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Review

Development of new treatments for Alzheimer's disease based on the modulation of translocator protein (TSPO)

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ABSTRACT

The increase in life expectancy of the world population is associated with a higher prevalence of neurodegenerative diseases. Alzheimer's Disease (AD) is the most common neurodegenerative disease, affecting currently 43 million people over the world. To date, most of the pharmacological interventions in AD are intended for the alleviation of some of its symptoms, and there are no effective treatments to inhibit the progression of the disease. Translocator protein (TSPO) is present in contact points between the outer and the inner mitochondrial membranes and is involved in the control of steroidogenesis, inflammation and apoptosis. In the last decade, studies have shown that TSPO ligands present neuroprotective effects in different experimental models of AD, both *in vitro* and *in vivo*. The aim of this review is to analyze the data provided by these studies and to discuss if TSPO could be a viable therapeutic target for the development of new treatments for AD.

1. Introduction

The increase in life expectancy of the world population is associated with a higher prevalence of neurodegenerative diseases. Alzheimer's disease (AD) is the most common neurodegenerative disease and the major cause of dementia in people aged 60 years or older, affecting around 43 million patients over the world (Querfurth e LaFerla, 2010; GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017). In the USA, the estimated cost of the care and treatment of patients with dementia, including non-AD associated dementia, is of 290 billion dollars for the year 2019 (Alzheimer's Association, 2019).

Despite the great impact caused by AD in the society, currently there are no available therapies that act on the pathogenic mechanisms of the disease to avoid its progression. The pharmacological treatment of AD is currently based on the use of two different drug classes: acetylcholinesterase inhibitors (*e.g.*rivastigmine, galantamine and donepezil) and the NMDA receptor antagonist memantine. However, the benefit associated with the use of these drugs is small, and they are only able to help in the control of some of AD symptoms (Sugino et al., 2015). Consequently, while the mortality rates associated with cardiovascular diseases and stroke in the USA have decreased by 11% and 16%, respectively, in the period between 2000 and 2015, the mortality rate associated with AD has increased by 123% in the same period. These numbers show that the available treatments for AD patients are ineffective. Indeed, AD is currently the sixth major cause of death in the USA and the fifth cause in people over 65 years old (Alzheimer's Association, 2019). Therefore, it is necessary to find new strategies for the treatment of AD and other diseases associated with dementia.

In the last decade, several clinical trials were performed trying to identify the efficacy of new drugs in the treatment of AD. However, despite the large investment, no disease-modifying drug for AD has been approved. Examples of drugs that presented promising effects in preclinical studies but that have failed when tested in phase 3 clinical trials include verubecestat (Egan et al., 2018) and semagacestat (Doody et al., 2013), which inhibit, respectively, β-secretase and γ-secretase, which are the enzymes involved in Aβ production, and the anti-Aβ antibodies bapineuzumab (Salloway et al., 2014; Vandenberghe et al., 2016) and solanezumab (Honig et al., 2018). Several factors have been associated with the failures of these trials, such as the inclusion of

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patients in late stages of AD in these trials, as well as an inadequate understanding of AD pathophysiology, leading to the selection of wrong treatment targets (Anderson et al., 2017). Therefore, in addition to studies aiming to increase our understanding of AD pathophysiology, it is important to search for agents acting not only in the amyloid cascade and in the reduction of Aβ accumulation, but also in other molecular mechanisms involved in AD progression, including neuroinflammation, oxidative stress and mitochondrial dysfunction.

Translocator protein (TSPO) is a mitochondrial membrane protein that participates in several cellular functions, including the regulation of inflammation, apoptosis, and mitochondrial respiration (Papadopoulos and Lecanu, 2009). TSPO is also involved in the regulation of the cholesterol transport inside the mitochondria, which is the rate limiting step in steroidogenesis (Rone et al., 2012; Fan et al., 2015; Barron et al., 2018; Fan et al., 2018).

In the last decade, several studies have reported that TSPO ligands are neuroprotective in different experimental pathological conditions, including models of traumatic brain injury and neurodegenerative diseases (Arbo et al., 2015). Specifically, considering AD, different research groups have shown that TSPO ligands are neuroprotective in both *in vitro* (Arbo et al., 2016b, 2017) and *in vivo* experimental models of this disease (Barron et al., 2013; Ma et al., 2016; Christensen and Pike, 2018). The aim of this review is to discuss recent findings regarding TSPO function and whether the modulation of this protein could be used for the development of new treatments for AD.

2. TSPO structure and functions

TSPO is an 18 kDa protein located in contact sites between the outer and inner mitochondrial membranes. It was formerly known as peripheral benzodiazepine receptor (PBR) due to its capacity of binding to diazepam (Papadopoulos et al., 2006). TSPO is formed by 169 amino acids that are arranged in five alpha-helical domains and presents a high affinity for cholesterol, due to the presence of a cholesterol recognition amino acid consensus (CRAC) domain in its C-terminal region (Li and Papadopoulos, 1998; Jaremko et al., 2014). It is important to mention that TSPO sequence is well conserved among different species (Jaremko et al., 2014), and this may have a positive impact on translational approaches towards the development of therapeutic agents targeting this protein.

Experimental studies have shown that, in healthy states, TSPO is expressed by several peripheral organs, including the gonads, adrenal, lung, kidney, spleen, liver and heart (Banati et al., 2014; Wang et al., 2016). In the brain, however, TSPO expression is relatively low, with this protein being found especially in glial cells and at lower levels in neurons (Chen and Guilarte, 2008; Papadopoulos and Lecanu, 2009; Betlazar et al., 2018). On the other hand, it is known that TSPO expression increases in reactive astrocytes and microglia at sites of neuroinflammation associated with pathological conditions, as for example in AD (McNeela et al., 2018).

Functionally, one of the most studied functions of TSPO is its role in cholesterol transport inside the mitochondria, thereby regulating steroidogenesis. In this context, studies have shown that TSPO associates with other mitochondrial proteins, such as the voltage-dependent anion channel (VDAC), the ATPase family AAA Domain-containing protein 3 (ATAD3) and steroidogenic acute regulatory protein (StAR), forming a structure called transduceosome, which regulates cholesterol transport into the mitochondria and its metabolism (Midzak et al., 2011; Rone et al., 2012). Recent studies based on crystal structure of TSPO have suggested that cholesterol transport by this protein complex may involve a sliding mechanism on the external protein surface (Li et al., 2015), challenging previous studies that hypothesized that TSPO could function as a channel (Lacapère and Papadopoulos, 2003).

As previously mentioned, the transport of cholesterol into the mitochondria is the first and the rate-limiting step in steroidogenesis. Since TSPO is thought to regulate the cholesterol entrance into the mitochondria, it is expected that this would lead to the regulation of steroidogenesis (Midzak et al., 2011; Rone et al., 2012). Indeed, this role was confirmed by studies showing decreased steroid formation after the knockout or silencing of TSPO in cell lines (Papadopoulos et al., 1997; Kelly-Hershkovitz et al., 1998; Hauet et al., 2005). Although TSPO is not essential for basal steroidogenesis in gonads and the adrenals (Banati et al., 2014; Tu et al., 2014; Fan et al., 2015), it is necessary for the action of the mechanisms that regulate steroidogenesis (Fan et al., 2015, 2018), its mutations in rats and a polymorphism in humans are associated with impaired steroidogenesis (Owen et al., 2017) and its deletion causes steroidogenic abnormalities that become exacerbated with aging (Barron et al., 2018).

TSPO is also involved in the control of other mitochondrial functions, including mitochondrial respiration, apoptosis and cell proliferation (Hirsch et al., 1989; Azarashvili et al., 2007; Veenman et al., 2007; Corsi et al., 2008). In addition, it has been suggested that TSPO participates in the opening of the mitochondrial permeability transition pore (MPTP) (Sileikyte et al., 2011), although some studies have questioned this function (Sileikyte et al., 2014). Although further research is required for a better understanding on TSPO functions, TSPO ligands have shown neuroprotective activity in different experimental models of neurodegenerative disease, suggesting that the modulation of this protein could represent a viable molecular target for the development of new treatments for AD.

3. TSPO as a molecular target for the development of new treatments for AD

The idea that TSPO could be used as a target for neuroprotective strategies is directly related with its location and functions. Compounds that act on the mitochondria have been suggested as potential therapeutic agents in neurodegenerative diseases, since the most prominent roles of this organelle are the regulation of cellular metabolism and energy production, which are essential to keep the integrity of brain structure and function (Lin and Beal, 2006; Lee, 2016). Indeed, mitochondrial dysfunction has been reported as an early event that leads to compromised energy supply and antioxidant response, contributing to neurodegeneration in AD (Hall et al., 2017).

Both *in vitro* and *in vivo* studies have been performed in order to evaluate the neuroprotective actions of TSPO ligands in experimental models of AD. Two studies *in vitro* have tested the neuroprotective activity of the TSPO ligand 4′-chlorodiazepam (4′-CD, also known as Ro5-4864) against Aβ neurotoxicity (Arbo et al., 2016b, 2017). In the first study, it was demonstrated that 4′-CD protects SH-SY5Y neuroblastoma cells against Aβ neurotoxicity (Arbo et al., 2016b). More recently, it has been shown that 4′-CD also protects organotypic hippocampal cultures against Aβ (Arbo et al., 2017).

The neuroprotective activity of 4′-CD has been also demonstrated in an *in vivo* model of AD. Actually, the first study suggesting that TSPO ligands could be neuroprotective against AD is the study of Barron et al. (2013). In this study, the authors have shown that the administration of 4′-CD attenuated hippocampal Aβ accumulation and decreased gliosis in 3xTgAD mice of 7 and 24 months of age. These changes were associated with improved spontaneous alternation behavior in the Y-maze, a hippocampal-dependent measure of attention and working memory, in 3xTgAD mice of both ages. The idea that TSPO may be associated with the regulation of Aβ levels has been also suggested by Tan et al. (2018), which showed that decreased levels of TSPO are associated with increased Aβ deposition in rats chronic treated with lorazepam.

Recently, Christensen and Pike (2018) showed that PK11195 presented anxiolytic effects and improved spontaneous alternation behavior in the Y-maze in female 3xTgAD mice. The study from Christensen and Pike (2018) is important since it extended to females the beneficial actions exerted by TSPO ligands against AD-related pathology. In addition, the fact that another TSPO ligand also presented beneficial effects in this experimental model of AD reinforces the idea that this

protein could be a viable target for the development of new drugs for the treatment of this disease. In this regard, it is important to mention that other TSPO ligands are also neuroprotective *in vitro* against Aβ neurotoxicity (Kim et al., 2017a, b).

Kim et al. (2017a, b) performed a series of studies involving the screening of the potential neuroprotective actions of a significant number of TSPO ligands in HT22 mouse hippocampal cells exposed to Aβ. The authors have shown that the thienopyrrolotriazine derivatives bind to TSPO in a similar way to 4′-CD, however, despite the excellent neuroprotective *in vitro* activity of some of these compounds, they presented a poor liver microsomal stability, and due to this reason, they were not evaluated *in vivo* (Kim et al., 2017a).

On the other hand, after a screening *in vitro* two benzimidazole derivatives (compounds 25 and 38) were evaluated in a mouse AD model, in which animals were treated with intracerebroventricular injections of Aβ. In this context, it was observed that the intraperitoneal administration of the two benzimidazole compounds for 6 days significantly improved the cognitive function of AD mice in the Y-maze spontaneous alternation test (Kim et al., 2017b). In addition, the authors orally administered one of these compounds (compound 25) to transgenic AD mice (APPswe/PSEN1 dE9 2X), and observed that it only partially restored fear-associated learning and memory. It should be mentioned that pharmacokinetic studies showed that this compound is BBB permeable, but presents a low oral bioavailability (3.7%), and this could explain its lower efficacy when orally administered, in comparison to its superior protective activity after intraperitoneal administration (Kim et al., 2017b). Therefore, further studies involving the development of potent thienopyrrolotriazine and benzimidazole derivatives with a more favorable pharmacokinetic profile may provide novel candidate compounds for the treatment of AD.

4. Potential cellular mechanisms involved in the neuroprotective actions of TSPO ligands

4.1. Cholesterol homeostasis

Although the brain represents only 2% of the total body mass, it contains almost 25% of the total amount of free cholesterol in the human body (Vance et al., 2006; Giudetti et al., 2016). Due to the presence of the blood-brain barrier (BBB), which prevents the import of cholesterol from the periphery, most of brain cholesterol is locally synthesized (Vance et al., 2006). Although it has been shown that neurons can synthesize cholesterol, astrocytes are the main source of cholesterol in the brain, delivering it to neurons through a cholesterol shuttle (Orth and Bellosta, 2012; Zhang and Liu, 2015).

Several studies have shown that changes in cholesterol levels and turnover may be involved in the development of AD. One key protein involved in cholesterol homeostasis is apolipoprotein E (apoE), which is also able to regulate Aβ aggregation and clearance in the brain (Sparks et al., 1996). The apoE gene presents three major alleles in humans: apoE ε2, apoE ε3, apoE ε4. Jiang et al. (2008) showed that apoE promotes the proteolytic degradation of Aβ, however, apoE ε4 seems to be less effective than other isoforms in the promotion of Aβ clearance, being particularly important in AD etiology. Therefore, the presence of copies of this allele increases the risk of developing AD. In fact, at least 50% of patients with AD present at least one apoE ε4 allele (Verghese et al., 2011; Liu et al., 2013; Giudetti et al., 2016).

TSPO influences cholesterol homeostasis in the brain by its role in steroidogenesis, facilitating the metabolism of cholesterol to synthesize neuroactive steroids. In addition, TSPO may protect cell viability by decreasing intracellular cholesterol accumulation and regulating its intracellular distribution, reducing its accumulation in the mitochondria (Taylor et al., 2014; Musman et al., 2017). For instance, TSPO ligands induce the redistribution of intracellular cholesterol in astrocytes, promoting its accumulation in lipid droplets in parallel to a decrease in cholesterol esterification (Falchi et al., 2007). In this regard, it is important to mention that Aβ homeostasis is affected by the balance between free and cholesterol esters. In the brain, cholesterol can be esterified by the enzyme acyl-CoA cholesterol acyltransferase (ACAT), and the accumulation of cholesteryl-esters is able to increase Aβ release, while genetic ablation or pharmacological inhibition of ACAT is associated with decreased levels of Aβ (Puglielli et al., 2001; Hutter-Paier et al., 2004; Bhattacharyya and Kovacs, 2010). Therefore, the reduction of the esterification of cholesterol by TSPO ligands may be involved in the action of these molecules in decreasing Aβ-levels in animal models of AD (see below).

4.2. Regulation of mitochondrial functions, oxidative stress and apoptosis

Mitochondrial dysfunction and oxidative stress are key etiopathological components of AD and aging-associated dementias (Ganguly et al., 2017; Hallé et al., 2017; Cheignon et al., 2018). Mitochondria play a central role in the control of death signals and mitochondrial dysfunction under uncontrolled production of reactive oxygen species (ROS) may result in the stimulation of apoptosis signaling pathways. In addition, studies have shown that increased production of Aβ is associated with impairments in cellular respiration, energy production, and mitochondrial chain complex activities (Rhein et al., 2009; Hallé et al., 2017).

TSPO is involved in the control of cellular bioenergetics, ROS production and apoptosis (Lin et al., 2014; Repalli, 2014; Baez et al., 2017; Hallé et al., 2017) and the neuroprotective action of TSPO ligands in models of AD may in part be mediated by the regulation of these cellular events (Fig. 1). For instance, Aβ promotes mitochondrial dysfunction through the decrease of the mitochondrial membrane potential and ATP production and by the increase of the generation of ROS, while some thienopyrrolotriazine and benzimidazole derivatives with a high binding affinity for TSPO were able to rescue these parameters (Kim et al., 2017a, b). Similar results were reported by Hallé et al. (2017), which showed that several TSPO ligands, including XBD173 and some new imidazoquinazolinone derivatives can increase ATP production in

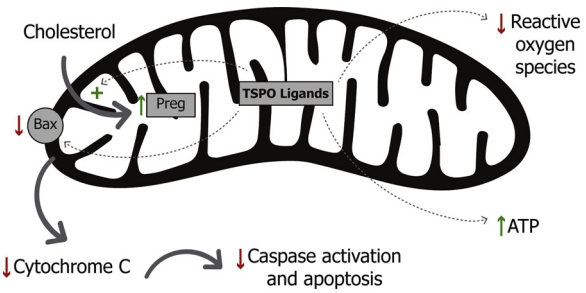

Fig. 1. Mitochondria as a target for the neuroprotective actions of TSPO ligands in Alzheimer's Disease (AD). AD-related neurodegeneration is closely associated with mitochondrial dysfunction. Typical mechanisms involved in the progression of AD include decreased mitochondrial membrane potential and ATP production and increased generation of reactive oxygen species (ROS), as well as increased expression of pro-apoptotic proteins such as Bax, which is associated with increased cytochrome C release, caspase activation and apoptosis. TSPO ligands could exert neuroprotective actions in AD by increasing ATP production and decreasing ROS generation in the mitochondria. In addition, TSPO ligands could decrease the expression of Bax and the release of cytochrome C, inhibiting caspase activation and apoptosis. The modulation of steroidogenesis is another mechanism possibly involved in the neuroprotective actions of TSPO ligands, since some *in vitro* studies demonstrated that the overexpression of amyloid precursor protein (APP) is associated with a decreased production of pregnenolone that could be at least partially restored by TSPO ligands. Pregnenolone then can be converted in other steroids with well-documented neuroprotective activity, such as estradiol, progesterone and allopregnanolone. Finally, it is important to mention that the neuroprotective actions of TSPO ligands in AD may involve other mechanisms not illustrated in this figure, including the regulation of neuroinflammation and Aβ deposition.

neuroblastoma cells overexpressing APP. In addition, the neuroprotective actions of 4′-CD against β-amyloid in organotypic hippocampal cultures seem to involve antioxidant effects (Arbo et al., 2017). Furthermore, TSPO regulates the antioxidant response pathway in different cells, decreasing ROS production and increasing the resistance against oxidative stress under different conditions (Carayon et al., 1996; Wang et al., 2014; Tu et al., 2016; Bonsack and Sukumari-Ramesh, 2018). These data are supported by a recent study from Lejri et al. (2019) showing that imidazoquinazolinone TSPO ligands improve mitochondrial respiration, reduce ROS and oxidative stress-induced cell death in neuroblastoma cells overexpressing APP.

The regulation of apoptosis by TSPO has implications for the development of TSPO-related drugs to promote apoptosis in cancer cells, such as gliomas (Veenman et al., 2014). However, some TSPO ligands, such as the new ligand 2-(2-chlorophenyl) quinazolin-4-yl dimethylcarbamate (2-Cl-MGV-1), have been shown to reduce neuronal apoptosis in different neurodegenerative models (Chen et al., 2017). In addition, *in vitro* studies have shown that the TSPO ligand 4′-CD protect SH-SY5Y neuroblastoma cells against Aβ neurotoxicity by a mechanism that involves an increased expression of anti-apoptotic proteins, such as survivin, and a decreased expression of pro-apoptotic proteins, such as Bax (Arbo et al., 2016b), which is related with increased cytochrome C release, caspase activation and apoptosis.

4.3. Regulation of neuroinflammation

As previously mentioned, although TSPO expression in the brain is low in healthy states, its expression increases in reactive astrocytes and microglia at sites of neuroinflammation associated with pathological conditions, as for example in AD (McNeela et al., 2018). The increase in TSPO expression in reactive glial cells has allowed the use of TSPO ligands as image markers of neuroinflammation, with a positive correlation with amyloid load and TSPO ligand binding (Parbo et al., 2017; Passamonti et al., 2018). Indeed, Tournier et al. (2019) recently demonstrated that the brain levels of TSPO increase before the apparition of amyloid deposits in the 3xTgAD mouse model of AD, and that there is a positive correlation between TSPO levels and amyloid deposits in the subiculum and the dorsal hippocampus of these animals.

Studies by Barron et al. (2013) have shown that the treatment of 3xTgAD mice with the TSPO ligand 4′-CD results in the downregulation of gliosis in the hippocampus, in parallel with a decrease in Aβ accumulation. In agreement with these findings, other authors have also shown that 4′-CD decreases reactive gliosis and presents neuroprotective actions in other experimental models, such as kainic acid excitotoxicity (Veiga et al., 2005).

In contrast, Christensen and Pike (2018) showed that the treatment of female 3xTgAD mice with another TSPO ligand, PK11195, was associated with increased glial activation, including increased levels of IL-6 (Christensen and Pike, 2018), a pro-inflammatory cytokine that has been associated with increased Aβ phagocytosis (Chakrabarty et al. 2010). It is important to mention, however, that PK11195 seems to play a complex role in the regulation of neuroinflammation. Actually, Ma et al. (2016) showed that PK11195 reduces neuroinflammation and Aβ generation and protects against the cognitive dysfunction induced by the systemic administration of lipopolysaccharide (LPS), a molecule found in the cellular wall of Gram-negative bacteria that is widely used for the experimental induction of inflammation. Moreover, Veiga et al. (2007) also reported that PK11195 decreases reactive gliosis in animals treated with LPS, corroborating the idea that this TSPO ligand may decrease glial activation under some circumstances. More recently, Lee et al. (2016) demonstrated that different TSPO ligands reduce the pro-inflammatory response elicited by toll-like receptors (TLR) ligands, with more pronounced effects on microglial cells than astrocytes. These data were actually confirmed by Azrad et al. (2019), which showed that PK11195 and other TSPO ligands are able to suppress the inflammatory response of BV-2 microglial cells exposed to LPS, decreasing the levels of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and nitric oxide (NO). Therefore, the data from these studies suggest that TSPO may present a dual role in the regulation of glial activation. Finally, the study of Baez et al. (2017) indicates that TSPO ligands, such as 4′-CD, activate protective mechanisms in glial cells to preserve its metabolic activity under pathological conditions. Since the impaired homeostatic response of astrocytes and microglia participates in the progression of AD-associated neuronal damage (Dzamba et al., 2016; Chun and Lee, 2018; Hansen et al., 2018), the neuroprotective action of TSPO ligands may be in part mediated by the maintenance of a proper adaptive functionality and immune response of glial cells, rather than by the control of the amount of reactive gliosis.

4.4. Regulation of brain steroidogenesis

TSPO has been classically associated with the regulation of steroidogenesis. Indeed, this protein is highly expressed in steroid-synthesizing tissues, such as the gonads and adrenals (Papadopoulos et al., 2006). The regulation of steroidogenesis seems to be highly relevant for the neuroprotective actions of TSPO ligands, since the brain is a steroidogenic tissue (Guennoun et al., 2015; Liere et al., 2017) and neuroactive steroids, such as estradiol, progesterone, allopregnanolone and dehydroepiandrosterone (DHEA) are neuroprotective under different conditions (Irwin and Brinton, 2014; Arevalo et al., 2015; Arbo et al., 2016a; Cspedes Rubio et al., 2018; De Nicola et al., 2018; Powrie and Smith, 2018; Bourque et al., 2019; Guennoun et al., 2019; Stein and Sayeed, 2019).

The modulation of neuroactive steroid levels by TSPO ligands is associated with its neuroprotective action in different experimental pathological conditions, including rodent models of peripheral nerve regeneration (Lacor et al., 1999), central and peripheral experimental diabetic neuropathy (Giatti et al., 2009; Mitro et al., 2012), neuroinflammation and cognitive dysfunction (Ma et al., 2016) and affective disorders (Costa et al., 2011). However, although 4′-CD modulates the production of neuroactive steroids under some of the studied conditions, there is not a clear correlation between the levels of these steroids with the neuroprotective actions of 4′-CD in a mouse model of AD (Barron et al., 2013), and more studies are needed to clarify if the neuroprotective actions of TSPO ligands in experimental models of this disease are related with the regulation of steroidogenesis. Indeed, trying to clarify this question, Hallé et al. (2017) reported that APP overexpression in neuroblastoma cells is associated with a remarkable decrease in pregnenolone production. Moreover, the authors found that different TSPO ligands, including 4′-CD, XBD173 and some imidazoquinazolinone derivatives are able to improve cellular bioenergetics and increase pregnenolone production (the first steroid that is produced in the steroidogenic pathway after the cholesterol import into the mitochondria) in these cells, corroborating a potential neuroprotective effect of TSPO ligands in AD (Fig. 1). However, it should be mentioned that there was a significant discrepancy in the active concentrations of TSPO ligands for the observation of these effects, since nanomolar concentrations were able to increase ATP production, but pregnenolone production was increased only in the micromolar range (Hall et al., 2017). Finally, it would be essential to identify which specific steroids could be mediating the neuroprotective actions of TSPO ligands, since some compounds, such as some glucocorticoids, could be neurotoxic under some conditions (Virgin et al., 1991; Wolkowitz et al., 2007).

4.5. Regulation of Aβ accumulation

AD is a pathology characterized by two major neuropathological findings: the senile plaques, formed by the aggregation of amyloid-beta (Aβ) peptide, and the neurofibrillary tangles, formed by the aggregation of hyperphosphorilated Tau protein (Querfurth and LaFerla, 2010). The Aβ peptide is formed by the sequential cleavage of the amyloid precursor protein (APP) by the enzymes β- and γ-secretase (Querfurth and

LaFerla, 2010). The amyloid cascade hypothesis for the pathogenesis of AD is receiving increasing support from experimental and genetic studies (Barage and Sonawane, 2015; Luo et al., 2016; Selkoe and Hardy, 2016). Therefore, it is important to determine whether TSPO ligands reduce β-amyloid load in experimental models of AD.

Barron et al. (2013) have shown that in 3xTgAD mice of 7 and 24 months of age, the administration of 4′-CD attenuates hippocampal Aβ accumulation. Similar results have been obtained by Christensen and Pike (2018), which showed that PK11195 decreased Aβ deposition in some brain regions of female 3xTgAD mice. These studies suggest that the neuroprotective action of 4′-CD in AD mouse models may be associated with a reduction in β-amyloid load. Furthermore, PK11195 has also been shown to reduce Aβ accumulation in a mouse model of cognitive dysfunction induced by peripheral LPS administration (Ma et al., 2016). Finally, Lejri et al. (2019) recently demonstrated that several TSPO ligands, including XBD173, SSR-180575 and imidazoquinazolinone derivatives are able to decrease Aβ in HEK SWE APP cells. All these findings suggest that regulation of Aβ may be one of the main mechanisms involved in the neuroprotective actions of TSPO ligands for AD.

5. Conclusion and future directions

In summary, findings from *in vitro* and *in vivo* preclinical studies suggest that TSPO could be a viable target for the development of neuroprotective drugs for the treatment of AD. Although the therapeutic effects of TSPO ligands in AD patients have not been investigated, the fact that TSPO sequence is well conserved among different species may encourage further clinical trials addressing this question. Since TSPO expression is higher in sites of inflammation, targeting this protein could be an interesting strategy for the treatment of diseases related to inflammation, including AD. TSPO activation by its ligands could be associated with decreased Aβ accumulation and AD-related neuroinflammation and mitochondrial dysfunction, leading to improved cognition. It is important to mention that other mechanisms of action may be also associated with the neuroprotective actions of TSPO ligands. More specifically, one important question to be answered by further studies is if the regulation of steroidogenesis is involved in the neuroprotective effects elicited by TSPO activation.

Another important aspect that needs to be adequately addressed is the possible sex differences in the neuroprotective actions of TSPO ligands in AD animal models, since most *in vivo* studies have used male animals. Although it was recently shown that TSPO ligands may be also neuroprotective in females (Christensen and Pike, 2018), more studies evaluating possible sex differences in the actions of TSPO ligands are needed before the conduction of possible clinical trials. Finally, as observed in the studies by Kim et al. (2017a, b), it is important the search and development of new compounds with high binding affinity to TSPO and good safety and pharmacokinetic profiles in order to increase the chances of success of possible clinical trials designed to test their efficacy in AD patients.

Declaration of Competing Interest

None.

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