MR-Guided Focused Ultrasound for Acute Stroke
A Rabbit Model

Thilo Hölscher, MD; Golnaz Ahadi, PhD; David Fisher, SRA; Eyal Zadicario, PhD; Arne Voie, PhD

Recent developments in thrombolysis therapy using tissue-type Plasminogen Activator (tPA) in combination with ultrasound for noninvasive stroke treatment are of great excitement. The first clinical trials have been successfully initiated, demonstrating the potential benefit of transcranial ultrasound in combination with tPA as an effective vessel recanalizing treatment strategy. The use of tPA, however, is limited because of its various exclusion criteria, its availability, and its costs. Depending on the literature, the number of patients with stroke receiving tPA treatment ranges between 1.6% and 9%, averaging 3% to 4% worldwide. Hence, novel treatment options in stroke are in high demand. Neurointerventional methods for mechanical clot retrieval in acute stroke show great potential but are currently limited to highly specialized centers. Alternative strategies to treat stroke noninvasively and in the absence of tPA might be provided by innovative ultrasound technologies, such as transcranial focused ultrasound (FUS). First investigations in this regard have emphasized the high potential of successful clot lysis in combination with17,18 but, more so, in the absence of tPA. Very recently, successful transcranial clot lysis within seconds and without the use of tPA has been demonstrated using one of the first transcranial FUS head systems worldwide, designed for therapeutic FUS applications in humans.

In the following report, the results of recent in vitro studies, first in vivo data using a novel sonothrombolysis model, and a future concept for potential transcranial FUS therapy in stroke will be presented.

Methods

The FUS System
An ExAblate 4000 High Intensity Focused Ultrasound headsystem (InSightec, Inc, Tirat Carmel, Israel), equipped with a transcranial hemispheric transducer, has been used for all studies. In the clinical setup, the FUS system is integrated into an MRI scanner (Figure 1). Because the ExAblate 4000 is a purely therapeutic device without imaging capability, MRI is used for focus beam visualization using MR thermometry and neuronavigation in the 3-dimensional space. The system has been developed for brain applications in humans. The key component of this system is a hemispheric phased array transducer with 1000 single piezo elements, which can be operated independently. The transmit frequency of the ExAblate 4000 is 220 kiloHertz. The hemispheric geometry of the FUS transducer allows ultrasound transmission across the entire skull. All 1000 beams are focused at the geometric center of the transducer, producing a sharp focus (radius, 2.0 mm in X/Y- and 3.0 mm in Z-orientation). The focus can be steered electronically in a radius of 3.0 cm in any direction without considerable phase distortion. Further focus translation beyond this radius can be achieved by mechanical steering of the hemispheric transducer using a stereotactical positioning system.

In Vitro
To mimic transcranial insonation, human calvaria were used and mounted upside down inside the degassed water-filled hemispheric transducer.

Venous whole blood was drawn from healthy, unmedicated donors, as approved by the UCSD Institutional Review Board. The thrombi were prepared following a standardized protocol. In brief, thrombi had an average weight of 0.2519 g ±7% after 3 hours incubation in a preheated (37°C) water bath. After incubation, blood clots were transferred into polyethylene test tubes (Advanced Polymers, Inc, Salem NH).

The polyethylene tubing with the clot inside was connected to a pulsatile flow system and positioned in such a way that the center of thrombus was aligned with the FUS focus location in the center of the hemispheric transducer.

In Vivo
A rabbit carotid artery model has been developed mainly because it provides arterial dimensions that are comparable with either a smaller sized proximal segment of a middle cerebral artery or a normal sized middle cerebral artery branch in humans. It further allows to potentially mimicking the intracranial bifurcation of the distal internal carotid artery or a first/second order bifurcation of the middle cerebral artery. The model includes the brain as the end organ, which can potentially be studied for downstream clot fragmentation and ischemic events related to this. The model allows testing for the impact of FUS sonothrombolysis on different thrombus models, including rabbit as well as human clot preparation methods. A detailed description of this model has been published recently.

Results

In Vitro
Impact of Skull
Using a standard operating parameter combination (acoustic output power: 270 W, FUS duration: 30 s, duty cycle: 50%,
pulse width: 200 ms) thrombolysis could be achieved within seconds. Depending on the skull specimen, the thrombolysis rates (preclot weight minus postclot weight) ranged between 42.12% and 10.32%. Computer tomography data analysis of the skulls showed variances in both bone thickness and bone density. The lowest amount of clot weight loss was seen in the sample with significantly greater bone thickness (mean 6.22 mm) and higher radio density (mean 1208 HU) measurements.

**Impact of Acoustic Output Power**
Whereas all other FUS parameters remained unchanged, the acoustic output varied between 100 and 400 W. It was found that clot weight loss was statistically significant. Higher intensities resulted in larger weight loss (Figure 2).

**Impact of Parameter Optimization**
Whereas the acoustic output power (235 W) and FUS duration (30 s) remained unchanged, different combinations of duty cycle (5% to 50%) and pulse width (0.1–100 ms) were tested against each other. Highest amount of weight loss (59%) was seen using the longest duty cycle in combination with the longest pulse width.

**Impact on Clot Fragmentation**
Clots were exposed to different acoustic output power levels (0–400 W), and different-sized mesh filters (180, 60, 11 µm) were used downstream to capture clot fragments. It was shown that no significant clot fragmentation occurred for acoustic output power levels <400 Watts.
In Vivo
The main goal of this preliminary in vivo work was to show feasibility of the rabbit carotid artery model for FUS sonothrombolysis research. Although overall recanalization rate was low (10% success rate), the model showed to be feasible, providing features that more closely mimic thrombotic occlusive event in humans. Furthermore, it allows studying flow mechanics in real time and provides insight into potential adverse mechanisms during sonothrombolysis, such as ultrasound-induced platelet activation. Further investigations are needed, and planned, to confirm reproducibility and reliability of this model.

Conclusions
We have demonstrated that transcranial sonothrombolysis can be achieved within seconds and in the absence of a lytic agent using the ExAblate 4000 FUS headsystem. Thrombolysis efficacy depends on the acoustic output power as well as other operating parameter combinations, such as duty cycle and pulse width. We have learned that flow mechanics contribute significantly to the thrombolysis rate as well as individual skull bone characteristics. The feasibility of a new sonothrombolysis animal model could be demonstrated, which provides features, anatomic as well as technical, which are considerably different from currently established in vivo sonothrombolysis models. In view of future noninvasive stroke treatment in humans using FUS, the current needs are (1) effective efficacy and safety studies, (2) better understanding of potentially counteracting mechanisms, such as platelet activation induced by FUS, and (3) investigations to what extent the ultrasound beam has to be focused on the target clot and, if so, what MR techniques might provide the appropriate sensitivity.

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None.

References

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