RAGE (Yin) Versus LRP (Yang) Balance Regulates Alzheimer Amyloid β-Peptide Clearance Through Transport Across the Blood–Brain Barrier

Rashid Deane, PhD; Zhenhua Wu; Berislav V. Zlokovic, MD, PhD

Abstract—Accumulation of amyloid β-peptide (Aβ) in the central nervous system (CNS) may initiate pathogenic cascades mediating neurovascular and neuronal dysfunctions associated with the development of cerebral β-amyloidosis and cognitive decline in patients with Alzheimer disease (AD) and with related familial cerebrovascular disorders. Whether Aβ-related pathology in the CNS is reversible or not and what key therapeutic targets are controlling Aβ/amyloid levels in the aging brain remain debatable. In this article, we summarize recent evidence why the receptor for advanced glycation end products and low-density lipoprotein receptor related protein 1 in the vascular CNS barriers are critical for regulation of Aβ homeostasis in the CNS and how altered activities in these 2 receptors at the blood–brain barrier may contribute to the CNS Aβ accumulation resulting in neuroinflammation, disconnect between the cerebral blood flow and metabolism, altered synaptic transmission, neuronal injury, and amyloid deposition into parenchymal and neurovascular lesions. We briefly discuss the potential of advanced glycation end products and low-density lipoprotein receptor related protein 1–based therapeutic strategies to control brain Aβ in animal models of AD and ultimately in patients with AD and related familial cerebrovascular β-amyloidoses. (Stroke. 2004;35[suppl I]:2628-2631.)

Key Words: acute care ■ Alzheimer disease ■ amyloid β-protein ■ blood–brain barrier

Continuous removal of amyloid β-peptide (Aβ) species from the central nervous system (CNS) is important for preventing their potentially neurotoxic accumulations in brain interstitial fluid (ISF). At the critical threshold concentrations in brain ISF, Aβ may initiate differential pathogenic cascades mediating neurovascular and neuronal stress and ultimately the development of cerebral and neurovascular amyloidosis and dementia in patients with Alzheimer disease (AD) and related Aβ-disorders. Aβ is produced by almost all cells in peripheral tissues and by all types of cells in the CNS, but Aβ’s physiological functions still remain unknown.

There is little evidence that normal brain aging results in local overexpression of the Aβ precursor protein (APP) and overproduction of Aβ. A relatively small number of AD patients may have increased Aβ production in the CNS because of inherited mutations in the APP gene nearby the Aβ coding region (ie, Swedish mutation) or presenilins 1 or 2 genes. However, the majority of patients with so-called nongenetic or late-onset AD and patients with familial forms of cerebrovascular β-amyloidosis do not have increased Aβ production or APP overexpression in the CNS. These patients likely exhibit a failure in Aβ clearance from the CNS because of either deficient transport efflux mechanisms for Aβ at the blood–brain barrier (BBB) or its faulty degradation in the CNS. Alternatively, an increased influx of circulating Aβ across the BBB may result in Aβ brain accumulation or its deposition in the CNS.

Regulation of Aβ levels in Brain ISF

Normal Aβ concentrations in brain ISF are carefully maintained by numerous pathways including (1) Aβ production in peripheral tissues, its systemic clearance and production in the CNS; (2) rapid receptor-mediated transport exchanges of free unbound Aβ between brain and blood and across the BBB; (3) the ability of Aβ carrier proteins (eg, apolipoprotein E [apoE], apoJ, α2-macroglobulin, transthyretin, and albumin) to bind and sequester Aβ in different extracellular fluid compartments, including plasma, brain ISF, and cerebrospinal fluid (CSF), or to influence its transport across the biological membranes isolating these compartments, including the BBB; (4) Aβ degradation by a variety of proteases, including enkephalinase, insulinase, plasmin, tissue plasminogen activator, or matrix metalloproteinases; (5) continuous slow removal of Aβ through the ISF-CSF bulk flow into the bloodstream; and (6) oligomerization of Aβ in the CNS.

Increased Aβ42 levels in brain ISF result in formation of neurotoxic Aβ oligomers and progressive synaptic, neuritic, and neuronal dysfunction. Alternatively, Aβ may form...
neurovascular or cerebral amyloid aggregates.\textsuperscript{14} In particular, missense mutations inside the Aβ sequence associate with vascular deposits and cerebral amyloid angiopathy.\textsuperscript{14} The development of cerebral amyloid angiopathy and parenchymal amyloid lesions in AD models is further substantially influenced by apoE and apoJ genes.\textsuperscript{15}

Studies in AD models suggested that Aβ brain efflux measurements may be useful for quantifying brain amyloid burden in patients at risk for or those who have been diagnosed with AD.\textsuperscript{8} For example, the intravenous administration of m266 monoclonal anti-Aβ antibody results in rapid efflux of Aβ from the CNS into plasma of plate-derived growth factor–driven mice increasing plasma Aβ to low nanomolar levels within 24 hours.\textsuperscript{8} The development of plaques in these mice\textsuperscript{16} and in senescent nonhuman primate models of cerebrovascular\textsuperscript{17} and parenchymal β-amyloidosis\textsuperscript{18} may shift the Aβ transport exchanges between the CNS and its peripheral pool toward the brain.

**Peripheral Aβ Pool**

Circulating Aβ pool reflects Aβ contributions from peripheral tissues and organs, on the one hand, and the CNS, on the other. Although the concentrations of free Aβ in brain ISF are ≈6-fold higher than in plasma under physiological conditions,\textsuperscript{19,20} the absolute amounts of free Aβ in body fluids available for transport exchanges at the BBB are ≈10-fold greater than the absolute amounts of Aβ in the brain ISF and CSF.

Increased levels of free Aβ in plasma have been reported in AD mouse models\textsuperscript{21} or after treatment with Aβ-peripheral binding agents.\textsuperscript{8,9,22,23} In AD patients, plasma Aβ levels are elevated and mainly incorporated into lipoproteins and different plasma proteins.\textsuperscript{24,25} The levels of circulating Aβ24 and Aβ40, after acid denaturation and chromatographic separation of Aβ carrier proteins in AD, are 54 nmol/L and 8.6 nmol/L, respectively,\textsuperscript{25} whereas free Aβ in the peripheral venous blood represents only a minute fraction of total plasma Aβ (ie, between 60 and 120 pmol/L).\textsuperscript{19}

**Aβ Transport at the BBB is Dominated by Receptor for Advanced End Glycation Products and Low-Density Lipoprotein Receptor Related Protein 1**

The BBB in vivo does not allow free exchanges of polar solutes such as Aβ between brain and blood, or between blood and brain, because of the presence of a continuous monolayer of brain endothelial cells that are zipped by tight junctions which effectively isolate brain ISF-CSF compartments from the plasma compartment.\textsuperscript{26} Therefore, specialized receptors at the BBB must exist to shuttle Aβ across the brain endothelium from CNS into the bloodstream or from blood into the CNS. The receptor for advanced end glycation products (RAGE) and low-density lipoprotein receptor related protein 1 (LRP) remain the most interesting targets, as demonstrated by their ability to rapidly transport circulating free Aβ into the CNS\textsuperscript{9} and brain-derived Aβ into the blood.\textsuperscript{7,30} (Figure 1).

Based on transport modeling with Aβ40,\textsuperscript{7,9} one can calculate that in healthy brain, if influx shut down, LRP efflux could remove all soluble Aβ at physiological levels from brain ISF in ≈1 minute (Figure 1). On the other hand, if efflux shut down, RAGE influx could replace all soluble Aβ in brain ISF by plasma Aβ in ≈40 minutes. The high excess capacity demonstrated in the LRP efflux system casts general doubt on theories of increased brain production of Aβ as the genesis of late-onset AD.

The possible implications for AD can also be seen from similar transport calculations. For example, if influx shut down, LRP efflux (assuming it remains normal) will be able to remove all soluble Aβ from brain ISF at increased levels as seen in AD at ≈12 nmol/L\textsuperscript{19} in ≈40 minutes. If one speculates that all insoluble Aβ at pathological levels in the brain could be resolubilized, then based on the transport modeling with Aβ40, it would take ≈65 hours to remove 4 μmol/L per kilogram of resolubilized Aβ (Figure 1). On the other hand, if efflux shut down, RAGE influx (if not blocked) could at ≈2 nmol/L Aβ plasma levels replace all soluble Aβ in brain ISF (Alzheimer levels) in <2 hours and may create an increment in Aβ levels in brain ISF at a rate of ≈0.15 μmol/L per kilogram per day.

**RAGE/Aβ Interactions at the BBB**

RAGE is a multiligand receptor in the immunoglobulin (IgG) superfamily, which binds soluble Aβ and mediates pathophysiologically relevant cellular responses consequent to ligation by a variety of ligands.\textsuperscript{27} RAGE is implicated in development of Alzheimer neurovascular disorder by mediating Aβ transcytosis across the BBB,\textsuperscript{9,20} by inflammatory and procoagulant responses in endothelium,\textsuperscript{9,27} and by promoting apoptosis through nuclear factor κB–dependent mechanism,\textsuperscript{28} as shown in Figure 2. Our recent study has demonstrated that RAGE mediates transport of pathophysiologically relevant concentrations of Aβ into the CNS.\textsuperscript{9} After
LRP/βamyloid Interactions at the BBB

LRP is a multiligand lipoprotein receptor which interacts with a broad range of secreted proteins and resident cell surface molecules (e.g., apoE, α2M, tissue plasminogen activator, plasminogen activator inhibitor-1, APP, factor VIII, and lactotransferrin), mediating their endocytosis or activating signal pathways through multiple cytosolic adaptor and scaffold proteins. LRP has been linked to AD genetically and may influence APP processing and metabolism and βamyloid uptake by neurons through α2M. Because APP mice overexpressing functional LRP minireceptors in neurons have increased levels of soluble βamyloid in the brain, it is unlikely that LRP on neurons in vivo mediates βamyloid clearance.

Recent studies indicate that LRP is expressed in brain capillary endothelium, and that LRP along the brain capillary membranes in vivo clears βamyloid into the blood. Because the formation of βamyloid complexes with either apoE or α2M has not been shown in the CNS in vivo during rapid clearance transport studies, it is possible that LRP interacts directly with βamyloid, and that this interaction may result in rapid clearance of βamyloid on brain capillaries.

RAGE and LRP-Based βamyloid-Lowering Strategies

Drugs that downregulate RAGE and upregulate LRP at the BBB may have a capability to readjust the transport equilibrium for βamyloid by promoting its efflux from brain into the bloodstream. In AD and in APP transgenic models of AD, RAGE is significantly upregulated at the BBB, whereas LRP is downregulated. Whether overexpression of RAGE influences the expression of LRP is currently unknown. Our recent findings demonstrate that RAGE-specific IgG can increase the expression of LRP in human brain endothelial cells exposed to βamyloid-rich environment by blocking the effect of βamyloid on RAGE receptor, suggesting the activities of the 2 receptors may be linked (Figure 3A). On the other hand, drugs that may upregulate LRP by acting directly on BBB endothelial cells, as we illustrate for the 2 statins (ie, simvastatin and lovastatin; Figure 3B and 3C), may also help in clearing βamyloid from brain.

The high binding affinity of βamyloid to RAGE and LRP may offer a basis for development of soluble products that can act as peripheral or central binding agents for βamyloid. An example of this is sRAGE, a truncated form of the RAGE receptor that does not contain the cytoplasmic domain of the receptor but does contain the V-type binding domain and is able to significantly reduce development of cerebral βamyloidosis in an AD mouse model. Another example could be soluble LRP fragments that bind directly βamyloid. Thus, future preclinical studies in APP animal models of AD should show...
whether RAGE and LRP-based strategies to modify Aβ transport exchanges at the BBB will have a major impact on clearing Aβ/amyloid across the BBB, with an ultimate goal to provide a safe therapy to control cognitive decline in AD and related cerebrovascular disorders.

Acknowledgments
This research was supported by grants from the US Public Health Service AG16223, NS34467, and AG23084 to B.V.Z.

References
RAGE (Yin) Versus LRP (Yang) Balance Regulates Alzheimer Amyloid $\beta$-Peptide Clearance Through Transport Across the Blood–Brain Barrier

Rashid Deane, Zhenhua Wu and Berislav V. Zlokovic

Stroke. 2004;35:2628-2631; originally published online September 30, 2004;
doi: 10.1161/01.STR.0000143452.85382.d1

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/35/11_suppl_1/2628

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/