Ann Vasc Dis Vol. 13, No. 2; 2020; pp 116–125

Online June 4, 2020
doi: 10.3400/avd.ra.20-00010

Review Article

Therapeutic Angiogenesis with Sound Waves

Tomohiko Shindo, MD, PhD and Hiroaki Shimokawa, MD, PhD

Along with the progress of global aging, the prognosis of severe ischemic heart disease (IHD) remains poor, and thus the development of effective angiogenic therapy remains an important clinical unmet need. We have developed low-energy extracorporeal cardiac shock wave therapy as an innovative minimally invasive angiogenic therapy and confirmed its efficacy in a porcine chronic myocardial ischemia model in animal experiments as well as in patients with refractory angina. Since ultrasound is more advantageous for clinical application than shock waves, we then aimed to develop ultrasound therapy for IHD. We demonstrated that specific conditions of low-intensity pulsed ultrasound (LIPUS) therapy improve myocardial ischemia in animal models through the enhancement of angiogenesis mediated by endothelial mechanotransduction. To examine the effectiveness of our LIPUS therapy in patients with severe angina pectoris, we are now conducting a prospective multicenter clinical trial in Japan. Furthermore, to overcome the current serious situation of dementia pandemic but with no effective treatments worldwide, we have recently demonstrated that our LIPUS therapy also improves cognitive impairment in mouse models of Alzheimer's disease and vascular dementia. Here, we summarize the progress in our studies to develop angiogenic therapies with sound waves.

Keywords: shock wave, ultrasound, angiogenesis, mechanotransduction, nitric oxide

1.1 Introduction

The endeavor of developing "therapeutic angiogenesis" is still halfway. The study on angiogenesis began in the 1800s when pathologists and anatomists made detailed morphological observations of blood vessels and reported the changes in blood vessel morphology. In the 20th century, an active approach to extracting angiogenic factors from cancer was extensively performed, among which basic fibroblast growth factor was first discovered in the 1980s.[1] Folkman found in 1971 that cancer growth depends on angiogenesis and suggested that inhibition of angiogenesis leads to cancer control.[2] This has led to dramatic progress in angiogenesis research from the perspective of cancer control. In 1989, Ferrara discovered the vascular endothelial growth factor (VEGF), which had a major impact on angiogenesis research.[3] In 1994, Isner and colleagues at Tufts University reported successful treatment with VEGF gene therapy in patients with lower limb ischemia.[4] In 1997, VEGF gene therapy was performed for patients with severe ischemic heart disease (IHD), which was difficult to treat surgically or medically, and the results were rather favorable.[4] Since then, various methods, such as transplantation of stem cells or endothelial progenitor cells, have been developed. Although many clinical trials were performed based on basic studies, none of them has yet reached established standard treatments. Under such circumstances, we aimed to develop a minimally invasive angiogenic therapy with sound waves (SWs) as a novel therapy. Here, we were able to develop innovative treatments with SWs.

2.1 Development of Extracorporeal Cardiac SW Therapy for IHD

When the expansion velocity of explosions such as volcanic eruptions and lightning exceeds the speed of sound, SWs are generated on the surface of explosions. From such characteristics of a longitudinal wave, SW contains a single short pressure pulse ($<1\,\mu s$), followed by a tensile portion with a lower amplitude (several μs). Since SW propagates through water, fat, and soft tissues, extracorporeal SW lithotripsy was clinically applied for the treatment of urolithiasis more than 30 years ago. In the treatment of urolithiasis, high-intensity SW is applied in order to break the urinary stones effectively. In contrast, we confirmed that low-energy SW, which is ~10% of that used for urolithiasis, was sufficient to enhance the expressions of VEGF and endothelial nitric oxide (NO)

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Received: January 20, 2020; Accepted: April 6, 2020
Corresponding author: Hiroaki Shimokawa, MD, PhD. Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan
Tel: +81-22-717-7152, Fax: +81-22-717-7156
E-mail: shimo@cardio.med.tohoku.ac.jp

synthase in cultured human umbilical vein endothelial cells (HUVECs).[5] Then, we conducted a series of animal experiments using a porcine model of chronic myocardial ischemia and clinical trials in patients with refractory angina to evaluate the efficacy and safety of this low-energy extracorporeal cardiac SW therapy.[5–7]

The number of patients with severe angina pectoris without indication of percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) has been increasing. Based on the data of our in vitro studies with HUVECs, we examined the effect on myocardial ischemia of low-energy SW therapy in a porcine model in vivo.[5] By placing an ameroid constrictor around the proximal segment of the left circumflex coronary artery (LCx), we made a porcine model of chronic myocardial ischemia. The ameroid constrictor gradually induced a total occlusion of the coronary artery with resultant sustained myocardial ischemia in 4 weeks. At 4 weeks after the surgery, low-energy SW therapy was applied 3 times in the first week in the SW-treated group, while the control group received anesthesia 3 times but without the SW therapy (n = 8, each). The extracorporeal cardiac SW therapy has a built-in echo diagnostic device in the SW generator (Storz Medical AG, Kreuzlingen, Switzerland) to specify the irradiation site under the echo guidance, and the SW was irradiated in synchronization with the R wave of electrocardiogram. The low-energy SW was irradiated for a total of 1800 shots for 9 spots (0.09 mJ/mm^2, 200 shots/spot) to the LCx region of the ischemic site of the left ventricle (LV).[5] Reduced LV wall motion in the LCx region was documented in both the control and the SW-treated groups at 4 weeks after the ameroid constrictor implantation. Importantly, the wall motion of the ischemic area was significantly improved in the SW group at 4 weeks after the SW therapy.[5] We also found that SW therapy increased myocardial capillary density associated with enhanced VEGF expression and normalized the regional myocardial blood flow in the ischemic myocardium in vivo.[5] In this experiment, no myocardial damage, arrhythmias, or LV perforation were detected after the SW irradiation. Altogether, we demonstrated that the low-energy extracorporeal cardiac SW therapy enhances effective angiogenesis in pigs in vivo and may have potential usefulness as a non-invasive angiogenic therapeutic approach for chronic myocardial ischemia.[5]

From the promising results in the experimental in vivo study, we moved to the next step and performed the first clinical trial in patients with refractory angina pectoris without indication of PCI or CABG[6] (**Fig. 1**). We applied low-energy SW for 4000–8000 shots to 20–40 spots (0.09 mJ/mm^2, 200 shots/spot) of the ischemic region 3 times in the first week with no anesthesia or analgesics. Importantly, we confirmed that the low-energy SW therapy showed significant improvement of symptoms, reduction in nitroglycerin use, and amelioration of myocardial perfusion in the ischemic area, as assessed by stress scintigraphy (**Fig. 2**). There were no complications or adverse effects associated with SW therapy. We then performed a second clinical trial of SW therapy in a randomized

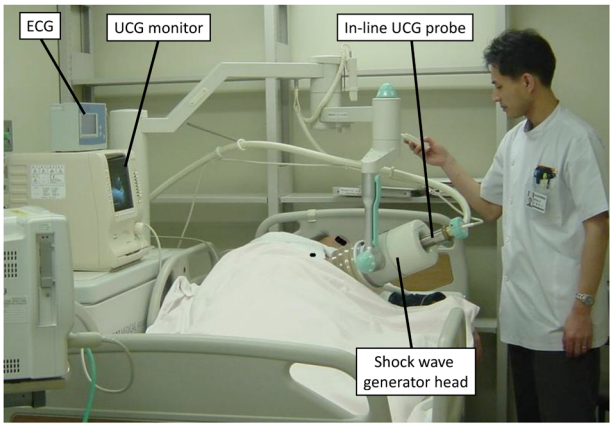

Fig. 1 Extracorporeal cardiac sound wave (SW) therapy in action in a patient with refractory angina pectoris.
The machine is equipped with a SW generator head and in-line echocardiography. The SW generator is attached to the chest wall of the patient when used. SW pulse is easily focused on the ischemic myocardium under the guidance of echocardiography. There is no need for anesthesia or analgesics.

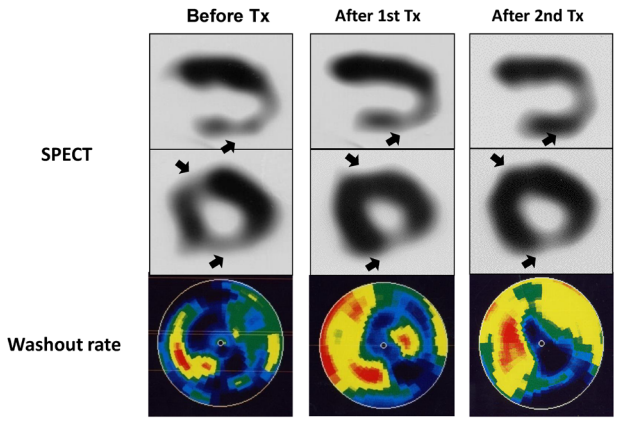

Fig. 2 Effects of the extracorporeal cardiac sound wave (SW) therapy on myocardial perfusion in a patient with refractory angina pectoris.
Dipyridamole stress thallium-201 single photon emission computed tomography (SPECT) imaging and polar map in a patient with severe 3-vessel coronary artery disease before and after SW therapy. As a result, it was clearly shown that myocardial perfusion was improved only at the myocardial ischemic site where the SW therapy was applied; in the anteroseptal wall after the first treatment (1st Tx) and in the lateral wall after the 2nd Tx (arrows) in a step-wise manner after the staged SW treatment. (from Ref. 6)

and placebo-controlled manner.[7] From the results of this second clinical trial, we demonstrated that the low-energy SW therapy improves LV function in severe IHD patients by inducing effective angiogenesis.[7] Following our initial clinical studies, clinical trials were subsequently performed worldwide with positive results.[8–14] Although it is still not clear whether it improves the long-term prognosis of angina patients, these results indicate that SW therapy improves the quality of life of those patients.

After the achievement of these positive results, we examined whether SW therapy is effective for treating a porcine model of LV remodeling after myocardial infarction in vivo.[15] We found that the LV ejection fraction and LV end-diastolic volume were significantly improved in the SW group compared with the control group at 4 weeks after SW therapy.[15] This was the first experiment that demonstrated SW therapy as an effective and non-invasive approach to ameliorate LV remodeling after myocardial infarction.[15] In another porcine acute myocardial infarction (AMI) model, which is induced by myocardial ischemia with 90 min coronary occlusion and afterward reperfusion, SW therapy showed significant improvement of the LV ejection fraction in a condition closer to the clinical setting.[16] From the promising results of the two AMI pig models in vivo, we performed the first clinical trial to evaluate the efficacy and safety of SW therapy in AMI patients.[17] In this trial, we included 17 patients with AMI who were successfully treated with PCI (peak creatine kinase $<$ 4,000 U/l), and we applied SWs to the border zone of the infarcted LV area as adjunctive therapy at 2, 4, and 6 days after AMI.[17] No procedure-related complications or any side effects were reported in this trial. We found that LV functions assessed by magnetic resonance imaging showed no signs of deleterious LV remodeling at 6 and 12 months after AMI.[17]

2.2 Additional Indications of SW Therapy

Based on the positive results mentioned previously, thereafter, various clinical trials were conducted to expand the indication of SW therapy. Generally, peripheral arterial disease (PAD), especially in patients with critical limb ischemia, is often associated with IHD, and the prognosis is poor.[18–20] Thus, we first examined the effectiveness of SW therapy in a rabbit hindlimb ischemia model induced by the surgical excision of the entire unilateral femoral artery. Low-energy SW was irradiated for a total of 6,000 shots to 30 spots (0.09 mJ/mm^2, 200 shots/spot) of the ischemic region 3 times a week for 3 consecutive weeks. Compared with the control group, blood flow and capillary density were significantly increased at 4 weeks after the operation in the SW group.[21] Based on these experimental results, to evaluate the effectiveness of SW therapy in PAD patients, we performed the next clinical trial on 12 PAD patients with intermittent claudication (Fontaine stage II).[22] Low-energy SW was irradiated for a total of 8000 shots to 40 spots (0.1 mJ/mm^2, 200 shots/spot) of the ischemic region 3 times a week for 3 consecutive weeks. We confirmed that patient symptoms, maximum walking distance, and peripheral perfusion were significantly improved in the SW group than in the control group.[22] A subsequent study confirmed the effectiveness of low-energy SW therapy in PAD patients in Fontaine stages III and IV.[23] As a result, by inducing effective angiogenesis, low-energy SW therapy could be a promising novel, non-invasive therapy for PAD patients.

Moreover, we further applied our low-energy SW therapy to patients with systemic sclerosis who suffer from Raynaud's phenomenon and digital skin ulcers.[24] In the pathogenesis of digital skin ulcers, not only immune activation but also the endothelial damage and persistent vasospasms are considered to be involved.[24] Several studies by our and other groups demonstrated that low-energy SW therapy enhances wound healing in rodents[25–28] and humans.[29,30] Interestingly, we confirmed that the local wound healing of skin ulcers in a mouse model was significantly enhanced by SW therapy, for which endothelial nitric oxide synthase (eNOS), VEGF, and subsequent angiogenesis may be involved in the process of wound repair.[27] To examine whether low-energy SW therapy is effective in patients with digital skin ulcers, a clinical trial of 9 patients was conducted, demonstrating that SW therapy may be effective for the treatment of refractory digital ulcers due to systemic sclerosis.[31]

In general, some cases of refractory lymphedema cannot be completely cured even by surgery, and such patients have limited treatment options. Thus, we next examined the beneficial effects of low-energy SW therapy on animal models of secondary lymphedema in vivo.[32,33] The results showed that SW therapy significantly enhanced the expressions of VEGF-C and basic fibroblast growth factor (bFGF), which improved the lymphatic system and lymphatic density. From these results, low-energy SW therapy may also induce therapeutic lymphangiogenesis in lymphedema through the upregulations of VEGF-C and bFGF, suggesting that SW therapy is a non-invasive therapy for patients with lymphedema.[32,33]

Finally, for the treatment of orthopedic diseases, such as bone non-unions, tendinosis calcarea, epicondylitis, and calcaneal spur, middle levels of SW have been widely used, expecting anti-inflammatory effects.[34–37] In the rat spinal cord injury model, we have previously demonstrated that low-energy SW therapy exerts beneficial effects on the locomotor functions in vivo.[38] Through the upregulation of VEGF, SW therapy attenuated nerve injury and promoted the recovery of locomotor function. This landmark study

demonstrated a new non-invasive therapeutic strategy with low-energy SW for spinal cord injury.[38]

3.1 Development of Low-Intensity Pulsed Ultrasound (LIPUS) Therapy for IHD

As mentioned above, we reported that low-energy cardiac SW therapy induces neovascularization and effectively ameliorates myocardial dysfunction in animals and humans. Since both SW and ultrasound are SWs and they similarly travel straight through body tissue (fats, muscles, body fluids, etc.), we then examined the potential feasibility of LIPUS for the treatment of IHD as the next generation of non-invasive angiogenic therapy.[39] Ultrasound is defined as the SW whose frequency is higher than the basic audible range for humans (>20 kHz), and diagnostic ultrasonography has been widely used for more than 50 years. Recently, ultrasound is also used for therapeutic applications, including tumor ablation, thrombolysis, bone regeneration, and drug delivery system.[39] The angiogenic potential of low-intensity ultrasound has been reported in several endothelial cells, chick chorioallantoic membrane, and a rat model of hind limb ischemia.[40–42] We examined the various conditions of LIPUS, such as acoustic pressure and the number of cycles (1, 16, 32, 48, and 64), with a needle hydrophone (**Fig. 3**). The number of acoustic waves per 1 pulse is called the number of cycles, and generally, 1 cycle is a condition used for diagnostic devices. To prevent the temperature rise of the ultrasound probe, the estimated spatial peak temporal average intensity of LIPUS was controlled to keep it under the upper limit of acoustic output standards (<720 mW/cm^2) for diagnostic ultrasound devices (U.S. Food and Drug Administration's Track 3 Limits). We identified that when LIPUS was applied to HUVECs, the maximal effect to upregulate VEGF messenger ribonucleic acid (mRNA) expression was noted at 32 cycles.[43] Although higher intensity ultrasound is used for thrombolysis or tumor ablation (high-intensity focused ultrasound), the intensity of the LIPUS in our study was within the diagnostic range.[44] With these findings, we were able to demonstrate that LIPUS therapy induces therapeutic angiogenesis and ameliorates myocardial ischemia in a porcine model of chronic myocardial ischemia.[43] In this study, at 8 weeks (post-treatment), left ventricle ejection fraction (LVEF) was normalized in the LIPUS-treated group, whereas it remained unchanged in the control group without the LIPUS (**Fig. 4**). Indeed, the LIPUS therapy normalized global and regional myocardial functions and increased capillary density and regional myocardial blood flow in the chronically ischemic region without any adverse effects, and significantly enhanced protein expressions of VEGF, eNOS, and bFGF in the ischemic myocardium but not in the non-ischemic myocardium in vivo.[43] Moreover, in a mouse model of AMI, LIPUS therapy enhanced angiogenesis, ameliorated post-myocardial infarction (MI) LV remodeling, and improved the mortality.[45] In this study, we used LIPUS to treat the animals on days 1, 3, and 5 and found enhanced

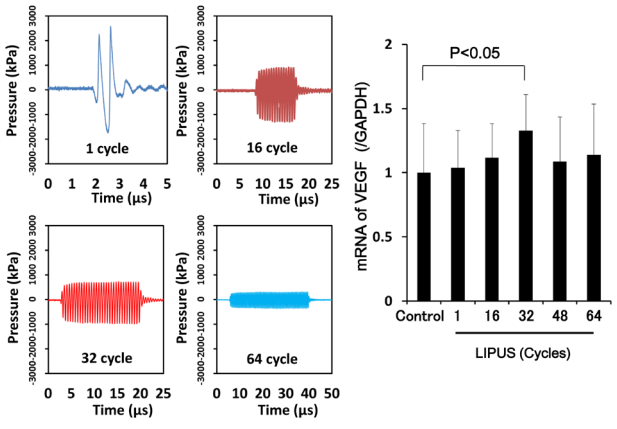

Fig. 3 Effects of low-intensity pulsed ultrasound (LIPUS) on human umbilical vein endothelial cells (HUVECs) in vitro. Acoustic pressure at various cycle numbers. LIPUS treatment upregulated mRNA expression of vascular endothelial growth factor (VEGF) in HUVECs in vitro with a maximum effect noted at 32 cycles. Results are expressed as mean±standard error of the mean (SEM) (n=9–11 each). (from Ref. 43)

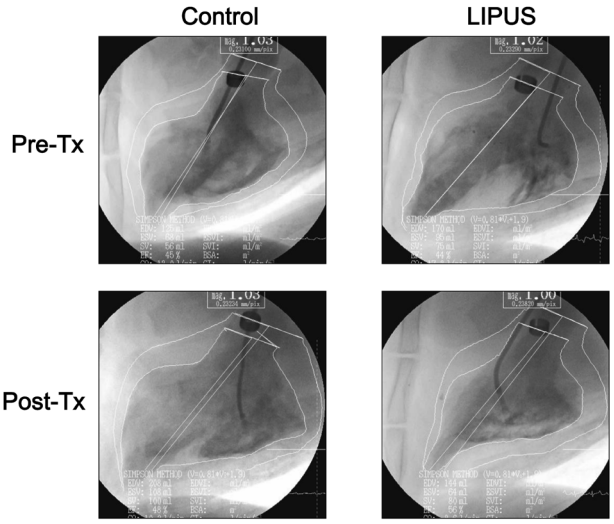

Fig. 4 Low-intensity pulsed ultrasound (LIPUS) therapy improves ischemia-induced myocardial dysfunction in pigs in vivo. At 4 weeks after implantation of an ameroid constrictor (pre-Tx), left ventricle (LV) wall motion of the left circumflex coronary artery (LCx) region was reduced to the same extent in both the control and the LIPUS groups. At 4 more weeks after the LIPUS therapy (post-Tx), left ventriculography showed marked improvement of LV wall motion only in the LIPUS group. (from Ref. 43)

phosphorylation of ERK1/2 and Akt on day 3 and the upregulation of VEGF and eNOS on day 6.[45] Although the LIPUS therapy was performed only in the acute phase of AMI associated with the upregulation of VEGF and eNOS, it enhanced capillary density and ameliorated post-MI LV remodeling in the chronic phase.[45] These results indicate that LIPUS therapy is an effective and safe therapeutic strategy for ischemia-induced myocardial dysfunction. Based on these encouraging results, in order to confirm the effectiveness and safety of LIPUS therapy in humans, we started a double blind, placebo-controlled trial in patients with severe angina pectoris at 10 cardiovascular institutes in Japan. We have already completed patient enrollment, and the trial will be completed in 2020.

3.2 Possible Mechanisms for the Beneficial Effects of LIPUS Therapy

Vascular endothelial cells cover the inner surface of blood vessels and are directly exposed to blood flow-induced mechanical stimuli, including shear stress. These stimuli invoke specific responses within the cells, leading to changes in their intrinsic structure and function.[46] Endothelial cells may sense these stimuli and convert them into a sequence of biological responses. Caveolae are flask-like invaginations of the plasma membrane 40–80 nm in diameter organized by caveolins.[47,48] One of the important functions of the caveolae is the conversion of mechanical stimuli into chemical signals by flow-sensing organelles transmitted into the cells, called mechanotransduction.[47] Caveolins bind to a variety of proteins involved in the signaling pathways such as G-protein subunits, tyrosine kinases, NO synthase, small GTPases, and growth factor receptors.[49–52] Caveolar membranes are also enriched in cholesterol, glycosphingolipids, and signaling enzymes such as Src kinase.[53] In addition, caveolae are reported to respond to cell stretch, thus contributing to stretch-induced signaling.[54] On the other hand, integrins are reported to regulate multiple pathways, including Erk, PI_3K, FAK, Src, and Rho GTPases.[55–57] Although caveolin-1 has no extracellular component, it plays an important role in sensing mechanical stress or the distortion of the extracellular membranes through interaction with β_1-integrin.[51,52,58–60] In cultured cell experiments, the degree of the LIPUS-induced upregulation of mRNA was higher in HUVECs than in human cardiomyocytes, suggesting that vascular endothelial cells may be the main player for the LIPUS-induced angiogenesis.[45] Microarray analysis showed that not only the VEGF signaling pathway but also the focal adhesion pathway were significantly affected by LIPUS.[45] The focal adhesion pathway contains several proteins on the cell membrane, including caveolin-1 and β_1-integrin, both of which are known to play key roles in the mechanotransduction process.[47,48] Especially, caveolin-1 is required to maintain the structure of caveolae.[47,48] The results of our siRNA experiments suggest that caveolin-1, β_1-integrin, Fyn, FAK, ERK1/2, and Akt are all involved in the LIPUS-induced upregulation of VEGF.[45] In addition, we found that the conformational changes of caveolae by either the knockdown of serum deprivation-response protein with small interfering RNA or administration of methyl-β-cyclodextrin suppressed the LIPUS-induced upregulation of VEGF.[45] Furthermore, we demonstrated that the beneficial effects of LIPUS therapy on post-MI LV remodeling were blunted in Cav-1-knockout (KO) mice.[45] LIPUS-induced upregulation of angiogenic molecules was also blunted in the eCav-1-KO mice, suggesting that endothelial cells play pivotal roles in the angiogenic effects of LIPUS therapy.[45] Taken together, these results suggest that acoustic streaming by LIPUS induces distortion of caveolae on endothelial cells, which then transmits the mechanical stimuli to intracellular signaling pathways with subsequent phosphorylation of Fyn, FAK, Erk1/2, and Akt and resultant enhanced expression of VEGF and angiogenesis[45] (**Fig. 5**). We also confirmed that the same mechanisms are involved in the angiogenic effects of low-energy SW[61] (**Fig. 5**).

The molecules mentioned above, such as Fyn, FAK, β_1-integrin and Caveolin-1, are also known to play key roles in cell proliferation and angiogenesis induced by mechanical stimuli (e.g., shear stress) on the surface of vascular endothelial cells.[51–53] Microarray analysis also suggested that LIPUS exerts biological effects on cell cycles, metabolic pathways, RNA transport, deoxyribonucleic acid (DNA) replication, mRNA surveillance, mismatch repair, and protein export in addition to its angiogenic effects.[45] Ultrasound has been reported to induce sonoporation and subsequent influx of calcium ion, which was correlated to LIPUS-induced bioeffects in cultured cells.[62] Thus, LIPUS may exert several biological effects through alteration of intracellular calcium ion levels, and this point remains to be fully elucidated in future studies. Finally, it is reported that the shear stress-induced intracellular signaling is mediated by the activation of β_1-integrin and concurrent caveolin-1 phosphorylation.[63] β_1-integrin-mediated activation of the ERK1/2 and PI_3-Akt pathways is mediated by caveolin-1.[64] In our study, endothelial expression of caveolin-1 was enhanced in the infarcted area early after AMI in mice and autopsy samples of AMI patients, suggesting that the abrupt reduction in coronary flow and shear stress affects endothelial cells in the ischemic myocardium to upregulate caveolin-1, leading to an increased sensitivity to LIPUS.[45] Moreover, we examined whether LIPUS therapy also ameliorates contractile dysfunction in LV pressure-overloaded hearts in vivo.[65] Chronic LV pressure overload was induced with transverse aortic con-

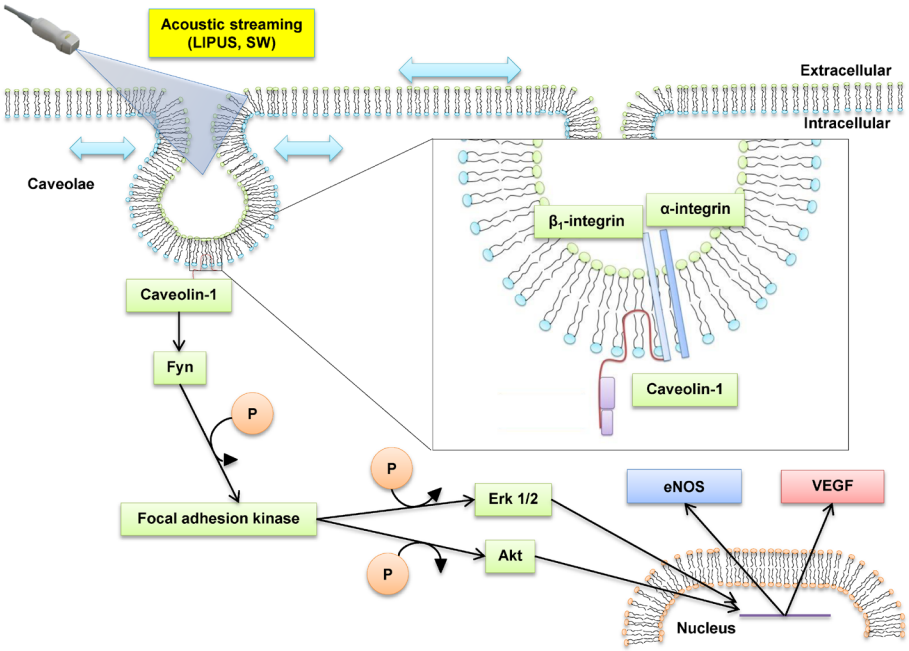

Fig. 5 Proposed molecular mechanisms for angiogenesis induced sound waves (SWs). Results from our gene-targeting studies suggest that the acoustic streaming by sound waves (low-intensity pulsed ultrasound (LIPUS) and low-energy SW) induces distortion of the caveolae with β_1-integrin and caveolin-1, which transmits the mechanical stimuli into intracellular signaling pathways and that the subsequent phosphorylation of Fyn and FAK induces Erk1/2 and Akt phosphorylation, leading to enhanced expression of endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) and subsequent angiogenesis. (from Refs. 45 and 61)

striction (TAC) in mice, and at 8 weeks after TAC, TAC-induced LV dysfunction was significantly ameliorated in the LIPUS-treated group compared with the control group.[65] LIPUS therapy may also ameliorate contractile dysfunction in chronically pressure-overloaded hearts through enhanced myocardial angiogenesis and attenuated perivascular fibrosis.[65]

3.3 Additional Important Indications of the LIPUS Therapy

Based on experimental studies of the heart, we aimed to expand the indications of our LIPUS therapy for other disorders, and one of them is dementia. In 2015, approximately 47 million patients worldwide were diagnosed as having dementia, and it is estimated that by 2050, more than 131 million patients will be diagnosed.[66] However, at this moment, no curative treatment is available for vascular dementia (VaD) or Alzheimer's disease (AD),[67,68] both of which comprise the most common causes of dementia. It was reported that low-intensity ultrasound (but not pulsed ultrasound like ours) increases the production of a brain-derived neurotrophic factor in astrocytes[69] and nerve growth factor in PC12 cells[70] and promotes nerve regeneration.[71] Recent studies have suggested that cerebral microcirculatory dysfunction, especially reduced NO bioavailability, plays an important role in the pathogenesis of AD in addition to VaD.[72,73] Based on our encouraging results in animal models of myocardial ischemia and these recent studies, we performed basic experiments using 2 mouse models of dementia (VaD and AD) to examine our hypothesis that whole-brain LIPUS therapy is useful for the treatment of dementia.[74] We used bilateral carotid artery stenosis as a VaD model,[74] and we used 5XFAD transgenic mice as an AD model.[74] Interestingly, our LIPUS therapy markedly ameliorated cognitive impairments (e.g., Y-maze test and/or passive avoidance test) associated with improved cerebral blood flow in both models.[74] In the VaD model, LIPUS therapy significantly increased CD31-positive endothelial cells and Olig2-positive oligodendrocyte precursor cells, while in the AD model, it reduced Iba-1-positive microglias and amyloid-β (Aβ plaque)[74] (**Fig. 6**). Mechanistically, in both models, RNA-sequencing showed that endothelium-related genes such as eNOS were significantly upregulated. Moreover, significant correlations were noted between the increases in glial cells and neurotrophin and eNOS expressions.[74] Importantly, these preferable effects of LIPUS were totally absent in eNOS-KO mice.[74] These results indicate the effectiveness and safety of the whole-

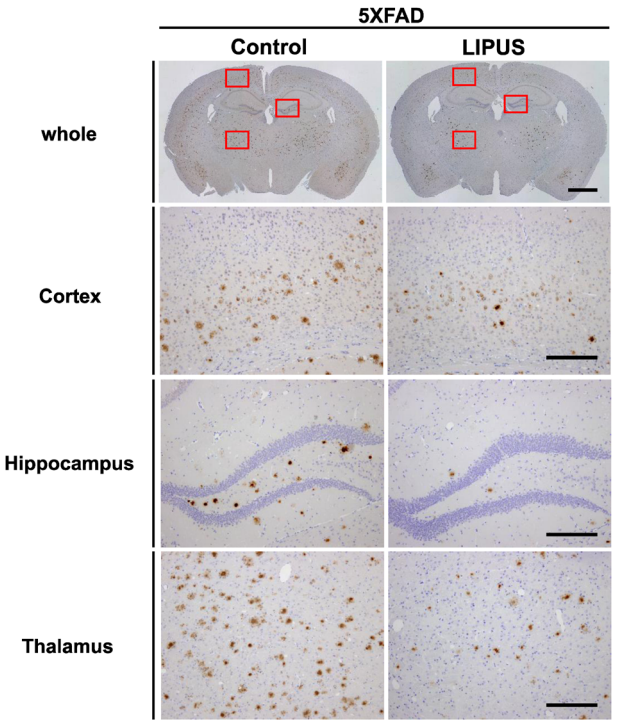

Fig. 6 Low-intensity pulsed ultrasound (LIPUS) therapy reduces Aβ plaques in the Alzheimer's disease model in mice.
Representative images of coronal sections immunostained for Aβ (stained in brown) on day 86 after LIPUS treatment; the lower 3 panels show magnified views of the cerebral cortex, hippocampus, and thalamus. As compared with the control group, LIPUS therapy markedly reduced the Aβ deposition throughout the brain. The scale bars represent 500 μm (whole-brain) or 50 μm (lower 3 panels). (from Ref. 70)

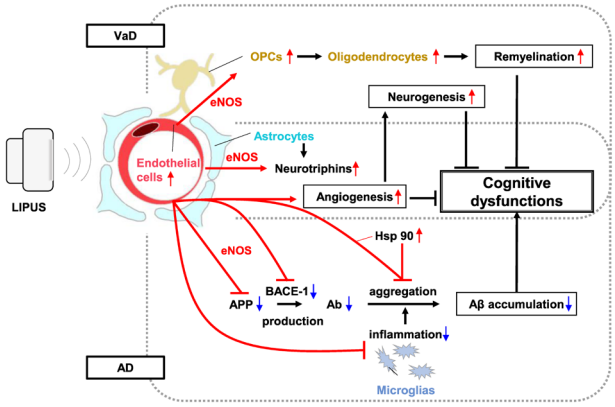

Fig. 7 Schematic diagram showing the proposed mechanisms underlying the beneficial effects of the whole-brain low-intensity pulsed ultrasound (LIPUS) therapy in two mice dementia models.
Our findings indicate that activation of endothelial nitric oxide synthase (eNOS) plays a key role for the beneficial effects of LIPUS therapy in both models. eNOS increases proliferation of oligodendrocyte precursor cells (OPCs) in the vascular dementia (VaD) model and decreases microglias in the Alzheimer's disease (AD) model. The proliferation of OPCs leads to an increase in mature oligodendrocytes, ultimately leading to re-myelination. eNOS also inhibits production and accumulation of Aβ. eNOS-induced angiogenesis plays an important role in neurogenesis via a change in cerebral blood flow. Finally, the cognitive dysfunction can be ameliorated by re-myelination, angiogenesis, and neurogenesis in the VaD model, and by reduced microgliosis and Aβ plaques and increased angiogenesis in the AD model. (from Ref. 70)

brain LIPUS for dementia and the involvement of eNOS as a common molecular mechanism[74] (**Fig. 7**). Based on these encouraging results, we have started a single-center, double blind, placebo-controlled trial with whole-brain LIPUS therapy for patients with AD. The trial will be completed in 2021.

4.1 Conclusion

Novel physiotherapy applying SW or LIPUS showed a new possibility for angiogenic therapy, and a series of evidence has been emerging. Interestingly, we were able to demonstrate that common intracellular mechanisms and pathways appear to be involved in the angiogenic effects of SW and LIPUS, including β_1-integrin/caveolin-1 in the endothelial caveolae, ERK1/2 and Akt phosphorylations, VEGF/eNOS expressions, and eventually, endothelial proliferation.[45,61] However, the physical characteristics are different between the two SWs; SW therapy produces strong shear stress, while LIPUS has low shear stress with some thermal effects. Thus, it is important to use the two SWs properly according to the target organ. For example, SW therapy may be more suitable for applying to hard tissues, such as bones and cartilage, while LIPUS may be more suitable for soft tissues, such as the brain and internal organs. Furthermore, although SW therapy has a potential risk of pulmonary hemorrhage, LIPUS has no apparent adverse effects independent of its target organ. The beneficial effects of SW therapy and LIPUS may be commonly mediated by enhanced various intrinsic pathways, where mechanotransduction and its downstream pathways appear to be involved. Although the precise intracellular mechanisms remain to be fully elucidated, low-energy extracorporeal SW and LIPUS therapies are promising as effective, safe, and non-invasive approaches for not only ischemic cardiovascular disorders but also a wide range of ischemic/inflammatory disorders.

Acknowledgments

The authors thank the collaborators at Kyushu University (Takeshita A, Nishida T, Oi K, Uwatoku T, Abe K, Eto M, Matsumoto M, Fukumoto Y, Ito A, Matoba T, Kishi

T) and those at Tohoku University (Ito K, Kikuchi Y, Ito Y, Shiroto T, Tsuburaya R, Aizawa K, Hao K, Fukumoto Y, Takahashi J, Takeda M, Nakayama M, Yasuda S, Kuriyama S, Tsuji I, Hanawa K, Hasebe Y, Hasegawa H, Kanai H, Kagaya Y, Nishimiya K, Ogata T, Kurosawa R, Eguchi K, Monma Y, Ichijo S, Hatanaka K, Miyata S, Kasukabe S, Taki H, Watanabe Y, Nishihara A, and Yamashita H).

Sources of Funding

This study was supported in part by the grants-in-aid for scientific research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, Tokyo, Japan, the Japanese Ministry of Health, Labor, and Welfare, Tokyo, Japan, and the Japan Agency for Medical Research and Development (AMED), Tokyo, Japan.

Disclosure Statements

None

Author Contributions

Study conception: HS
Data collection: TS
Investigation: all authors
Writing: all authors
Funding acquisition: HS
Critical review and revision: all authors
Final approval of the article: all authors
Accountability for all aspects of the work: all authors

References

1) Gospodarowicz D, Neufeld G, Schweigerer L. Fibroblast growth factor: structural and biological properties. J Cell Physiol Suppl 1987; **133 Suppl 5**: 15-26.
2) Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971; **285**: 1182-6.
3) Ferrara N. Vascular endothelial growth factor and the regulation of angiogenesis. Recent Prog Horm Res 2000; **55**: 15-35; discussion, 35-6.
4) Vale PR, Isner JM, Rosenfield K. Therapeutic angiogenesis in critical limb and myocardial ischemia. J Interv Cardiol 2001; **14**: 511-28.
5) Nishida T, Shimokawa H, Oi K, et al. Extracorporeal cardiac shock wave therapy markedly ameliorates ischemia-induced myocardial dysfunction in pigs in vivo. Circulation 2004; **110**: 3055-61.
6) Fukumoto Y, Ito A, Uwatoku T, et al. Extracorporeal cardiac shock wave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease. Coron Artery Dis 2006; **17**: 63-70.
7) Kikuchi Y, Ito K, Ito Y, et al. Double-blind and placebo-controlled study of the effectiveness and safety of extracorporeal cardiac shock wave therapy for severe angina pectoris. Circ J 2010; **74**: 589-91.
8) Khattab AA, Brodersen B, Schuermann-Kuchenbrandt D, et al. Extracorporeal cardiac shock wave therapy: first experience in the everyday practice for treatment of chronic refractory angina pectoris. Int J Cardiol 2007; **121**: 84-5.
9) Prinz C, Lindner O, Bitter T, et al. Extracorporeal cardiac shock wave therapy ameliorates clinical symptoms and improves regional myocardial blood flow in a patient with severe coronary artery disease and refractory angina. Case Rep Med 2009; **2009**: 639594.
10) Vasyuk YA, Hadzegova AB, Shkolnik EL, et al. Initial clinical experience with extracorporeal shock wave therapy in treatment of ischemic heart failure. Congest Heart Fail 2010; **16**: 226-30.
11) Wang Y, Guo T, Cai HY, et al. Cardiac shock wave therapy reduces angina and improves myocardial function in patients with refractory coronary artery disease. Clin Cardiol 2010; **33**: 693-9.
12) Wang Y, Guo T, Ma TK, et al. A modified regimen of extracorporeal cardiac shock wave therapy for treatment of coronary artery disease. Cardiovasc Ultrasound 2012; **10**: 35.
13) Yang P, Guo T, Wang W, et al. Randomized and double-blind controlled clinical trial of extracorporeal cardiac shock wave therapy for coronary heart disease. Heart Vessels 2013; **28**: 284-91.
14) Schmid JP, Capoferri M, Wahl A, et al. Cardiac shock wave therapy for chronic refractory angina pectoris. A prospective placebo-controlled randomized trial. Cardiovasc Ther 2013; **31**: e1-6.
15) Uwatoku T, Ito K, Abe K, et al. Extracorporeal cardiac shock wave therapy improves left ventricular remodeling after acute myocardial infarction in pigs. Coron Artery Dis 2007; **18**: 397-404.
16) Ito Y, Ito K, Shiroto T, et al. Cardiac shock wave therapy ameliorates left ventricular remodeling after myocardial ischemia-reperfusion injury in pigs in vivo. Coron Artery Dis 2010; **21**: 304-11.
17) Kagaya Y, Ito K, Takahashi J, et al. Low-energy cardiac shockwave therapy to suppress left ventricular remodeling in patients with acute myocardial infarction: first-in-human study. Coron Artery Dis 2018; **29**: 294-300.
18) Hiatt WR. Medical treatment of peripheral arterial disease and claudication. N Engl J Med 2001; **344**: 1608-21.
19) Wennberg PW. Approach to the patient with peripheral arterial disease. Circulation 2013; **128**: 2241-50.
20) Jaff MR, White CJ, Hiatt WR, et al. An update on methods for revascularization and expansion of the TASC lesion classification to include below-the-knee arteries: a supplement to the inter-society consensus for the management of peripheral arterial disease (TASC II): The TASC steering committee. Catheter Cardiovasc Interv 2015; **86**: 611-25.
21) Oi K, Fukumoto Y, Ito K, et al. Extracorporeal shock wave therapy ameliorates hindlimb ischemia in rabbits. Tohoku J Exp Med 2008; **214**: 151-8.
22) Serizawa F, Ito K, Kawamura K, et al. Extracorporeal shock wave therapy improves the walking ability of patients with peripheral artery disease and intermittent claudication. Circ J 2012; **76**: 1486-93.

23) Tara S, Miyamoto M, Takagi G, et al. Low-energy extracorporeal shock wave therapy improves microcirculation blood flow of ischemic limbs in patients with peripheral arterial disease: pilot study. J Nippon Med Sch 2014; **81**: 19-27.
24) Abraham DJ, Varga J. Scleroderma: from cell and molecular mechanisms to disease models. Trends Immunol 2005; **26**: 587-95.
25) Stojadinovic A, Elster EA, Anam K, et al. Angiogenic response to extracorporeal shock wave treatment in murine skin isografts. Angiogenesis 2008; **11**: 369-80.
26) Yan X, Zeng B, Chai Y, et al. Improvement of blood flow, expression of nitric oxide, and vascular endothelial growth factor by low-energy shockwave therapy in random-pattern skin flap model. Ann Plast Surg 2008; **61**: 646-53.
27) Hayashi D, Kawakami K, Ito K, et al. Low-energy extracorporeal shock wave therapy enhances skin wound healing in diabetic mice: a critical role of endothelial nitric oxide synthase. Wound Repair Regen 2012; **20**: 887-95.
28) Weihs AM, Fuchs C, Teuschl AH, et al. Shock wave treatment enhances cell proliferation and improves wound healing by ATP release-coupled extracellular signal-regulated kinase (ERK) activation. J Biol Chem 2014; **289**: 27090-104.
29) Saggini R, Figus A, Troccola A, et al. Extracorporeal shock wave therapy for management of chronic ulcers in the lower extremities. Ultrasound Med Biol 2008; **34**: 1261-71.
30) Moretti B, Notarnicola A, Maggio G, et al. The management of neuropathic ulcers of the foot in diabetes by shock wave therapy. BMC Musculoskelet Disord 2009; **10**: 54.
31) Saito S, Ishii T, Kamogawa Y, et al. Extracorporeal shock wave therapy for digital ulcers of systemic sclerosis: a phase 2 pilot study. Tohoku J Exp Med 2016; **238**: 39-47.
32) Kubo M, Li TS, Kamota T, et al. Extracorporeal shock wave therapy ameliorates secondary lymphedema by promoting lymphangiogenesis. J Vasc Surg 2010; **52**: 429-34.
33) Serizawa F, Ito K, Matsubara M, et al. Extracorporeal shock wave therapy induces therapeutic lymphangiogenesis in a rat model of secondary lymphedema. Eur J Vasc Endovasc Surg 2011; **42**: 254-60.
34) Ogden JA, Alvarez RG, Levitt R, et al. Shock wave therapy (Orthotripsy) in musculoskeletal disorders. Clin Orthop Relat Res 2001; **387**: 22-40.
35) Birnbaum K, Wirtz DC, Siebert CH, et al. Use of extracorporeal shock-wave therapy (ESWT) in the treatment of nonunions. A review of the literature. Arch Orthop Trauma Surg 2002; **122**: 324-30.
36) Wang CJ. Extracorporeal shockwave therapy in musculoskeletal disorders. J Orthop Surg Res 2012; **7**: 11.
37) Al-Abbad H, Simon JV. The effectiveness of extracorporeal shock wave therapy on chronic Achilles tendinopathy: a systematic review. Foot Ankle Int 2013; **34**: 33-41.
38) Yamaya S, Ozawa H, Kanno H, et al. Low-energy extracorporeal shock wave therapy promotes vascular endothelial growth factor expression and improves locomotor recovery after spinal cord injury. J Neurosurg 2014; **121**: 1514-25.
39) ter Haar G. Therapeutic applications of ultrasound. Prog Biophys Mol Biol 2007; **93**: 111-29.
40) Barzelai S, Sharabani-Yosef O, Holbova R, et al. Low-intensity ultrasound induces angiogenesis in rat hind-limb ischemia. Ultrasound Med Biol 2006; **32**: 139-45.
41) Mizrahi N, Seliktar D, Kimmel E. Ultrasound-induced angiogenic response in endothelial cells. Ultrasound Med Biol 2007; **33**: 1818-29.
42) Ramli R, Reher P, Harris M, et al. The effect of ultrasound on angiogenesis: an in vivo study using the chick chorioallantoic membrane. Int J Oral Maxillofac Implants 2009; **24**: 591-6.
43) Hanawa K, Ito K, Aizawa K, et al. Low-intensity pulsed ultrasound induces angiogenesis and ameliorates left ventricular dysfunction in a porcine model of chronic myocardial ischemia. PLoS One 2014; **9**: e104863.
44) Maloney E, Hwang JH. Emerging HIFU applications in cancer therapy. Int J Hyperthermia 2015; **31**: 302-9.
45) Shindo T, Ito K, Ogata T, et al. Low-intensity pulsed ultrasound enhances angiogenesis and ameliorates left ventricular dysfunction in a mouse model of acute myocardial infarction. Arterioscler Thromb Vasc Biol 2016; **36**: 1220-9.
46) Mizrahi N, Seliktar D, Kimmel E. Ultrasound-induced angiogenic response in endothelial cells. Ultrasound Med Biol 2007; **33**: 1818-29.
47) Parton RG. Cell biology-life without caveolae. Science 2001; **293**: 2404-5.
48) Parton RG, del Pozo MA. Caveolae as plasma membrane sensors, protectors and organizers. Nat Rev Mol Cell Biol 2013; **14**: 98-112.
49) Sedding DG, Hermsen J, Seay U, et al. Caveolin-1 facilitates mechanosensitive protein kinase B (Akt) signaling in vitro and in vivo. Circ Res 2005; **96**: 635-42.
50) Sonveaux P, Martinive P, DeWever J, et al. Caveolin-1 expression is critical for vascular endothelial growth factor-induced ischemic hindlimb collateralization and nitric oxide-mediated angiogenesis. Circ Res 2004; **95**: 154-61.
51) del Pozo MA, Balasubramanian N, Alderson NB, et al. Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization. Nat Cell Biol 2005; **7**: 901-8.
52) Zemljič Jokhadar Š, Majhenc J, Svetina S, et al. Positioning of integrin β1, caveolin-1 and focal adhesion kinase on the adhered membrane of spreading cells. Cell Biol Int 2013; **37**: 1276-84.
53) Wary KK, Mariotti A, Zurzolo C, et al. A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. Cell 1998; **94**: 625-34.
54) Yu J, Bergaya S, Murata T, et al. Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. J Clin Invest 2006; **116**: 1284-91.
55) Salanueva IJ, Cerezo A, Guadamillas MC, et al. Integrin regulation of caveolin function. J Cell Mol Med 2007; **11**: 969-80.
56) Shyy JY, Chien S. Role of integrins in endothelial mechanosensing of shear stress. Circ Res 2002; **91**: 769-75.
57) Bock-Marquette I, Saxena A, White MD, et al. Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. Nature 2004; **432**: 466-72.
58) Yeo MG, Oh HJ, Cho HS, et al. Phosphorylation of Ser 21 in Fyn regulates its kinase activity, focal adhesion targeting, and is required for cell migration. J Cell Physiol 2011; **226**: 236-47.
59) Gervasio OL, Phillips WD, Cole L, et al. Caveolae respond to cell stretch and contribute to stretch-induced signaling. J Cell Sci 2011; **124**: 3581-90.

60) Stein CK, Qu P, Epstein J, et al. Removing batch effects from purified plasma cell gene expression microarrays with modified ComBat. BMC Bioinformatics 2015; **16**: 63.
61) Hatanaka K, Ito K, Shindo T, et al. Molecular mechanisms of the angiogenic effects of low-energy shock wave therapy: roles of mechanotransduction. Am J Physiol 2016; **311**: C378-85.
62) Rizzo V, Sung A, Oh P, et al. Rapid mechanotransduction in situ at the luminal cell surface of vascular endothelium and its caveolae. J Biol Chem 1998; **273**: 26323-9.
63) Echarri A, Del Pozo MA. Caveolae internalization regulates integrin-dependent signaling pathways. Cell Cycle 2006; **5**: 2179-82.
64) Jasmin JF, Rengo G, Lymperopoulos A, et al. Caveolin-1 deficiency exacerbates cardiac dysfunction and reduces survival in mice with myocardial infarction. Am J Physiol Heart Circ Physiol 2011; **300**: H1274-81.
65) Ogata T, Ito K, Shindo T, et al. Low-intensity pulsed ultrasound enhances angiogenesis and ameliorates contractile dysfunction of pressure-overloaded heart in mice. PLoS One 2017; **12**: e0185555.
66) Prince M, Adelina CH, Knapp M, et al. World Alzheimer Report 2016. London: Alzheimer's Disease International, 2016.
67) Scheltens P, Blennow K, Breteler MM, et al. Alzheimer's disease. Lancet 2016; **388**: 505-17.
68) O'Brien JT, Thomas A. Vascular dementia. Lancet 2015; **386**: 1698-706.
69) Liu SH, Lai YL, Chen BL, et al. Ultrasound enhances the expression of brain-derived neurotrophic factor in astrocyte through activation of TrkB-Akt and Calcium-CaMK signaling pathways. Cereb Cortex 2017; **27**: 3152-60.
70) Zhao L, Feng Y, Hu H, et al. Low-intensity pulsed ultrasound enhances nerve growth factor-induced neurite outgrowth through mechanotransduction-mediated ERK1/2-CREB-Trx-1 signaling. Ultrasound Med Biol 2016; **42**: 2914-25.
71) Chang CJ, Hsu SH, Lin FT, et al. Low-intensity-ultrasound-accelerated nerve regeneration using cell-seeded poly(D,L-lactic acid-co-glycolic acid) conduits: an in vivo and in vitro study. J Biomed Mater Res B Appl Biomater 2005; **75B**: 99-107.
72) Katusic ZS, Austin SA. Endothelial nitric oxide: protector of a healthy mind. Eur Heart J 2014; **35**: 888-94.
73) Iadecola C. Untangling neurons with endothelial nitric oxide. Circ Res 2016; **119**: 1052-4.
74) Eguchi K, Shindo T, Ito K, et al. Whole-brain low-intensity pulsed ultrasound therapy markedly improves cognitive dysfunctions in mouse models of dementia—crucial roles of endothelial nitric oxide synthase. Brain Stimul 2018; **11**: 959-73.