formation takes place. Glial scar includes alterations in molecular expression and functional features.

The different physiological functions of astrocytes are listed in Table 1. For instance, in a healthy and uninjured CNS, astrocytes play an essential role in the modulation of potassium, sodium, calcium and glutamate levels in the extracellular space, thus modulating synaptic activity and preventing excitotoxic cell death [58–60]. Astrocytes have cell processes that contact with blood vessels, neuronal perikarya, axons, and synapses. Thus, these cells uptake glucose from blood vessels and supply energy metabolites, such as lactate, to neurons [61]. Besides the production gliotransmitters and growth factors, astrocytes

also have the capacity of supplying neuroactive steroids, which modulate synaptic activity [61, 62]. It is noteworthy that astrocytes have a dual role: (1) these cells can be a direct target of several endocrine molecules and (2) also they can be regulatory-endocrine cells that exert influences on nervous tissue. For instance, studies demonstrate that in different physiological and pathological conditions astrocytes are able to synthesize a variety of endogenous neurosteroids [63, 64], which have regulatory effects and affect the neurosecretory activity of other cells of the CNS, such as neurons, microglia, and tany-

Table 3. Specific signaling cascades involved in the morphological and functional modifications of reactive astrocytes

Astrocyte morphological and functional changes	Signaling pathways implicated	References
Hypertrophy	STAT3, Endothelin-1	[76, 247]
Proliferation	Endothelin-1; JAK/STAT3, JNK/c-Jun and Olig2	[104, 248]
Migration	CEPB1	[249]
Scar formation	STAT3; EphB2, fibrinogen, TGF β , FGF2	[76, 250]
Release of pro-inflammatory molecules	NF- κ B; P2X7; MAP-kinase	[75, 251, 252]
Release of anti-inflammatory molecules	STAT3; Nrf2; SOCS1 and SOCS3	[76, 253–255]
Increased release of cytotoxic molecules (NO and NOS)	IF γ ; TGF β	[256]
Protection by glutamate transport and extracellular homeostasis	Cyclic adenosine monophosphate, FGF, PI-3K/Akt and EGF	[257, 258]
Protection by antioxidant response	Nrf2	[254, 255]

cytes [65]. Likewise, the role of astrocytes may be supported by tanycytes and microglia. For instance, tanycytes are cells with morphological and molecular characteristics similar to those of astrocytes and are responsible for transporting substances from the cerebral spinal fluid and the blood portal [30, 66, 67]. Estrogen receptors (ERs) have been identified in tanycytes and microglial cells and it has been observed that they help in estrogenic communication, as estradiol stimulates the secretory activity of these cells by releasing prostaglandin E, which is related to synaptic alterations in the hippocampus, as well as in astrocytes [67, 68]. As discussed later, the interaction of astrocytes with other brain cells plays a fundamental role in neuroprotection [68].

Under pathological conditions, astrocytes undergo a variety of phenotypic and functional changes, known as reactive astrogliosis. Generally, this pathological phenomenon is related to an enhanced proliferation and secretion of inflammatory mediators, adhesion molecules and growth factors [69–74]. Reactive astrogliosis is elicited by a variety of molecular signals (Table 2) associated with different forms of CNS injuries and diseases such as trauma, ischemia, infection, stroke, neurological disorders and neurodegenerative diseases [71]. Compared to non-reactive astrocytes, reactive astrocytes show various alterations in gene expression, activation of specific signalling cascades, such as signal transducer and activator of transcription 3, suppressor of cytokine signalling 3 or nuclear factor κ B (NF- κ B) and exhibit morphological and functional changes (Fig. 1) [75–78]. These changes depend on the severity of the damage (Table 3) [51, 72, 79–85]. Previous histopathological studies of the human brain have shown various grades of reactive astrogliosis [84]. Mild to moderate astrogliosis are caused by different insults including TBI, and viral and bacterial infections. When the brain tissue presents mild or moderate injury, astrocytes experiment hypertrophic astrogliosis and pro-

cess hypertrophy [84]. These changes include modifications in the expression of the glial fibrillary acidic protein (GFAP), vimentin, nestin and other proteins [85], which alter the normal cellular structure, energy metabolism, intracellular signalling, and pump activities of astrocytes [84]. In severe cases of brain injury, astrocytes undergo progressive cellular hypertrophy, proliferation and scar formation [7].

Astrogliosis can be interpreted as a homeostatic response aimed to reduce and repair the initial damage, maintaining brain function [51, 74]. However, depending on the different stages of the insult and its progress over time, astrocytes are capable of playing beneficial or detrimental actions [84, 86]. A number of experimental studies have shown that astrogliosis has an essential role in the recovery after a TBI or ischemia, due to their ability to isolate the still health brain tissue [74, 87–89]. Reactive astrocytes have a beneficial role in the acute response after brain trauma because they may limit the extension of damage, and separate the harmful lesion from the rest of the CNS thus preventing its spreading. Studies using genetic ablation of GFAP and vimentin at the time of injury have shown a significant reduction in astrogliosis, a slower response to damage and a decrease in the integration of new neuronal synapses after TBI [82]. Interestingly, this acute ablation increases the number of leukocytes that cross the BBB, leading to neuronal degeneration [87]. On the other hand, it seems like that reactive astrocytes respond to brain injury by expressing aromatase. This enzyme is involved in the synthesis of different estrogens in humans (E1, E2, E3). The increase in the local expression of estrogens may be involved in the neuroprotective and regenerative effect in the brain [90]. It is important to point out that astrogliosis progresses over the time and becomes harmful in chronic stages. In these stages, reactive astrocytes are known to inhibit axonal regeneration and the integration of neurons after brain injury [86, 91–93].

Reactive astrocytes also can release chemokines, growth factors, proteases and danger-associated molecular patterns, which could be beneficial or detrimental for the BBB integrity in various ways [7, 94, 95]. For example, different studies have shown that astrocytes secrete apolipoprotein E and the vasoactive endothelial growth factor in response to stimulation with inflammatory mediators [96], increasing BBB permeability and causing leukocyte infiltration [7, 94, 97, 98].

Astrocytes and the Glial Scar

Scar formation takes place in most lesions that cause severe tissue damage, such as penetrating trauma. In this process, astrocytes undergo severe morphological changes with the spread of long branched processes that overlap [84]. In the pathologic process, reactive astrocytes cooperate with the different brain cells and interact with the extracellular matrix to generate the glial scars [76, 84, 99]. These scars persist for long periods of time even when the causes of the brain damage have disappeared [76, 87, 89, 100]. The glial scar is formed in damaged zones that contain surviving neurons where these glial formations can interact with neuronal and non-neuronal cells in the area around the lesion [87, 101–103]. It has been proposed that in this process of scar formation are involved some molecular triggers that initiate the spread of reactive astrocytes in vivo such as ATP, the signal transducer and activator of transcription 3, fibroblast growth factor, epidermal growth factor, and endothelin 1, which can diminish or aggravate the brain damage [104–106]. However, the origin of newly scar-forming astrocytes is not well established. Different hypotheses have been postulated, such as that mature astrocytes have the capability of starting the cell cycle and multiply in the moment of scar formation [87, 104, 107]. There is also evidence that some of the proliferating astrocytes after brain damage derive from polydendrocytes, cells that express the neural/glial antigen 2 proteoglycan [108], in the brain parenchyma and progenitors of ependymocytes [109, 110]. Besides, it is also postulated that other source of scar-forming astrocytes could be multipotent progenitors in ependymal and subependymal tissues expressing GFAP [84]. These progenitors might produce cells with the capacity of migrating to locations of injury [111].

Recent evidence indicates that the glial scar seems to have a beneficial role by forming a compact barrier that isolates the healthy tissue of damaged tissue and does not allow the red and white blood cells to have contact with the healthy tissue [80]. Glial scar plays also a significant role in the regulation and the control of the propagation of CNS

inflammation [84]. By contrast, there are studies showing the negative effects of reactive gliosis and glial scar. For instance, experiments in animal models suggest that the suppression of glial scar formation might improve the outcome after diverse types of CNS injuries by reducing excitotoxic neurodegeneration [87, 112, 113], inducing inflammation [76, 87–89, 114, 115] and improving axonal regeneration [116]. The glial scar can also exert other negative effects. For example, there is also evidence showing that glial scar impedes axonal regeneration and reduces axonal re-growth. As described above, the negative effects of astrogliosis and glial scar have been related to late chronic phases, specifically, when reactive astrocytes are not able to appropriately respond to damage during post-acute and early chronic stages after TBI [116]. Consequently, nerve cells are exposed to harmful molecules for a long time, which also have some deleterious long-term effects in the injured brain. Altogether, accumulating evidence has suggested that astrocytes seem to play a critical determinant role in acute and chronic stages after TBI. Their beneficial and detrimental responses can directly affect neuronal survival [117]. For this reason, astrocytes (and astrogliosis) have been postulated as an important therapeutic target for the action of different neuroprotective substances, such as estrogenic compounds. These compounds have been implicated in the activation of different protective cellular pathways in the CNS.

The CNS as a Target for Neuroprotective Estrogenic Compounds

Numerous studies have shown that synthetic or natural estrogens, such as estradiol, phytoestrogens, selective ER modulators (SERMs) and tibolone, exert neuroprotective actions in vitro and in vivo (Fig. 2) [10, 118–137]. Thus, there is evidence showing that estrogens reduce neuronal death in rodent models of TBI [138] and prevent damage in several pathological conditions affecting the CNS such as cerebral ischemia and Alzheimer's disease [139]. This opens the possibility of the development of neuroprotective estrogenic drugs to support cognitive function and to decrease damage after brain injury [10, 128, 140, 141].

Endogenous Estrogens

Endogenous estrogens are steroid hormones recognized for their participation in the development and maintenance of the female sexual characteristics. There are mainly 3 types of hormonal estrogens: E2, which oc-

Fig. 2. Estrogenic actions on astrocytes. Estrogenic compounds (EC) exert different actions on astrocytes through ER α , ER β and GPR30. (1) Estrogenic compounds may have beneficial effects after brain injury through the regulation of reactive gliosis; (2) increase in the production and release of different molecules, such as neuroestrogens and neurotrophic factors (TrkA, BDNF and ChAT), with the subsequent decrease of neuronal death after brain injury; (3) regulate glutamate transporter GLT-1 and GLAST expression in astrocytes, thus maintaining optimal glutamate levels; (4) reduce the formation of ROS and decrease the loss of antioxidant glutathione, with the subsequent recovery of the mitochondrial membrane potential, ATP production and decreasing cell death; (5) preserve mitochondrial function in astrocytes through ER α / β . Likely, ERs may inhibit (a) apoptotic signalling and (b) regulate the expression of mitochondrial proteins, proton channels and subunits of key enzymes of the respiratory chain, as well as by eliminating radical oxygen species (ROS); and (6) decrease the inflammatory response in astrocytes reducing neuroinflammation through a decrease in the expression of IL-1 β , IL-6, tumor necrosis factor (TNF- α) and TNF- β .

curs in higher concentrations in women of fertile age, estrone (E1) that predominates in menopausal women and estriol (E3), which is the primary estrogen during pregnancy [142]. The most potent endogenous estrogen is estradiol, which is produced by the growing follicle and its levels vary across the menstrual cycle, peaking just before ovulation [143]. The placenta, adrenal glands, testicles and adipose tissue are also estrogen-secreting organs [143]. Estrogens are also reported in men in the form of estradiol, which is produced by peripheral aromatization of testosterone in adipocytes and tissues that require the action of estrogens such as bones and the brain [144]. Hormonal estrogens act through their binding to the classical alpha and beta ERs (ER α , ER β). These classical ERs are ligand-activated transcription factors that once bound to estrogens form dimers that interact with ER response elements (EREs) located in the promoter regions of some genes [145]. In addition, classical ERs can modulate other genes without interacting with ERE regions by the regulation of different signalling pathways [142]. Furthermore, hormonal estrogens may also activate different signalling pathways acting on G-protein coupled ER [146].

ERs are expressed in different tissues, being predominant the ER α in women with greater expression not only in the uterus, but also in liver, adipose tissues, skeletal muscle, pituitary and hypothalamus, while ER β is present in ovaries, prostate and in the brain [147], suggesting an important role of ERs in different tissues [148].

For many years, it has been known that hormonal estrogens act in different organs such as bones, heart and brain, exerting effects beyond the control of reproductive function [149]. ERs have been identified in diverse brain regions such as the neocortex, amygdala and hippocampus, suggesting an effect of hormonal estrogens on cognition, mood and other general brain functions. Furthermore, the expression of ERs has been identified in microglia, neurons and astrocytes [122, 149–151].

Phytoestrogens

Phytoestrogens are estrogenic molecules produced by plants that are involved in vegetal development, healing and reproduction. Phytoestrogens are mainly found in the Leguminosae family and occur in different parts of the plant such as seeds and fruits [152]. Phytoestrogens are

classified into 4 groups: isoflavones, lignans, coumestans and stilbenes, and these can be obtained through diet [153]. Isoflavones have been extensively studied, among which daidzein and genistein are the most recognized compounds and are found mainly in soy and red clover. Lignans are found in linseed, in grains such as wheat, rye and oat, and in various types of berries. Coumestans are present in small amounts in red clover and alfalfa, and lower concentrations are found in lima bean and sunflower seeds [154]. Of the stilbenes, the most recognized is resveratrol that is present in the skin of grapes, peanuts and cranberries [153, 154].

Once in the intestine, intestinal bacteria hydrolyze phytoestrogen to the aglycone form by removing the sugar residue(s). The aglycones are absorbed by the enterocytes, conjugated in the liver and released into the blood. In some cases, metabolites of phytoestrogens are absorbed easier than the parent compound; for example, phytoestrogen enterolactone is produced from lignanosecoisolariciresinol by the action of colon bacteria, which is then absorbed by the enterocytes and released into the bloodstream [152].

Phytoestrogens have attracted the attention because of their structural similarity with estradiol. This particular characteristic has positioned the phytoestrogens as natural alternatives for the treatment of menopausal symptoms. Phytoestrogens are also considered potential complementary treatments for osteoporosis, cardiovascular diseases and CNS diseases [154]. The neuroprotective activity of phytoestrogens may be mediated by the affinity of these compounds to ERs, in particular to ER β [154].

Few studies have focused on determining the effect of phytoestrogens on astrocytes. Among phytoestrogens, the most studied have been genistein and daidzein, which increase iron transport, increased as a result of oxidative stress [155], protect DNA and mitochondrial function [156], and exert anti-inflammatory effect by inducing the expression of antioxidant enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase (COXs) [157] and peroxisome proliferator-activated receptor (PPARs) [158] in different astrocytic cell lines.

Studies performed by Soltani et al. [159] have revealed a protective effect of the isoflavone genistein against TBI by inhibiting the development of cerebral oedema, suppressing the increase of intracranial pressure and diminishing motor damage. Genistein has also shown to reduce excitotoxic neuronal death in the rat hippocampus, induced by the systemic administration of kainic acid [122]. Similarly, resveratrol has been shown to protect brain

functions against hypoxia-ischemia induced by neonatal rat brain injury that causes impairment of sensorimotor abilities, neurological reflexes, learning and memory [160]. Despite the above-mentioned evidence, more research is needed to better understand the mechanisms underlying the action of phytoestrogens in the brain, in order to implement the use of these compounds for controlling the symptoms of estrogen deficiency and protecting brain function in postmenopausal women.

Synthetic Estrogenic Compounds

Selective ER Modulators

SERMs are synthetic molecules, which depending on the tissue act as ER agonists or antagonists. The first synthesized SERM was tamoxifen. This molecule was first considered an antagonist of ERs due to its antagonistic effect in ER-positive breast cancers. Later, it was discovered that tamoxifen acts as an ER agonist in other tissues and the concept of SERM was developed. Nevertheless, it is possible to find in the market first-generation SERMs, such as tamoxifen and toremifene that are used in the treatment of breast cancer. The second-generation drug, raloxifene, is an antagonist of ERs in ER-positive uterine and mammary cancer cells, while it is used as an ER agonist in bone for the prevention of osteoporosis. Finally, there are also third-generation SERMs including bazedoxifene, lasofoxifene and ospemifene that are being used primarily in combination therapies with other estrogenic compounds for the treatment of postmenopausal symptoms [161, 162].

The binding of SERMs to the ligand-binding domain of ERs causes specific conformational changes in the receptors that determine which tissue-specific coregulators of transcription could be recruited to the transcriptional complex [162]. Thus, the transcriptional response to SERMs varies according to the specific SERM, the specific ER and the presence of tissue-specific transcriptional cofactors, resulting in different transcriptional outcomes in different tissues and organs [162, 163].

SERMs have been extensively studied for their agonist activities and selective effects in the brain, acting on neurons, glial cells, astrocytes and microglia. Some SERMs have neuroprotective effects by acting as antioxidants, decreasing neuroinflammation and gliosis, stimulating neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF), regulating mitochondrial functions and promoting neuronal survival [8, 128, 164]. In addition, SERMs, such as raloxifene and tamoxifen, have been shown to be neuroprotective in animal models of Parkinson's disease by stimulating the phosphatidyl-

inositol 3-kinase (PI3K)/Akt/GSK3 β signalling pathway [161, 165]. Furthermore, tamoxifen promotes functional recovery in animal models of spinal cord injury [270].

Tibolone

Tibolone is a synthetic steroid that belongs to a group of molecules so-called selective tissue estrogenic activity regulators (STEARs), which have estrogenic, progestogenic and androgenic properties. Selective tissue estrogenic activity regulators, in particular tibolone, are also used in hormone therapy aiming at the reduction of postmenopausal symptoms [166].

When tibolone is metabolized, 3 steroid compounds are produced: delta-4 isomer, a 3-alpha-hydroxy metabolite and a 3-beta-hydroxyl metabolite. The 3-OH metabolites bind to ER, and the delta-4 isomer binds to both androgen and progesterone receptors [167]. A study performed on ovariectomized cynomolgus monkeys treated orally with 0.5 mg/kg/day tibolone for 36 days showed an increase of estrogenic metabolites in the hypothalamus that was correlated with a reduction in the number and severity of hot flushes and perspirations. The combined effect of tibolone on androgen receptor and ERs is responsible for improving the libido, mood and general well-being [168]. Also, it has been reported in an in vivo study that an increase in antioxidant activity may underlie the neuroprotective effects of tibolone. Specifically, the treatment of Wistar adult rats with 1 mg/kg of tibolone for 30 days prevented the oxidation of membrane lipids and proteins in the hippocampus. Furthermore, the administration of the same dose of tibolone for 60 days decreased neuronal cell death caused by ozone in the CA3 region of the hippocampus [264]. Given the above-mentioned findings, tibolone has also been proposed as a therapeutic agent for brain damage including TBI because of the ability of its metabolites to act on different sex steroid receptors, such as ER α and ER β , and improving physiological functions. In this regard, astrocytes are postulated as mediators of the action of tibolone in the CNS [118]. Indeed, a recent study has shown that tibolone reduces reactive astrogliosis in a model of TBI in ovariectomized female mice [169].

Neuroprotective Actions of Estrogenic Compounds after TBI

Estrogenic compounds reduce the extent of the injury, the sensorimotor and working memory deficits, and reactive astrogliosis after TBI [123, 124, 133, 170–175]. For

instance, estradiol has been shown to reduce intracranial pressure, oedema formation and BBB permeability in female rats after TBI [176]. The SERM raloxifene increases neurotransmitter receptor binding in brain regions associated with cognition and memory, and enhances the transcription and protein levels of neurotrophic factors such as tropomyosin receptor kinase A (TrkA), BDNF and ChAT in experimental models of TBI in rats [177]. Another SERM, tamoxifen, has been shown to reduce tissue damage and promote functional recovery after traumatic injury of the spinal cord [202]. The phytoestrogen resveratrol reduced neuronal loss in all ipsi- and contralateral hippocampal regions while improving exploratory activity and memory abilities in albino Wistar rats submitted to TBI [178]. Another phytoestrogen, genistein, reduced cerebral oedema, BBB permeability and intracranial pressure while improving motor conditions [159]. Similar protective actions have been reported for puerarin, an isoflavone glycoside isolated from the Chinese herb *Radix Puerariae* [179, 180].

Mechanisms Involved in the Neuroprotective Actions of Estrogenic Compounds after TBI That Are Mediated by Astrocytes

In vitro studies indicate that astroglial cells are directly or indirectly regulated by estrogenic compounds. Direct actions have been demonstrated in primary rodent astrocytes cultures where, for instance, estradiol regulates the activation of ERK1/2, PI3K and NF- κ B signalling [181, 182]. That astrocytes may be direct targets of estrogenic compounds is also suggested by the finding that they express ERs both in vitro [181, 183–188] and in vivo, in particular after brain injury [14, 128, 151, 164, 189–195]. In addition, there is evidence that estradiol anti-inflammatory and protective effects in experimental autoimmune encephalomyelitis is dependent on the expression of ER α in astrocytes [196–198], indicating that these cells may mediate neuroprotective actions of estrogenic compounds. In the next subsections, we review the mechanisms regulated by estrogenic compounds in astrocytes that may mediate neuroprotection after TBI (Table 4).

Regulation of Astrogliosis

Estrogenic compounds may have beneficial effects after brain injury through the regulation of reactive gliosis and the production of different molecules by reactive astrocytes [123]. In vivo studies have shown that several estrogenic compounds, such as estradiol, phytoestrogens (genistein), some SERMs and tibolone, decrease reactive

Table 4. Estrogenic compounds and mechanisms of astrocytic-induced neuroprotection in TBI

EC	Compound	Model	Mechanisms	Protective effect	References
Natural Estrogens	Estrogen (33.3 mg/kg and 1 mg/kg)	Albino N Mary rats	Inflammation by IL-1 β , TGF- β	Preventing BBB disruption, decreasing the brain level of IL-1b and increasing the level of TGF- β Co-administration has an inhibitory effect on brain edema	[226, 259]
	17 β -estradiol (1–5 mg/kg)	Sprague Dawley rats model of TBI (Induction of lateral fluid percussion)	PI3K/Akt1 mediated by GPER IL-1 β , IL-6, TNF, TGF- β caspase-3 GFAP	Decreases neuronal degeneration, astrogliosis, programmed cell death and the cleavage of caspase-3 in the cortical pericontusional zone, enhances ER α expression Regulation of mitochondrial calcium sequestration Reduce oxidative stress and levels of proinflammatory cytokines	[143, 228, 260, 261]
	Estradiol	Rodent model of TBI	estrogen receptors	Decreases cortical reactive astrogliosis and reduce the extent of injury	[123]
Synthetic estrogens	Tibolone (10 nM)	Human Astrocyte cell model and microglial cells subjected to inflammatory stimuli	Neuroglobin and NF- κ B mediated by ER β	Decreases cell death, nuclear fragmentation, superoxide ion production, mitochondrial membrane potential and cytoplasmic calcium concentration	[120, 121, 130, 131]
	Tibolone (1 mg/kg)	Rats exposed to brain oxidative stress	Increased SOD through the ER	Reduces the formation of ROS and the oxidation of lipids and proteins in CNS Increased the overall antioxidant capacity in the cerebral cortex and hippocampus Prevents cognitive deficits in short- and long-term memory and enhances cognitive performance	[262–264]
	Tibolone (2.5 mg/day)	Women who for 10 years had been taking tibolone	ND	Improves the semantic memory	[265]
SERMS	Raloxifene (<10 μ M)	Primary cultures of Astrocytes	Inhibitory action on CCL20 expression and NF- κ B ER α	Reduction of neuroinflammation and reactive astrogliosis Reduces neuronal injury and increase anti-apoptotic caspase-3-independent actions	[172, 173, 225]
	Raloxifene (1 μ M)		ERK, EGFR, and CREB NF- κ B, MAPK and PI3K mediated by ER- α , ER- β , and GPR30	Exerts neuroprotection through increased glutamate uptake and expression of GLT1 mRNA and protein levels in astrocytes	[185, 196, 197, 266]
	Tamoxifene (1 μ M)		TGF- α signaling, CREB, NF- κ B and PKA mediated by ER- α , ER- β , and GPR30	Neuroprotection by enhanced of astrocytic GLT-1 expression	[196, 197, 267]
	Raloxifene (3.0 mg/kg)	Rodent model of TBI (bilateral cortical contusion) injury	MAPK/ERK1/2	Reduces sensorimotor and working memory deficits following TBI Enhances neurogenesis and spine density	[12, 133, 268, 269]
	Tamoxifen (1 mg/kg)	Rodent model of TBI	$\uparrow$ P-ERK1/2, Bcl2 and $\downarrow$ Bax mediated by ER- α	Improved motor function, increased spared tissue, reduced TBI-induced cerebral infarction volume and Decrease the formation of ROS, cell apoptosis and neuronal loss	[174, 270]
	Raloxifen and Tamoxifen (1 mg/kg)		ND	Decrease the number of reactive astrocytes after TBI Decreases microglial activation and production of pro-inflammatory cytokines	[48, 123, 170, 271]

Table 4 (continued)

EC	Compound	Model	Mechanisms	Protective effect	References
Phyto-estrogens	Resveratrol (100 mg/kg)	Wistar Albino rats	Antioxidant activity by Xanthine oxidase, glutathione, lipid peroxidation	Improved locomotor activity, memory maintenance, decreased anxiety, avoid cell death Reduced MDA, XO and NO levels, increased GSH level	[178, 217]
	Genistein (15 mg/kg)	Male Wistar rats	Antioxidant and anti-inflammatory mechanisms by ERs and Glucocorticoid receptor	Reversal of brain edema formation, restores BBB disruption, decreases ICP levels, recovered motor function Prevented the excessive activation of microglia	[159, 272]
	Puerarin (200 mg/kg)	Sprague-Dawley rats	Antioxidant activity, Regulation of apoptotic molecules by PI3K-Akt	Reduced TBI-induced neuronal degeneration, increase the Akt phosphorylation, improve endogenous antioxidant defense, ameliorate the cellular membrane damage, decreases neuroinflammation, restores the redox balance	[179]
	3'-Daidzein sulfonate (0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg)	Male Sprague-Dawley rats	Apoptosis by Bcl-2, Bax and caspase-3	Preventing BBB disruption and reduced swelling of astrocytes and pericytes in the BBB, enhanced the expression of Bcl-2, and inhibited the expression of Bax and caspase-3	[273]

IL, Interleukin; TGF- β , Transforming growth factor-beta; PI3K/Akt, Phosphatidylinositol 3-kinase pathway; GFAP, Glial Fibrillary Acidic Protein; NF- κ B, Nuclear factor κ B; SOD, Superoxide Dismutase; CCL20, C-C motif chemokine ligand 20; EGFR, epidermal growth factor receptor; CREB, cAMP response element-binding.

astrogliosis and glial scar formation after TBI [123, 169–171, 199–201]. After spinal cord injury, tamoxifen has been shown to first increase astrogliosis at day 2 after injury and then gradually decrease astrogliosis [202]. This suggests that tamoxifen may accelerate the process of astrogliosis, increasing reactive astrocytes in the acute injury phase, where astrocytes may release trophic factors to protect neurons, and decrease reactive astrocytes in the chronic injury phase.

Regulation of Excitotoxicity

Glutamate transporters are related to excitotoxic brain injury and neurodegenerative diseases. Excitotoxic damage is produced by a dysregulation of astrocytic glutamate carriers, such as glutamate transporter-1, which is responsible for absorbing the majority of glutamate from the synaptic cleft, maintaining optimal glutamate levels [203]. Excessive extracellular glutamate leads to a dysregulation in calcium, sodium and potassium fluxes, which may open the mitochondrial permeability transition pore. The opening of the mitochondrial pore could liberate ROS and proteins that may lead to apoptosis [203, 204].

Estradiol, other several estrogenic compounds and genistein have been shown to decrease excitotoxic damage

in the brain in vivo [122, 126, 205–209]. Estradiol and tamoxifen increase the expression of the glutamate transporters glutamate aspartate transporter (GLAST) and glutamate transporter-1, increasing glutamate uptake by astrocytes [181, 196, 197]. This estrogenic action is mediated by ERs, including G-protein coupled ER, and the PI3K and Mitogen-activated protein kinase signalling pathways [185, 196, 197, 210]. The phytoestrogen resveratrol increases glutamate uptake and glutamine synthetase activity in primary cortical astrocytes [211]. This action may be involved in the protective actions of phytoestrogens against excitotoxic damage [159, 211, 212].

Regulation of Oxidative Stress

Different estrogenic compounds, such as tamoxifen, hydroxytamoxifen, genistein and raloxifene, have been shown to control oxidative stress in the brain tissue [155, 159, 213, 214]. This action is exerted by increasing the activities of several detoxifying enzymes such as superoxide dismutase, catalase and glutathione peroxidase [156, 159, 212, 215]. In astrocytes, estrogenic compounds modulate mitochondrial gene expression, increase clearance of ROS and decrease the loss of antioxidant glutathione [10, 179]. In an in vitro model of ischemia (oxy-

gen-glucose deprivation/reperfusion) in primary astrocytes 17 β -estradiol and tibolone decreased the production of ROS, recovered the mitochondrial membrane potential and ATP production and decreased cell death induced by mitochondrial dysfunction [216]. The actions of estrogenic compounds on oxidative stress after brain injury may be associated with the reduction in edema formation [217]. For instance, resveratrol decreases lipid peroxidation in the trauma zone and it is known that lipid peroxidation contributes to ischemia and cerebral edema [160, 217], and worsens the prognosis of patients with TBI. Additionally, genistein and daidzein increase iron transport, as a result of oxidative stress [155], protect DNA and mitochondrial function [156], and exert anti-inflammatory effect by inducing the expression of antioxidant enzymes such as thioredoxin, MnSOD, iNOS and COXs [157] and PPARs in different astrocytic cell lines [158].

Regulation of Mitochondrial Function

An important neuroprotective mechanism of estrogenic compounds is the regulation of mitochondrial function [218–220]. Mitochondria are vital for the maintenance and homeostasis of different brain cells and their dysfunction has been associated with several neurological disorders [221, 222]. Some investigations suggest that mitochondria can be regulated by estrogenic compounds and their interactions with ERs. For example, it has been demonstrated that ER β is located in the mitochondria of various brain cells including astrocytes [223]. Therefore, it has been proposed that the neuroprotective actions of estrogenic compounds linked to mitochondria are mediated by ER β [223]. ER β located in the mitochondria may prevent mitochondrial expression of apoptotic molecules and regulate the function and production of other proteins involved in the reduction of ROS [219].

Regulation of Neuroinflammation

Astrocytes and microglia play an important role in inflammatory responses after TBI, as these cells release growth factors, cytokines and chemokines. These molecules could act as modulators for the beginning and progression of post-traumatic inflammation. Thus, the participation of these cells may be causing extra cell death and neurological damage [8, 164, 224]. Estrogenic compounds, like estradiol, SERMs and phytoestrogens, act on astrocytes and microglial cells reducing neuroinflammation through a decrease in the expression of IL-1 β , IL-6, tumour necrosis factor (TNF- α) and (TNF- β) [131, 225–

228]. The anti-inflammatory actions of estrogenic compounds on these cells begin with the activation of signaling pathways (Mitogen-activated protein kinase, PI3K, and Akt), which in turn control the activity of transcription factors such as cAMP response element-binding and NF- κ B [119, 225], a powerful transcriptional regulator of several inflammatory genes [224].

Concluding Remarks

It is clear that the role of astrocytes on TBI is dependent upon the stage of the pathological mechanism. This is especially important when designing potential protective strategies aimed at controlling reactive astrogliosis and astrocytic double-edged functions. This review highlights the neuroprotective actions of estrogenic compounds and their action on astrocytes in the context of TBI. The response of the wounded nervous tissue to estrogenic compounds is contingent on the interface of multiple cell types, like microglia, tanycytes and astrocytes. One of the most important cells is astrocytes that play vital roles in TBI and in post-TBI synaptic plasticity. This response is determined by specific signalling mechanisms and depends on the nature and severity of tissue damage. Besides, astrocytes have shown to be targets of estrogen and estrogenic compounds and are postulated to have a key role in estrogen-mediated protection of the brain. These compounds have a promising potential as a target for therapeutic approaches for the prevention of cognition decline and neurodegeneration caused by brain trauma. However, further studies are needed to deepen the mechanisms of action of estrogenic compounds in astrocytes and how they trigger a neuroprotective response in TBI.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

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