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Inflammation at the blood-brain barrier: the role of liver X receptors

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Abstract

The blood-brain barrier (BBB) is indispensable for the maintenance of brain homeostasis and proper neuronal functioning. Dysfunction of the BBB significantly contributes to the pathogenesis of neuroinflammatory and neurodegenerative diseases like stroke, multiple sclerosis (MS), and Alzheimer's disease (AD). The neuroinflammatory environment that characterizes these disorders propagates chronic impaired function of the BBB, processes that will be discussed in this review. Limiting dysfunction of the BBB may be an attractive target for treatment of neurological disorders. To date, no current treatments are directly targeting the function of the BBB. In this review, we will specifically discuss the potential protective role of nuclear liver X receptors (LXRs) as a promising therapeutic target to reverse or prevent BBB impairment in neurological diseases.

Keywords**:** Liver X receptor, blood-brain barrier, neuroinflammation, neurodegenerative disease, tight junction

1. The blood-brain barrier

The blood-brain barrier (BBB) is responsible for maintaining brain homeostasis by controlling the environment of the central nervous system (CNS), the entry of nutrients, and by protecting it against xenobiotics. The physical barrier is formed by specialized endothelial cells, which have unique properties. Brain endothelial cells exhibit low pinocytotic activity and are sealed with adherens junctions (AJs) like VE-cadherin and E-cadherin that are connected to the cytoskeleton via catenins. Barrier function is instigated by complex tight junctions (TJs) formed by the interaction of proteins such as claudins (1,3,5), occludin and the intracellular TJ proteins zona occludens (ZO) that firmly seal the paracellular route for ions and small molecules [1, 2]. To regulate the transport of nutrients as well as the efflux of toxins and waste products, brain endothelial cells express highly polarized, specific transporters that actively regulate these processes. Key transporters include the glucose transporter (GLUT-1) and members of the ATP binding cassette (ABC) transporters, in particular P-glycoprotein (P-gp) [3].

The barrier properties of brain endothelial cells are strongly supported by pericytes and astrocytic end-feet. Pericytes are contractile cells located between brain endothelial cells and astrocytic end-feet, and are enclosed by the endothelial basement membrane. They play a major role in the induction of BBB function by controlling TJ protein expression and microvascular stability during development [4-6]. The loss of pericyte coverage leads to a disruption of BBB function, underscoring their importance [6]. Astrocytes provide further barrier support through their end-feet that form a network of fine lamellae opposed to the outer surface of the endothelium, which together with the parenchymal membrane form the glia limitans [7, 8]. They are of critical importance for the continuous maintenance of BBB function by controlling TJ formation and the expression and polarization of transporters. The term neurovascular unit (NVU) is often used to refer to these complex cellular interactions between the different cell types, creating a highly dynamic vascular structure in the brain, which closely regulates the neuronal environment (FIGURE 1).

As part of maintaining brain homeostasis, the BBB supports the relative immune privileged nature of the CNS by regulating immune cell migration. Under healthy conditions, leukocyte trafficking into the CNS is relatively low compared to peripheral organs and immune cells rarely enter the parenchyma [9]. During this basal immune surveillance, immune cells may encounter antigen-presenting cells in the perivascular space, which can be found at post capillary venules between the endothelium and the glia limitans [10]. However, upon inflammation, leukocyte trafficking across the inflamed brain endothelium may increase considerably. The production of cytokines and chemokines by the inflamed endothelium is the first leading event for extravasation of leukocytes into the CNS and subsequent neuroinflammation, since these cytokines and chemokines attract leukocytes to the BBB [11-13].

The upregulation of cell adhesion molecules (CAMs) by brain endothelial cells such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 further increases firm adhesion of leukocytes to the endothelium [14]. A next step in leukocyte migration is the crawling of the leukocytes across the endothelium in search for a permissive site for diapedesis. To enter the CNS parenchyma, immune cells must finally traverse the glia limitans, which requires the induction of matrix metalloproteinase (MMP) production [15]. By penetrating the glia limitans, leukocytes infiltrate the parenchyma causing major CNS damage. Moreover, chronic neuroinflammation severely affects brain homeostasis and the continuous exposure to inflammatory mediators is frequently associated with neurodegenerative diseases [16].

1.1. The BBB in neuroinflammatory diseases

In numerous pathological conditions of the CNS, including ischemic stroke, multiple sclerosis, and Alzheimer's disease, neuroinflammation contributes to the progression of clinical symptoms and may lead to neurodegeneration. Moreover, a dysfunction of the BBB is associated with these disorders. BBB dysfunction is characterized by endothelial cell junctional alterations and increased permeability. This can be induced by inflammation and vasoactive compounds in a disease-specific manner and ultimately lead to detrimental neuroinflammation and consequent neurodegeneration. The role of BBB dysfunction and consequent neuroinflammation in the above-mentioned disorders is discussed in detail below.

1.1.1. Stroke

Stroke is a frequent cause of long-term disability and accounts for ~12% of all deaths worldwide [17]. Stroke may be caused by a blocked artery (ischemic stroke) or by the leaking or bursting of a blood vessel (hemorrhagic stroke) [18]. Approximately 80% of all strokes are ischemic and during acute ischemic stroke a sudden occlusion of a blood vessel leads to an almost immediate loss of oxygen and glucose to the cerebral tissue, due to the lack of proper perfusion. The ischemic core of a stroke area contains cells that are irreversibly damaged while the penumbra, the area surrounding the core, can potentially be rescued by improving blood flow. Within hours after the ischemic insult, a whole array of events occur throughout the infarcted region such as ATP depletion, ionic imbalance, oxidative stress, and activation of inflammatory processes, which may lead to neurodegeneration in the longer term [19].

The molecular cues that are generated by cerebral ischemia activate components of the innate immune system and promote inflammation, which contributes to the underlying pathophysiology and have an effect on BBB function [20]. Moreover, the different cell types of the NVU differ in their sensitivity to oxygen deprivation and ischemia, possibly contributing at different levels to BBB dysfunction [21, 22]. After a stroke, microglia, the resident immune cells of the brain, secrete proinflammatory cytokines including tumor necrosis factor α (TNFα) and interleukins (ILs). In addition, neurons and other damaged brain cells release danger associated molecular patterns (DAMPs) and cerebral endothelial cells upregulate their expression of CAMs. Together with the secretion of chemokines by activated microglia and the endothelium, these changes facilitate the recruitment of leukocytes to the ischemic brain [23]. Furthermore, inflammation induces the production of proteases, including MMPs, which may degrade the basal lamina leading to BBB disruption and consequently the infiltration of leukocytes into the brain parenchyma [24].

After the early opening of the BBB directly after a stroke, a second phase of severe BBB disruption occurs within 24-72 hours after infarction. Although reperfusion, i.e. the reestablishment of cerebral blood flow, is necessary for tissue survival in the penumbra, it causes MMP release, leukocyte infiltration, and an acute opening of the TJs of the BBB [25]. Together, these events promote ischemic neuroinflammation as a result of BBB disruption.

1.1.2. Multiple sclerosis

Multiple sclerosis (MS) is a chronic neuroinflammatory and demyelinating disease of the CNS [26]. Worldwide, approximately 2.5 million people are affected with MS [27]. The underlying mechanisms for MS are considered autoimmune due to the pivotal role of the body's own immune system in disease progression [28]. The immunopathological process in MS is mediated by T and B cells and activated macrophages that are involved in the demyelination of axons, thereby inducing severe tissue damage and neuronal dysfunction [29, 30].

As observed in stroke, MS is also marked by prominent BBB breakdown. The ongoing inflammatory response in MS causes the production of harmful mediators, including cytokines, which interrupt the architectural organization of AJs and TJs of the BBB [31]. In that way, changes in BBB structure occur in the early phase of lesion formation resulting in BBB hyperpermeability in active MS lesions [32]. Furthermore, brain endothelial cells produce cytokines and chemokines upon inflammation and express adhesion molecules. The inflammatory status, together with BBB disruption, induces leukocyte migration into the CNS parenchyma as well as oedema, axonal loss and gliosis leading to neurodegeneration. Although the breakdown of the BBB is thought to be transient in MS, it is a recurrent and even chronic event during disease progression that eventually leads to permanent neurological deficits [33].

1.1.3. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia affecting 48 million people in the world [34]. It is a progressive neurodegenerative disorder characterized by the formation of amyloid beta (Aβ) plaques, which are released by sequential proteolytic cleavage of the amyloid precursor protein (APP), and the appearance of intracellular neurofibrillary tangles in the brain [35].

It has become apparent that Aβ plaques are associated with inflammatory mediators and adhesion molecules in the initial stages of AD pathology. In isolated brain microvessels obtained from AD patients, endothelial cells express high levels of cell adhesion molecules and release significantly higher levels of inflammatory factors including nitric oxide (NO), thrombin, TNFα, ILs, and MMPs upon interaction with Aβ [36, 37]. Moreover, Aβ enhances microglia and macrophage activation, which further induces secretion of proinflammatory cytokines and chemokines, indicating a major role for neuroinflammation in AD. The observed neuroinflammation is accompanied by chronic dysfunction of the BBB. Histopathological studies have demonstrated that BBB changes are evident in the cerebral microvascular endothelial cells, showing a loss of TJs and a reduced expression of the transporters GLUT-1 and P-gp [38-40].

Although BBB dysfunction seems to be evident, there remains controversy about whether there is apparent BBB breakdown in AD [41]. *In vitro* studies have shown that TJs of endothelial cells are disrupted upon stimulation with Aβ via the induction of MMPs, which was also shown in an animal model of AD [42]. In post-mortem tissue of AD patients, blood-derived proteins such as albumin and thrombin have been found to accumulate in the hippocampus and cortex indicating the possibility of BBB breakdown followed by serum leakage [43]. However, an extensive study using multiple AD mouse models and human AD post-mortem samples found no increase in BBB permeability, indicating a lack of widespread BBB disruption in AD [44].

Currently, in vivo imaging is being used to assess BBB impairment in human AD patients. So far, imaging studies revealed an impaired P-gp function using positron emission tomography (PET) but not a leaky or damaged BBB as visualized by magnetic resonance imaging (MRI) [45, 46]. Therefore, there is a need to improve the characterization of BBB physiology to better understand the molecular processes involved in AD.

1.2. BBB alterations in neuroinflammation: cellular changes

The tight regulation of BBB function and its specific interactions with the environment ensures the preservation of CNS homeostasis. As mentioned, BBB dysfunction during disease can involve disruption of TJs, increase in transcytosis, changes in transport properties, and increased leukocyte infiltration, which are processes that are orchestrated by all cell types of the NVU. Their individual role in neuroinflammation is discussed below.

1.2.1. Endothelial cells

Brain endothelial cells form the first line of defence of the CNS. Under healthy conditions, they express low levels of adhesion molecules and secrete low levels of pro-inflammatory cytokines and chemokines [47]. However, upon inflammation brain endothelial cells become activated, resulting in the activation of the nuclear transcription factor NF-κB. NF-κB is a heterodimer that resides in the cytoplasm and is bound by its inhibitor IκBα. Activation of the NF-κB pathway leads to degradation of IκBα resulting in translocation of NF-κB to the nucleus and transcription of the pro-inflammatory target genes including cytokines, chemokines and adhesion molecules [48].

In combination with the expression of CAMs, brain endothelial cells loosen their TJs in response to inflammatory stimuli. Both AJs and TJs are expressed ubiquitously, but primarily the alteration of TJs by phosphorylation induced by MMPs and protein tyrosine kinase (PTK) activities may result in disruption of the BBB [49]. Together with leukocyte cytoskeletal changes mediated by a family of signalling molecules, the Rho GTPases, loosening of TJs results in transmigration of leukocytes across the BBB [50, 51]. Leukocytes are potentially also able to migrate into the brain via the transcellular route through the endothelial cell body by the formation of a channel or a pore [52]. Once infiltrated in the CNS, leukocytes contribute to tissue damage by releasing pro-inflammatory cytokines and other cytotoxic products.

1.2.2. Astrocytes

Astrocyte interaction with the brain endothelium is important for the maintenance of BBB function. The physical contact between astrocytic end-feet and the endothelial basement membrane is important for vessel development and BBB integrity [53]. For instance, ablation of astrocyte-secreted laminin leads to down-regulation of junctional proteins and a leaky BBB [54]. In addition, the endothelium-astrocytic end-feet cross-talk provides factors necessary for the formation of appropriate TJs [55].

However, during inflammation, cytokines such as TNFα and IL-1β can be detrimental for astrocyte survival and thereby negatively affect TJ formation resulting in BBB disruption [56-58]. Furthermore, astrocytes are shown to upregulate ICAM-1 and VCAM-1 expression in response to interferon gamma (IFNγ) and TNFα, thereby promoting leukocyte extravasation [59]. Vice versa, astrocytes also produce a repertoire of inflammatory mediators facilitating leukocyte migration into the CNS parenchyma [60]. CC-chemokine ligand 2 (CCL2) and CXC-chemokine ligand 10 (CXCL10) are important recruiters of leukocytes and are released specifically by astrocytes [7]. In addition, it is suggested that astrocytes act as semi-antigen-presenting cells (APCs) that directly (via MHCII and B7 molecules) or indirectly (by stimulating microglia) may activate T cells. However, microglia are provisional APCs, which efficiently process and present antigens and activate T cells [59].

Although astrocytes are considerable contributors to inflammation and the migration of leukocytes into the CNS, they are also involved in the resolution of inflammation. Astrocytes can release anti-inflammatory mediators, such as transforming growth factors and retinoic acid that attenuate inflammation and protect BBB function [61, 62]. Moreover, angiopoietin-1 is able to reduce BBB permeability and suppress neuroinflammation [63]. In addition, astrocytes also promote junctional protein expression by releasing Sonic hedgehoc (Shh) [64]. Not only does Shh tighten the BBB, it also acts as an endogenous anti-inflammatory effector by reducing chemokine and CAM expression by endothelial cells. Moreover, Shh directly interacts with leukocytes by lowering their binding capacity, thereby preventing the immune cells from crossing the BBB. Taken together, astrocytes are important regulators of BBB function in a context-dependent manner that is regulated by specific signalling events.

1.2.3. Pericytes

In contrast to brain endothelium, microglia and astrocytes, the contribution of pericytes to BBB performance during neuroinflammation is less clear. As mentioned, pericytes are wrapped around endothelial cells and play an important role in the induction of BBB properties [4, 5]. Whereas astrocytes induce TJ expression, pericytes suppress endothelial transcytosis, thereby reducing BBB permeability and contributing to BBB function and integrity [65].

In the brain, it has been shown that in response to inflammation, activated pericytes release MMP-9 and generate pro-inflammatory cytokines and chemokines [66]. In addition, pericyte loss is observed under pathological conditions such as stroke and AD, affecting the BBB. For instance, during acute stroke in animal models as well as in humans, pericyte loss appears to precede and exceed vessel degeneration [67]. Moreover, experimental mouse models of AD showed pericyte rearrangement and degradation during microvascular inflammation and Aβ accumulation, which correlated with BBB breakdown [68, 69]. In AD patients, accelerated pericyte loss occurs particularly in individuals carrying the apolipoprotein E4 (*APOE4*) gene [70]. Here, consistent with animal data, pericyte degeneration also correlates with BBB breakdown, which might be related to higher levels of the proinflammatory cytokine cyclophilin A and MMP-9. These data indicate that pericytes are able to disrupt the BBB and contribute to neuroinflammation.

2. The Liver X Receptor; the missing link?

Interestingly, apart from neuroinflammation, a dysregulation of brain cholesterol homeostasis has been observed in the above-described pathological conditions. Cholesterol is a major component of cell membranes and as such it contributes to the regulation of cellular structure and function. While the brain on average only comprises 2.5% of the total body mass, it contains about 23% of the body's total cholesterol, underlining its importance in brain function [71]. Almost all cholesterol content in the brain can be accounted for by *de novo* synthesis and its metabolism is separated from the periphery by the BBB. Under healthy conditions, a constant level of cellular cholesterol is maintained through a balance in cholesterol synthesis and efflux. However, high levels of cholesterol are related to neurodegeneration, implying a shift in the balance of brain cholesterol content under pathological conditions [72].

The nonsteroidal nuclear liver X receptor (LXR) may provide a link between the observed neuroinflammation and cholesterol dysregulation. LXRs belong to a large family of nuclear transcription factors that modulate gene transcription. They exist in two isoforms, termed LXRα (NR1H3) and LXRβ (NR1H2), which share 77% identity in their DNA and ligand binding domain amino acid sequences [73]. LXRα and LXRβ have identical ligands but differ in their tissue expression pattern. While LXRα is highly expressed in the liver, adipose tissue, adrenal glands, intestine, kidney, and macrophages, LXRβ is more ubiquitously expressed. Yet, both LXR isoforms are expressed in the CNS [74].

LXRs are the major regulators of cholesterol homeostasis. Both LXR isoforms are activated by naturally occurring cholesterol metabolites, known as sterols and oxysterols, and regulate the expression of target genes like the aforementioned ABC transporters. Besides the capacity of ABC transporters to efflux waste products, they are also involved in cholesterol absorption, efflux, transport, and excretion thereby regulating a process called reverse cholesterol transport [75]. In the nucleus, LXRs form a heterodimer with the retinoid X receptor (RXR) which, being in an inactive state, inhibits transcription processes by the recruitment of nuclear co-repressors (FIGURE 2). Ligand binding first induces the dissociation of the co-repressor complex followed by the recruitment of co-activators to start gene transcription [75]. As mentioned, activation of LXRs occurs through the binding of the natural occurring oxysterols

and intermediates of the cholesterol synthesis pathway. However, to investigate LXR function, synthetic agonists that activate both LXR isoforms, such as T0901317 and GW3965, are widely used in experimental studies as well as LXR knock-out mouse models [76]. In addition, LXR/RXR transcriptional activity can also be induced by retinoids, which are ligands for the RXR. Ligand binding to LXR or RXR directly correlates to an upregulation of downstream gene transcription.

LXRs gained attention as potential therapeutic targets because of their diverse physiological functions. In addition to their central role in cholesterol homeostasis, LXRs are involved in inflammatory gene expression and innate immunity [77]. Interestingly, LXRs are able to indirectly suppress the expression of inflammatory NF-κB target genes in macrophages by a process called transrepression [78]. Under inflammatory conditions, LXR activation results in SUMOylation, which is the covalent attachment of small ubiquitin-like modifier proteins to specific target proteins that can regulate and modify protein function and cellular pathways [79]. The SUMOylation-dependent targeting of the nuclear receptor to corepressor complexes prevents their signal-dependent clearance. Thus, this process results in preservation of the co-repressor complex at the NF-κB downstream inflammatory gene promoter, thereby inhibiting the inflammatory gene expression. Therefore, LXRs may provide the missing link between cholesterol dysregulation, neuroinflammation and neurodegeneration.

2.1.1. LXRs and the endothelium

Brain endothelial cells express functional LXRs and their downstream targets, the ABC transporter proteins [80]. Since the 90s, several studies describe a role for oxysterols in LXR activations in peripheral endothelial cell (dys)function. These studies demonstrated an elevation in endothelial permeability for albumin, i.e. decreased barrier function, across vascular endothelial monolayers after exposure to oxysterols [81, 82]. Furthermore, oxysterol incubation of human umbilical vein endothelial cells (HUVECs) resulted in alterations in the activity of membrane-bound enzymes leading to increased calcium influx and endothelial cell toxicity [82, 83]. Moreover, an increase in cell apoptosis, IL-1β cytokine expression and ICAM-1 and VCAM-1 expression was found after exposure of HUVECs to oxysterols, including 7β-hydroxycholesterol and 7-ketocholesterol [84]. Exposure of human aortic endothelial cells to another oxysterol, 25-hydroxycholesterol, induces VCAM-1 expression and monocyte adhesiveness, all demonstrating a pro-inflammatory role of LXR-activating oxysterols in endothelial cell function [85].

In contrast to the effects of endogenous activation, studies using the synthetic LXR agonists T0901317 and GW3965, revealed anti-inflammatory actions of LXR activation in peripheral endothelial cells. Synthetic activation of LXRs attenuated the lipopolysaccharide (LPS)-induced upregulation of ICAM-1, VCAM-1 and E-selectin adhesion molecules in HUVECs [86]. Furthermore, it was shown that the inhibition of cellular senescence of HUVECs by LXR agonists is associated with a decline in ROS production and an increase in endothelial nitric oxide synthase (eNOS) activity, mediating the production of NO [87]. Finally, synthetic LXR activation in HUVECs provokes a protein-protein interaction of LXRβ and estrogen receptor (ER)α resulting in the activation of eNOS, thereby retaining monolayer integrity [88]. The contradictory results between the pro-inflammatory effects of endogenous LXR ligands compared to the anti-inflammatory effects of the synthetic LXR agonists might be explained by the finding that oxysterol-induced inflammation in peripheral endothelial cells is LXR-independent [86]. Here, it was shown that the upregulation of inflammatory markers by oxysterols was unaffected during LXR antagonism.

Multiple studies describe a cross talk between LXRs and the proliferator peroxisome activated receptor (PPAR), another important member of the nuclear hormone transcription

factor family. Three subtypes of this receptor have been identified, i.e. PPAR-alpha, -delta, and -gamma, and these receptors regulate endothelial cell function and inflammation level, possibly indirectly via an interplay with LXRs. For instance, PPARα activation with fenofibrate increases eNOS activity and protein expression in aortic endothelial cells in a concentration-dependent manner [89]. In human vascular endothelial cells, PPARα-specific activators inhibit TNFα-induced IL-6, cyclooxygenase-2 (COX-2) and VCAM-1 expression by repressing NF-κB signaling [90-92]. Furthermore, PPARδ activation in HUVECs significantly decreases TNFα-upregulated adhesion molecule expression, including VCAM-1 and E-selectin, intracellular reactive oxygen species (ROS) generation, and leukocyte adhesion [93]. Finally, PPARγ stimulation augmented calcium ionophore-induced NO release from HUVECs [94]. In summary, multiple PPAR isoforms interfere with peripheral endothelial inflammatory status, possibly through PPAR-LXR interaction. However, the interaction between LXRs and PPARs is complex and probably cell specific, giving rise to differential responses from cell to cell.

Surprisingly, research into the PPAR-LXR axis in brain endothelium and how it determines its function is lacking. The above discussed biological processes of LXR agonism on peripheral endothelial cell permeability, ROS and cytokine production, and adhesion molecule expression are key in the protection of the CNS from the entry of harmful inflammatory leukocytes. However, one must keep in mind the functional and morphological differences between peripheral endothelial cells and brain endothelial cells as being part of the BBB, which may lead to differential endothelial responses. Endothelial cell differentiation largely depends on the local environment and interaction with surrounding cells. As mentioned, brain endothelial cells are highly specialized by possessing developed intracellular tight junction structures and expressing specific markers and transport systems like P-gp and Glut-1. Morphologically, brain endothelial cells differ in the absence of fenestrae in their plasma membrane and because of the presence of high densities of mitochondria in the cytosol [95]. Therefore, before extrapolating above LXR-dependent mechanisms, investigations into the role of LXRs in brain-specific endothelial cells are of great importance.

2.1.2. LXRs and astrocytes

In addition to endothelial cells, both LXRα and LXRβ are expressed in astrocytes and mediate the efflux of 24S-hydroxycholesterol-induced cholesterol to ApoE under the control of ABCA1 and ABCG1 transporters [96]. LXR activation in primary murine astrocytes by the oxysterols 7-ketocholesterol and 22R-hydroxycholesterol suppresses inducible nitric oxide synthase (iNOS) expression and NO release by inhibiting LPS-upregulated inflammatory mediators, including IFN-β and interferon regulatory factor (IRF)-1 [97]. In addition, LXR activation in LPS-stimulated primary mouse astrocytes inhibits the production of the proinflammatory cytokines IL-1β and IL-6 and of the chemotactic cytokine CCL2 by modulating the NF-κB signaling pathway [98]. Finally, LXR ligands are able to inhibit signal transducer and activator of transcription 1 (STAT1)-mediated inflammatory responses in IFNγ stimulated astrocytes. Here, the SUMOylated LXRs bind to STAT1 and prevent the binding of this transcription factor to the promoter regions of its target genes [99]. Collectively, these results suggest a transrepressive pathway of LXRs in astrocytic cells.

2.2. LXRs and the BBB in neuroinflammatory diseases

Unlike the contribution of LXRs to the function of peripheral endothelial cells, the link between LXRs and the inflammatory status or integrity of brain endothelial cells is only discussed to a limited extent. In this section, we elaborate on the current knowledge of the impact of LXRs on BBB function with the focus on stroke, MS and AD pathology (FIGURE 3).

The involvement of LXRs in BBB function is mainly studied in stroke models. The effect of LXR activation on brain inflammation in an animal model of stroke was investigated by analyzing the expression of NF-κB target genes such as iNOS, COX-2 and MMP-9 and the pro-inflammatory cytokines TNFα and IL-1β [100]. Activation of LXRs by the synthetic agonists T0901317 and GW3965 resulted in a decreased expression of iNOS, COX-2 and MMP-9 in the brain compared to sham animals. In addition, IL-1β levels were also decreased, whereas the expression of TNFα did not change. The involvement of LXRs in these anti-inflammatory effects was validated using LXR knock-out mice. Here, the LXR agonist GW3965 inhibited NF-κB target genes including IL-6, IL-12, and CCL-2 in the brain of wild-type mice but not in the LXR knock-out animals. The suppression of NF-κB target genes by LXR activation under stroke conditions was confirmed, indicating anti-inflammatory effects of LXR agonists in stroke pathology [101]. Therefore, the anti-inflammatory property of LXRs may be beneficial for the BBB phenotype in stroke disease pathogenesis.

Another study focused more specifically on LXRs and BBB integrity after stroke [102]. Here, synthetic LXR activation decreases serum IgG permeability in the ischemic brain. Furthermore, LXR activation prevents the downregulation of the TJ proteins occludin and ZO-1 in ischemic vessels thereby promoting BBB preservation. Apart from the preservation of TJ complexes, LXR activation also increases the expression of the ABCA1 and ABCG1 transporters on brain endothelial cells after stroke, confirming that LXRs have an important function in the brain endothelium. Finally, brain deficiency of ABCA1, a key target of LXRs, increased albumin leakage in ischemic brain reinforcing the idea of LXR involvement in BBB function [103]. Therefore, LXRs may gain interest as a possibly therapeutic approach to protect the vasculature and improve the clinical outcome of patients after a stroke.

In contrast to stroke, less is known about the potentially protective role of LXRs in BBB function in the context of MS or AD pathology. Inflammation is an important feature in the pathogenesis of MS and is responsible for BBB damage and leads to leukocyte infiltration into the CNS [31]. Harmful mediators such as ROS, cytokines, and chemokines play a pivotal role in the disruption of BBB junctional complexes in MS. Previous sections highlighted the suppression of NF-κB downstream cytokines and chemokines by LXRs in macrophages and astrocytes or the inhibition of ROS and iNOS production in astrocytic cells and the endothelium [87, 97, 98]. The transrepressive phenotype of LXRs on these mediators may protect BBB composition and therefore be effective in MS treatment. Indeed, there are several studies that focused on synthetic LXR activation in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS and showed that activation of LXRs during EAE development ameliorated disease outcome. Moreover, LXR activation reduced leukocyte infiltration, IL-17, TNFα, IFNγ cytokine secretion and MMP-9 expression in the CNS of the EAE animals [104-106]. Therefore, LXRs may be promising in protecting BBB function in MS pathology.

In AD, it is suggested that inflammation precedes Aβ deposition and vice versa, Aβ promotes the release of inflammatory mediators [107]. This inflammatory burden may promote BBB breakdown and leukocyte extravasation. To date, only one study investigated the role of the LXR agonist GW3965 in reversion of BBB changes and astrogliosis in AD [108]. The authors showed that LXR treatment of an AD mouse model partially restored BBB integrity, increasing the length of the microvasculature in the hippocampus. Furthermore, this process was associated with a decreased deposition of perivascular Aβ and a reduction in reactive astrocytes. Other studies also show that the activation of LXRs modulates the production of inflammatory molecules together with Aβ burden in AD [109, 110]. Zelcer and colleagues demonstrated that LXR activation inhibits glial cell activation in response to inflammatory stimuli including Aβ [111]. In an AD mouse model, LXR agonists attenuate pro-inflammatory cytokine levels and COX-2 and

iNOS expression compared to vehicle treated mice [106]. Accumulating evidence underlines BBB disruption in AD pathology. However, future research is necessary to understand whether LXRs effectively restore BBB function and inhibit inflammation in AD models.

3. Future perspectives

Activation of LXRs may be considered a promising therapeutic target in stroke, MS, and AD. LXR agonists decrease brain inflammation and exert protective effects of BBB function. Yet, there are some important points to consider before the administration of LXR agonists may become a novel therapeutic approach.

For instance, the specific roles of the individual LXRα and LXRβ subtypes are at present unknown and research focusing on LXR function in the brain rarely makes a distinction between the two isotypes. The difference in tissue distribution implies a possible distinct function for LXRα versus LXRβ, as suggested in experimental animal studies [112-114]. However, although both receptors are expressed in the brain, it is to date unclear whether they have different functions in the CNS.

Secondly, there is a lack of specific agonists for both the LXR isoforms. Part of the difficulty resides in the fact that the two receptors share a high degree of homology. The often-used LXR agonists T0901317 and GW3965 both activate LXRα and LXRβ [73]. Thus, the only way to investigate the possible different functions of the LXRs is by creating tissue or cell specific knock out models using mouse models or cell lines. If LXRα and LXRβ have discreet functions in the CNS, the next challenge would be to develop LXR-specific agonists.

In conclusion, LXR activation is a promising therapeutic target in diseases with BBB dysfunction. Moreover, apart from BBB recovery there seem to be additional beneficial effects observed in stroke, MS and AD after LXR activation. However, there is currently a lack of studies investigating the role of the LXR pathway in the function of BBB during neuroinflammation. Specifically, research into the relationship between LXRs and pericytes is missing. To that end, further research is needed in animal models of disease or human cell cultures on the interaction between LXR activation and BBB function and integrity, specifically for the two isoforms separately. A better understanding of the underlying mechanism will bring the use of (specific) LXR agonists as therapeutic agents closer in the battle against neurodegeneration.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure 1. Structural representation of the BBB. The BBB is a complex system composed of specialized endothelial cells, supported by pericytes, astrocytic end-feet and the basement membrane. The endothelial cells are sealed together with adherens junctions like VE-cadherin and E-cadherin and by complex tight junctions formed by the interaction of proteins such as claudins (1,3,5) and occludin. Key transporters include the glucose transporter (GLUT-1) and members of the ATP binding cassette (ABC) transporters.

Figure 2. Molecular mechanisms of LXR activation and transrepression. **A)** LXRs form a transcriptional unit as permissive heterodimers with retinoid X receptors (RXRs) bound to a specific DNA sequence, called the LXR response element (LXRE). Unliganded LXR/RXR actively suppresses transcription by recruiting co-repressors. **B)** LXR activation leads to a dissociation of the corepressor complex and subsequent recruitment of the coactivator complex, resulting in stimulation of gene transcription. **C)** Under inflammation, LXR activation results in SUMOylation. SUMOylated LXRs dock to corepressor complexes at the NF-κB downstream inflammatory gene promoter, suppressing the expression of inflammatory genes.

Figure 3. The involvement of LXRs in BBB function during neuroinflammation. BBB dysfunction during neuroinflammatory disease can involve disruption of tight junctions, increase in transcytosis, changes in transport properties, and increased leukocyte infiltration. The beneficial effects of LXR activation include **1)** reducing leukocyte infiltration, **2)** preventing the downregulation of tight junction proteins, and **3)** suppressing the expression of inflammatory NF-κB target genes.