Therapeutic potential of translocator protein ligands for age-related macular degeneration

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Age-related macular degeneration: Age-related macular degeneration (AMD) is a retinal degenerative disorder, characterized by the irreversible loss of the central vision during ageing. This chronic, progressive disease has been estimated to currently affect around 196 million people worldwide and will increase to 288 million in 2040 (Wong, et al., 2014). Early AMD is defined by the presence of drusen underneath the retinal pigment epithelial (RPE) layer. Late AMD can be divided into two groups, "wet AMD" and "dry AMD", depending on the underlying clinical features. Wet AMD demonstrates a clinic feature of choroidal neovascularization (CNV) in which new blood vessels protrude from the choroid through the Bruch's membrane and interfere with the morphological architecture of RPE and the superficial retina. Wet AMD accounts for approximately 10% of AMD patients, but for around 90% of resultant blind registration. Targeting vascular endothelial growth factor treatment greatly suppresses CNV progression in most wet AMD patients. Dry AMD is characterized by geographic atrophy and no effective therapy is available for dry AMD patients (Pikuleva and Curcio, 2014; Wong et al., 2014).

The major pathological feature of AMD is the abnormal accumulation of extracellular deposits (called drusen) in the macular area, underneath the RPE layer. These deposits block normal exchange of nutrition and waste between choroidal capillary and RPE cells, resulting in initial dysfunction and later death of the RPE cells and subsequent photoreceptor degeneration. With age, the Bruch's membrane becomes thicker with progressive accumulation of lipids; there is also a progressive increase in intracellular lipid deposits in the RPE cells, which is proposed to damage retinal metabolism. Histopathological analyses of the eyes of AMD patients have demonstrated the presence of apolipoproteins, cholesterol and cholesteryl ester deposits beneath the RPE, implicating abnormal cholesterol transport in the progression of the disease (Pikuleva and Curcio, 2014). Genome-wide association studies have also shown that hepatic lipase C and cholesteryl ester transfer protein, key genes involved in the metabolism of triglycerides and high-density lipoproteins (HDL), are implicated in the pathogenesis of AMD.

Removal of additional cholesterol from retina and RPE is mediated by reverse cholesterol transport which helps to return cellular cholesterol to the liver for either storage or excretion. The translocator protein (TSPO) localizes to mitochondrial outer membrane with five transmembrane domains. TSPO can bind cholesterol via the cholesterol recognition amino acid consensus on the fifth transmembrane domain and is mainly responsible for transferring cholesterol from the mitochondrial outer membrane to the mitochondrial inner membrane, where cholesterol is converted into pregnenolone in steroidogenic cells and into oxysterols in non-steroidogenic cells such as the RPE and macrophages. Oxysterols can activate the LXRα signaling pathway, upregulating cholesterol transporting genes and increasing cholesterol efflux (Li et al., 2016; Biswas et al., 2017). TSPO is also associated with other cellular functions such as oxidative stress, inflammation and apoptosis. Deletion of TSPO in rodents results in divergent characteristics possibly due to the methodology of creating the knockout models or the difference of genetic background between the knockout models (Li et al., 2016). Immunochemistry has shown TSPO is expressed at high levels in a human RPE cell line and the mouse RPE layer (**Figure 1**). Its expression is decreased in aged mouse neuroretina and RPE. TSPO is proposed to mediate cholesterol efflux in the RPE and loss of TSPO in RPE cells results in cholesterol efflux deficiency and intracellular accumulation of cholesterol (Biswas et al., 2017).

TSPO ligands promote cholesterol removal in RPE and choroid endothelial cells: TSPO ligands contain endogenous and synthetic ligands, which are divided into at least 16 chemical classes (Rupprecht et al., 2010). A previous study reported that TSPO ligands enhanced cholesterol efflux in ARPE-19 cells. RPE cells treated with FGN-1-27 or XBD173 have a significant increase in cholesterol efflux to ApoE, ApoAI, HDL and human serum (HS). Another ligand, Etifoxine, also significantly increased cholesterol efflux to HDL and HS in RPE cells (Biswas et al., 2017). Similarly, Etifoxine and XBD173 markedly increased cholesterol efflux to HDL and HS in choroid endothelial cells (Biswas et al., 2018). These ligands significantly decreased total cholesterol and triglycerides, and lowered lipogenesis in both RPE and choroid endothelial cells, possible via upregulating expression of cholesterol transport and metabolism genes (Biswas et al., 2017, 2018).

TSPO ligands inhibit oxidative stress and inflammation in the retinal cells: The retina is one of highest oxygen-consuming tissues in the body and endures oxidative stress. It is well known that oxidative stress induces inflammation. Oxidative stress and inflammation are proposed to contribute to the pathogenesis of AMD (Datta et al., 2017) and a number of recent reports have demonstrated how this may be reversed by TSPO ligands. Rashid et al. (2020) examined the effect of TSPO ligands on ARPE-19 cells in which inflammatory effects were induced by active microglial supernatant and L-leucyl-L-leucine methyl ester (a lysosomal destabilizer). They found that PK11195, XBD173 or Ro5-4864 suppressed the generation of reactive oxygen species (ROS) and activated the NRF2 pathway. These ligands also inhibited expression of proinflammatory genes and inflammasome-mediated caspase-1 activation. Ligand-exposed RPE cells also had less lipid accumulation.

Wang et al. (2014) reported that the TSPO ligand, triakontatetraneuropeptide, decreased ROS production and tumor necrosis factor alpha (TNFα) expression, and inhibited microglial activation in cultured primary mouse retinal microglia and a microglial cell line (BV2) treated with lipopolysaccharide (LPS). In mice with intravitreal injection of LPS, triakontatetraneuropeptide suppressed ROS and TNFα production in the retinas. PK11195 and Ro5-4864 also inhibited ROS generation and TNFα secretion in LPS-treated BV2 cells (Wang et al., 2014). Karlstetter et al. (2014) demonstrated that XBD173 inhibited microglial activation and suppressed expression of proinflammatory genes [C-C Motif Chemokine Ligand 2 (*Ccl2)*, interleukin-6 (*Il-6*) and inducible nitric oxide synthase (*iNos*)] in LPS-treated BV2 cells. XBD173 also increased the phagocytic capacity of human and mouse microglial cell lines. In a mouse model of white-light-induced retinal degeneration, XBD173 decreased expression of proinflammatory genes, inhibited retinal microglial activation and reversed light-induced retinal damage (Scholz et al., 2015).

The laser-induced CNV model is the widely used *in vivo* model for wet AMD. XBD173 treated mice had less activated-microglia in a laser-induced lesion. Activated microglia are considered to be an abundant source for ROS in degenerative retina. XBD173, FGIN-1-27, Etifoxine, PK11195 and Ro5-4864 suppressed ROS production in cultured mouse primary microglia incubated with phorbol myristate acetate or photoreceptor debris. XBD173 also decreased the levels of CCL2 and IL-6

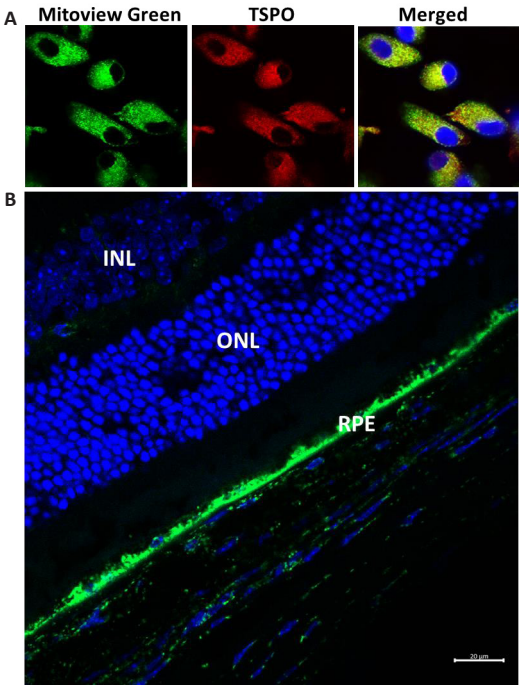

Figure 1 | **TSPO is localized to RPE cells.**
(A) Co-localization of TSPO with mitochondrial marker, Mitoview green, in human retinal pigment epithelial cells (ARPE-19). (B) Immunohistochemistry showing TSPO expressed in mouse RPE layer at high level. INL: Inner nuclear layer; ONL: outer nuclear layer; RPE: retinal pigment epithelial layer; TSPO: translocator protein. Unpublished data.

in the retina and CCL2, IL-6 and IL-1β in the RPE/choroid of the laser-induced CNV mice. In addition, XBD173 lowered laser-induced vascular leakage intensity and area, through reducing the levels of proangiogenic growth factors in both the retina and RPE (Wolf et al., 2020).

Future directions: There are endogenous and synthetic TSPO ligands, which are divided into 16 classes. There are over 60 different ligands of which only a few have been examined in the retina. So it is necessary to assess the effects of other ligands on cholesterol efflux and anti-inflammatory and anti-oxidative stress properties in retinal cell lines and in animal models of retinal degeneration, which will lead to the identification of more effective ligands. Current data show some ligands, such as Etifoxine and XBD173, have an effective capacity to enhance cholesterol efflux and to inhibit production of ROS and proinflammatory cytokines in retinal cell lines and preclinical animal models. Etifoxine (trade name Stresam®) has been used for anxiety disorders since the 1960s in approximately 40 countries (Nguyen et al., 2006). XBD 173 (AC-5216, Emapunil) has gone through Phase I and Phase II clinical trials in patients with anxiety disorder (Rupprecht et al., 2009; https://www.clinicaltrials.gov/ct2/show/NCT00108836). Both ligands also have shown protective effects in other neurodegenerative diseases, e.g. Parkinson's disease and multiple sclerosis, in preclinical studies. Etifoxine has also been shown to decrease body weight in a mouse model of obesity and thus may have additional benefits in patients with obesity-associated syndromes that are frequently found to be associated with AMD (Ibrahim et al., 2020). So it may be worthwhile to start clinical trials with Etifoxine and XBD173 in AMD patients. In conclusion, TSPO ligands have the capacity to promote cholesterol efflux, inhibit oxidative stress and suppress inflammation, which will offer a novel therapeutic option for AMD patients.

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