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Is RAGE still a therapeutic target for Alzheimer's disease?

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Abstract

The receptor for advanced glycation end products (RAGE) is a multiligand receptor involved in inflammatory disorders, tumor outgrowth, diabetic complications and Alzheimer's disease (AD). RAGE transports circulating amyloid- β toxins across the blood–brain barrier (BBB) into the brain. RAGE–amyloid- β toxin interaction at the BBB leads to oxidative stress, inflammatory responses and reduced cerebral blood flow. Thus, regulating RAGE activity at the BBB and/or within brain could be beneficial to AD patients. Herein, the structure–function relation for RAGE–ligand interaction and the role of RAGE as a potential target in the development of treatments for AD and other RAGE-associated disorders are discussed. Despite recent setbacks in the development of RAGE-based therapies for AD, a new generation of compounds that regulate RAGE activity could be efficacious. Careful studies are needed in rodent and nonrodent animal models of AD with new the generation of RAGE antagonists to ensure safety and efficacy in chronic treatment before clinical trials.

Background

The receptor for advanced glycation end products (RAGE) is a 35–45 kD pattern recognition multiligand receptor and member of the immunoglobulin superfamily of receptor [1–3]. Since its discovery as a cell surface receptor in bovine lungs [4], it was shown to be ubiquitously expressed in many cell types, including various brain cell types, such as endothelial cells, pericytes, astrocytes and microglia [5–7]. While RAGE is a receptor for advanced glycation end products (which is the product of non-enzymatic glycation and oxidation of proteins and lipids [8]), it also interacts with several other ligands that are structurally unrelated, including HMGB1 (also called amphoterin), S100/calgranulins family of polypeptides (e.g., S100B, S100P, S100A1, S100A2, S100A4, S100A5, S100A6, S100A7, S100A8/A9, S100A12 and S100A13) [2,9,10], macrophage-1 antigen (Mac-1) [11], phosphatidylserine [12], lipopolysaccharides [13], transthyretin [14,15] and amyloid- β toxins (A β) (Table 1) [1,5,6,16]. In addition to cell surface RAGE, soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE) are present in human plasma but may [17] or may not be present in mouse plasma [18], depending on the methods used to detect RAGE.

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With the exception of lungs, the basal expression of RAGE is low but increases with the levels of its ligands and at sites of stress and injury [1,4–8].

RAGE is a cargo transporter [5,19] and a transmembrane cell-signaling receptor [1,20–23]. RAGE binds free A β and mediates pathophysiological cellular responses [1,5,21,22,24], including transport of circulating plasma A β across the **blood–brain barrier** (BBB) into the brain, oxidative stress and reduction in the cerebral blood flow (CBF) by increasing cerebral ET-1 levels and increasing levels of proinflammatory cytokines, such as TNF- α and IL-6 [5]. Thus, compounds that block or regulate A β /RAGE interaction at the BBB and/or cells within the brain may have multiple effects, such as reducing levels of oxidative stress, **neuroinflammation** and brain A β levels; this may improve CBF and learning and memory, all of which should have beneficial therapeutic effects in **Alzheimer's disease** (AD).

RAGE is also associated with other chronic diseases, including diabetes [24,25], cancer growth and metastasis [26], cardiovascular disease [27,28] and inflammation [29,30]. Pharmaceutical agents that antagonize RAGE or RAGE-knockout mice have suggested that blocking RAGE-mediated effects can slow the progression of **neurodegeneration**, chronic inflammation and oxidative stress [5,6]. Therefore, RAGE is a major therapeutic target for inhibiting pathophysiological consequences of ligand/RAGE interactions. Indeed, to antagonize ligand binding to RAGE, small compounds (FP-04494700; mono- and bicyclicazole derivatives) have been used in Phase II clinical trials for mild to moderate AD (ClinicalTrials.gov identifier: NCT00566397 [101]) and diabetes nephropathy (ClinicalTrials.gov identifier: NCT00287183 [102]). However, recently it was suggested that these studies in AD were ineffective but the reasons are unclear, at present. The results are not posted on ClinicalTrials.gov nor published in peer-reviewed journals. Despite this setback, other **RAGE blockers** with a possible different site of interaction with RAGE, and therefore different mode of action, may be effective in regulating RAGE activity in a safe and efficacious manner.

Structure–function relationship of RAGE

RAGE consists of three main regions: the extracellular domain (approximately amino acid residues 23–342) that interacts with the ligands, a transmembrane domain (residues 343–363) and a short intracellular domain (residues 364–404) involved in transmembrane signaling (Figure 1A). The extracellular region is composed of three immunoglobulin-like domains, a V-type domain and two C-type domains (C1 and C2) [31,32]. The amino acid sequences of the V-domain are residues 23–119, the C1-domain residues 120–233 and the C2-domain residues 234–325. High-resolution x-ray crystallography has shown that the V-C1 domains exist as an elongated structure at an angle (a bend) of 144/145° [2,33]. The V-C1 tandem domain is connected to the C2 domain by a flexible linker with a minimum of 7 amino acids [2,33]. The C2 region is structurally independent of the V-C1 domains. As a result, V-C1 forms a function unit with unique binding sites for most of RAGE ligands [2,3,33].

The forces involved in protein–protein interaction include hydrophobic, electrostatic, H-bond, disulfide bridge and van der Waals. The structural configuration of the V-C1 domains

is held together by several interdomain hydrogen bonds and hydrophobic interactions [2,26]. For example, the amino acid Gln-119 in the V-type domain bind via a pseudo hydrogen bond with Tyr-150 in the C1-type domain. In addition, the carboxylate oxygen of Glu-94 in the V-type domain binds with the hydroxyl hydrogen of Tyr-150 in the C1-type domain via hydrogen bonds [2]. However, hydrogen bonds are not the only force keeping the V-type domain bonded to the C1-type domain; hydrophobic interactions exist as well. There are hydrophobic interactions between the side chain of Pro-215 from the C1-type domain and the side chain of Tyr-118, and between the side chain of Tyr-150 and Ile-91 from another area of the C1-type domain [2]. Thus, the V-C1 complex exists as an integrated rigid structural unit for the interaction with ligands [2,33,34].

The V-C1 domains exist with some Gly and Pro residues in sequence that results in a loop structure with the occurrence of many Arg and Lys residues in the V-domain [2,3,33]. The large number of Arg and Lys residues means the V-domain is highly positively charged in these regions. Thus, there are elevated levels of ligand interaction in this binding domain that led to the theory that RAGE bind to ligands by electrostatic interaction, recognizing regions that are highly negative charged on the ligands. Thus, RAGE, a pattern recognition receptor [35–37], may recognize negative charge and/or hydrophobicity on its ligands. There are several positively charged patches on the V-domain of RAGE, including Arg-29, Lys-37, Lys-39, Lys-43, Lys-44, Arg-48, Lys-52, Arg-98, Arg-104, Lys-107, Lys-110, Arg-114 and Arg-116 [33,38,39], which may form two main regions [39] that interact with negatively charged regions of RAGE ligands (e.g., A β , S100 proteins, age-modified compounds and HMGB1) (Figure 2B). RAGE also has a number of hydrophobic pockets, which include residues Ile-26, Ala-28, Ile-30, Pro-33, Leu-34, Val-35, Leu-36, Trp-61, Val-63, leu-64, Trp-72, Val-75, Val-78, Leu-79, Pro-80, Phe-85, Leu-86, Pro-87, Ala-88, val-89, Ile 91 and Tyr-118 [33,39]. There is one hydrophobic cavity close to the C1 terminal formed by Ile-30, Pro-87, Ala-88, Ile-91 and Tyr-118 (Figure 2) [39].

The main ligands that bind to RAGE in the V-C1 ligand-binding pockets are AGEs, A β and S100 proteins [2]. While the molecular basis for the interaction of each ligand to RAGE is unclear, all RAGE ligands have a net negative charge at neutral pH and most tend to oligomerize [2,3]. At physiological pH, A β has a net negative charge. The surface of amyloid fibrils has patches of negative charges [2,3,16,35]. Residues 17–23 (LVFFAED) of A β interact with the V-domain of RAGE, a 30 amino acid residue N-terminal sequence (Figure 2A). This is a highly hydrophobic string of residues flanked by two negatively charged residues (ED) in A β [40]. Thus, compounds that interact with amino acid residues of the RAGE ligand-binding pockets, positively charged area or hydrophobic regions, may prevent A β interaction.

In the case of S100B, it is attracted to the receptor entropically by hydrophobic interactions dependent on calcium ions bound to S100B and on residues 54–67 of the V-domain [33,41]. To study the mechanism of RAGE–S100B interaction, isothermal titration calorimetry was used to better understand the thermodynamics of the interaction [33]. The isothermal titration calorimetry showed that the RAGE and S100B interaction features unfavorable positive enthalpy but favorable entropy [33]. Thus, the interaction is entropy driven. There are three factors that contribute to entropy-driven binding reactions: hydrophobic salvation

effects, conformational changes in protein structure and changes in rotation and translation. Two of these factors are unfavorable; conformation changes within the protein and changes in rotation and translation. The only favorable factor is binding in the hydrophobic ligand-binding clefts in RAGE that interact with S100B dimer, tetrameric and octameric protein [33]. Thus, RAGE antagonists that penetrate the BBB and achieve effective therapeutic levels in the brain could regulate interaction of all RAGE ligands binding to RAGE, including A β and S100B.

In the case of AGEs, RAGE does not recognize the specific amino acids that are modified, but instead it may recognize the negatively charged patches of the protein surface of AGE. In the formation of AGEs, the positive charge located on the protein surface may be removed or the negatively charged area could be introduced [3,33,42]. RAGE can also bind directly to DNA and RNA via the sulfate region within RAGE, which allows the phosphate group on nucleic acids to bind to RAGE [33]. Thus, electrostatic and hydrophobic interactions appear to be the main forces involved in RAGE–ligand binding. Compounds that blocks ligand binding to RAGE may interact with the positive charged regions or the hydrophobic areas.

RAGE isoforms

While RAGE is encoded by one gene, *AGER* [4], there are splice variants of this gene that encode for several forms of mutant RAGE [32], such as endogenous secretory RAGE (esRAGE or RAGE_V1), which contains a unique peptide at the C2-immunoglobulin domain [6,8,29], and dominant negative RAGE, which is cell surface bound but lacks the cytoplasmic domain [3]. In addition, there is an sRAGE formed by ectodomain shedding due to the proteolytic action of matrix metalloproteinase and/or ADAM 10 presumably on the cell surface of RAGE (Figure 1B) [6,32]. In human plasma/serum, levels of sRAGE and esRAGE may be associated with inflammatory disorders, diabetes and vascular disorders [6,10,17]. Using *in vivo* models, it was shown that sRAGE can block the effects of RAGE ligands on levels of inflammatory markers and can protect against complications in diabetes and other chronic illnesses [5,6]. In a mouse model of AD, PD-hAPP, sRAGE increased CBF and reduced neuroinflammation and brain A β levels by acting as a decoy receptor that sequesters the A β and preventing it from interacting with membrane bound RAGE [5]. However, RAGE is known to self-associate and forms oligomers via the C1 domain [2,38]. Ligand binding may stabilize the RAGE oligomers that lead to transmembrane cell signaling. It is also possible that binding of sRAGE to cell membrane bound RAGE, due to RAGE hetero-oligomerization, prevents A β binding to the V-C1 domain [2,38]. Levels of sRAGE may play a role in chronic disorders, such as cardiovascular, inflammatory, diabetes and AD. As a result, RAGE blockers and sRAGE may be used to regulate the effect of RAGE activation, and hopefully prevent or delay the onset and progression of these chronic disorders. In addition to a potential therapeutic target, these soluble RAGE molecules could be potential biomarkers, but further work is needed to elucidate their specificity and sensitivity as biomarkers of AD.

RAGE & AD

Accumulation of A β in the brain is associated with AD but its role in the etiology of the disease is unclear [43,44]. In the aging brain, faulty A β clearance via low-density lipoprotein related-receptor 1 (LRP1) and/or increased A β influx into brain from the circulation, via RAGE-mediated transport across the BBB, may be responsible for its progressive accumulation in sporadic AD [45–51]. Thus, there is bidirectional transport of A β across the BBB. Transport from blood to brain is mediated by RAGE expressed on the luminal surface of the BBB [5,52,53]. In contrast, transport from brain to blood is mediated by LRP1 expressed mainly on the abluminal surface of the BBB [45,47,50]. The transport of A β from blood to brain by LRP2 is mediated by apoJ but this is saturated at blood levels of apoJ, and therefore, plays a negligible role in the control of brain A β levels [45,49].

RAGE binds free A β in the low nanomolar range and mediates pathophysiological cellular responses [1,5,20–23]. These responses include transport of circulating plasma A β into the brain, oxidative stress, reduction in the CBF by increasing cerebral ET-1 levels and increasing levels of proinflammatory cytokines, such as TNF- α and IL-6 (Figure 3) [5]. In the brains of AD patients and mouse models of AD, RAGE expression is increased on cells of the neurovascular unit, particularly in an A β -rich environment [5,51–53]. Thus, compounds that block A β –RAGE interaction at the BBB and/or cells within the brain may have multiple actions, such as reducing neuroinflammation, improving CBF and cognitive decline, and reducing brain A β levels, which should have beneficial therapeutic effects in AD (Figure 3). In addition, RAGE antagonists that cross the BBB may reduce RAGE-mediated neuroinflammation, mainly via microglia, and A β generation, mainly by neurons, via inactivation of NF- κ B [54] and BACE1 expression (Figure 3) [55].

In APP^{sw+/-} mice and in AD patients, the increased levels of proinflammatory mediators in the brain are associated with the degree of A β deposits [56–59]. Microglia activity and their response to A β deposits may be regulated by inflammatory cytokines [59,60–63]. While the role of microglia in the clearance of A β deposits is unclear, they are a major source of these cytokines [61,64,65]. Reducing RAGE-mediated activity in microglia may reduce the secretion of proinflammatory cytokines, via inhibition of NF- κ B activation [1,54]. Consequently, microglia activity is reduced in the vicinity of A β deposits [64,65]. In addition to residential microglia, trafficking of blood-derived bone marrow stem cells or monocytes, which differentiate into microglia, would be affected by reducing the activity of RAGE at the BBB [66] or decreasing the secretion of chemotactic-attractant cytokines by microglia. In addition, reducing the levels of cytokines, such as TNF- α , a major proinflammatory cytokine secreted by microglia in AD and APP^{sw+/-} mice, may reduce neuronal cell death [67,68]. Taken together, reducing neuroinflammation may improve neuronal function, which in turn, improves learning and memory [69]. Thus RAGE antagonists that enter the brain may be efficacious in regulating microglia activity. Studies are needed to establish the level of microglia activity that is needed to control the accumulation of A β . However, RAGE antagonists that regulate both the production of A β and microglia activity might be effective in controlling levels of brain A β (Figure 3).

RAGE may also induce AD-like effects through astrocytes and another RAGE ligand, S100B. Activated astrocytes produce acute-phase reactants and proinflammatory molecules (such as S100B), which decreases neuronal survival. The overexpression of S100B acts to accelerate AD [70,71]. S100B can cause neuronal damage through overexpression of NO as seen with elevated levels in AD models. S100B also influence neurite extension [71]. Thus, RAGE blockers may act on several cell types within the brain to reduce AD pathology and improve learning and memory.

RAGE as a therapeutic target

While RAGE is associated with many vascular and inflammatory disorders as well as AD, its normal function is unclear. Thus, regulating RAGE activity could be more efficacious than blocking its action. This can be achieved by using antagonists that interact with cell surface RAGE or the use of sRAGE to modulate RAGE activity. sRAGE may act as a decoy receptor, which reduces ligand binding to cell surface RAGE, and thereby regulating the activity of RAGE. It may also form hetro-oligomers by interacting with cell surface bound RAGE [2,38]. While the use of sRAGE as a potential therapeutic approach has been tested in rodent modes of AD and diabetes, its efficacy and safety in larger animal models is yet to be determined. The effects of oligomerization between membrane bound RAGE and sRAGE is unclear [2,38].

Recently, a small compound (FP-04494700) has been used to antagonize ligand binding to RAGE in Phase II clinical trials for mild to moderate AD (ClinicalTrials.gov identifier: NCT00566397 [101]), but this clinical trial was terminated. It was suggested that this RAGE blocker was ineffective but the reasons are unclear. It is important that the data are published so that second generation compounds or other antagonist can be developed that could regulate RAGE activity without toxic effects.

There are two groups of small molecules that have been used as blockers of RAGE–A β interaction. One compound is anazole derivative (FP-04494700) that was used in the Phase II clinical. The molecule weight for one of these compounds is approximately 532 Da, and it has one H-bond donor (assuming the tertiary amine is protonated at the physiological pH), three H-bond acceptors, a logP of 7.46 and a 2D polar surface area of 40.72 at physiological pH. These properties were estimated since details of the structure of this compound is not published. The other compound is a tertiary amide that is still in preclinical development. The molecule weight is 327 Da, and it has one H-bond acceptor, a logP of 4.309, 2D polar surface area of 20.31 Å² at pH 7.4 and is uncharged at physiological pH. It appears as if the tertiary amide contains an electron-rich aromatic group, an electron-poor aromatic group and a hydrophobic group. It is possible that the hydrophobic group and/or the electron-rich aromatic group interact with RAGE. The IC₅₀ for inhibiting A β –RAGE interaction for theazole compound is in the high nanomolar range, while it is in the low nanomolar range for the tertiary amide. Thus, the properties of these compounds are different, which may indicate that their mode of action is different. Details on their molecular basis for inhibition of ligand binding to RAGE are needed.

Recently, it was shown that a type of tertiary amide, identified by library screening, competitively antagonizes A β -RAGE interaction at the BBB, it also improves functional CBF responses to brain stimulation and cognition, and reduces brain A β levels in a mouse model of AD, APP^{sw-/+} mice. A smaller second generation analog, with greater BBB permeability and potency than the parent compound, was more effective in reducing the levels of proinflammatory cytokines and A β production by inhibiting NF- κ B nuclear translocation and BACE1 activity, respectively. In turn, this improved CBF responses to brain stimulation and cognitive deficits, especially in APP^{sw+/-} mice with prominent A β deposits. These multiple beneficial effects of this class of compound may have therapeutic applications, especially in AD [72-76]. During production of this review on RAGE an article was published on the preclinical studies using the tertiary amide in a mouse model of AD [77].

Small organic chemical molecules may be more feasible given the cost associated with GMP production. Oral administration is convenient and compliance is good. The major unmet need in the AD therapeutic market is for disease-modification and -prevention drugs. RAGE is still a potential target in the development of therapies to regulate levels of brain A β , and in AD.

Future perspective

The following approaches should be applied in order to further develop RAGE as a therapeutic target:

- While the clinical trials (Phase II) with an azole derivative were terminated, it does not mean that RAGE blockers, in general, would not work. The reasons for the publicized failure are unknown, since the data were not published. Nevertheless, given the complexities of the RAGE structure and its ability to bind ligands that are structurally unrelated, it is possible that other RAGE blockers or second-generation compounds with a different mode of action may work. Further work is needed to explore the use of RAGE blockers. It is possible that a second-generation derivative of the azole compound or new generation of chemical compounds may be effective. A type of tertiary amide, which has different chemical properties to the azole, blocks A β -RAGE interaction with higher affinity and therefore needs further investigation. The search for a treatment of AD must continue, although the risk of failure in clinical trials seems high;
- The recent studies using x-ray crystallography to identify the molecular basis of ligand/RAGE interaction should lead to a better understanding on the mechanism for binding of multiple ligands to RAGE. This technique could be used to identify the site or sites of interaction of RAGE blockers to RAGE, and therefore the molecular basis for controlling RAGE activity. This should lead to the development of more specific and effective RAGE antagonists of A β ;

- Detailed pharmacokinetics are needed to establish bioavailability, metabolism and half-life ($t_{1/2}$) in plasma and tissues, such as brain;
- Further studies are needed in larger animal models of AD to demonstrate safety and efficacy before progression to clinical trials;
- In addition, the normal role of RAGE needs to be addressed further. RAGE knockout mice appear to have increased home cage activity and enhanced sensitivity to auditory stimulation [78]. Rather than blocking A β -RAGE interaction, it might be possible to modulate its interaction;
- Role of small organic molecules on oligomerization of RAGE and its effect on transmembrane cell signaling may help in determining the effectiveness of this therapy;
- Since activation of microglia may be a good and bad thing, its levels may need to be regulated but not blocked. The levels of RAGE blockers that enter the brain may need to be controlled to ensure that microglia activity is not completely blocked. This may also be the case for A β production by neurons, since the normal function of brain A β remains unclear.

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Key Terms

Blood-brain barrier	A cellular barrier that separates blood and brain by restricting the transport of polar molecules
Neuroinflammation	An inflammatory response in brain that mobilizes the body immune mechanisms
Alzheimer's disease	An irreversible, progressive brain disease that slowly destroys nerve cells, which leads to a loss in memory, thinking and behavior
Neurodegeneration	Structural and functional loss of nerve cells
RAGE blockers	Compounds that block the cell surface receptor called RAGE

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Executive summary

Background

- Receptor for advanced glycation end products (RAGE) is a multiligand receptor that binds structurally unrelated ligands. It is present as a cell surface bound receptor and as soluble RAGE (sRAGE) in plasma.
- The extracellular region is made up of three domains; V, C1 and C2. Raised levels of RAGE are associated with Alzheimer's disease (AD), inflammatory disorders and diabetes.
- At the blood-brain barrier, amyloid- β ($A\beta$)-RAGE interaction causes a reduction in cerebral blood flow, neuroinflammation and increased levels of brain $A\beta$.
- Thus, compounds that regulate $A\beta$ -RAGE interaction could be beneficial in AD patients. However, a recent Phase II clinical trial was terminated, but the reason is unclear since the data are not published.

Structure–function relationship of RAGE

- Recently, x-ray crystallography studies have revealed details of RAGE structure and possible sites for ligand interaction. These studies have shown that the V- and C1-domains function as a single fixed unit (V-C1 domain) while the C2-domain functions independently.
- These studies have also shown that RAGE has patches of positive charge and regions of hydrophobicity that may interact with ligands. Thus, ligands with patches of negative charge or containing regions of hydrophobicity may interact with RAGE by pattern recognition.
- $A\beta$ has regions of negative charge and areas of hydrophobicity that may interact with RAGE. Thus, compounds that block the positive-charge patches on RAGE that interact with $A\beta$ may prevent $A\beta$ -RAGE-mediated effects. Also, compounds that block the hydrophobic regions on RAGE that interacts with $A\beta$ may prevent $A\beta$ -RAGE-mediated effects.
- These are at least two ways by which RAGE blockers may interfere with the pattern recognition mechanism of $A\beta$ /RAGE interaction.

RAGE isoform

- There are many isoforms of RAGE. Cell membrane bound RAGE may interact, via the C1 domain, with itself forming self oligomerization, or with sRAGE forming hetero-oligomers. These oligomerizations may be enhanced with ligand-RAGE interactions that could stabilize the complex and mediate transmembrane cell signaling.
- sRAGE maybe a possible biomarker.

- The role of RAGE blockers on RAGE oligomerization is unclear.

RAGE & AD

- Levels of RAGE are increased with aging and in AD brains. This would increase the transport of A β across the blood-brain barrier into brain, reduce cerebral blood flow and increase neuroinflammation. In the brain, RAGE may increase A β production by neuron, and neuroinflammation mainly by microglia.
- Thus regulating A β /RAGE interaction with RAGE blockers may be beneficial in AD.

RAGE as a therapeutic target

- To date there are two classes of small organic compound that have been shown to block A β -RAGE interaction:azole-derivatives and a type of tertiary amide.
- Clinical trials (Phase II) with theazole compound were terminated, but the reasons for this are not published.
- Preclinical studies with the tertiary amide in a mouse model of AD have shown that this compound was effective, but further work is needed before it should be used in clinical trials.
- These two compounds are chemically different, and therefore there mechanism of action may also be different.
- sRAGE may act as a decoy receptor that prevents ligands binding to the cell surface RAGE. It may do so by forming hetero-oligomers with cell surface bound RAGE, which could prevent ligand binding to cell surface RAGE.

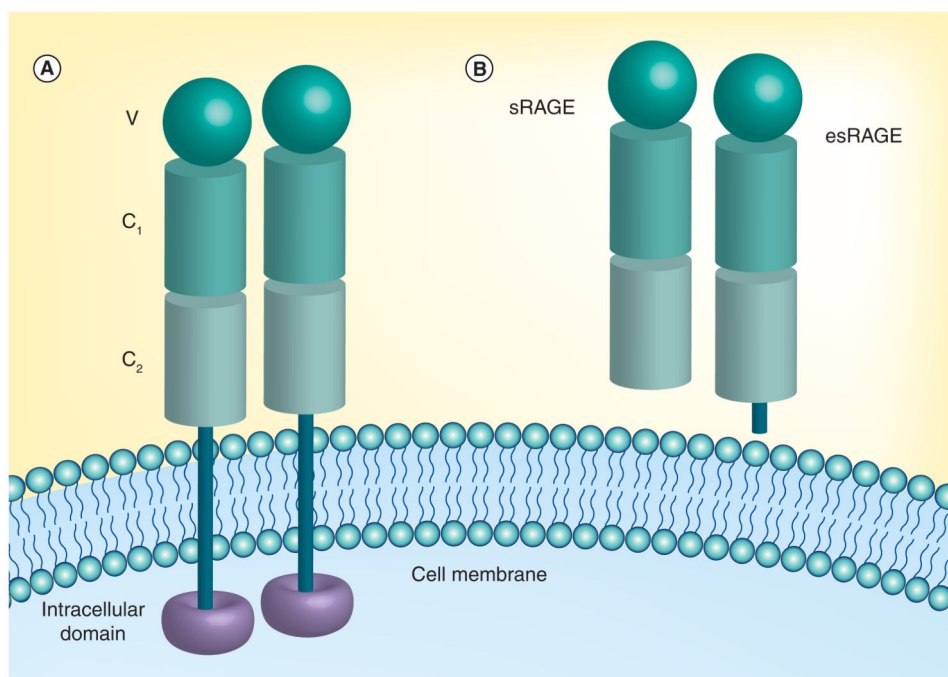


Figure 1. Structure and location of receptor for advanced glycation end products isoforms
(A) Structure of RAGE. RAGE consists of three main regions: the extracellular domain that interacts with the ligands, a transmembrane domain and a short intracellular domain involved in transmembrane signaling. The extracellular region consists of three immunoglobulin-like domains, a V-type domain and two C-type domains (C₁ and C₂). RAGE may be oligomerized via the V-domain. **(B)** sRAGE. There are splice variants of the gene that encode for several forms of mutant RAGE, such as esRAGE or RAGE_V1, which contains a unique peptide at the C₂-immunoglobulin domain. There is an sRAGE formed by ectodomain shedding due to the proteolytic action of matrix metalloproteinases and/or ADAM 10, presumably on the cell surface of RAGE.
 esRAGE: Endogenous secretory receptor for advanced glycation end products; sRAGE: Soluble receptor for advanced glycation end products.

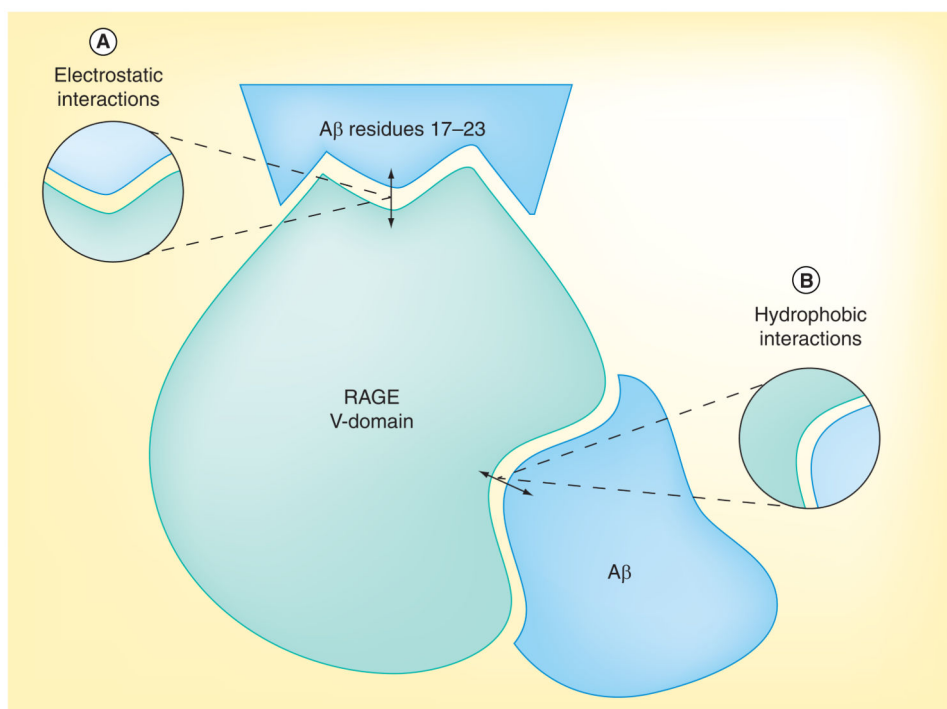


Figure 2. Interaction sites between receptor for advanced glycation end products and its ligands Residues 17–23 (LVFFAED) of A β interact with the V-domain of RAGE. This is a highly hydrophobic string of residues flanked by two negatively charged residues (ED) in A β . **(A)** RAGE, a pattern recognition receptor, may recognize negatively charged regions on its ligands. There are several positively charged patches on the V-domain of RAGE, including Arg-29, Lys-37, Lys-39, Lys-43, Lys-44, Arg-48, Lys-52, Arg-98, Arg-104, Lys-107, Lys-110, Arg-114 and Arg-116, which may interact with negatively charged regions on A β . **(B)** RAGE also has a number of hydrophobic pockets, which includes residues Ile-26, Ala-28, Ile-30, Pro-33, Leu-34, Val-35, Leu-36, Trp-61, Val-63, leu-64, Trp-72, Val-75, Val-78, Leu-79, Pro-80, Phe-85, Leu-86, Pro-87, Ala-88, Val-89, Ile-91 and Tyr-118. There is one hydrophobic cavity close to the C1 terminal formed by Ile-30, Pro-87, Ala-88, Ile-91 and Tyr-118.

A β : Amyloid- β ; RAGE: Receptor for advanced glycation end products.

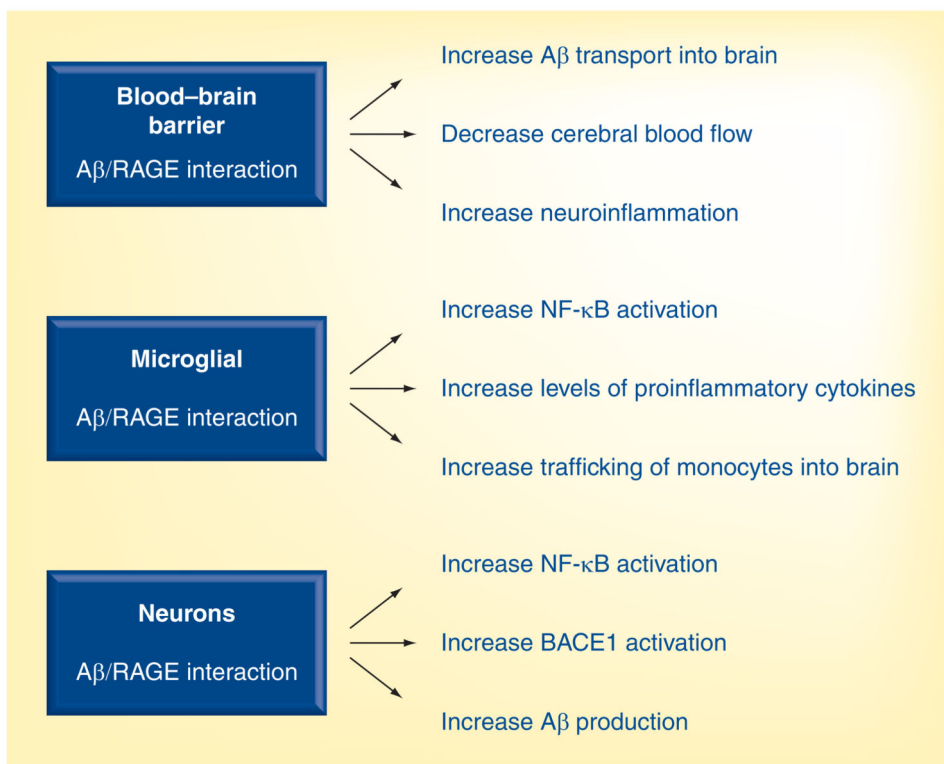


Figure 3. Amyloid β /receptor for advanced glycation end products-mediated effects at the blood-brain barrier, microglia and neuron

These effects will be regulated with new second-generation RAGE antagonists.

A β : Amyloid β RAGE: Receptor for advanced glycation end products.

Table 1

Receptor for advanced glycation end products main ligands and their functions.

Ligand	Ligand family	Ligand-binding region	Function	Related disease
Advanced glycation end products	AGEs	V-type	Advanced glycation endproducts are present in high levels in diabetes	Diabetes
HMGB1	HMGB1	V-type	Involved in cell stress mechanisms. It also induces cellular migration	Tumor outgrowth
S100b	S100 calgranulins	V-type	Calcium-binding protein that is related to Alzheimer's disease	Alzheimer's disease
S100A6	S100 calgranulins	C2-type	Calcium-binding protein that binds to a different domain	Alzheimer's disease
A β	A β	A β /oligomer – V-type Aggregate – C1 type	Oligomer species appears more pathogenic. A β transport at the blood–brain barrier, cerebral blood flow reduction and neuroinflammation	Alzheimer's disease
Mac-1	β 2-integrin	VC1 subunit	Involved with leukocyte recruitment relevant in inflammatory disorders	Diabetes
Transthyretin	Transthyretin	VC1 subunit	Associated with extracellular amyloid deposits that lead to degeneration of neurons in the peripheral nerve	Alzheimer's disease
LPS	LPS	VC1 subunit	A bioactive component of bacterial cell walls, it can bind with RAGE to regulate inflammatory responses	LPS-induced septic shock
Phosphatidylserine	Phospholipid	Bind on soluble RAGE	Assists in the clearance of apoptotic cells	Diabetes and atherosclerosis

A β : Amyloid- β ; LPS: Lipopolysaccharide; RAGE: Receptor for advanced glycation end products.