Alzheimer's Disease (AD) and eNOS

1. It has been reported that nitric oxide (NO) derived from eNOS suppresses amyloid β (Aβ) deposition in the brain (Katusic ZS, et al. *Eur Heart J*. 2014;35:888-94).

2. Shimokawa hypothesized that AD could be caused by cerebral microvascular dysfunction at the onset and that the LIPUS therapy could suppress the progression of AD through eNOS.

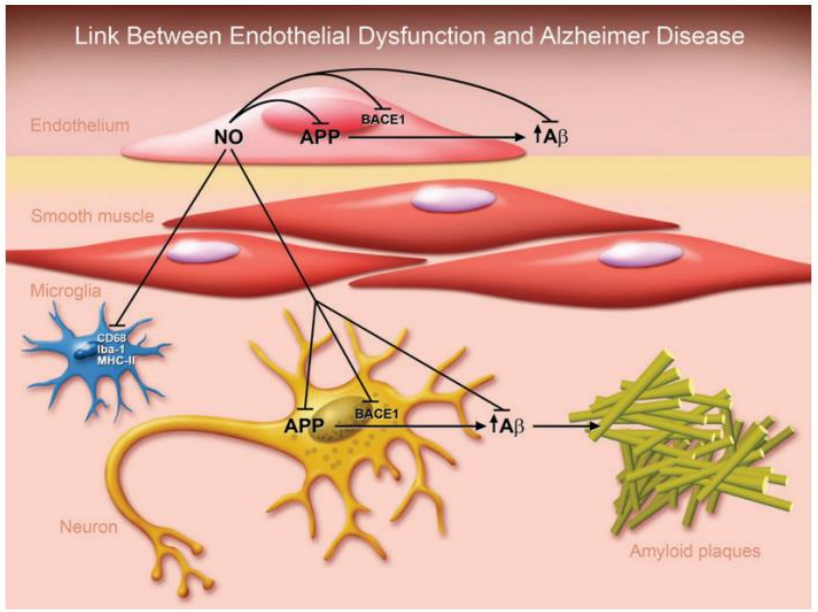


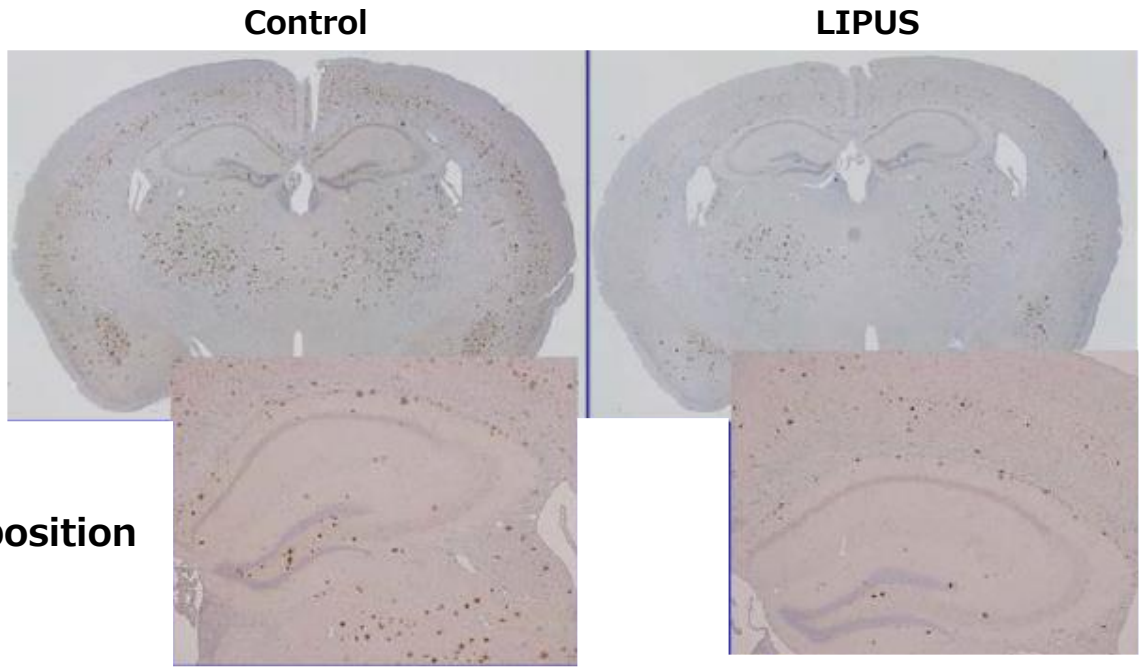
Figure. Schematic diagram of the inhibitory effect of endothelial NO on APP, BACE1, and Aβ in cerebral vascular endothelium and nerve cells

(Katusic ZS, et al. *Eur Heart J*. 2014;35:888-94.)

Preclinical Evidence (Mouse Model of AD)

LIPUS significantly reduces Aβ accumulation in the mouse model of AD.

AD model mouse (5xFAD mice)

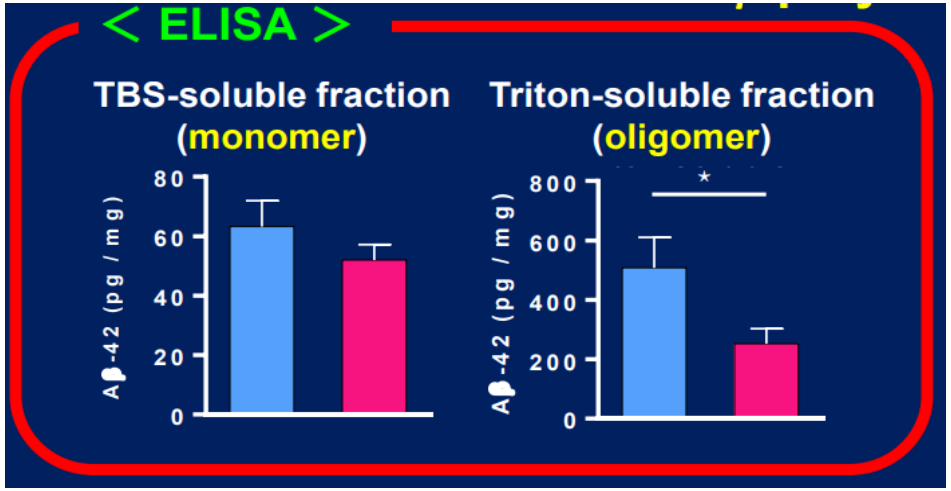


LIPUS therapy
(1.875MHz, 6.0 kHz, 32cycles)

- Enhanced expression of eNOS
- Reduced accumulation of amyloid β

Suppression of cognitive decline

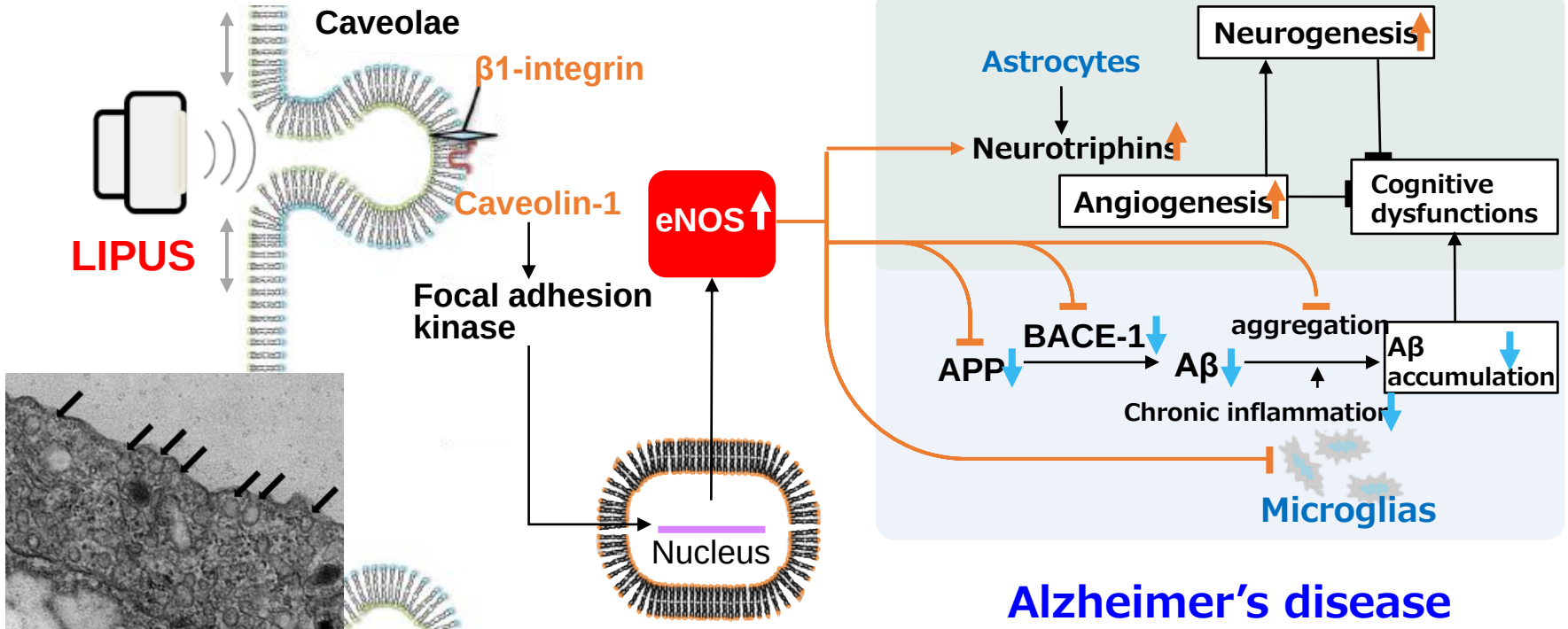
Suppression of Aβ production



(EguchiK, ShimokawaH, et al. *Brain Stimulation*. 2018;11:959-973.)

Mechano-transduction Mechanism

The molecular mechanisms of the LIPUS therapy include mechano-transduction mediated by β1-integrin/ caveolin-1 complex, resulting in eNOS upregulation and improved cognitive dysfunction.



Electron micrograph of caveolae (mouse mesenteric artery)

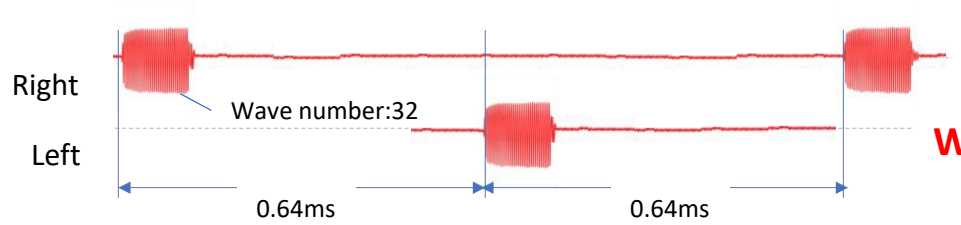
(Shindo T, Shimokawa H, et al. *ATVB*. 2016;36:1220-9.)

(Eguchi K, Shimokawa H, et al. *Brain Stimulation*. 2018;11:959-973.)

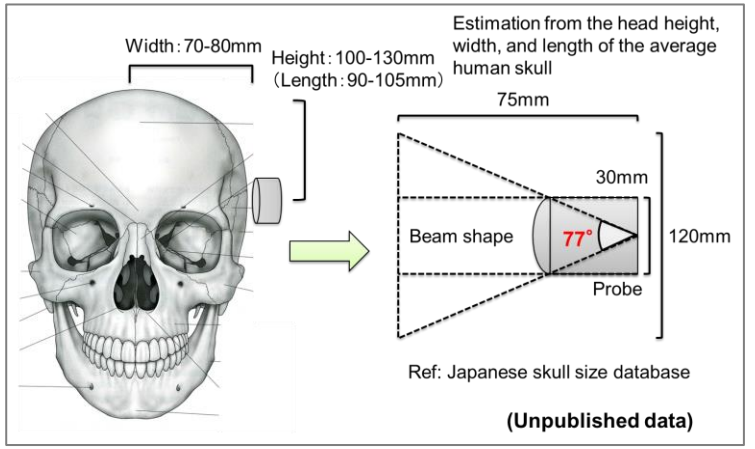
Pilot Clinical Trial of the LIPUS Therapy for AD

Operating principles

- **Whole-brain irradiation:** LIPUS is irradiated for the whole brain at regular intervals through bilateral temporal bones alternatively as follows;
- Irradiation conditions
  - Frequency: 0.5 MHz
  - Intensity: 0.25 W/cm^2
  - Cycle Number: 32 cycles
  - PRF (Pulse repetition frequency) 7.1kHz



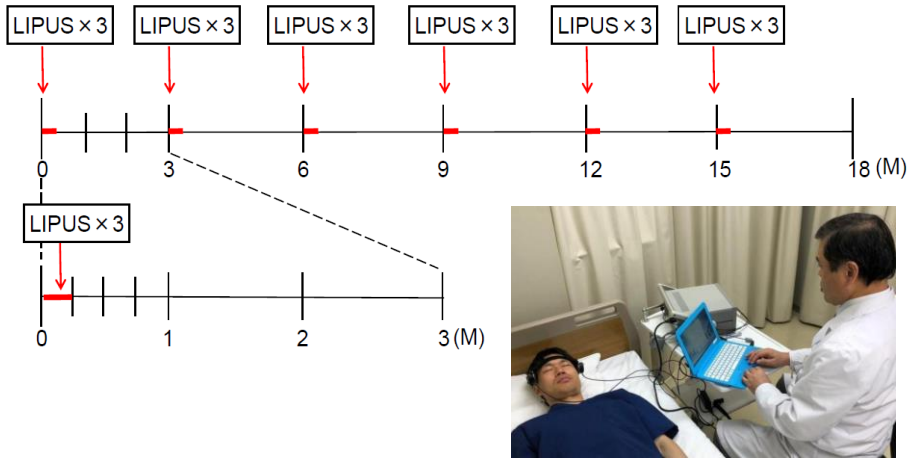
Wave condition



Whole-brain irradiation

Clinical trial protocols

1. Put the headset on the patient's head
2. Perform the LIPUS therapy for total 60 min (20min x 3 times)
3. Perform 3 times a week, every other day (1 course)
4. This course is performed every 3 months for a total of 6 courses.
5. The placebo group receive a placebo therapy in the same manner but without LIPUS.



LIPUS therapy