SCIENCE BEHIND THE STUDY
Elizabeth G. Phimister, Ph.D., *Editor*

Sounding Out the Blood–Brain Barrier

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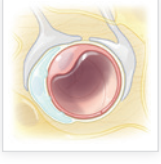
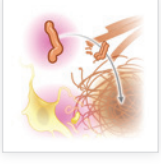
The blood–brain barrier (see Key Concepts) safeguards the brain from harmful substances while allowing essential nutrients to pass through. However, it also impedes the delivery of drugs to the brain.[1] This challenge is especially prominent when treating Alzheimer's disease, a neurodegenerative disorder with limited treatment options that imposes a major burden on health care, due to an aging global population. In this issue of the *Journal*, Rezai et al.[2] report a proof-of-concept trial involving three patients with Alzheimer's disease who were treated with aducanumab, an amyloid-binding monoclonal antibody with limited penetration of the blood–brain barrier.[3] The experimental treatment involves the creation of an opening in this barrier by magnetic resonance imaging (MRI)–guided focused ultrasound to enhance drug delivery. Rezai et al. observed a reduction in the load of cerebral amyloid-beta (Aβ) in the targeted areas.

Key Concepts



Blood–brain barrier

A physical and biochemical boundary between the bloodstream and the parenchyma of the central nervous system (CNS). Composed of tightly bound vascular endothelial cells, surrounded by a basement membrane, and supported by astrocytes, pericytes, and microglia, the blood–brain barrier shields the CNS from viruses, bacteria, and neurotoxins. It also controls the movement of ions, molecules, and cells between blood and the CNS. The blood–brain barrier prevents 98% of small-molecule drugs and 100% of large-molecule drugs from entering the CNS through the bloodstream.



Amyloid-beta (Aβ)

A protein derived from the amyloid-beta precursor protein (APP) that is produced in the brain throughout life. Sequential cleavage of this protein by β- and γ-secretases produces two major isoforms of the toxic peptide amyloid-beta ($A\beta_{40}$ and $A\beta_{42}$). Overproduction of APP, which occurs in Down's syndrome, also drives an excess of Aβ. In older persons and in persons with Alzheimer's disease, Aβ accumulates in brain parenchyma and blood-vessel walls. These molecules aggregate as misfolded oligomers, which leads to the creation of amyloid plaques. Plaques and soluble Aβ are harmful to neurons and synapses, causing their dysfunction and loss.

An expanded illustrated glossary is available at NEJM.org

HOW DOES FOCUSED ULTRASOUND BREACH THE BLOOD–BRAIN BARRIER?

Focused ultrasound generates a mechanical wave, inducing oscillations in the medium that transitions between compression and rarefaction. Gas bubbles, when injected into the bloodstream and exposed to the ultrasound field, undergo greater compression and expansion than surrounding tissues and blood. These oscillations create mechanical stress on blood-vessel walls, leading to the stretching and opening of tight junctions between endothelial cells (Fig. 1A). (They also stimulate active vacuole transport through these cells.[4]) Thus, the integrity of the blood–brain barrier is compromised, allowing molecules to diffuse into the brain. The barrier reseals itself within approximately 6 hours — with less time for mild exposures or larger molecules and more time after higher exposure levels (which may be associated with histologic damage).

Focused ultrasound–induced opening of the blood–brain barrier was first demonstrated in experiments in animals in 2001,[7] followed by preclinical studies showing enhanced drug delivery and therapeutic effects.[8] Later, focused ultrasound was shown to safely open the blood–brain barrier in the absence of drug delivery in patients with Alzheimer's disease[9] and to deliver antibodies into brain metastases that originated from breast cancer.[10]

HOW WERE THE MICROBUBBLES DELIVERED?

Microbubbles are an ultrasound contrast agent routinely used in diagnostic ultrasonography to

visualize blood flow and vasculature. In this trial, a nonpyrogenic suspension of phospholipid-encapsulated perfluoropropane bubbles was infused intravenously during sonications (Fig. 1B). The microbubbles have high polydispersity, with diameters ranging from less than 1 μm to more than 10 μm. Perfluoropropane is a stable gas that is not metabolized and that exits the body through the lungs. The lipid shell surrounding the gas, which stabilizes the bubble, comprises three naturally occurring human blood lipids, which are metabolized in a similar manner to endogenous phospholipids.

HOW WAS FOCUSED ULTRASOUND DELIVERED?

Focused ultrasound is generated with the use of a hemispherical transducer helmet encircling the patient's head (Fig. 1C). The helmet is equipped with 1024 independently controllable ultrasound sources that naturally focus at the center of the hemisphere. These sources, driven by sinusoidal radiofrequency voltage, emit ultrasound waves under MRI guidance. The patient wears the helmet: degassed water circulating around the head facilitates wave propagation. Ultrasound waves pass through the skin and skull to reach the brain target.

Variability in skull thickness and density affects wave propagation, causing the waves to arrive at the focus at slightly different times. This distortion can be corrected by obtaining data from high-resolution computed tomography, thus yielding information about skull shape, thickness, and density. A computer simulation model calculates compensating phase shifts for each driving signal, restoring a sharp focus.[11] Controlling the phase of radiofrequency signals allows electronic focus placement for sonications, covering a large tissue volume without moving the array of ultrasound sources. The location of tissue for targeting is determined through MRI of the head with the helmet in place. The target volume is filled with a three-dimensional grid of sonication locations, each sonicated for 5 to 10 msec and repeated every 3 seconds. Ultrasound power is gradually increased until the desired emissions from bubbles are detected; these are then maintained for 120 seconds. This process is repeated with new grids until the target volume is fully covered.

SOUNDS DELICATE, RIGHT?

Opening the blood–brain barrier requires the amplitude of the sound wave to exceed a threshold, beyond which the permeability of the barrier increases with pressure amplitude until tissue damage occurs, evident by erythrocyte extravasations, bleeding, apoptosis, and necrosis, all of which are often associated with bubble collapse (called inertial cavitation).[12] These thresholds depend on microbubble size and shell material. Maintaining exposure within a safe range is essential and can be achieved through detection and interpretation of the ultrasound signals scattered by microbubbles.[13]

During sonications, Rezai et al. used scattered signals from microbubbles to determine appropriate acoustic power levels. After sonications, they used T1-weighted MRI with a contrast agent to determine opening of the blood–brain barrier in targeted locations and T2*-weighted images to confirm that extravasation or bleeding did not occur. These observations guided modifications (if necessary) of remaining treatments.

HOW DID THE AUTHORS GAUGE EFFECT?

Rezai et al. quantified the effect of treatment on cerebral Aβ load by comparing ^{18}F-florbetaben positron-emission tomographic scans before and after the treatment series, assessing the difference between the volume of Aβ in the treated region and that in the analogous region of the other hemisphere. Previous studies by this group, however, showed that focused ultrasound alone slightly reduced Aβ levels. The reduction observed in the current trial was numerically greater than in the previous studies.

WHAT'S NEXT?

The trial by Rezai et al. involves small tissue volumes, which were not systematically chosen, in one side of the brain of three patients only. Expanding treatment to clinically significant volumes on both sides of the brain is crucial for assessing its efficacy in slowing disease progression. Moreover, additional studies are needed to establish long-term safety and efficacy, and cost-effective treatment devices that are not reliant on online MRI guidance must be developed for broader accessibility. That all being said, the results spark optimism that this approach to

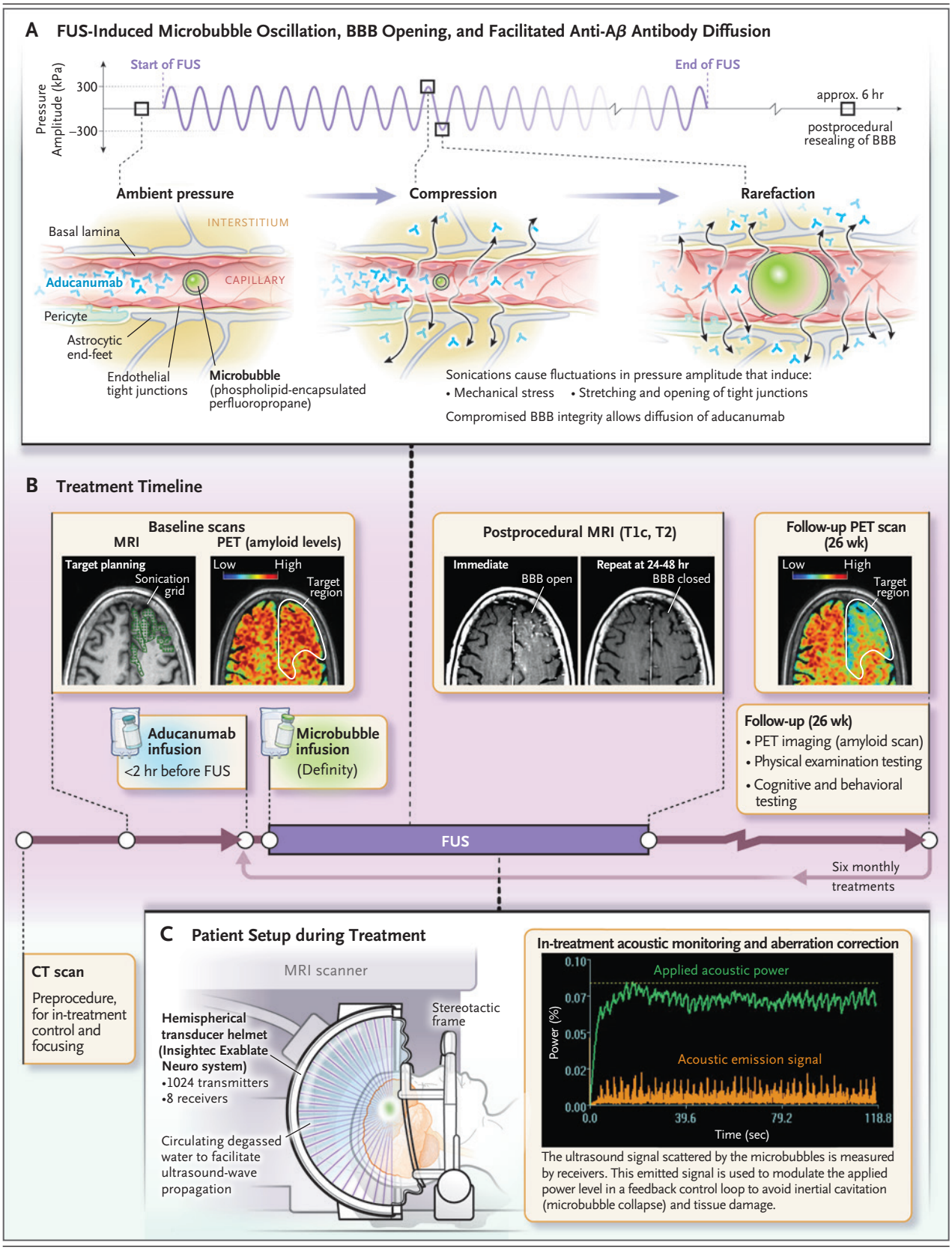


Figure 1 (facing page). Drug Delivery by Focused Ultrasound.

In this issue of the *Journal*, Rezai et al.[2] report the results of treating patients with Alzheimer's disease with the antiamyloid antibody aducanumab during opening of the blood–brain barrier (BBB) by magnetic resonance imaging (MRI)–targeted local focused ultrasound (FUS). Afterward, they observed a reduction in amyloid-beta (Aβ) levels in the targeted brain locations. Panel A shows the effect of targeted ultrasound waves on the capillary wall when a micrometer-size gas bubble is present inside the vessel. Owing to the high compressibility of gas, the bubble contracts and expands with the pressure wave more than the surrounding tissue, causing mechanical stresses on the endothelial cells. This process results in the opening of tight junctions and perhaps the detachment of astrocytic endfeet from the vessel wall, compromising the integrity of the blood–brain barrier and facilitating antibody diffusion. In addition, exposure to focused ultrasound increases active vacuole trafficking across endothelial cells[4] and has been shown to inhibit efflux pumps, decreasing the clearance of the antibodies from the brain.[5,6] Panel B shows the treatment timeline, which includes previous computed tomography (CT) and MRI for sonication planning, an ^{18}F-florbetaben positron-emission tomographic (PET) scan at baseline, antibody infusion before the focused ultrasound treatment and microbubble infusion during the treatment, and acoustic monitoring of the ultrasound signal scattered from the microbubbles used to control the treatment. Imaging obtained after the focused ultrasound treatment included T1-weighted contrast-enhanced MRI, showing opening of the blood–brain barrier in the sonicated locations; an image of the same area, 24 to 48 hours after the focused ultrasound treatment, showing complete healing of the barrier; and an ^{18}F-florbetaben PET scan 26 weeks later during the follow-up of one of the patients, showing a reduction in Aβ levels in the treated brain volume. Panel C shows the MRI-guided focused ultrasound setup during the treatment. The hemispherical transducer helmet incorporates more than 1000 ultrasound sources that converge on a focal point in the brain, using real-time MRI guidance.

treatment, together with agents that remove Aβ, could eventually slow the progression of Alzheimer's disease.

Disclosure forms as provided by the author are available with the full text of this editorial at NEJM.org.

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