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Whole-brain low-intensity pulsed ultrasound therapy markedly improves cognitive dysfunctions in mouse models of dementia - Crucial roles of endothelial nitric oxide synthase

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ABSTRACT

Background: Therapeutic focused-ultrasound to the hippocampus has been reported to exert neuroprotective effects on dementia. In the present study, we examined whether the whole-brain LIPUS (low-intensity pulsed ultrasound) therapy is effective and safe in 2 mouse models of dementia (vascular dementia, VaD and Alzheimer's disease, AD), and if so, to elucidate the common underlying mechanism(s) involved.

Methods: We used bilateral carotid artery stenosis (BCAS) model with micro-coils in male C57BL/6 mice as a VaD model and 5XFAD transgenic mice as an AD model. We applied the LIPUS therapy (1.875 MHz, 6.0 kHz, 32cycles) to the whole brain.

Results: In both models, the LIPUS therapy markedly ameliorated cognitive impairments (Y-maze test and/or passive avoidance test) associated with improved cerebral blood flow (CBF). Mechanistically, the LIPUS therapy significantly increased CD31-positive endothelial cells and Olig2-positive oligodendrocyte precursor cells (OPCs) in the VaD model, while it reduced Iba-1-positive microglias and amyloid-β (Aβ) plaque in the AD model. In both models, endothelium-related genes were significantly upregulated in RNA-sequencing, and expressions of endothelial nitric oxide synthase (eNOS) and neurotrophins were upregulated in Western blotting. Interestingly, the increases in glia cells and neurotrophin expressions showed significant correlations with eNOS expression. Importantly, these beneficial effects of LIPUS were absent in eNOS-knockout mice.

Conclusions: These results indicate that the whole-brain LIPUS is an effective and non-invasive therapy for dementia by activating specific cells corresponding to each pathology, for which eNOS activation plays an important role as a common mechanism.

1. Introduction

Novel, effective strategies for treating dementia are urgently needed, particularly given alarming increases in the global prevalence of dementia. According to the current estimates, nearly 47 million patients worldwide had dementia in 2015, and this number is estimated to exceed 131 million by 2050 [1]. However, no curative treatment is yet available for vascular dementia (VaD) or Alzheimer's disease (AD) [2,3], both of which comprise the most common causes of dementia. VaD and AD share risk factors, such as hypertension, hypercholesterolemia, and diabetes mellitus [2,3]. Long-term exposure to these risk factors results in common outcome, i.e., impairment of vascular endothelial functions [4].

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Abbreviations

Aβ	amyloid-beta
AD	Alzheimer's disease
APP	amyloid precursor protein
BACE-1	β-site APP-cleaving enzyme-1
BCAS	bilateral carotid artery stenosis
BDNF	brain-derived neurotrophic factor
CBF	cerebral blood flow
CREB	cAMP response element-binding protein
CXCR4	CXC chemokine receptor 4
DCX	doublecortin
eNOS	endothelial nitric oxide synthase
FGF-2	fibroblast growth factor-2
GFAP	glial fibrillary acidic protein
GO	gene ontology
Iba-1	ionized calcium-binding adapter molecule-1
iNOS	inducible nitric oxide synthase
LIPUS	low-intensity pulsed ultrasound
MAPK	mitogen-activated protein kinase
NGF	nerve growth factor
nNOS	neuronal nitric oxide synthase
OPC	oligodendrocyte precursor cell
Hsp 90	heat shock protein 90
RNA-seq	RNA-sequencing
VaD	vascular dementia
VEGF	vascular endothelial growth factor
WML	white matter lesions

Thus, the endothelium has been recognized as an important and common therapeutic target for prevention and treatment of dementia [4].

Among several reports on therapeutic applications of ultrasound technology, low-intensity pulsed ultrasound (LIPUS) has emerged as a non-invasive therapy for several diseases. We have previously demonstrated that vascular endothelial cells substantially contribute to the therapeutic effect of LIPUS, inducing therapeutic angiogenesis in myocardial ischemia [5,6]. Meanwhile, LIPUS has also been reported to increase the production of brain-derived neurotrophic factor (BDNF) in astrocytes [7] and nerve growth factor (NGF) in PC12 cells [8], and to promote nerve regeneration [9]. Also, focused LIPUS to the hippocampus could ameliorate dementia (VaD and AD) in mice via increasing endogenous neurotrophins and vascular endothelial growth factor (VEGF) [10,11]. However, it is still unknown what kind of cell type contributes to the LIPUS-induced cognitive improvement, and whether there is a common factor in the two disorders. Moreover, from clinical point of view, we hypothesized that whole-brain LIPUS, rather than focused ultrasound, is effective and feasible since VaD is characterized by the widespread white matter lesions (WML) and AD by the widespread deposition of amyloid-β (Aβ) in the brain [2,3]. Thus, in the present study, we aimed to examine the effectiveness and safety of whole-brain LIPUS therapy that has never been used before. We first examined whether the whole-brain LIPUS is effective in different mouse models of dementia (VaD and AD), and if so, to elucidate the common mechanism underlying the beneficial effects of LIPUS.

2. Methods

See Supplementary Methods.

3. Results

3.1. LIPUS therapy improves cognitive dysfunctions in the VaD model

In the VaD model, the LIPUS therapy (Fig. 1A and B, Supplementary Figs. 1A and B) had no effects on body weight or systolic blood pressure (Supplementary Fig. 1C). LIPUS-treated mice showed no signs of cramps, paralysis, cerebral hemorrhage, hypothermia, hyperthermia, or increased mortality compared with control mice, which underwent the same procedure without LIPUS treatment (data not shown). In the Y-maze test, the number of entries was comparable between the LIPUS and control groups, suggesting that LIPUS causes no hyperactivity (Fig. 1C). Next, the LIPUS-treated BCAS group had significantly improved performance for cognitive functions, including spontaneous alternation task in the Y-maze and retention in the passive avoidance test (Fig. 1C). In the novel object recognition test, the LIPUS-treated BCAS group tended to have improved cognitive function compared with the control group (Fig. 1C). Similar results were also obtained at 56 and 84 days after the LIPUS therapy (Supplementary Fig. 1D), and there were no side effects of LIPUS for 3 months (data not shown). The LIPUS therapy significantly improved cerebral blood flow (CBF) in the BCAS group for up to 28 days after the therapy, whereas it had no effect in the control groups (Fig. 1D). Taken together, these results suggest that the cognitive improvement by the LIPUS therapy is associated with increased CBF and can last for at least 3 months.

3.2. LIPUS therapy improves WML in the VaD model

Next, we examined brain histology to determine the mechanism of the beneficial effects of the LIPUS therapy in the VaD model. We focused on the constituent cells of neurovascular unit possibly contributing to multifaceted actions of LIPUS. The results showed that the number of cells in the corpus callosum that were positive for GST-π, a marker of mature oligodendrocytes, was significantly higher in the LIPUS-treated group compared with the control group (Fig. 2A). In addition, Klüver-Barrera staining showed that the LIPUS therapy reduced the severity of WML following BCAS (Fig. 2A). Although there was no difference in CD31-positive cells in the corpus callosum (for assessment of capillary density), significantly more CD31-positive cells were present in the hippocampus of LIPUS-treated group compared with control group (Fig. 2A). Compared with control group, LIPUS-treated group showed no difference in the number of cells positive for GFAP (glial fibrillary acidic protein; for astrocytes) or Iba-1 (ionized calcium-binding adapter molecule-1; for microglias) (Supplementary Fig. 2). Moreover, doublecortin (DCX) staining of the hippocampus showed significantly more newly formed neurons in the dentate gyrus of LIPUS-treated group compared with control group (Fig. 2B, Supplementary Fig. 3). These results suggest that the LIPUS-induced angiogenesis ameliorates cognitive dysfunctions through neurogenesis and reduction of WML. Since we found no significant difference between the LIPUS-treated sham group and the control group, we focused on the BCAS group in the subsequent biochemical analyses.

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