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

Xing Li, Zhiming He, Xinhua Shu

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). Immunohistochemistry 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 XB173 had 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 XB173 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, XB173 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 XB173 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. XB173 also increased the phagocytic capacity of human and mouse microglial cell lines. In a mouse model of white-light-induced retinal degeneration, XB173 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. XB173 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.XB173, FGIN-1-27, Etifoxine, PK11195 and Ro5-4864 suppressed ROS production in cultured mouse primary microglia incubated with phorbol myristate acetate or photoreceptor debris. XB173 also decreased the levels of CCL2 and IL-6.
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., 2003; 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.

The present work was supported by the Rosetrees Trust, National Eye Research Centre, Tavenss Scotland and the Lotus Scholarship Program of Hunan Province (2019) (to XS). XS is a visiting professor to Shaoyang University, Shaoyang, China.

**Xing Li, Zhiming He, Xinhua Shu** *School of Basic Medical Sciences, Shaoyang University, Shaoyang, Hunan Province, China (Li X, He Z, Shu X) Department of Biological and Biomedical Sciences; Department of Vision Science, Glasgow Caledonian University, Glasgow, UK (Shu X).

*Correspondence to:* Xinhua Shu, PhD, Xinhua.Shu@gcu.ac.uk.

https://orcid.org/0000-0003-3760-3019 (Xinhua Shu)

**Date of submission:** December 26, 2020
**Date of decision:** February 2, 2021
**Date of acceptance:** March 16, 2021
**Date of web publication:** August 30, 2021

https://doi.org/10.4103/1673-5374.322460

How to cite this article: Li X, He Z, Shu X (2022)

Therapeutic potential of translocator protein ligands for age-related macular degeneration. Neural Regen Res 17(4):793-794.

Copyright license agreement: The Copyright License Agreement has been signed by all authors before publication.

Plagiarism check: Checked twice by iThenticate.

Peer review: Externally peer reviewed.

Open access statement: This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

References

Biswas L, Farhan F, Reilly J, Bartholomew C, Shu X (2018) TSPO ligands promote cholesterol efflux and suppress oxidative stress and inflammation in choroidal endothelial cells. Int J Mol Sci 19:3740.

Biswas L, Zhou X, Dhillon B, Graham A, Shu X (2017) Retinal pigment epithelium cholesterol efflux mediated by the 18 kDa translocator protein, TSPO, a potential target for treating age-related macular degeneration. Hum Mol Genet 26:4327-4339.

Datta S, Cano M, Ebrahimi K, Wang L, Handa JT (2017) The impact of oxidative stress and inflammation on RPE degeneration in non-vascular AMD. Prog Retin Eye Res 60:201-218.

Ibrahim KS, Craft JA, Biswas L, Spencer J, Shu X (2020) Etifoxine reverses weight gain and alters the colonic bacterial community in a mouse model of obesity. Biochem Pharmacol 180:11451.

Karlstetter M, Nothdurfter C, Aslanidis A, Moeller K, Horn F, Scholz R, Neumann H, Weber BH, Rupprecht R, Langmann T (2014) Translocator protein (18 kDa) (TSPO) is expressed in reactive retinal microglia and modulates microglial inflammation and phagocytosis. J Neuroinflammation 11:1.

Li F, Liu J, Liu N, Kuhn LA, Garavito RM, Ferguson-Miller S (2016) Translocator protein 18 kDa (TSPO): an old protein with new functions? Biochemistry 55:2821-2831.

Nguyen N, Fakra E, Pradel V, Jouve E, Alquier C, Le Guern ME, Micaleff J, Blin D (2006) Efficacy of etifoxine compared to lorazepam monotherapy in the treatment of patients with adjustment disorders with anxiety: a double-blind controlled study in general practice. Hum Psychopharmacol 21:139-149.

Pikkarainen IA, Cursio CA (2014) Cholesterol in the retina: the best is yet to come. Prog Retin Eye Res 41:64-89.

Rashid K, Verhoven M, Taiwo M, Langmann T (2020) Translocator protein (18 kDa) (TSPO) ligands activate Nrf2 signaling and attenuate inflammatory responses and oxidative stress in human retinal pigment epithelial cells. Biochem Biophys Res Commun 528:261-268.

Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M (2010) Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. Nat Drug Discov 9:97-106.

Rupprecht R, Rammes G, Eser D, Baghai TC, Schüle C, Nothdurfter C, Troxler T, Gentisch C, Kalkman HD, Chaperon F, Uzunov V (2009) Translocator protein (18 kDa) as target for anxioytics without benzodiazepine-like side effects. Science 325:490-493.

Scholz R, Rammes G, Bhuckory MB, Rashid K, Chen M, Xu H, Grimm C, Langmann T (2015) Targeting translocator protein (18 kDa) (TSPO) dampens pro-inflammatory and oxidative stress in patients with adjustment disorders with anxiety. Eur J Pharmacol 752:86-93.

Wong WT (2014) Macroglia-microglia interactions via TSPO signaling regulates microglial activation in the mouse retina. J Neurosci 34:3793-3806.

Wool A, Herb M, Schramm M, Langmann T (2020) The TSPO-NOX1 axis controls pathoge-triggered pathological angiogenesis in the eye. Nat Commun 11:1-17.

E-Editors: Zhao M, Liu WJ, Qiu Y T-Editor: Jia Y