Dysfunction of Cerebral Endothelium in Alzheimer's disease

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Global burden of dementia

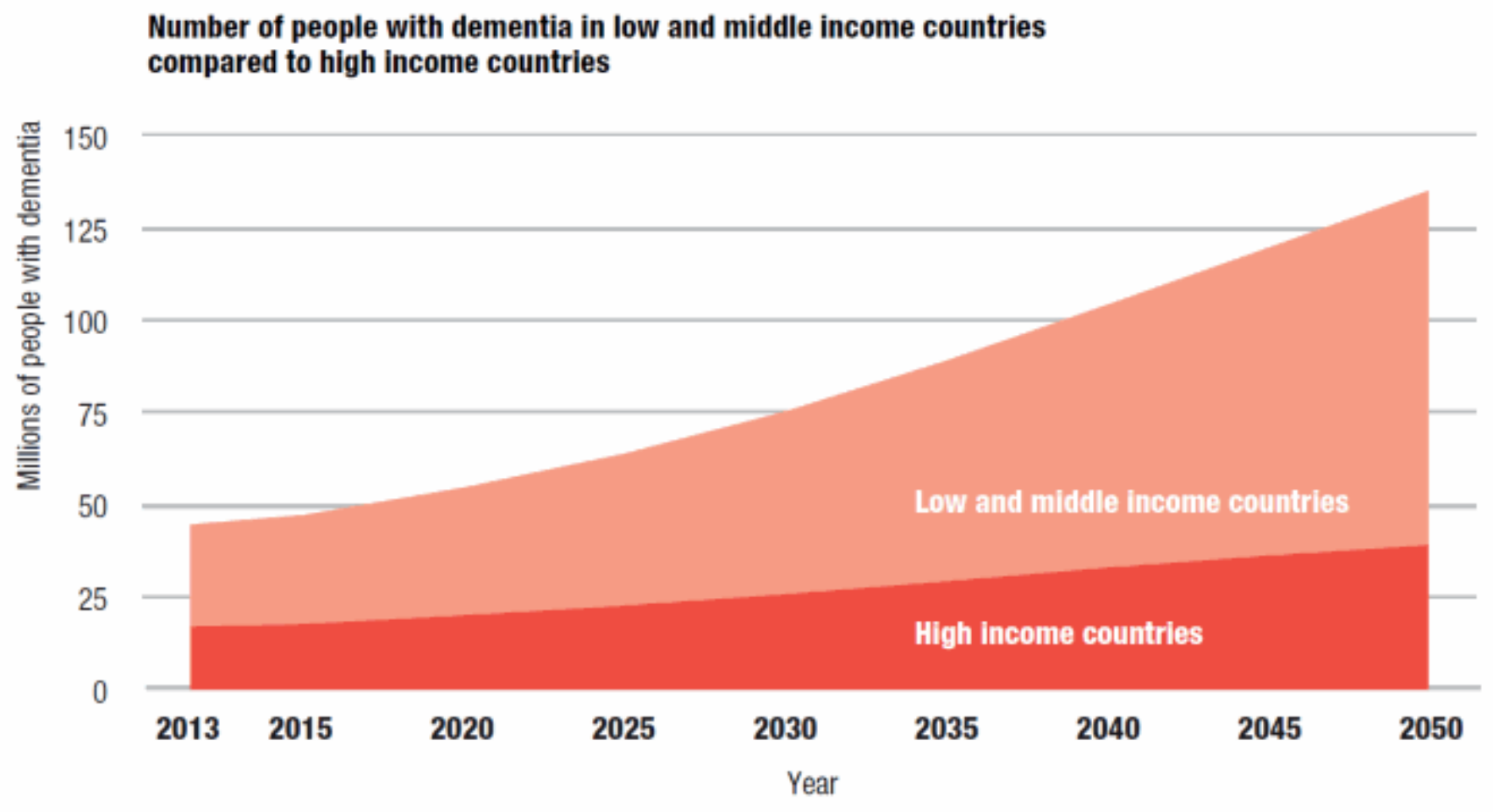
As of 2013, there were an estimated **44.4 million** people with dementia worldwide.

This number will increase to an estimated **75.6 million** in 2030, and **135.5 million** in 2050.

Much of the increase will be in developing countries.

Source: http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf

Already **62%** of people with dementia live in developing countries, but by 2050 this will rise to **71%**.



Source: http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf

In China, Hong Kong and Taiwan

about

8.4

million

people living with dementia

In the United States

more than

5

million

people living with dementia

In Russia

more than

1.5

million

people living with dementia

In the United Kingdom

815,827

people living with dementia

Kazakhstan

???

Alzheimer's disease (AD)

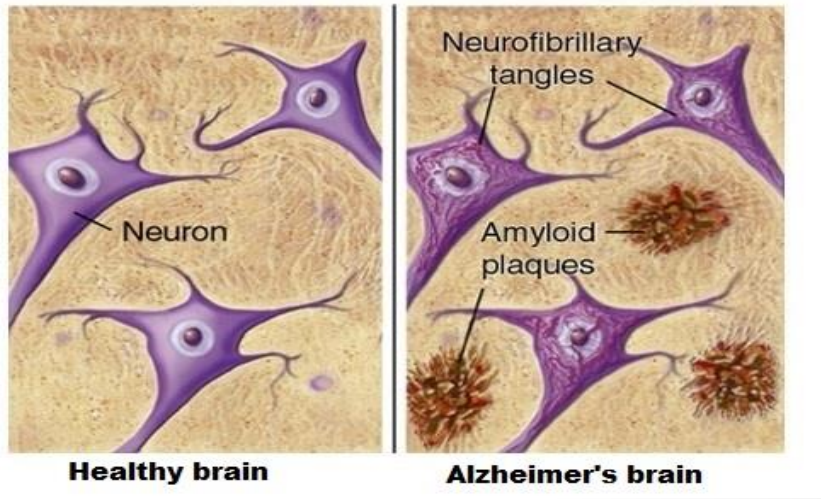
- Alzheimer's disease is the most common form of dementia in the elderly, which accounts up to 80 % of all cases.
- Clinical manifestation of the disease includes cognitive impairments such as attention deficit, spatial disorientation, speech disorders, and progressive loss of memory.
- In the late stage of Alzheimer's, people generally lose the ability to communicate coherently, experience a decline in physical abilities and require total assistance with personal care.
- Those with Alzheimer's live an average of eight years after their symptoms become noticeable to others, but survival can range from four to 20 years, depending on age and other health conditions.

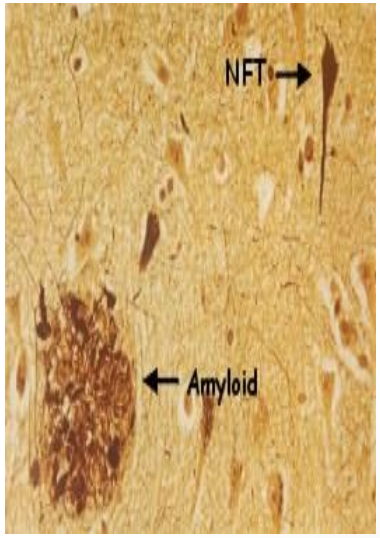
http://www.youtube.com/watch?v=2iHUXSVv8Mg

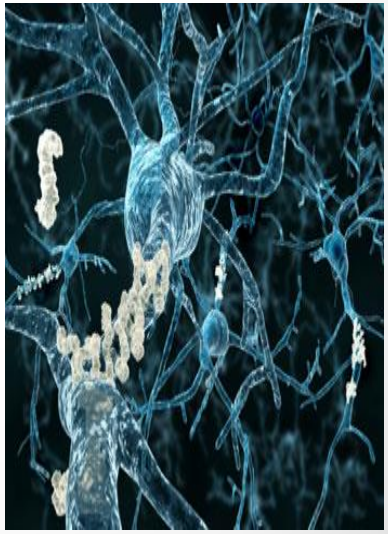
- **Late-onset (sporadic) Alzheimer's.** This is the most common form of Alzheimer's disease, accounting for about 90% of cases, and usually occurs after age 65. It may or may not be hereditary.
  - The ApoE4 variant of gene encoding Apolipoprotein E (ApoE) is the largest known genetic risk factor for late-onset sporadic Alzheimer disease
- **Familial Alzheimer's disease (FAD)** is a rare form of disease that is known to be entirely inherited. In affected families, members of at least two generations have had Alzheimer's disease. FAD is extremely rare, accounting for less than 1% of all cases of Alzheimer's disease. It has a much earlier onset (often in the 40s).
  - Early-onset Alzheimer's is linked with a genetic defects on chromosome 14 (PSEN 1 gene), chromosome 1 (PSEN 2 gene), chromosome 21 (APP gene) to which late-onset Alzheimer's is not linked.
  - People with Down syndrome (trisomy 21) are at risk for a form of early onset Alzheimer's disease as well. Adults with Down syndrome are often in their mid- to late 40s or early 50s when symptoms first appear.

Pathophysiology of AD

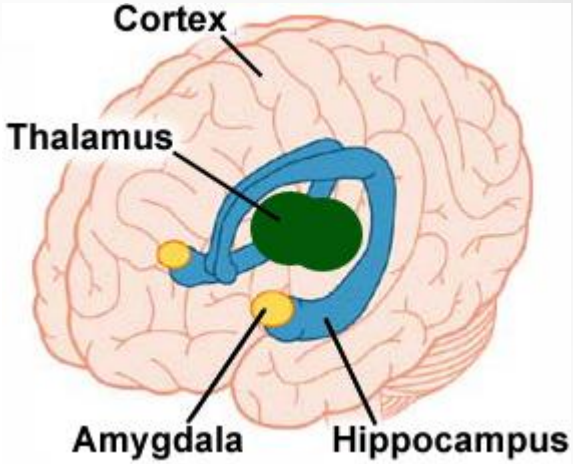
- AD is characterized by the accumulation of amyloid plaques and neurofibrillary tangles accompanied by impairment of the neurons in specific regions of the brain

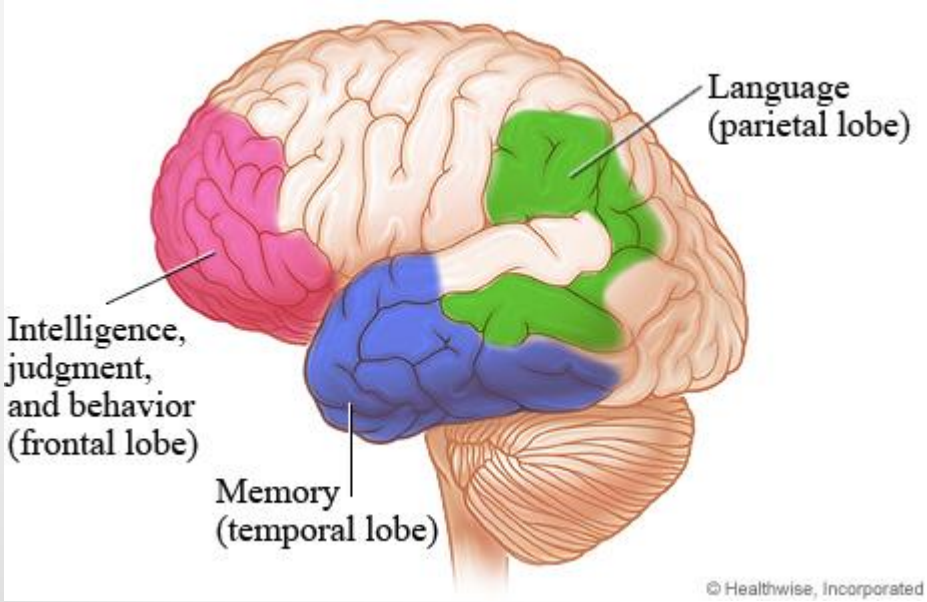




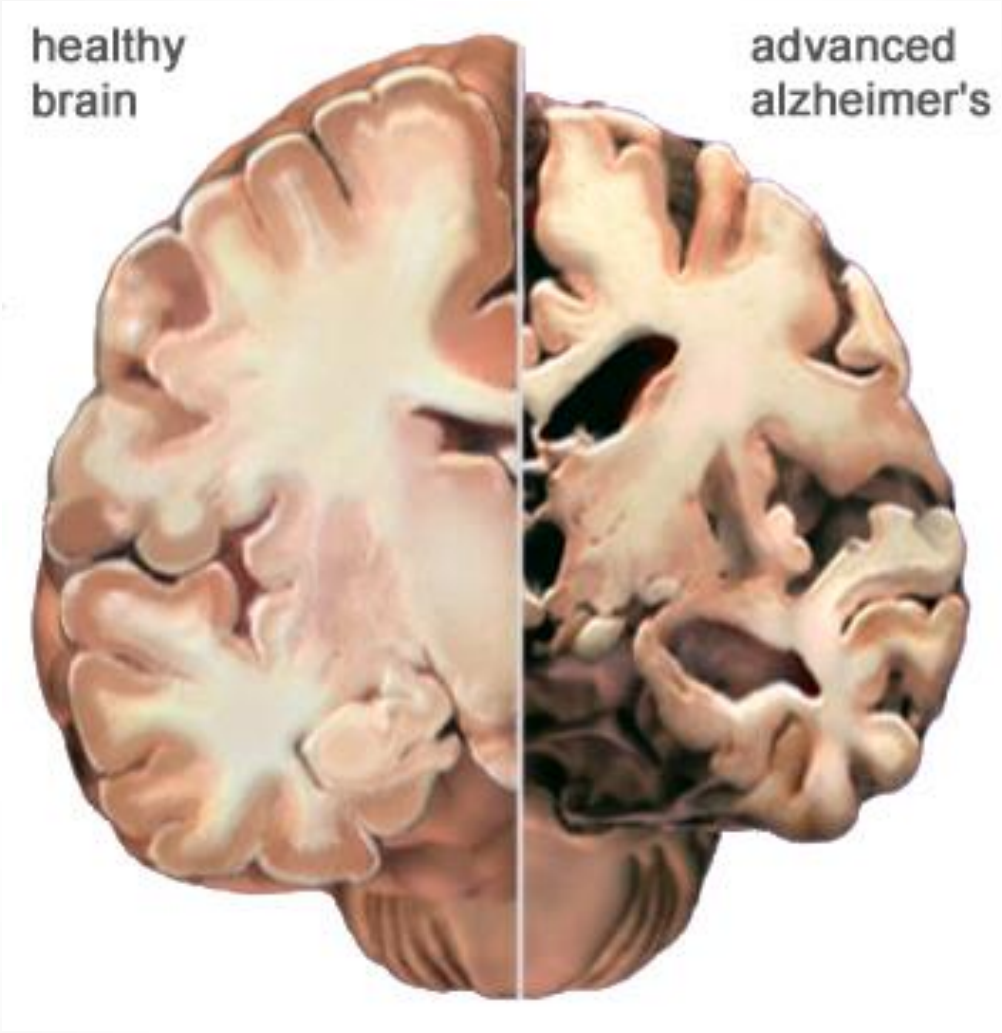


- The hippocampus and amygdala are among the first brain structures to be affected by Alzheimer's disease which is correlated with emotional instability and short-term memory disturbance.





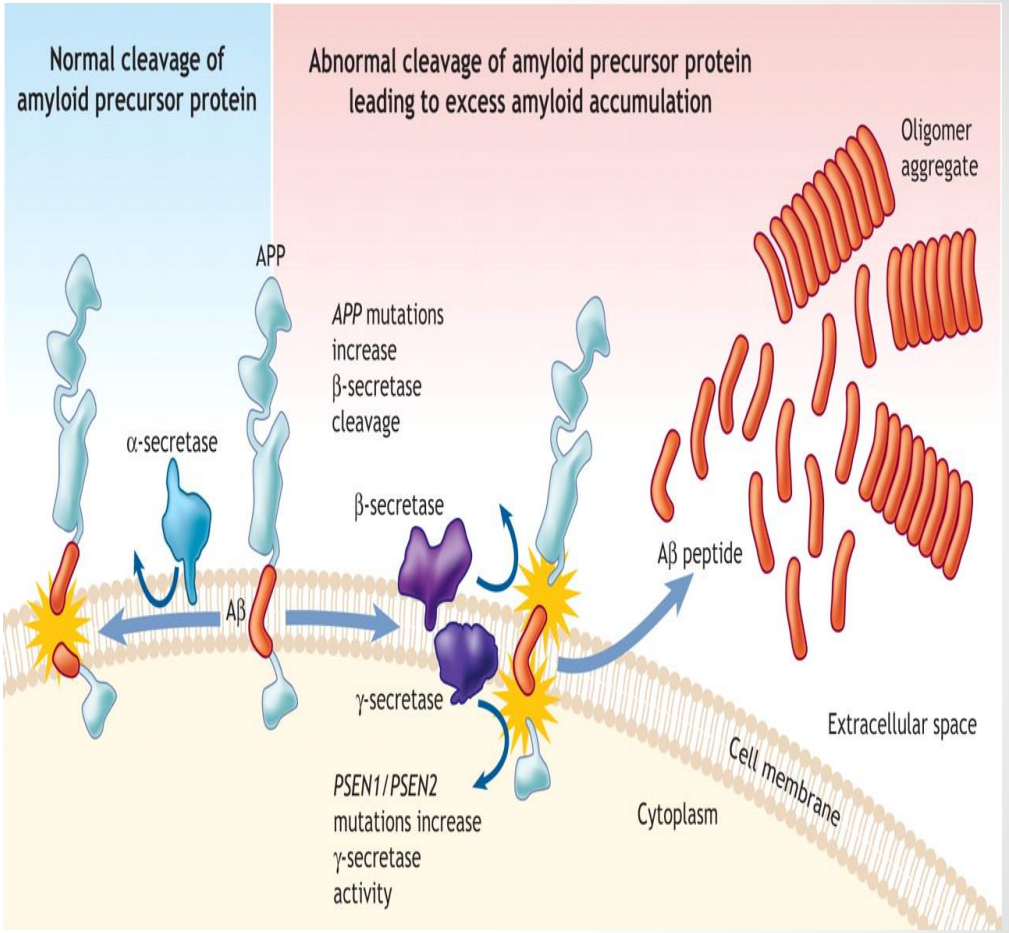
- Progressive atrophy of the frontal, parietal and temporal lobes is associated with loss of language and long-term memory, changes in personality and behavior.



By the final stage, plaques and tangles spread throughout the brain, and brain tissue shrinks significantly

Amyloid Cascade Hypothesis

- An increased deposition of amyloid-β peptide (Aβ), the main constituent of senile plagues, initiates a sequence of events that ultimately lead to AD dementia.
- Aβ is derived from amyloidogenic cleavage of membrane bound amyloid precursor protein (APP) by β- and γ-secretase.
- Amyloidogenic processing of the APP leads to the production of Aβ peptides of different length
- $Aβ_{1-40}$ is the major species and the $Aβ_{1-42}$ is the most fibrillogenic and predominant component in AD plaques.
- Recent research has indicated that smaller Aβ aggregates, which are soluble in the intercellular fluid and may form at an earlier stage, are the most toxic form of the peptide.



- While Aβ is a key molecule in AD, epidemiological studies have shown that vascular disorders like atherosclerosis, ischemia, hypertension, stroke, transient ischemic attacks, microvessel pathology, and diabetes mellitus are among the risk factors for AD

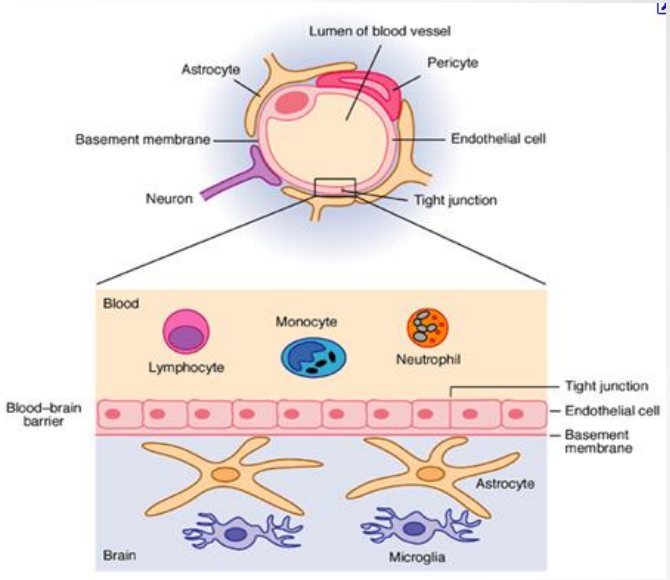
- In fact, cerebral hypoperfusion precedes cognitive decline, and neurodegeneration in AD.

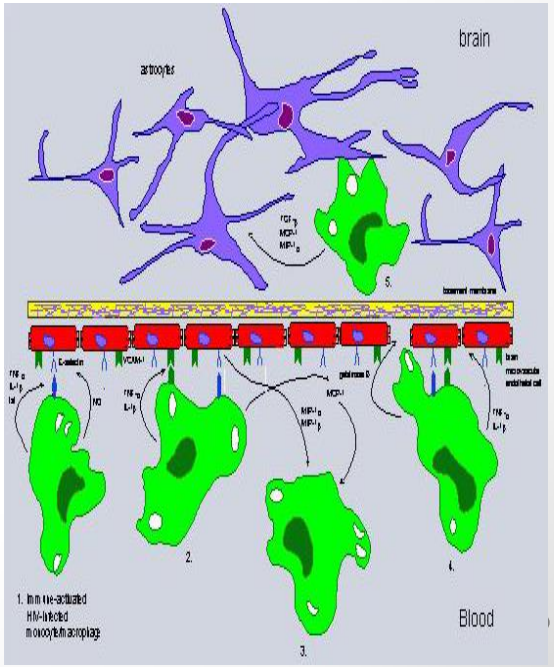
- Therefore, disturbance of cerebrovascular system is likely a major contributor to AD pathogenesis.

Little is known about mechanisms that underlie Aβ induced damage of cerebrovascular cells.

Blood-Brain Barrier Dysfunction in AD

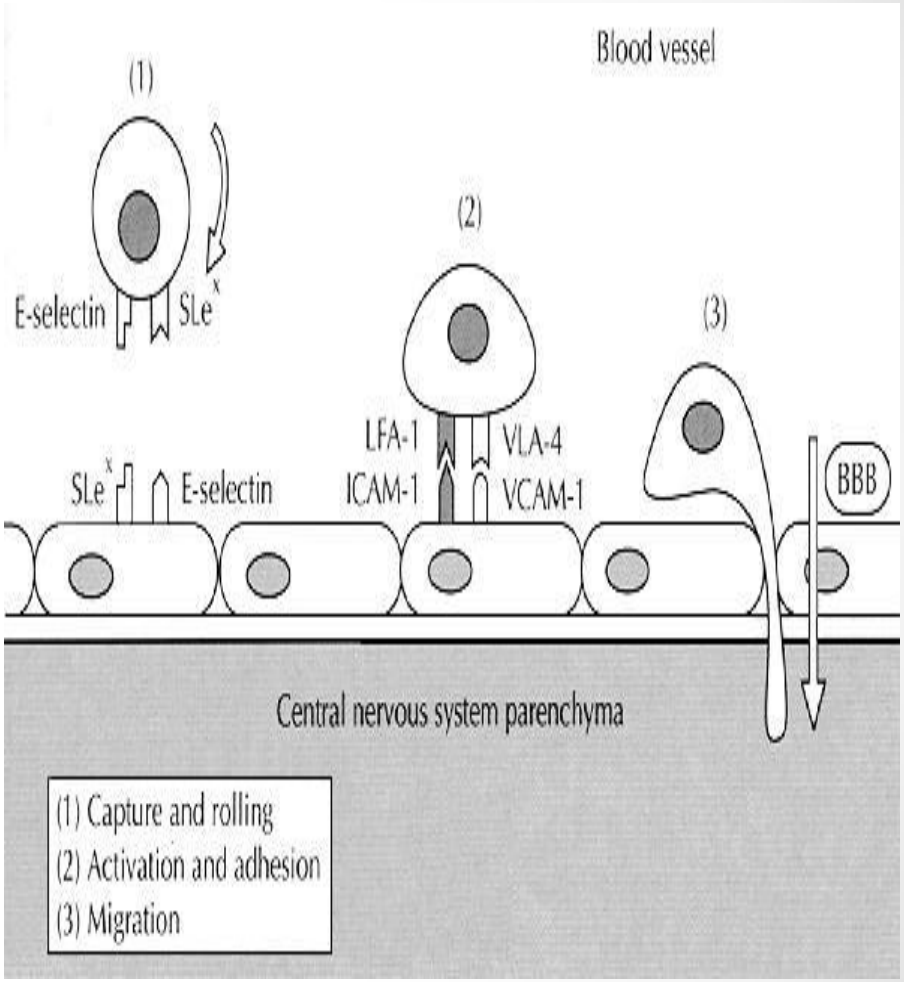
- Recent *in vitro* studies have demonstrated that amyloid-β peptide enhances adhesion and transmigration of monocytes across BBB.
- Cell-cell adhesion is governed by the expression of adhesion molecules as well as the mechanical properties of the cells
- However, the Aβ -enhanced adhesion between endothelial cells and monocytes has not been fully understood.
- We aimed to study the direct effects of $A\beta_{42}$ oligomers on expressions of adhesion molecules and cell mechanical properties of CECs



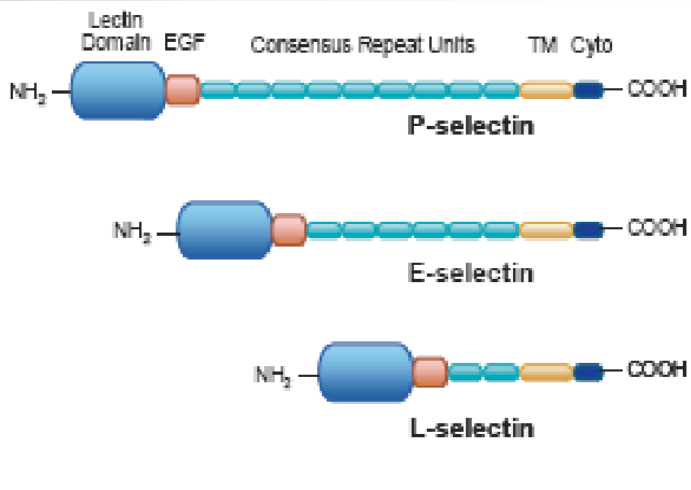


- At least five steps of the adhesion cascade are capture, rolling, slow rolling, firm adhesion, and transmigration

- Rolling adhesion is an initial step in transmigration of monocytes governed by dynamic bond formation and rupture between adhesion molecules selectins and their ligands.

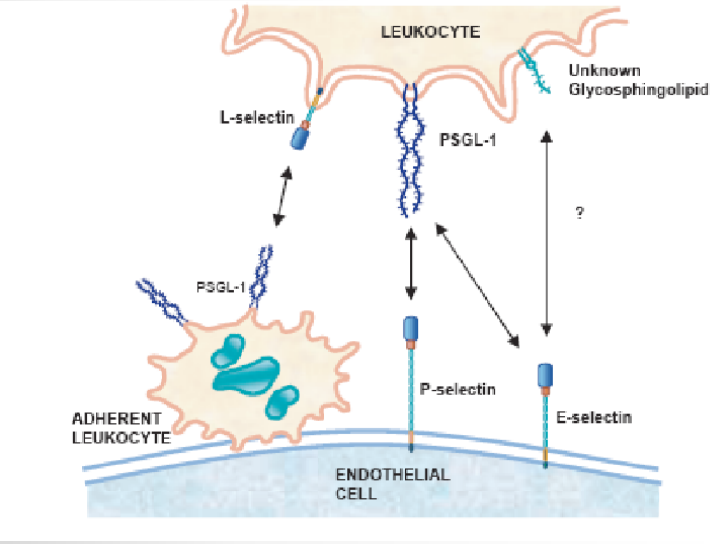


http://www.youtube.com/watch?v=297HcgDxb7k

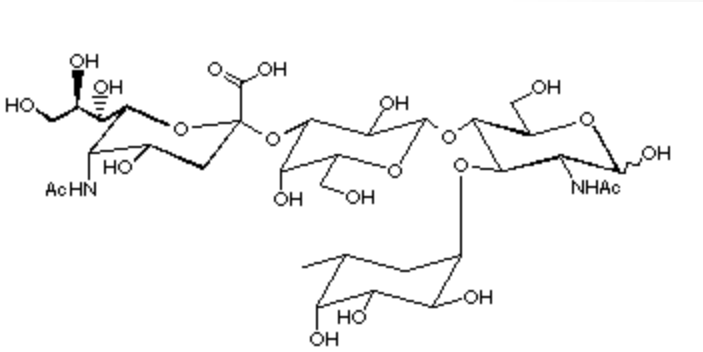


Ligands for selectins:

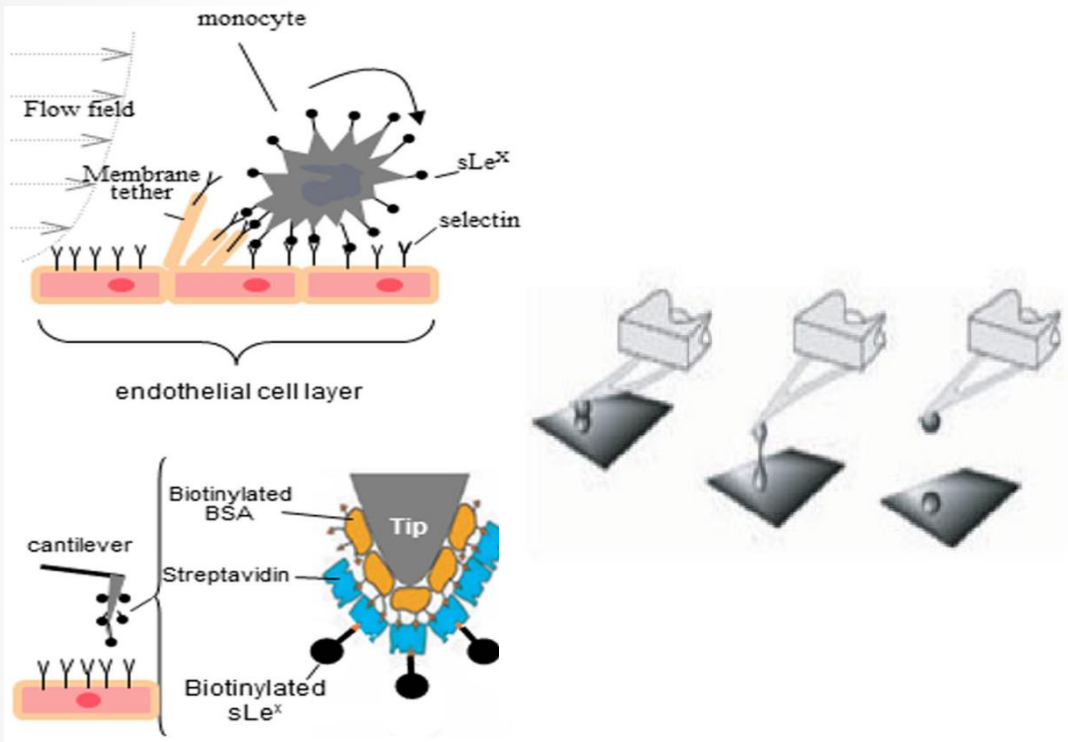
- P-selectin glycoprotein ligand 1 (PSGL-1)
- E-selectin ligand 1 (ESL-1)
- Glycosylation-dependent cell adhesion molecule 1 (Gly-CAM-1)
- CD34

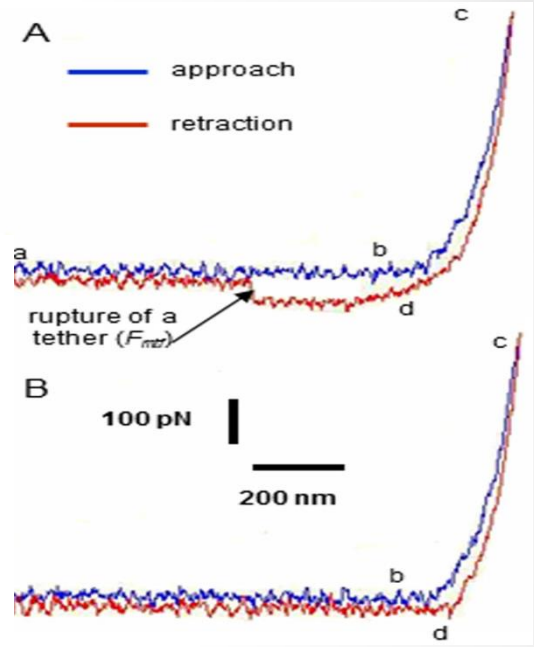


Sialyl Lewis X (sLe^x).



AFM cantilever tips bio-functionalized by sLe^x were applied to examine the sLe^x-selectin tethering at the cerebral endothelial cell surface

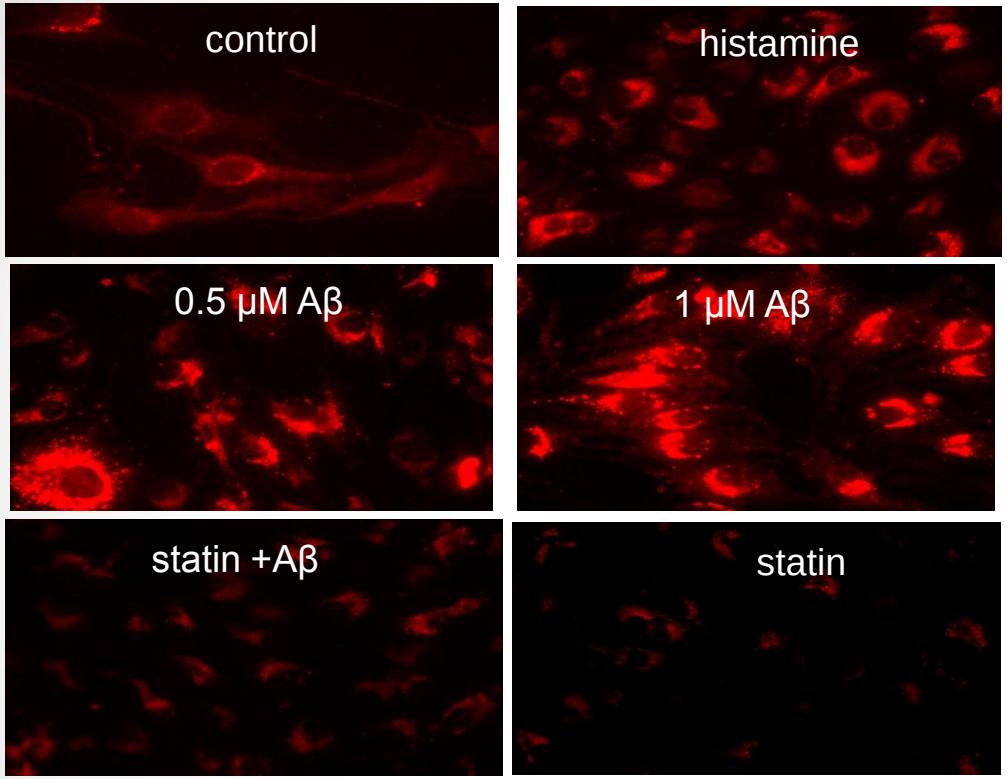




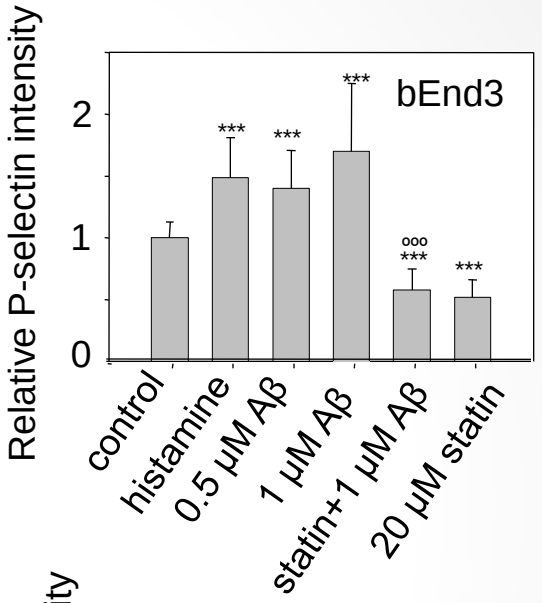
Schematic descriptions of membrane tether formation and biofunctionalization for the AFM cantilever tip. Membrane tether formation mediated by sLex-selectin bonding during a monocyte rolling on the endothelial layer (upper) and the strategy using AFM cantilever tips bio-functionalized by sLex to characterize the mechanics of membrane tether adhesion (lower).

Typical force curves obtained from AFM measurement. (A) Approach and retraction force curves with adhesion; and (B) without adhesion. The cantilever approaches (a to b), touches (b), makes indentation (b to c) and retracts (d) from the cell. Force of membrane tether formation (Fmtf) was measured at the sudden drop of force when a rupture of a membrane tether occurred (denoted by an arrow).

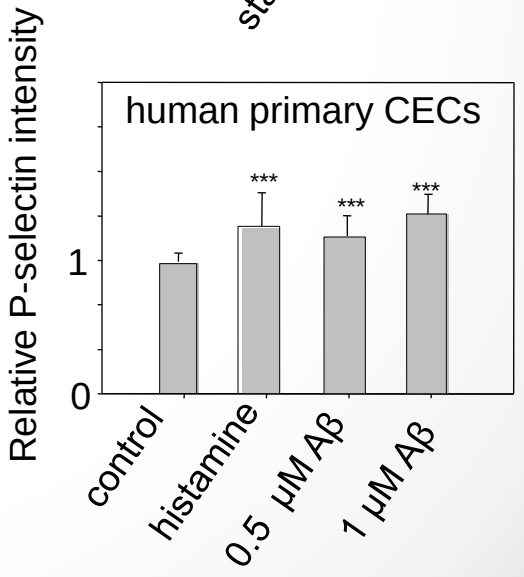
Aβ42 enhanced expression of P- selectins on the cells surface and these effects were counteracted by lovastatin



B



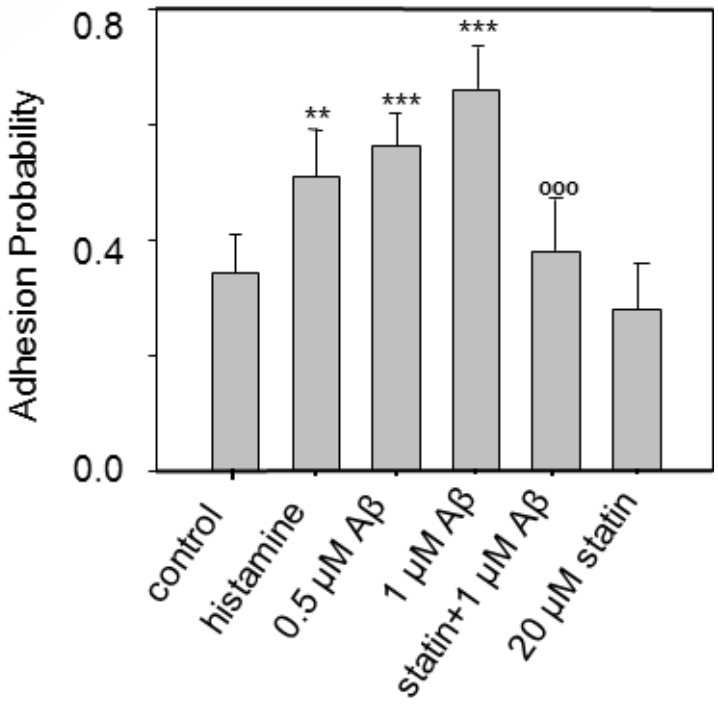
C



Effects of Aβ, histamine, and lovastatin on P-selectin expression at the CEC surface. (A) Fluorescent micrographs of fluorescently-labeled P-selectin at the bEnd3 cells. (B) Relative P-selectin intensity at the bEnd3 cell surface and (C) the human primary CEC surface.

*** - $p \leq 0.001$, ** - $p \leq 0.01$ compared to the control;
ooo - $p \leq 0.001$ compared to the 1 μM of Aβ.

Adhesion probability characterized by AFM



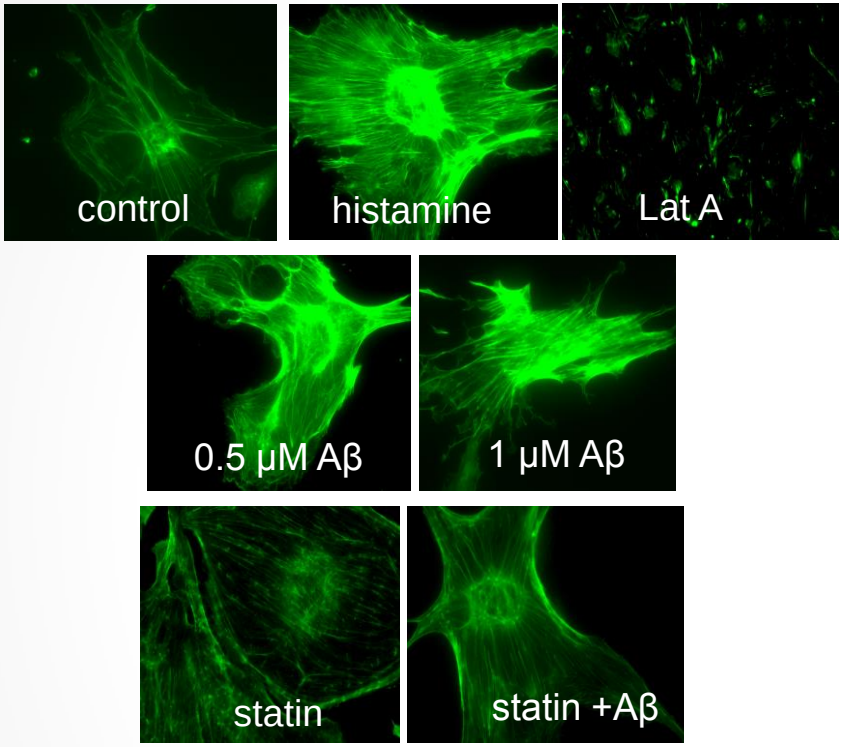
Aβ and histamine increase number of adhesion events for AFM cantilever tips bio-functionalized by sLe^x at the surface of the bEND3 cells, while lovastatin decreases adhesion probability

Adhesion probability was calculated by normalizing the number of force curves with adhesion events by the total number of force curves.

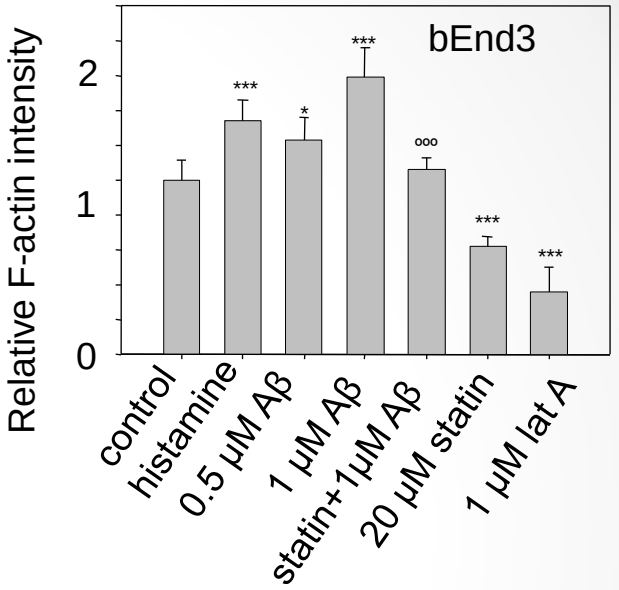
***p ≤ 0.001, **p ≤ 0.01 compared to the control;
°°°0.001 compared to the Aβ (1 μM) treatment group.

Aβ42 promoted actin polymerization within CECs and lovastatin decreased F-actin fluorescence

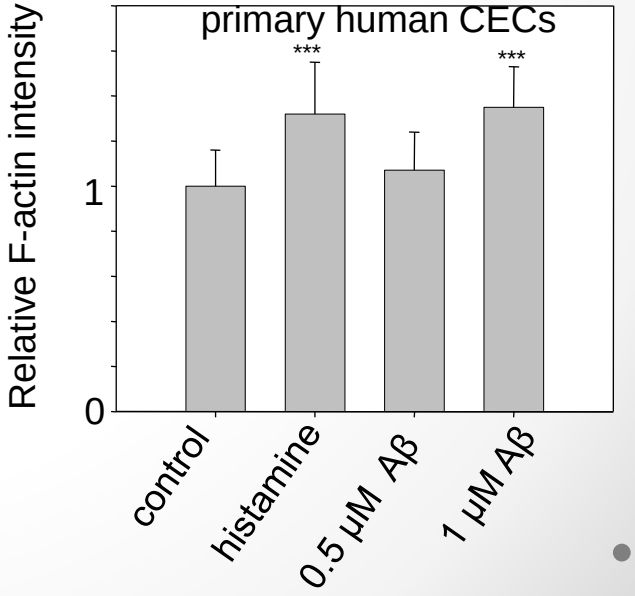
A



B



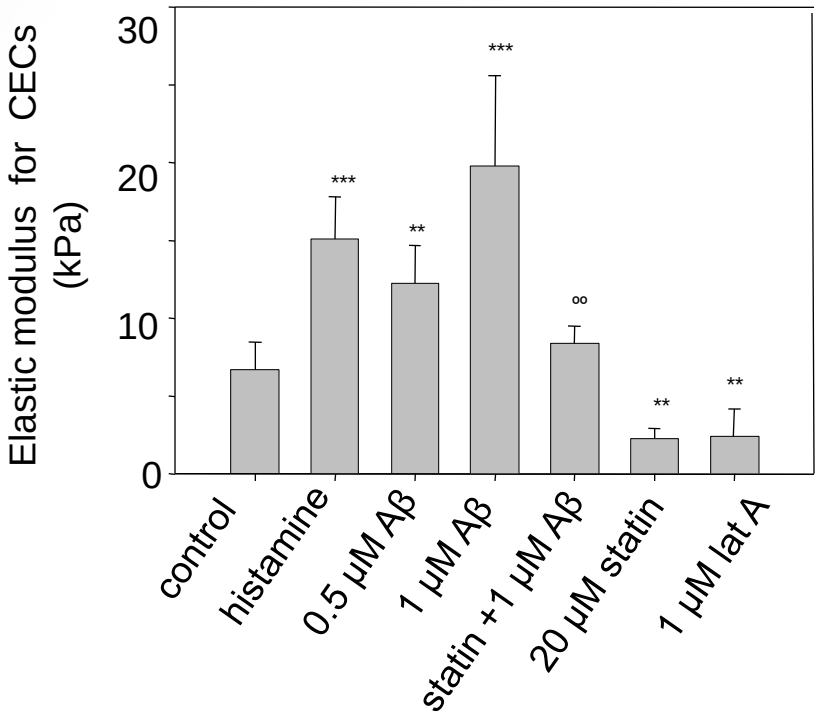
C



Effects of Aβ, and histamine on actin polymerization in CECs. (A) Fluorescent micrographs of Oregon-green phalloidinlabeled F-actin in bEnd3 cells. (B) Relative F-actin intensity in bEnd3 cells and (C) primary human CECs.

*** - p ≤ 0.001, ** - p ≤ 0.01, * - p ≤ 0.05 compared to the control; ooo - p ≤ 0.001 compare to the Aβ (1 μM) treatment.

AFM data showed that Aβ42 and histamine increased, and statin and latrunculin A decreased the overall cell stiffness.

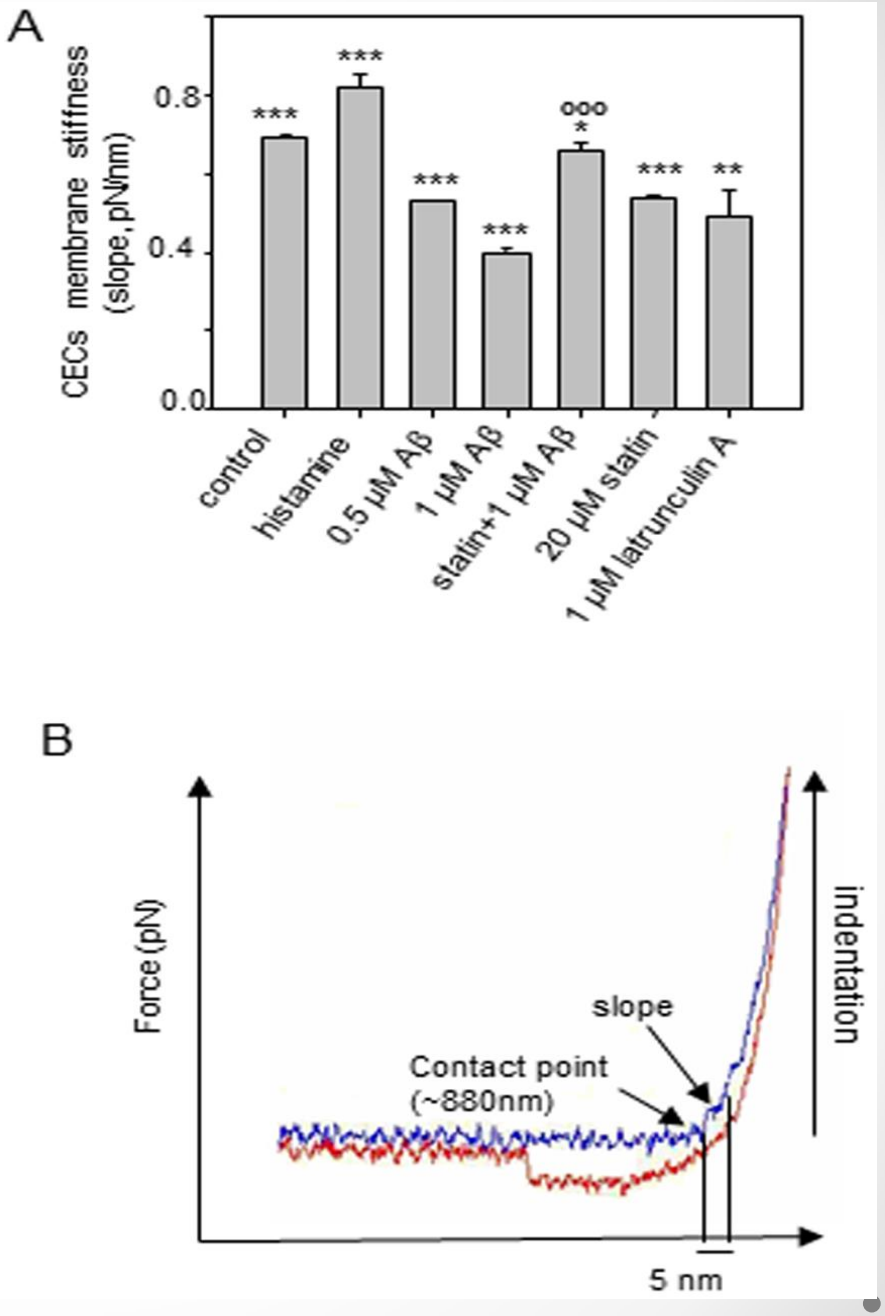


The elastic modulus was calculated by fitting the cell indentation part of the force curves with Hertz model.

*** - $p \leq 0.001$, ** - $p \leq 0.01$ compared to the control;

^{oo} - $p \leq 0.01$ compared to the Aβ (1 μM) treatment.

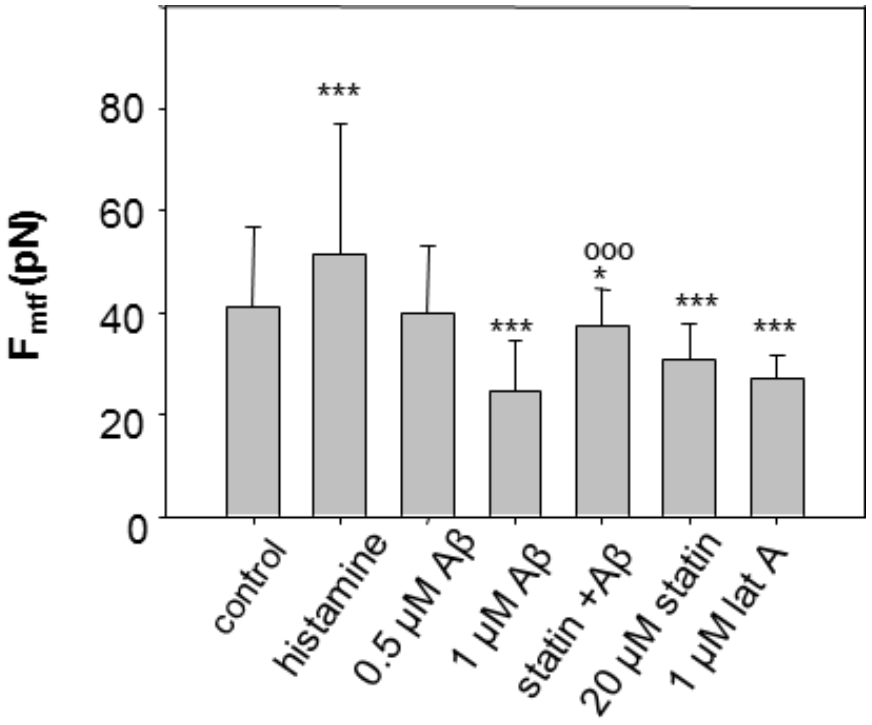
Aβ decreases membrane stiffness



Membrane stiffness is measured by calculating the slope (denoted by the arrow) from 5 nm indentation at the cell surface.

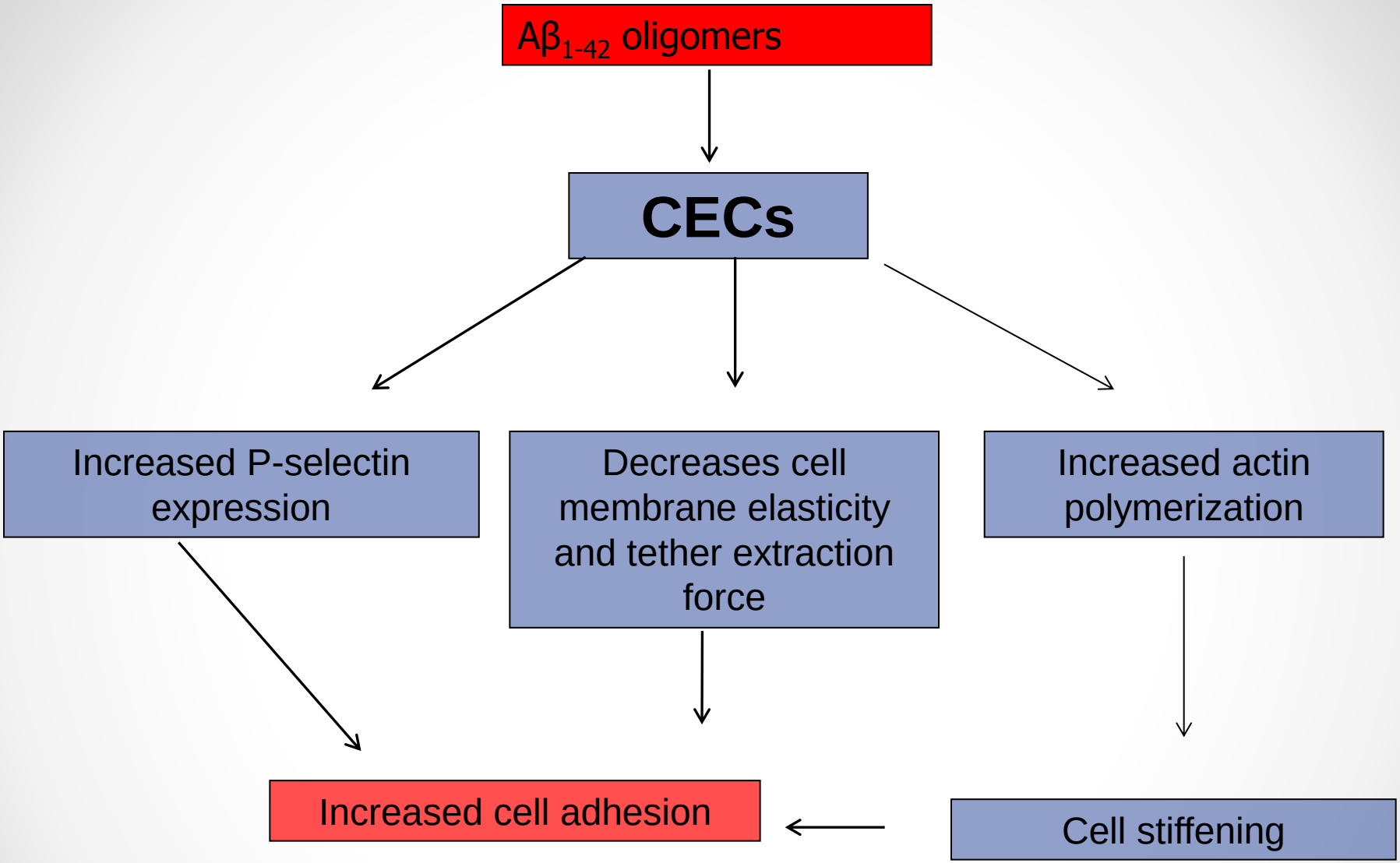
***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05 compared to the control;
^{ooo}p ≤ 0.001 compared to the Aβ (1 mM) treatment group.

Aβ decreases membrane tether formation force (Fmtf) mediated by sLex-selectin bonding

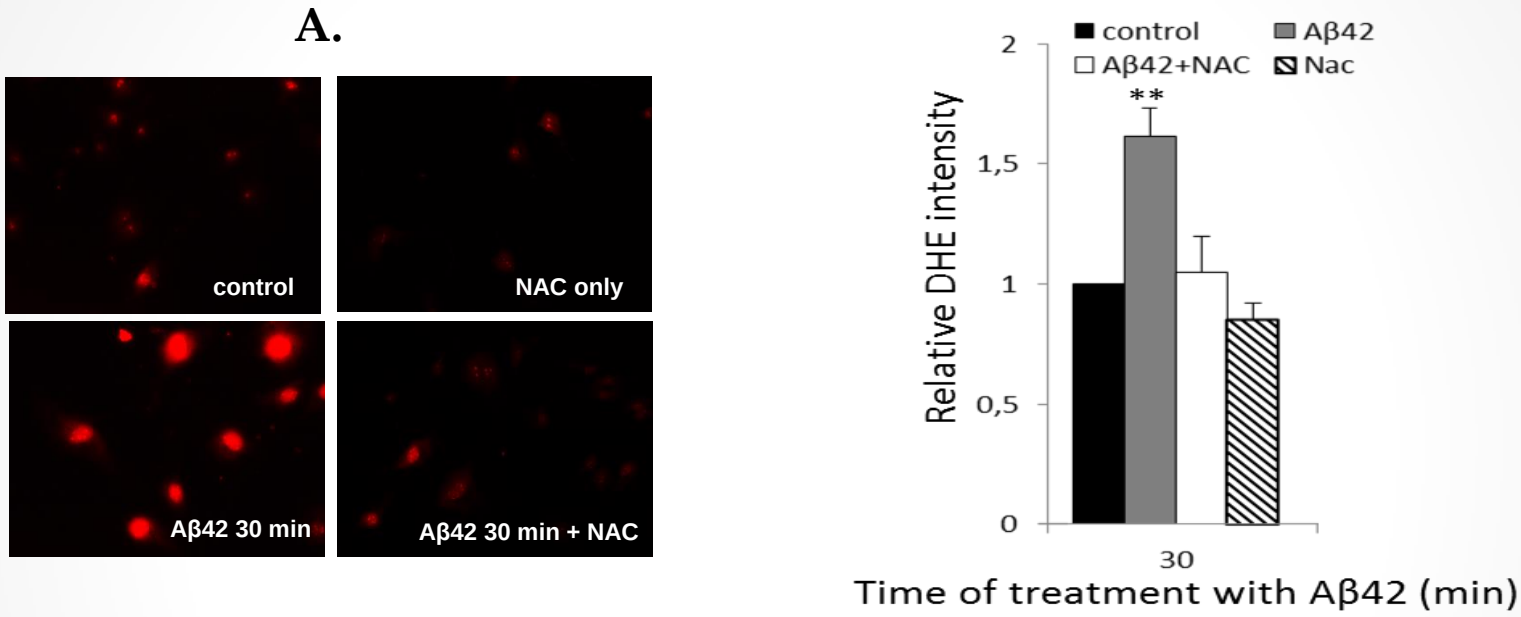


Fmtf was measured at the sudden drop of force when a rupture of a membrane tether occurred.

***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05 compared to the control;
^{oo}p ≤ 0.01 compare to the Aβ (1 mM) treatment group


Aβ1-42 oligomers
CECs
Increased P-selectin expression
Decreases cell membrane elasticity and tether extraction force
Increased actin polymerization
Increased cell adhesion
Cell stiffening

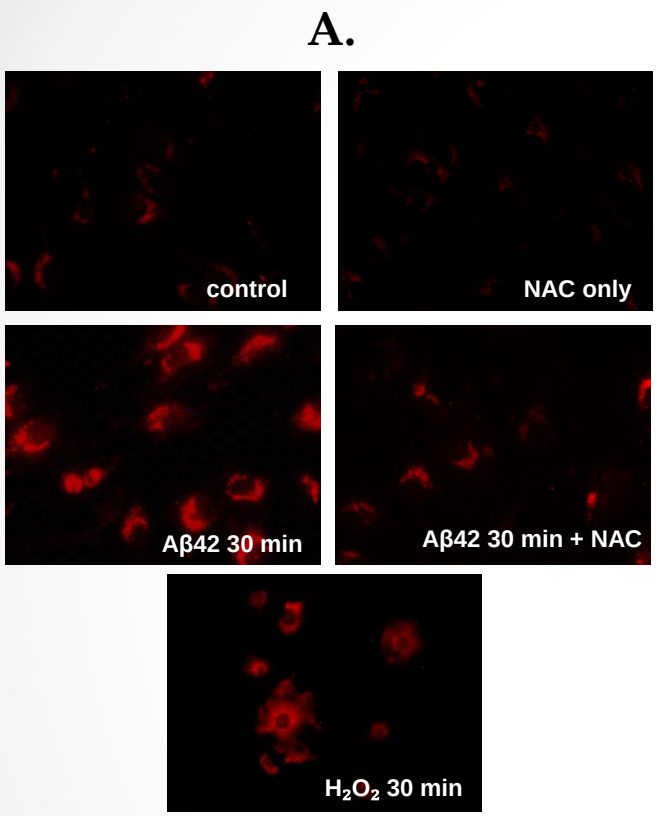
$A\beta_{42}$ induced ROS generation in bEnd3.

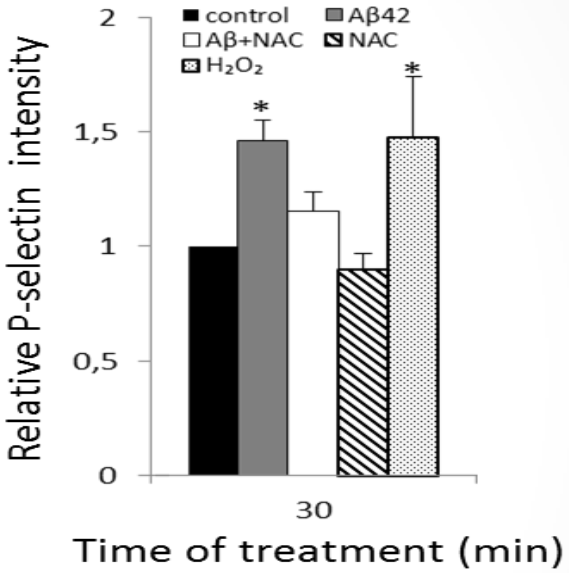


Images of DHE-stained bEnd3 (A); relative DHE fluorescent intensity (B).

** - $p \leq 0.01$ compared to the control in each experimental group (unpaired T-test analysis)

Aβ42 enhanced P-selectin fluorescence on the cell surface through elevated ROS



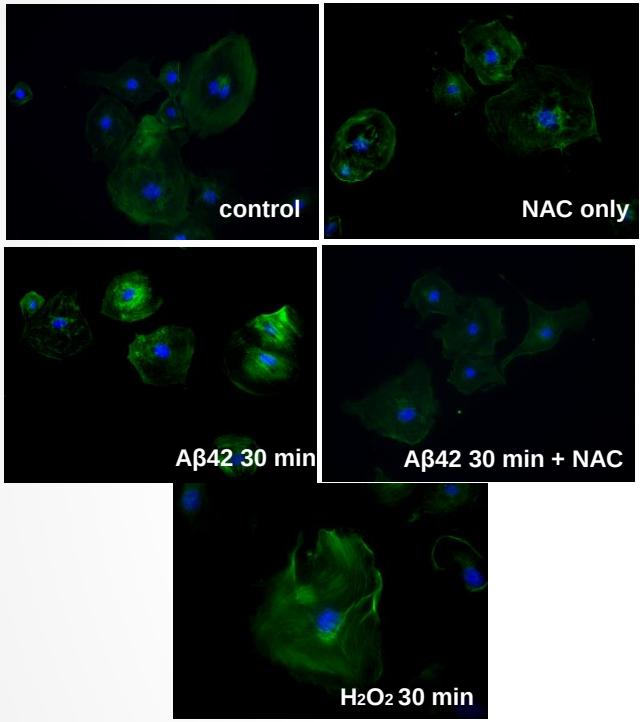


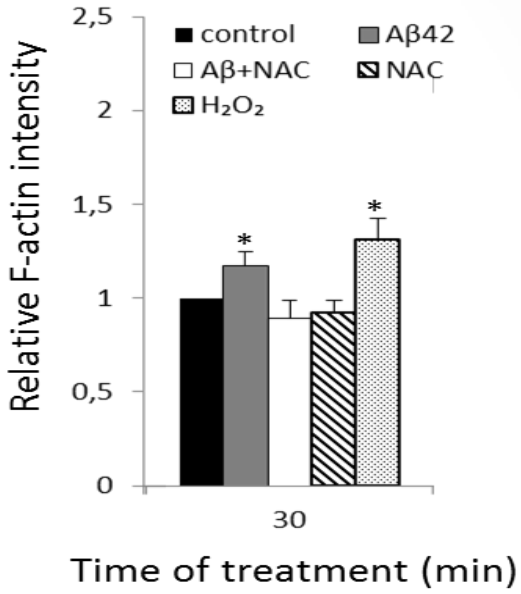
Fluorescent images of anti-P-selectin labeled bEnd3 cells (A); relative P-selectin intensity in the mouse bEnd3 cells (B).

*- $p \leq 0.05$ compared to the control in each experimental group (unpaired T-test analysis)

Aβ42 induced actin polymerization in mouse bEnd3 cells through elevated ROS

A.



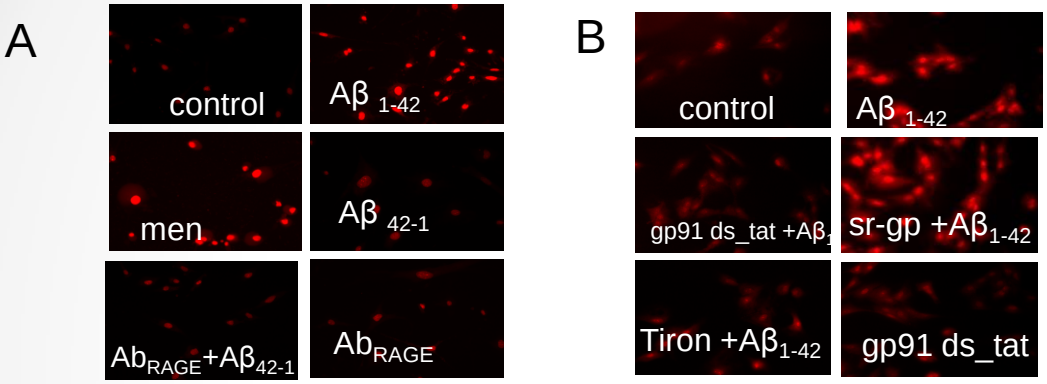


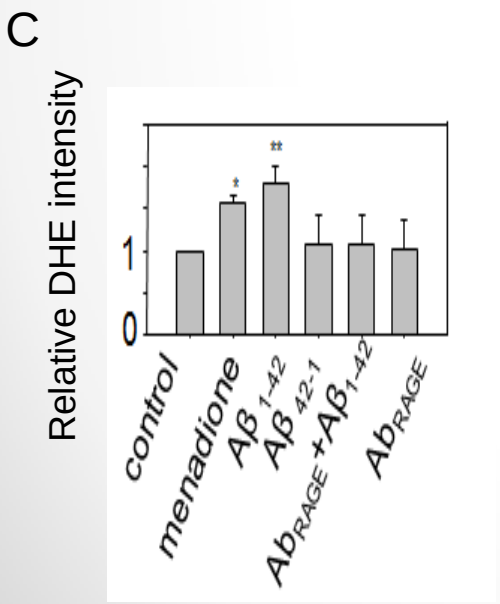
Fluorescent images of Oregon-green phalloidin-stained bEnd3 cells (A); relative F-actin intensity in mouse bEnd3 cells (B).

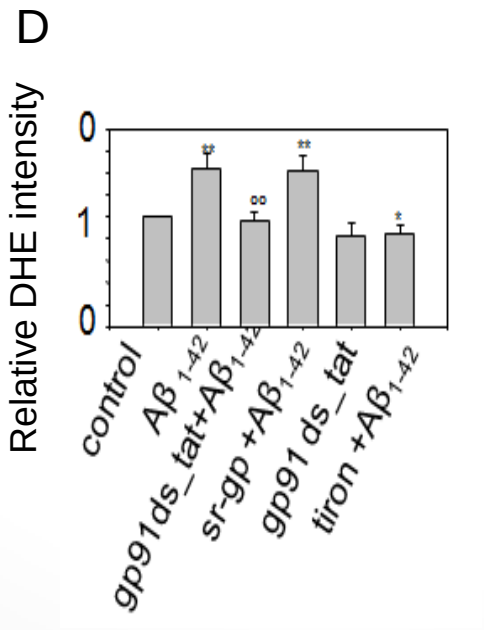
* - $p \leq 0.05$ compared to the control in each experimental group (unpaired T-test analysis).

- Recent studies have indicated that the receptor for advanced glycation endproducts (RAGE) is a binding site for Aβ
- RAGE is a multiligand cell surface receptor which is normally expressed in brain endothelium and, at low levels, in microglia and neurons.
- In AD brains RAGE expression is increased by several-fold in cerebral endothelial cells, astrocytes, microglia, and neurons.

Images of dihydroethidium (DHE)-stained CECs treated with Aβ oligomers, polyclonal antibody to RAGE, ROS scavenger, and NADPH oxidase inhibitor



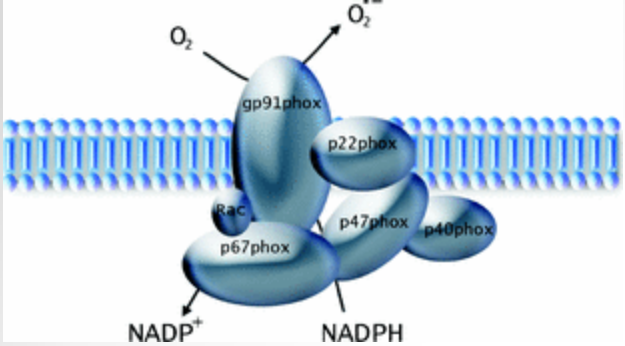




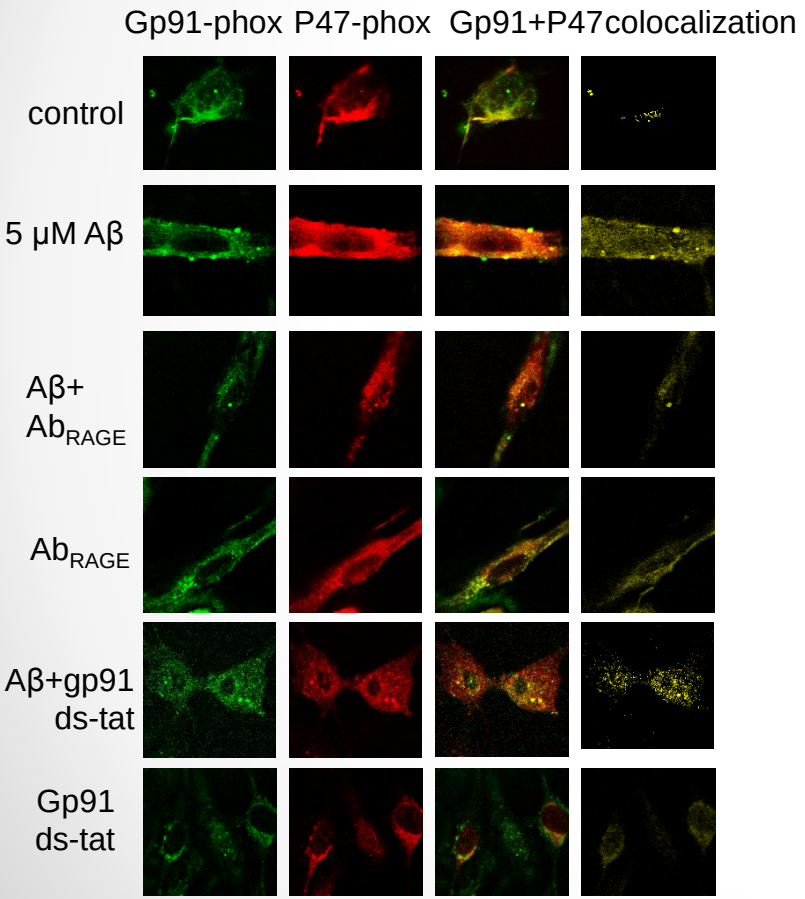
Both polyclonal antibody to RAGE and NADPH oxidase inhibitor significantly suppress oxidative stress induced by Aβ in CECs.

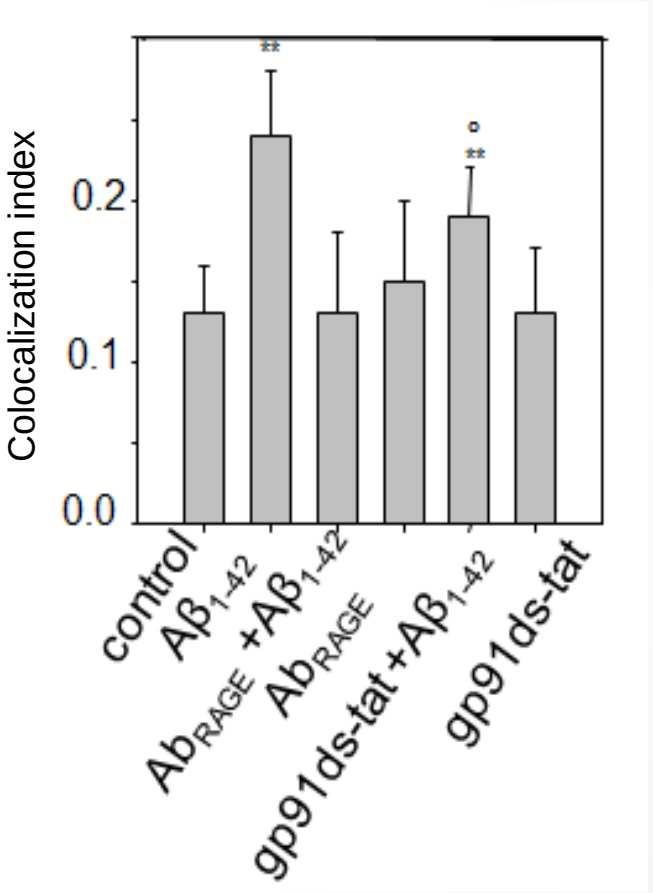
*- p≤0.05; ** - p ≤ 0.01; compared to the control;

°° - p ≤ 0.01 compared to the Aβ treatment



Polyclonal antibody to RAGE and NADPH oxidase inhibitor suppressed Aβ 1-42 induced colocalization of p47-phox to gp91-phox.

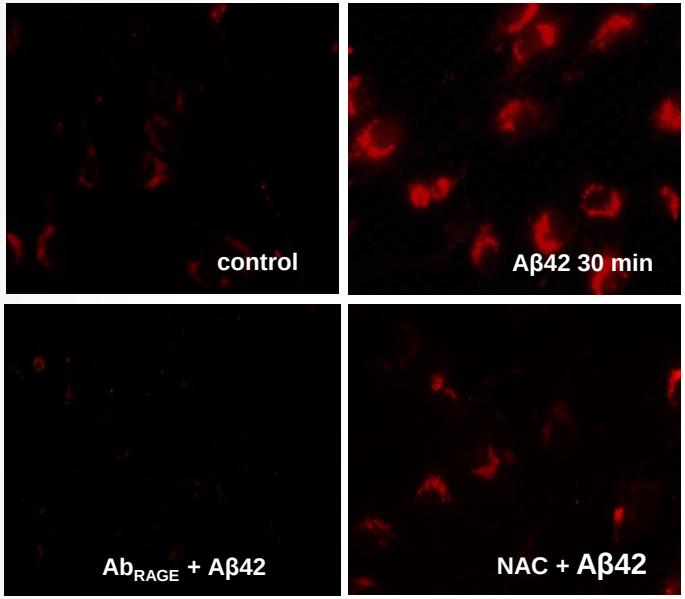


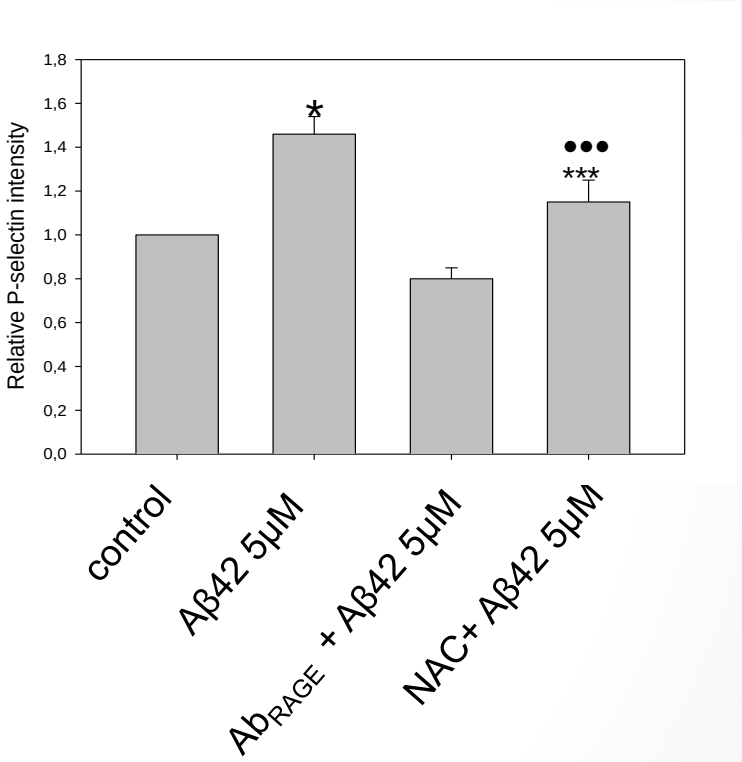


** - p ≤ 0.01 compared to the control;

° - p ≤ 0.05 compared to the Aβ treatment

Polyclonal antibody to RAGE suppressed oligomeric Aβ42 –induced upregulation of P-selectin at the bEnd3 cell surface

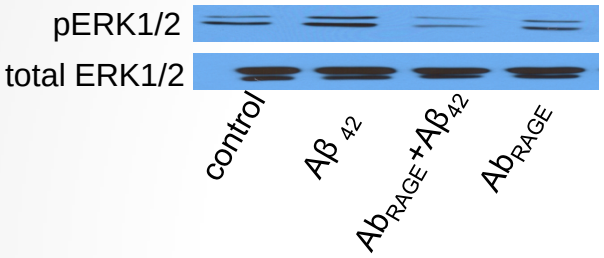


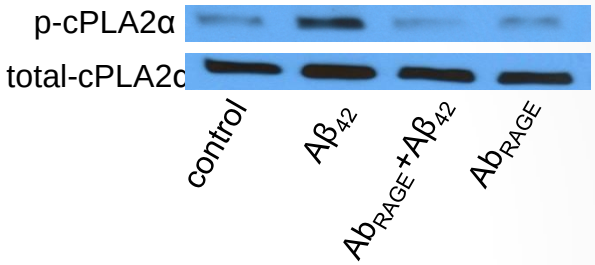


*** - p ≤ 0.001compared to the control;

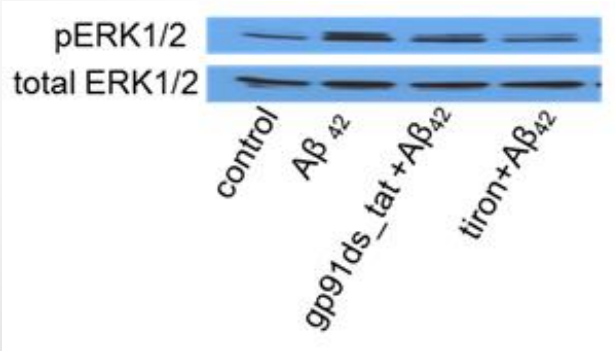
ooo - p ≤ 0.001 compared to the Aβ treatment.

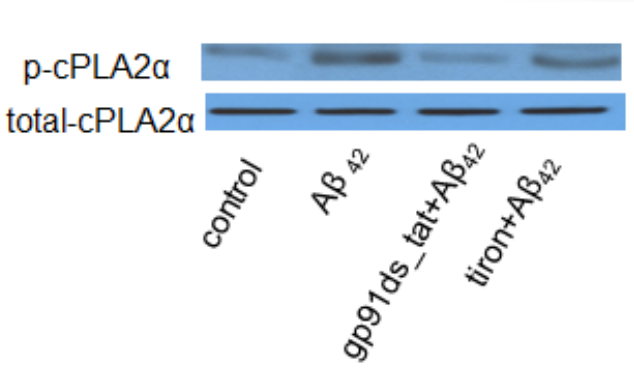
Polyclonal antibody to RAGE inhibits ERK1/2 and cPLA2α phosphorylation in CECs

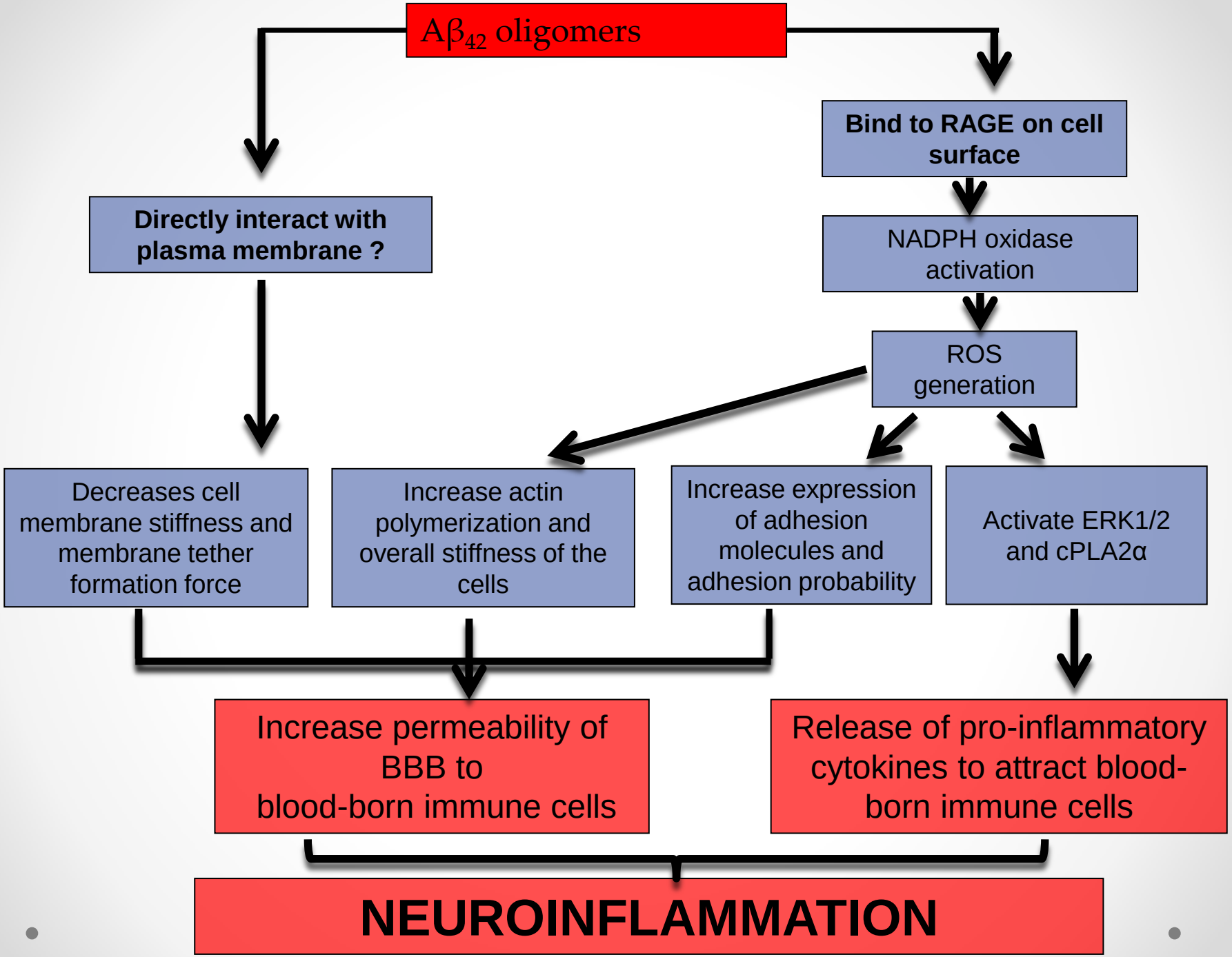




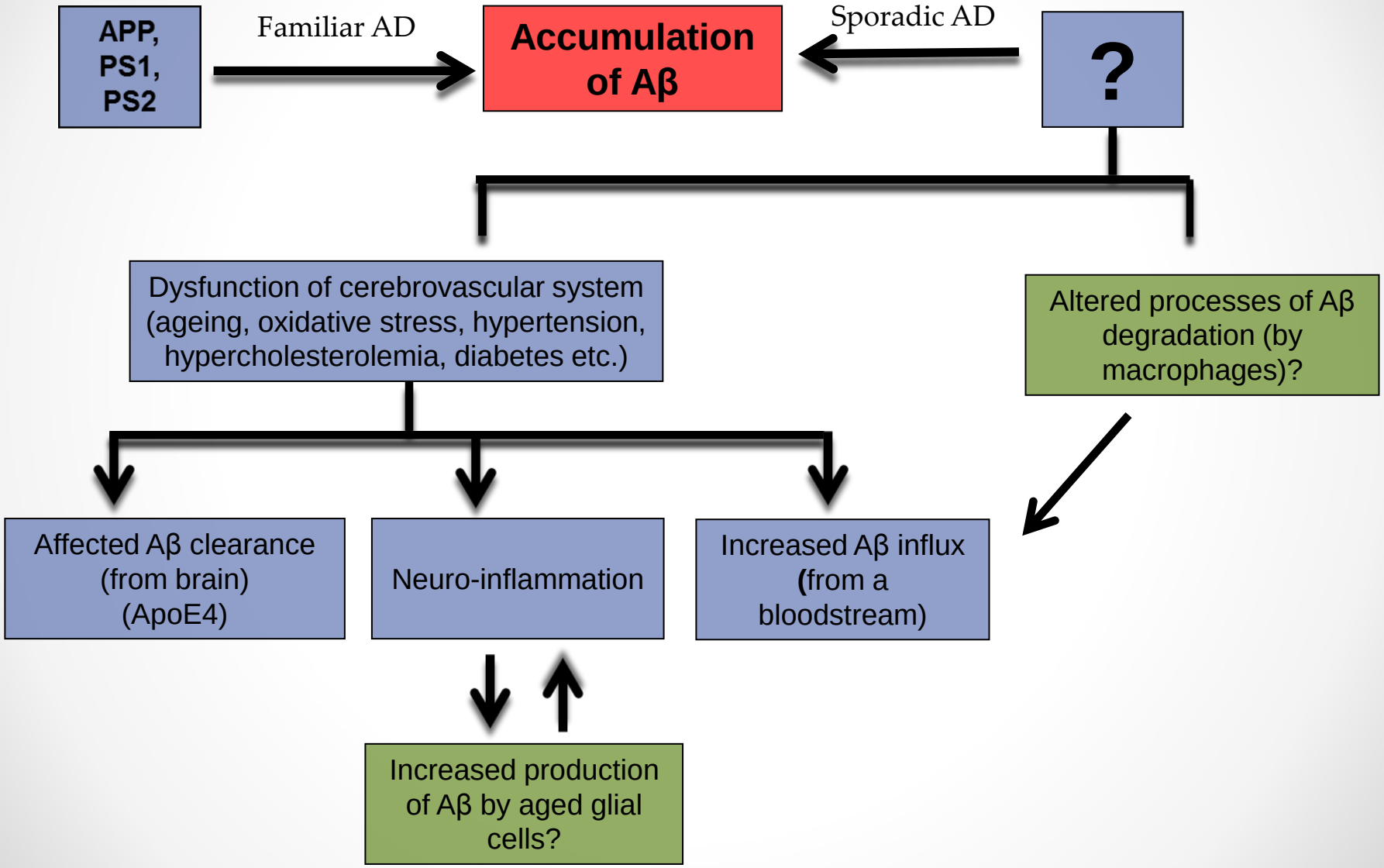
NADPH oxidase inhibitor and ROS scavenger inhibit ERK1/2 and cPLA2α phosphorylation in CECs






$A\beta_{42}$ oligomers
Directly interact with plasma membrane ?
Bind to RAGE on cell surface
NADPH oxidase activation
ROS generation
Decreases cell membrane stiffness and membrane tether formation force
Increase actin polymerization and overall stiffness of the cells
Increase expression of adhesion molecules and adhesion probability
Activate ERK1/2 and cPLA2α
Increase permeability of BBB to blood-born immune cells
Release of pro-inflammatory cytokines to attract blood-born immune cells
NEUROINFLAMMATION

FUTURE RESEARCH DIRECTIONS



THANK YOU