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The Role of the Cell Surface LRP and Soluble LRP in Blood-Brain Barrier A β Clearance in Alzheimer's Disease

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Abstract

Low-density lipoprotein receptor related protein-1 (LRP) is a member of the low-density lipoprotein (LDL) receptor family which has been linked to Alzheimer's disease (AD) by biochemical and genetic evidence. Levels of neurotoxic amyloid -peptide (A β) in the brain are elevated in AD contributing to the disease process and neuropathology. Faulty A clearance from the brain appears to mediate focal A β accumulations in AD. Central and peripheral production of A β from A β -precursor protein (APP), transport of peripheral A β into the brain across the blood-brain barrier (BBB) *via* receptor for advanced glycation end products (RAGE), enzymatic A β degradation, A β oligomerization and aggregation, neuroinflammatory changes and microglia activation, and A β elimination from brain across the BBB by cell surface LRP; all may control brain A β levels. Recently, we have shown that a soluble form of LRP (sLRP) binds 70 to 90 % of plasma A β , preventing its access to the brain. In AD individuals, the levels of LRP at the BBB are reduced, as are levels of A β binding to sLRP in plasma. This, in turn, may increase A β brain levels through a decreased efflux of brain A β at the BBB and/or reduced sequestration of plasma A β associated with re-entry of free A β into the brain *via* RAGE. Thus, therapies which increase LRP expression at the BBB and/or enhance the peripheral A β "sink" activity of sLRP, hold potential to control brain A β accumulations, neuroinflammation and cerebral blood flow reductions in AD.

Keywords

LRP; sLRP; blood-brain barrier; Alzheimer's disease; A β clearance

INTRODUCTION

Transport of polar solutes across the cerebrovascular endothelial cells is restricted by the presence of tight junctions, which form the blood-brain barrier (BBB) [1,2]. Amyloid -peptides (A β) are small peptides (~ 4.5 kDa) of which the most common isoforms contain 40 (A β 40) or 42 (A β 42) amino acids. A circulates in plasma, cerebrospinal fluid (CSF) and brain interstitial fluid (ISF) mainly bound to its chaperone molecules [2]. A smaller fraction of A β in these body fluids remains unbound and circulates as free A β in equilibrium with bound A β . In human plasma, a soluble form of the low-density lipoprotein receptor related protein-1 (LRP), sLRP, is a major binding protein for circulating A β [3]. In human CSF, lipocalin-type prostaglandin D synthase/ -trace appears to be a major A β chaperone [4]. In addition, apolipoprotein J (apoJ), apoE, transthyretin and α 2-macroglobulin (α 2M) bind A β in the CSF and ISF, and may influence its clearance, metabolism and aggregation [5–9]. In normal human

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CSF and plasma, A β 40 levels are greater than those of A β 42 by about 10-fold and 1.5-fold, respectively [10].

A β concentration in normal brain ISF is regulated by its rate of production from the A β -precursor protein (APP), influx or re-entry into the brain mainly *via* receptor for advanced glycation end products (RAGE) [11] and by A β clearance at the BBB [12–13] (Fig. 1) and within the brain itself [14]. APP, a type I transmembrane protein, is expressed in almost all cell types in the body and in the brain, including brain endothelial cells, astrocytes and neurons [2,15]. APP proteolysis is mediated by two major pathways; a nonamyloidogenic pathway involving α - and γ -secretase, and amyloidogenic pathway involving β - and γ -secretase [15]. Clearance of A β from brain ISF involves non-enzymatic transport mechanisms and enzymatic degradation. There are two pathways for the nonenzymatic A β clearance. The major one is transcytosis across the BBB (BBB clearance) which is mediated mainly by the cell surface LRP localized predominantly on the abluminal side of the cerebral endothelium [16,17]. The second pathway involves bulk flow of the ISF into the CSF followed by drainage into the blood across the perivascular Virchow-Robin arterial spaces which under physiological conditions accounts for about 10–15% of total A β clearance in mice [16,18]. The enzymatic clearance involves a number of proteases, including insulin-degrading enzyme, neprilysin and endothelin-converting enzyme (cellular clearance) [19–21]. Importantly, degradation of free A in brain ISF appears to be insignificant [16].

Circulating A β can enter into the brain in guinea pigs, mice, monkeys and rats across the BBB by a saturable specialized transport mechanism in addition to diffusional transport [11,22–28]. In guinea-pigs, A β 40 permeability across the BBB is greater than that of immunoglobulin G (IgG) [29], arginine-vasopressin [30] and leucine-enkephalin [31,32] by 11-fold, 2.5-fold and 1.5-fold, respectively (Table 1), but is lower by about 5-fold than transport of amino acid tyrosine across either the BBB or the choroid plexus [33,34]. In AD models, expression of RAGE, which mediates A β influx across the BBB, is increased [11,35]. Similarly, in AD individuals, expression of RAGE in brain endothelial vascular cells is increased [11,36,37], whereas that of LRP is reduced [16,17,37] which, together, favor A β accumulation in brain. Increased RAGE vascular expression may also mediate A β -induced migration of monocytes across the human brain endothelial cells [38]. Interestingly, exercise reduces RAGE expression at the BBB and A β influx, and increases LRP BBB expression and A β efflux in an animal model of AD [39]. A β accumulation in brain leads to its gradual oligomerization and greater levels of neurotoxic A β oligomer species [40–42]. Thus, continuous removal of A β from the CNS is the most important mechanism for preventing potentially neurotoxic accumulations of A β in brain [43].

LRP

LRP is a large multifunctional scavenger and signaling receptor and a member of the LDL receptor family [44,45]. It plays a major role in the transport and metabolism of cholesterol associated with apoE-containing lipoproteins. LRP is synthesized as a single polypeptide precursor (600 kDa) that is processed into two heterodimeric peptides, the α and β chains, by furin in the trans-Golgi network [46]. The heavy α -chain of LRP (515 kDa) forms the extracellular domain and is non-covalently coupled to the 85 kDa transmembrane and cytoplasmic light β -chain. The β -chain contains four ligand-binding domains (clusters I–IV), consisting of 2, 8, 10, and 11 cysteine-rich complement-type repeats, respectively [47]. Between these clusters there are epidermal growth factor (EGF) receptor-like repeats and YWTD -propeller repeats that are typical of the LDL receptor family [47]. LRP binds a diverse array (~40) of structurally and functionally unrelated ligands, such as apoE, α 2M, tissue plasminogen activator (tPA), proteinase-inhibitors (plasminogen activator inhibitor-1), APP, blood coagulation factors (e.g., factor VIII, protein S), growth factors, bacteria and viral proteins, lactoferrin [45] and A β wild

type and mutant peptides [17]. Although LRP is mainly an endocytic receptor, it has been shown that LRP mediates transcytosis of both A β [16,17] and tPA [48] across the BBB. The majority of ligands bind to clusters II and IV [44,49]. RAP (receptor-associated protein), an endoplasmic reticulum (ER) molecular chaperone for the LDL receptor family, binds LRP with high affinity, and not only prevents premature ligand binding to LRP but is also required for proper LRP folding [50].

The light chain of LRP (85 kDa) contains the transmembrane domain and a cytoplasmic tail which contains both tyrosine and serine phosphorylation sites [51]. Phosphorylation of LRP in response to nerve growth factor accelerates the endocytic activity of the receptor [52], whereas phosphorylation by platelet-derived growth factor does not have a clear effect on endocytosis [53]. The cytoplasmic tail contains two NPXY and one YXXL motifs and two di-leucine motifs [54]. The YXXL motif and distal di-leucine repeat have been shown to be the main feature required for rapid LRP endocytosis or internalization (< 0.5 s) [55]. This rapid endocytotic rate of LRP indicates its major role as a cargo transporter. In addition, the cytoplasmic domain interacts with a number of adaptor proteins, such as disabled-1, FE65 and PSD-95, suggesting a signal transduction role of LRP [56,57]. Thus, LRP has a dual role as a rapid cargo transporter and the transmembrane cell signaling receptor.

LRP AND ALZHEIMER'S DISEASE

LRP has been linked to AD by genetic and biochemical studies. Polymorphisms in the *LRP* gene on chromosome 12 in a 5' tetranucleotide repeat and a single base pair change within exon 3 (C766T) may be associated with late-onset AD [58–60] and cerebral amyloid angiopathy [61], but this has not been confirmed by all studies [62]. The C to T change in exon 3 has been identified in some ethnic groups [59,63]. LRP and its ligands have been detected in senile plaques [64,65]. LRP interacts with APP in neurons and may influence APP processing and metabolism, and thereby production of A β , the neurotoxic species central to the pathogenesis of AD [66,67]. LRP expressed in neurons may regulate A β uptake (cellular uptake within brain) *via* LRP ligands α 2M and apoE [68–71]. However, LRP expression on neurons does not appear to mediate A β clearance *in vivo* since APP mice overexpressing functional LRP mini-receptors on neurons have increased levels of soluble A β in brain [72, 73]. LRP is expressed in brain capillary endothelium [16,17]. Its reduced brain capillary expression has been observed during normal aging in rodents, non-human primates and in AD patients associated with positive staining of cerebral vessels for A β 40 and A β 42 [16,17,37, 39]. Since LRP is the main receptor for A β transport across the BBB from brain to blood, its down regulation in brain endothelium in AD individuals and patients with Dutch-type cerebrovascular β -amyloidosis likely reduces A β clearance and promotes A β cerebrovascular and brain focal accumulations. In an AD mouse model (TgCRND8), exposure to enriched environment for 120 days beginning at 30 days of age promoted upregulation of LRP associated with reductions in A β levels [39]. This demonstrates that LRP expression can be regulated by environmental and behavioral factors.

LRP AND BBB A β CLEARANCE

In a recent study, human A β microinjected into the mouse brain was detected in plasma using human specific ELISA for intact A β [8]. This clearly demonstrates intact A β transcytosis from brain ISF into blood. A β clearance may be influenced by apoE and α 2M, known ligands for LRP, but formation of A β complexes with either of those ligands has not been shown in the CNS *in vivo* during clearance studies [16,17]. Direct LRP/A β interaction *in vitro* has been demonstrated by surface plasmon resonance (SPR) analysis and ELISA assays, as well as the direct binding of A β to the abluminal side of brain capillaries, which suggests that this interaction is the first step of A β transcytosis across the mouse BBB *in vivo* [8,17]. The affinity

of A β 40 for LRP is greater than that of A β 42 and the vasculotropic mutants, e.g., Dutch/Iowa (DI) A β 40. Compared to A β 40, the A β peptides with higher β -sheet content and/or more negative charges (A β 42 and DI A β 40) are cleared less efficiently from brain. Despite extremely low levels of human APP in brain and low A β production from neurons, the transgenic TgDI/Swe mice (Dutch, Iowa and Swedish mutations, Thy-1 APP DI/Swe mice) develop robust A β brain accumulation much earlier than Tg2576 A β -overproducing mice [17,74]. The mutant Dutch A β , E22Q, is also cleared less efficiently from brain to blood [75]. This clearly demonstrates that genetic mutations resulting in errors in A β clearance significantly contribute to A β accumulation in brain. Other lipoprotein receptors, such as LDLR, VLDLR, LRP2, appear to have no major role in transport of free A β across the BBB into blood [17], but their roles in transporting A β bound to its chaperone proteins apoE and apoJ remain to be elucidated. LRP2/megalin is typically expressed on the apical membrane of epithelial cells [76]. It has been shown to transport RAP, an endoplasmic reticulum chaperone molecule for the LDL receptor family, *in vitro* across polarized epithelial cells from the apical to the basolateral chamber, and *in vivo* into the brain in mice [77]. In addition, LRP2/megalin transports apoJ and A β /apoJ complexes from blood to brain [78]. However, this transport process is completely saturated at physiological levels of plasma apoJ, and therefore unlikely to play a key role in the regulation of A β levels in brain ISF. A recent study using an *in vitro* kidney epithelial monolayer as a transport model has shown that cells transfected with LRP minireceptor containing cluster IV of LRP (mLRP4) mediate A β endocytosis followed by its degradation [79]. This study revealed no role for P glycoprotein (Pgp) in A β transport, which had been previously reported to influence A β brain levels *in vivo* [80]. Although epithelial transport models mimic most physicochemical properties of endothelial monolayer, genomic and proteomic studies suggest a unique molecular makeup of brain endothelial monolayers [81], which differ from both systemic endothelial cells and epithelial cells. This might account, in part, for the lack of measurable transcytosis of LRP ligands in epithelial kidney monolayers [79], whereas, in brain endothelial monolayers, LRP-mediated transcytosis of tPA across a bovine BBB model has been reported [48]. Also, in contrast to typical *in vivo* and *in vitro* BBB studies demonstrating relatively rapid clearance of A β [8,16,17,80] [48], the A β uptake across the kidney epithelial monolayer required much longer equilibration time (up to 48 h) [79] which might account for the observed differences compared to LRP-mediated transcytosis demonstrated across brain endothelial monolayers [48].

LRP AND SYSTEMIC A β CLEARANCE

sLRP is the truncated extracellular domain of LRP which is normally present in plasma [82]. sLRP is released into the extracellular fluid after beta-secretase cleavage of the LRP β -chain [83]. Our recent data show that endogenous native sLRP is a major peripheral A β binding protein in humans and mice [3]. In humans, sLRP normally sequesters ~70–90% of circulating A β . However, in AD we showed decreased binding of A β to sLRP due to lower sLRP levels. In addition, we detected increased oxidation of sLRP, which was associated with a 3–4-fold increase in free A β 40 and A β 42 levels in plasma. These findings suggest that the endogenous sLRP “peripheral sink” for A β is compromised in AD, leading to an elevated free A β fraction in plasma that may contribute to increased A β levels in the brain *via* RAGE-mediated transport [11,37].

Continuous systemic A β clearance and sLRP sequestration of plasma A β are essential components of peripheral A β “sink” action which regulates A β removal from brain. In turn, liver LRP mediates systemic elimination of A β from plasma [84]. We also showed that recombinant LRP clusters could effectively sequester plasma A β (both 40 and 42) in human AD plasma and in AD mice. In the mice, this sequestration resulted in reductions of A β parenchymal and vascular accumulations and amyloid load, as well as improvements in memory, learning and CBF responses [3]. Curiously, sLRP is also protective against peripheral

nerve injury and neuropathic pain in mice [85]. Other A β -binding agents, such as anti-A β antibody [86,87], can also promote clearance of brain-derived A β in APP overexpressing mice, but are not endogenous A β sink agents and therefore are often associated with unwanted side effects. Since oxidation of LRP is associated with aging [3], antioxidants with angiogenic and vasculoprotective properties under hypoxic conditions, such as activated protein C [88], might prove beneficial against A β -induced oxidant stress associated with LRP oxidation.

CONCLUSION

LRP in brain capillaries plays a key role in A β elimination from the brain by mediating its clearance across the BBB. In plasma, a soluble form of LRP, sLRP, maintains a native A β peripheral 'sink' activity, which is critical for efficient A β efflux from the brain. In AD, both LRP levels at the BBB and peripheral sLRP binding of A β are compromised, favoring A β accumulation in the brain. Thus, therapies focused on upregulation of the cell surface LRP in brain endothelial cells and on replacement of sLRP in plasma, as, for example, with recombinant high affinity A β binding LRP clusters, represent promising new mechanisms to therapeutically control A β levels in brain.

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Fig. 1. Schematic diagram showing the blood and brain compartments, and the roles of the cell surface LRP and soluble LRP (sLRP) in the regulation of Aβ transport across the blood-brain barrier (BBB). See text for details. RAGE (receptor for advanced glycation end products). LRP (low-density lipoprotein receptor related protein 1). APP (amyloid-precursor protein).

Table 1

Blood-Brain Barrier Permeability for A β 40, Leucine-Enkephalin, Arginine-Vasopressin and IgG Expressed as Unidirectional Transfer Constant (K_{in}) in Guinea-Pigs

Molecules	$K_{in}(\mu\text{l/g/min})$	References
A β 40	6.01 ± 0.80	25
Leucine enkephalin	3.65 ± 0.11	31,32
Arginine-vasopressin	2.37 ± 0.25	30
IgG	0.55 ± 0.04	29