

Ultrasound attacks Alzheimer's disease?

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The rise in the incidence of dementia has become a major public health concern all over the world (1). Alzheimer's disease (AD), characterized by progressive cognitive deterioration, is known to be the most common cause of dementia. The brains of AD patients have an abundance of amyloid plaques, in the form of extracellular aggregates of amyloid- β peptide ($A\beta$), which are believed to contribute to the progressive neurodegeneration observed in the AD brain (2,3). Despite decades of widespread research into approaches to slow progression of the disease, scientists have had no luck developing promising treatments. In a recent article published in *Science Translational Medicine*, Leinenga *et al.* reported use of ultrasound as a promising approach to clear neurotoxic amyloid plaque from the AD brain (4).

$A\beta$ is a 38- to 43-amino-acid peptide generated from amyloid precursor protein (APP) by sequential proteolytic processing (5). Abnormal accumulation of toxic species of $A\beta$ is assumed to trigger a cascade of pathological events resulting in synaptic damage, neuronal dysfunction, and, eventually, neuronal cell death. This "amyloid cascade hypothesis", although it does not explain all features of the AD pathogenesis, has dominated AD research for the past 20 years and provided the basis for the development of new therapeutic strategies (6). Treatments to reduce excess $A\beta$ in the AD brain, so-called "anti-amyloid therapies" such as gamma-secretase inhibitors and anti- $A\beta$ immunotherapy, have been developed and numerous clinical trials have been launched to test the safety and efficacy of those strategies. However, despite the promising outcomes of most pre-clinical studies in animal models, some earlier clinical trials uncovered unfavorable potential side effects of $A\beta$ -lowering agents (7,8). Off-target effects of secretase inhibitors

or neuro-inflammatory side effects of immunotherapy, although relatively uncommon, are now considered to be potentially important adverse effects to overcome for successful treatment.

Leinenga *et al.* utilized a unique "non-pharmacological" strategy to safely remove cerebral deposition of $A\beta$ plaque in a mouse model of AD (4). They used repeated scanning ultrasound (SUS) treatment, which is known to trigger transient opening of the blood-brain barrier (BBB), to remove cerebral amyloid depositions in the mouse brain. Ultrasound irradiation combined with intravenous (i.v.) injection of microbubbles causes a range of effects on brain blood vessels, including inducing dilatation and contraction of blood vessels, opening tight junctions, and facilitating transport across the BBB (9). The researchers treated APP23 mice with repeated SUS combined with microbubble injection for several weeks. APP23 mice, a well-validated mouse model for AD, overexpress human-type mutant APP derived from familial AD and develop AD-like amyloid plaque in the brain with age (10). The effect of SUS on amyloid pathology was drastic. Histological and biochemical analysis using postmortem brain tissues revealed a roughly 50% reduction of dense core amyloid plaque and soluble species of toxic $A\beta$ peptides. More importantly, repeated SUS successfully rescued memory deficit in Alzheimer APP23 mice. The authors confirmed the favorable effect of SUS treatment on cognitive function in three different behavioral tasks, showing that the spatial memory deficit in APP23 mice was completely rescued to non-transgenic control levels.

Notably, repeated SUS irradiation with i.v. injection of microbubbles did not cause any apparent damage to the brain: histological examination after SUS treatment

showed no evidence of neuronal death, edema, erythrocyte extravasation, or ischemic changes. Unfavorable adverse effects on normal brain structures or physiological functions have been major obstacles for the before-mentioned A β -lowering agents. SUS appears to be considerably superior to pharmacological approaches in terms of invasiveness. Indeed, the safety and usefulness of ultrasound irradiation of the central nervous system (CNS) has been well investigated and validated. Shimamura *et al.* demonstrated that a microbubble-enhanced ultrasound method successfully delivered therapeutic genes into the CNS with no evidence of brain damage (11). It is important to find the right balance between “thorough cleaning of toxic aggregates” and “securing intact brain structures.”

What specific mechanisms are involved in the successful removal of cerebral amyloid deposition by ultrasound? Leinenga *et al.* looked at the contribution of microglia because microglial phagocytosis has been postulated to contribute to reduction of amyloid plaque in A β immunotherapy (12). Postmortem histological assessment revealed that more A β plaques were engulfed by microglia and sorted into lysosomes in SUS-treated APP23 mice compared to control mice, suggesting that SUS enhanced microglial uptake of A β plaque and subsequent intracellular degradation. This was not due to an increase in the total number of microglia in the brain: there was no difference in the number or size of microglia between SUS-treated and non-treated APP23 mice. Microglia is known to be morphologically dynamic cells, and morphological changes are strongly associated with their functional activities (13). Resting microglia exhibit a ramified morphology (i.e., highly branched processes) and become amoeboid when stimulated. Microglia in the brain of SUS-treated APP23 mice exhibited more “activated” morphology compared to non-treated mice. Taken together, these findings indicate that ultrasound treatment somehow triggered microglial activation and enhanced A β phagocytosis.

Given that ultrasound treatment induces microglial activation, what links ultrasound-induced BBB opening with microglial activation and A β phagocytosis? Leinenga *et al.* postulate that albumin entry into brain parenchyma via an “opened” BBB can mediate A β uptake by microglia. It is known that albumin binds to A β , which inhibits self-association and prevents further aggregation (14). The authors tested this hypothesis in a cell culture system and found that the presence of albumin enhanced facilitated microglial uptake of A β . However, the authors did not show any direct evidence that the same phenomenon can happen

in vivo. Further work will be needed to demonstrate the more specific mechanisms linking BBB opening and A β clearance.

Another possible mechanism for the reduction of amyloid deposits in SUS-treated APP23 mice may be a direct impact of ultrasound on amyloid plaque. Sato *et al.* reported that ultrasonic irradiation can induce the dissociation of soluble A β from fibrils *in vitro* (15). The soluble form of low molecular weight A β is more readily cleared from the brain than high molecular weight A β oligomer (16). It may be that ultrasound breaks amyloid plaque into small soluble species and facilitates efflux of A β into peripheral circulation.

No convincing conclusion regarding the mechanisms for A β reduction is available at this stage. Knowing the precise mechanisms underlying the drastic reduction of cerebral A β after SUS is critical in order to avoid the kinds of unexpected adverse effects experienced in clinical trials of other A β -lowering agents. Given that BBB opening and subsequent entry of serum albumin into the brain contributed to the reduction of A β , great care must be taken to guard against cerebral edema or entry of other neurotoxic molecules into the CNS, which could potentially damage, not cure, the AD brain.

There are some obstacles to overcome for this technology to be considered for application in human patients. The larger amount of brain tissue and thicker skull in humans can make it challenging for the ultrasound to cover broad brain regions with amyloid plaques. The hippocampus and medial temporal lobe are known to play an important role in memory formation and also to be among the most affected brain regions in AD. Their location deep within the brain may preclude efficient irradiation of ultrasound through the skull or even via cranial windows.

Overall, the work reported by Leinenga *et al.* provides a promising “non-pharmacological” approach to remove toxic A β from the AD brain. Further work is needed to elucidate the mechanisms and confirm the safety of this technology. It should be emphasized, however, that ultrasound treatment was able to reduce cerebral A β to the same degree as A β immunotherapy (17). It is possible that this approach may be applied to other neurodegenerative diseases involving toxic protein aggregation. The impact of ultrasound treatment on tau pathology, which is known to be more tightly linked to cognitive decline in AD patients, should be further investigated using a tau-transgenic mouse model.

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Footnote

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