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High intensity focused ultrasound sonothrombolysis: the use of perfluorocarbon droplets to achieve clot lysis at reduced acoustic powers

Daniel Pajek, BASc[1,2], **Alison Burgess, PhD**[1], **Yuexi Huang, PhD**[1], and **Kullervo Hynynen, PhD**[1,2]

[1]Physical Sciences Platform, Sunnybrook Research Institute, Toronto, Canada

[2]Department of Medical Biophysics, University of Toronto, Toronto, Canada

Abstract

The purpose of this study was to evaluate use of intravascular perfluorocarbon (PFC) droplets to reduce the sonication powers required to achieve clot lysis using high intensity focused ultrasound (HIFU). HIFU with droplets was initially applied to blood clots in an *in vitro* flow apparatus and inertial cavitation thresholds were determined. An embolic model for ischemic stroke was used to demonstrate the feasibility of this technique *in vivo*. Recanalization with intravascular droplets was achieved *in vivo* at 24±5% of the sonication power without droplets. Rabbits receiving 1 ms pulsed sonication during continuous intravascular droplet infusion recanalized in 71% of cases (p=0.041 vs controls). Preliminary experiments showed that damage was contained to the ultrasonic focus, suggesting that safe treatments would be possible with a more tightly focused hemispherical array that allows the whole focus to be placed inside of the main arteries in the human brain.

Keywords

sonothrombolysis; HIFU; inertial cavitation; stroke; focused ultrasound

Introduction

Acute ischemic stroke (AIS) is the third leading cause of death in North America (Roger *et al* 2011). The primary method for treating AIS – the administration of the thrombolytic drug tissue plasminogen activator (tPA) – is associated with relatively low recanalization rates and increased risk of intracerebral hemorrhage (ICH) (Saqqur *et al* 2007, Medel *et al* 2009). Furthermore, 95–97% of patients are contraindicated against tPA due to a restrictive time

Corresponding Author: Daniel Pajek, C713 – 2075 Bayview Avenue, Toronto, ON Canada M4N3M5, dpajek@sri.utoronto.ca, Tel: (416) 480-6100 x89414, Fax: (416) 480-4696.

window and the drug's systemic side effects (Lindsay *et al* 2010). Delays in treatment can significantly affect patient outcomes (Marler *et al* 2000), making the time-to-reperfusion an important factor in AIS treatment. Treatments that are faster and better localized are required to improve outcomes and potentially permit treatment of currently contraindicated patients.

Transcranial focused ultrasound is an emerging therapeutic modality that has been used in clinical trials for the treatment of glioblastomas (McDannold *et al* 2010), Essential Tremor (Lipsman *et al* 2013, Elias *et al* 2013), Parkinson's disease (Elias 2013), and neuropathic pain (Jeanmonod *et al* 2012). Low intensity ultrasound is being investigated for the treatment of AIS in combination with injected tPA or ultrasound contrast agents (Alexandrov *et al* 2004, Culp *et al* 2011a, Molina *et al* 2006, Hitchcock *et al* 2011), with an early trial demonstrating a significant increase in recanalization rates at 2 h (Alexandrov *et al* 2004).

The use of high intensity ultrasound (HIFU) to break up a blood clot has been demonstrated both *in vitro* (Rosenschein *et al* 1994, Maxwell *et al* 2009, Wright *et al* 2012) and *in vivo* (Wright *et al* 2012, Maxwell *et al* 2011, Burgess *et al* 2012). The benefits of this approach are that treatments are localized, clot lysis occurs within minutes, and thrombolytic drugs are not required.

The primary mechanism responsible for HIFU clot lysis is inertial cavitation (IC) (Rosenschein *et al* 1994). IC refers to the inception and inertially dominated collapse of a cavity under very high acoustic pressures (Noltingk and Neppiras 1950), which has been shown to cause mechanical tissue erosion (Brujan *et al* 2005). HIFU clot lysis has been demonstrated without vessel damage in a rabbit stroke model at 1.5 MHz (Burgess *et al* 2012), however the application of this technique transcranially is not yet feasible due to the high acoustic powers required (Pajek and Hynynen 2012).

Sonication of perfluorocarbon (PFC) droplets with sufficiently high pressures has been shown to cause vaporization and the formation of microbubbles (Giesecke and Hynynen 2003, Suslick and Grinstaff 1990, Kripfgans *et al* 2000). Furthermore, the presence of droplets provides nucleation sites from which cavities can grow, allowing IC to occur at lower a threshold intensity. Schad and Hynynen demonstrated a 52% reduction in the IC threshold intensity at 1.7 MHz (2010).

The use of droplets allows better spatial control of the induced bioeffect (Phillips *et al* 2013), when compared to microbubbles, which are acoustically active throughout the ultrasound field (McDannold *et al* 2006). At the relatively low frequencies used in this study, it has been shown that droplets do not vaporise below the IC threshold (Schad and Hynynen 2010), so it is expected that the induced bioeffect should be contained within the ultrasonic focus, since off-focus regions with pressures below the cavitation threshold should not produce droplet vaporization. Due to a lower solubility and diffusivity, droplets remain in blood longer than microbubbles, which makes them more suitable for *in vivo* use (Giesecke and Hynynen 2003, Kripfgans *et al* 2002, Rapoport *et al* 2009). Microbubbles have a circulation half-life on the order of minutes (Mullin *et al* 2011), while a half-life of 2

h has been observed for droplets (Rapoport *et al* 2011). Furthermore, the small size of droplets (< 1 μm) may make them small enough to fit within the fibrin matrix of a blood clot (Couture *et al* 2006, Carr and Hardin 1987).

The goal of this study was to investigate the feasibility of using intravascular droplets to reduce the sonication power required to achieve IC-mediated clot lysis. The application of this method was investigated *in vitro* and IC thresholds were determined. The feasibility of this technique was also assessed in preliminary *in vivo* experiments.

Methods

In Vitro

Ultrasound Parameters—An in-house constructed spherically focused HIFU transducer (1.513 MHz, 10 cm diameter, 10 cm radius of curvature, 55% efficiency), connected to driving electronics, was positioned 10 degrees to the water surface to minimize standing waves and an acoustic absorber was placed at the water surface to reduce reflections (Figure 1). Alignment was achieved by temporarily connecting the HIFU transducer to a pulser/receiver (DPR300; JSR Ultrasonics, Pittsford, NY). The transducer produced a -6dB focus of 0.9 mm × 7.1 mm. Free-field peak negative pressures, measured previously using a calibrated fibre-optic hydrophone (Precision Acoustics Ltd., Dorset, UK), were measured up to 3.6 MPa – due to limitations of the hydrophone – and extrapolated to estimate focal pressures at higher powers (Wright 2010).

An in-house constructed passive cavitation detector (PCD; 0.52 MHz, 11% −6dB fractional bandwidth, 4 cm diameter, 10 cm radius of curvature) was mounted at the side of the therapeutic transducer and their focal regions were aligned. PCD signals were recorded at 200 MS/s using a personal computer through a GPIB connector (GPIB-USB-HS; National Instruments, Austin, TX).

Clot Preparation—Blood clots were prepared based on the methods described by Wright et al (2012). Rabbit blood was withdrawn from the auricular artery of New Zealand White Rabbits into sodium citrate Vacutainer tubes (0.109 M, 3.2% sodium citrate; Stryker, Hamilton, ON). 600 μL of citrated blood was combined with 75 μL of degassed $CaCl_2$ (100 mmol/L in deionized water; Sigma Aldrich, St. Louis, MO) and 40 μL of degassed bovine thrombin (25 NIH units/ml in saline; Sigma Aldrich, St. Louis, MO).

Polyester tubing (2 mm ID, 0.01 mm wall thickness; Advanced Polymers Inc., Salem, NH) was flushed with saline (0.9% NaCl), secured at one end using a 2 mm vessel clamp (Fine Science Tools, Vancouver, BC, Canada), and the preformed clot was injected using an 18-gauge spinal needle. A second clamp was placed approximately 5 cm away from the first clamp. The sealed tubes were incubated at 37°C for 1 h and then left at room temperature for no more than 3 h.

Droplet Preparation—Droplets were created by combining 300 μL of liquid dodecafluoropentane (DDFP; Synquest Labs, Alachua, FL), 20–25 μL of fluorosurfactant (Zonyl FSO; DuPont, Wilmington, DE), and 10 mL of double distilled water, and

emulsifying for 10 min using a tip sonicator (1 s pulses, 50% duty cycle, 4 W power; Sonicator 3000, Misonix, Farmingdale, NY). The method was similar to that described in Williams et al (2013). The tube was then centrifuged for 1 h at 900g at 4°C within a refrigerated centrifuge and the excess supernatant removed. Droplets were stored at 4°C and used within 3 days. The droplets were measured on the day of use using a Zetasizer (Malvern, Worcestershire, UK), and a range of droplet sizes were used to evaluate the effects on threshold intensity during the *in vitro* experiments.

Flow Apparatus and Experimental Method—The tube, containing the prepared clot, was placed above the HIFU transducer and degassed saline was flowed through using a gravity drip apparatus at a volume flow rate of approximately 1.6 mL/s. A collateral flow tube, the same length and diameter as the main tube, bypassed the occluded portion and functioned as a pressure release (Figure 1). 4–5 targets, each 7 mm apart, were chosen along the length of the clot. 1 ms burst sonications were performed at a duty cycle of 0.1% to minimize heating. To assess the integrity of the clot, each target was sonicated for 20 s at burst-peak acoustic powers of 19 W, 40 W, and 58 W prior to the addition of droplets. These sonication powers are below the reported 120–160 W IC threshold of the clot (Wright *et al* 2012) and if IC occurred, it was assumed that an air bubble had escaped into the system and that clot was not used.

Droplets were combined with degassed saline in a 10:1 ratio and 250 μL of the solution was injected into the flow system. After 5 min, to allow the droplet solution to flow past the clot, the target was sonicated at 19 W for 20s. If IC was not observed on the PCD, the power was increased and a subsequent 20 s sonication was applied. The IC threshold was defined as where broadband noise was observed for at least 10 pulses in a row, since single pulses of broadband noise were often seen at the beginning of sonication, as was similarly observed by Vykohdeseva et al (1995). Broadband noise was identified as both an order of magnitude increase in signal and a non-uniform signal amplitude. This threshold agrees with prior studies, where a signal amplitude increase of at least an order of magnitude was observed when inertial cavitation occurred during HIFU clot lysis (Wright *et al* 2012) and with droplets (Schad and Hynynen 2010). Pulsed sonication was continued for 1 min at the threshold power. The most distal target was sonicated first, with sonications continuing proximally, so that byproducts from initial exposures did not affect subsequent sonication outcomes.

Clots were visually inspected for observable erosion between each sonication. Optical images of each clot were taken before and after sonication. Erosion widths were calculated as the largest width that could fit within at least 50% of the erosion area.

In Vivo

Animal Preparation—Male New Zealand White rabbits were obtained from Charles River Laboratories (Sherbrooke, QC). A 2 cm × 4 cm piece of bone was removed from the parietal surface of the skull. The skin was sutured back over the cranial window and allowed to heal for two weeks prior to experiments. Animal procedures were approved by the institutional animal care committee and conformed to the National Animal Care guidelines.

***In Vivo* Apparatus and Experimental Method—**Following the same procedure as Burgess et al (2012), rabbits were anesthetized using ketamine (50 mg/kg) and xylazine (5 mg/kg) and 1 mL of blood was drawn. Clots were formed following the same recipe as above, with the addition of 10 μL of superparamagnetic iron oxide (1.5×10^7 particles/mL; Bang's Laboratories Inc, Fischers, IN) to make the clots visible on MR images.

A 20-gauge plastic catheter was filled with heparinised saline (33 U/mL) and inserted into the internal carotid artery (ICA). A 2 mm × 2 mm × 2 mm embolus was inserted into an injection hub, attached to the inserted catheter, and flushed with 1–2 mL of saline. X-ray angiograms (OEC, GE Healthcare, Milwaukee, WI) were acquired while injecting 0.5–1.0 mL of Omnipaque (350 mg I/mL, GE Healthcare, Milwaukee, WI). Angiograms were performed prior to and after injection to confirm blockage of the MCA. If the MCA was not blocked, a maximum of two repeat injections were performed until MCA blockage was confirmed.

MR Guidance and Focused Ultrasound Sonications—The rabbits were placed on a custom MR-compatible motorized positioning and sonication system (Chopra *et al* 2009) within a 3T MRI scanner (MR750; GE Healthcare, Milwaukee, WI). The positioning system was registered with the MR bed's coordinate system by sonicating a gel phantom (15 W for 30 s) and registering the obtained MR thermometry images (MR thermometry scan parameters: GRE, TR = 39 ms, TE = 20 ms, slice thickness = 1 mm). The gel phantom was removed prior to placing the first rabbit. The same transducer was used in the *in vivo* experiments as was used in the *in vitro* experiments, described above.

Rabbits were positioned supine with their head coupled to the degassed water, such that the cranial window faced downwards and was aligned with the centre of the water tank. Time of flight (TOF) images (TE=4.7 ms, TR = 16 ms, slice thickness = 0.5 mm, NEX=1, 256×192) were acquired to identify the location of the iron-loaded blood clot (Figure 2). MR images were loaded into the motorized positioning system's image-guided targeting software. Sonication locations were planned along the length of the clot, 1 mm apart, starting proximally and moving distally and droplets were injected through the rabbit's ICA catheter.

Three separate treatment protocols were explored, their parameters summarized in Table 1. For group A (n=2), the axial centre of the focus was placed on the clot. 250 μL of droplets (1:1 with degassed saline) were injected, followed by 500 μL of degassed saline. After a 2 min wait, the first target was sonicated using 1 ms pulses for 20 s. After a 5 minute wait, this was repeated at the next sonication location. If recanalization did not occur, the procedure was repeated at a higher power.

For groups B and C, the 7.1 mm long focus was moved 1.5–2 mm away from the skull to minimize reflections at the skull base, resulting in the axial centre of the focus being placed in front of the targeted vessel. Furthermore, droplets were infused during sonication, to reduce the potential for droplets to extravasate prior to sonications. For group B (n=7), 250 μL of droplets (1:1 with degassed saline), followed by 500 μL of degassed saline, were injected during sonication of three targets using 1 ms pulses. If recanalization did not occur, the procedure was repeated at a higher power.

For group C (n=2), the group B protocol was repeated with a lower, 1:5 droplet-to-degassed saline ratio and shorter, 0.1 ms pulses. These changes were made to make this treatment more conservative with respect to safety.

The sonication power was chosen based on the IC thresholds determined *in vitro*, after derating for 2 cm of brain tissue, as was done in Vykhodtseva et al (1995). Untreated control rabbits (n=8) were given 250 μL of droplets (1:1 with degassed saline, followed by 500 μL of degassed saline) at 25–30 minutes and assessed for recanalization at 1 h following clot injection.

Imaging and Histological Analysis—TOF scans were acquired to confirm changes in blood flow and detect micro-hemorrhages. Hypo-intense regions on the MR images were assumed to be indicative of deoxygenated blood within tissue, which would cause a susceptibility artefact (Lee *et al* 1999). Angiograms were acquired to determine whether MCA recanalization had occurred and whether recanalization was accompanied by any major vessel damage. Following the completion of the imaging, animals were sacrificed with Euthanyl, the brains were removed, and fixed in a 10% buffered formalin solution for at least 7 days. Following tissue processing, each brain was embedded in paraffin wax and 4 μm thick coronal sections were cut at 250 μm intervals, parallel to the beam path, from a 1 cm block of tissue. Sections were stained with Hematoxylin and Eosin (H&E) to assess and localise the damage done during sonication. A Mirax Scan system (Carl Zeiss MicroImaging, GmbH, Germany) was used to capture high resolution digital images of tissue sections.

Statistical Methods

Recanalization in treatment groups were compared to controls using Fisher's exact test. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA). A preliminary power analysis using G*Power (Faul *et al* 2007) revealed that approximately 14 animals in total between any compared groups would be required, assuming an 80% success rate in the treatment group, a 0% success rate in the control group, an alpha of 0.05, and a power of 85%.

Result

In Vitro

Clot lysis was only observed after the IC threshold was reached. IC was identified by observing rolling amplitude increases in the received PCD signal (Figure 3b, d). Analysis of these data showed that broadband signal was increased over an order of magnitude, as shown in Figure 3d, which is indicative of IC. Figures 3e and 3f illustrate the sharp increase in fundamental and broadband signal content once the IC threshold was reached.

The thresholds of acoustic power required for clot lysis were determined within the *in vitro* flow apparatus for a range of droplet radii from 136 nm to 552 nm (Figure 4a). The mean IC threshold did not significantly change with droplet size. This trend is in agreement with prior *in vitro* studies, which indicated that droplet IC thresholds are more dependent on acoustic parameters – such as frequency and pulse length – and less dependent on properties of the

droplet itself – such as core composition and droplet size (Giesecke and Hynynen 2003, Schad and Hynynen 2010). Across the range of droplet sizes, the mean IC threshold was 49±9 W, or 5.8±0.5 MPa, and the highest IC threshold was 75 W, or 7.1 MPa. Clot erosion was only observed after the occurrence of IC. An example of a blood clot, both pre- and post-sonication, is shown in Figure 4b. The mean erosion width was 1.3±0.4 mm and did not appear to vary systematically with droplet size nor sonication power used (Figure 4c, d).

In Vivo

Angiograms were taken of the brain vasculature prior to and following clot injection in order to confirm vessel blockage (Figure 5). There were originally 15 non-control rabbits in this study, however 4 of them were excluded: 1 from group C where the rabbit was not treated due to an unsuccessful MCA blockage after three clot injections and 3 cases where the subject died prior to treatment due to the onset of stroke (1 from group A, 2 from group B). These cases were not sonicated and so excluded from the data presented below. A narrower range of droplets was used for the *in vivo* experiments. The radii ranged from 156 nm to 207 nm over all *in vivo* experiments, with the mean radius being 182 nm.

Combining all sets of treated rabbits together, recanalization was achieved in 73% of cases (Table 2) and bleeding was not observable on the post-treatment angiograms. Recanalization was demonstrated using 1 ms pulses and 0.1 ms pulses. For treatment group A, IC and recanalization was observed in one rabbit at 88 W and one rabbit at 111 W. For treatment group B, recanalization was achieved in four rabbits at 88 W and one at 137 W. Both rabbits in group C only received sonications at 88 W.

Hypo-intense regions, indicative of tissue micro-extravasations, were observed on MR images following sonication in all treatment groups (Figure 6). This was later confirmed through H&E staining (Figure 7), which showed evidence of red blood cells in the tissue in corresponding areas. The damage extended approximately 3 mm towards the transducer from the targeted vessel, indicating that the bioeffect was confined axially within the 7.1 mm focus. Group C, designed to be a more conservative treatment protocol, was halted after 2 subjects, since the 0.1 ms pulses still resulted in red blood cell micro-extravasations outside of the target vessel.

The brains from 8/11 treated rabbits were removed for histological analysis. H&E staining revealed blood pooling, potentially caused by the rupturing of a major vessel, in 2/2 cases where the focus was placed closer to the skull (group A) and 0/6 cases (groups B & C) where the focus was placed away from the skull ($p=0.036$) (Figure 7). Group A was halted after 2 subjects due to the observance of blood pooling. Recanalization was observed in 4/6 rabbits where an absence of blood pooling was confirmed through H&E staining. All treated rabbits survived through MR imaging and x-ray angiography and were then sacrificed.

A recanalization rate of 12% (n=8) was observed in control rabbits that were given intravascular droplets, but not treated with HIFU. Statistical analysis confirmed that recanalization rates were significant versus controls (5/7; $p=0.041$) in rabbits receiving 1 ms pulsed sonication and continuous droplet infusion (group B).

Since groups A and C were halted, significance was not demonstrated when comparing recanalization rates to controls, which resulted in p-values of 0.067 and 0.378 for groups A and C, respectively. In order to demonstrate significance, assuming groups A and C both reflect the 71% treatment ratio obtained in group B, each group would require approximately 5 additional animals.

Discussion

This study demonstrated that IC-induced clot lysis could be achieved at reduced acoustic powers with the addition of intravascular droplets. *In vitro* clot lysis using droplets was demonstrated at a range of powers from 40 W to 75 W, corresponding to estimated pressures of 5.2 to 7.1 MPa. Using the same apparatus and transducer, prior *in vitro* work by Wright et al demonstrated consistent HIFU clot lysis without droplets at 160 W and no clot lysis at 120 W (2012). This reduction in required acoustic power is consistent with the findings by Schad and Hynynen, who demonstrated an approximately 52% reduction of the cavitation threshold intensity when droplets were used over water alone (Schad and Hynynen 2010). Clot lysis with ultrasound and injected microbubbles has been demonstrated previously at even lower pressures (< 1 MPa), however erosion appeared to be constrained to the surface of the clot and occurred over the span of an hour (Petit *et al* 2012), instead of minutes in the current study.

In vivo recanalization was demonstrated with droplets at 88 W to 137 W. Prior experiments without droplets, using the same experimental model and operators, achieved recanalization in 2/4 cases at 415 W, and in 0/3 cases at 275 W (Burgess *et al* 2012). The addition of droplets resulted in IC-mediated *in vivo* clot lysis at 24±5% of the acoustic power used previously. The substantial decrease in the *in vivo* case is indicative of the increased attenuation at higher amplitudes, where the propagating wave is more nonlinear (Duck 2002). In the current study, inertial cavitation activity was often observed throughout the entire 0.1–1 ms pulse, indicating that the process occurred relatively quickly. This agrees with the microsecond timescales for inertial cavitation and droplet vaporization that have been shown previously (Noltingk and Neppiras 1950, Kripfgans *et al* 2004).

Blood pooling was observed in the two cases (group A) where the focus was placed directly on the target vessel in the axial direction. In rabbits, the MCA lies at the bottom of the brain, directly above the skull base and there is potential for reflections from the skull base to increase the amplitude at the focus. In all other cases, the focus was placed away from the skull, such that the target would still rest well within the 7.1 mm focus, but that the focus would not extend as far into the skull. Blood pooling was not observed in any of these cases, however micro-extravasations were observed in the tissue in the vicinity of the targeted vessel. On both the MR and histology images, damage extended into the brain, but not more than approximately 3 mm, indicating that the extravasations were contained within the focal region.

We created a cranial window in the rabbit skull in order to focus enough energy into the brain for effective clot lysis. The transducer therefore had to have an aperture small enough to fit its entire beam through that window and this meant using a more directed transducer

with an elongated focus. The use of a hemispherical array would result in an axial focus approximately ¼ the size of that used in this study, which would be small enough to completely fit inside the major cerebral arteries of humans, as was shown by our earlier simulation study (Pajek and Hynynen 2012).

With the HIFU clot lysis approach, there is concern that clot debris could cause distal occlusions, as Wright et al observed that particles up to 60 μm in size were produced, however the majority of particles were below 10 μm in size (2012). However, the use of phased arrays, which would allow rapid scanning over an entire clot, may mitigate this risk. Furthermore, Maxwell et al have shown that clot debris can be trapped by creating an acoustic trap downstream from the initial clot (2009). The long-term effect of recanalization through HIFU clot lysis alone has not been systematically investigated. Future studies to address the safety and efficacy of this approach by measuring ischemic infarct volume and behavioural analysis would be required once the ultrasound equipment and parameters were established. Longer duration survival studies, similar to those conducted for lower intensity sonothrombolysis therapy (Culp *et al* 2011b), are required to validate HIFU clot lysis as an effective AIS therapy.

One limitation of this study is the relatively low animal numbers used in groups A and C. These treatment groups were halted because it was believed that they would not generate additional information of interest, and so further animal sacrifice was not justified. In order to demonstrate significant recanalization rates versus controls, approximately 5 additional animals would need to be treated for each of treatment groups A and C. However, this effect was demonstrated within group B, and demonstrating it again within the other two treatment groups would not produce incrementally valuable information.

Group A was halted because the occurrence of blood pooling when the focus was centred on the vessel was shown significant versus groups where the focus was placed in front of the target vessel. Future investigations regarding the sonication of vessels close to the skull base would be useful to optimize targeting for droplet-mediated HIFU clot lysis.

Group C was designed to be a more conservative treatment than group B, however it was halted after 2 treatments, since red blood cell extravasations were still seen. A power analysis revealed that a total combined sample size of 8 would be required to compare the treatments in group B versus the treatments in group C, given perfect red blood cell extravasation proportions of 100% and 0%, respectively, and a test with a power of 80%. The 2 treatments in group C produced red blood cell extravasations, however there may still be smaller a difference between groups B and C that this study was under-powered to detect (for example, a test with a power of 80% and proportions of 50% and 100% would require a combined sample size of 24 subjects). It was decided that the detection of this lesser effect did not justify further animal sacrifice and so treatment group C was halted.

Faster and better localized AIS treatments are required to improve outcomes and potentially permit treatment of currently contraindicated patients. HIFU sonothrombolysis represents a potential treatment modality that is both fast and targeted, however our simulations demonstrate that the construction of a clinical array for HIFU sonothrombolysis alone would

require over an order of magnitude more elements than current arrays contain, making the technology technically infeasible (Pajek and Hynynen 2012). The use of droplets represents a method for enabling clot lysis at one-quarter the power of HIFU alone, which would reduce the technical complexity required in a transcranial clinical array. The powers required for transcranial HIFU clot lysis with intravascular droplets could be achieved with the currently available technology, making this a potentially viable treatment.

Currently, a transcranial focused ultrasound system operating at 650 kHz is being used clinically to treat several diseases of the CNS (McDannold *et al* 2010, Lipsman *et al* 2013, Elias 2013, Jeanmonod *et al* 2012, Elias 2012). This system is capable of producing a focal spot of approximately 3 mm × 4 mm (Martin and Werner 2013), which could be appropriate for treating axially oriented ICA and vertibro-basilar occlusions. Following feasibility studies targeting these regions, a higher frequency array would be required to produce a more precise focal spot to target occlusions within the laterally oriented MCA.

Conclusion

This study demonstrated that droplets reduce the IC threshold and enable IC-mediated clot lysis to occur at lower powers. IC thresholds were determined *in vitro* and recanalization was demonstrated *in vivo*. This reduction in acoustic power requirements brings HIFU sonothrombolysis one step close to its use in the clinic. The results suggest that a transducer with a smaller focus would be capable of administering this technique without bleeding.

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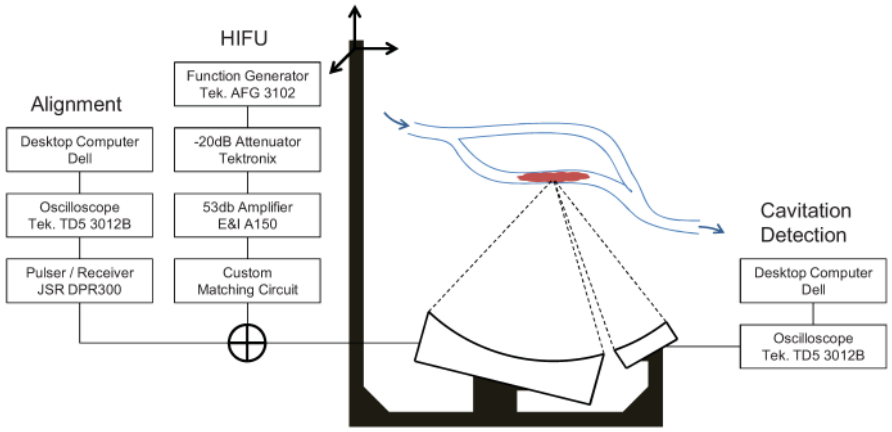

Figure 1.
In vitro schematic contains both a therapy HIFU transducer and PCD for acoustic monitoring.

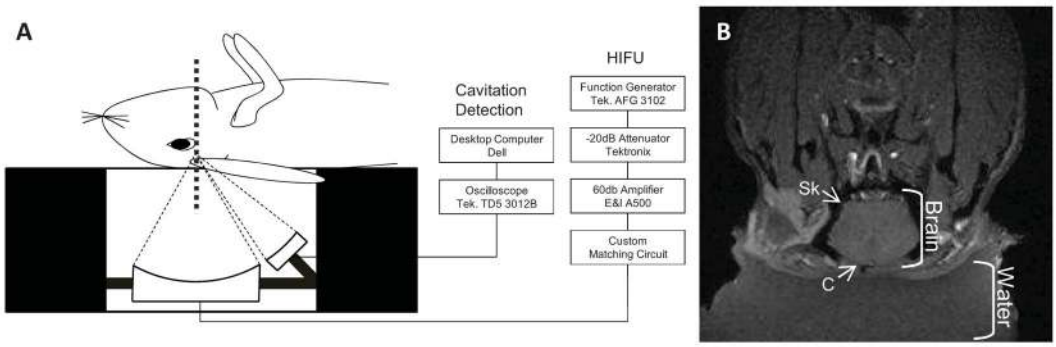

Figure 2.

In vivo schematic and coronal image of the rabbit brain, taken axially through the MR bore. The approximate MR slice plane is denoted with a dotted line in the schematic image. The water bath, physical coupling to the skin, skull (Sk), and cranial window (C) can be seen.

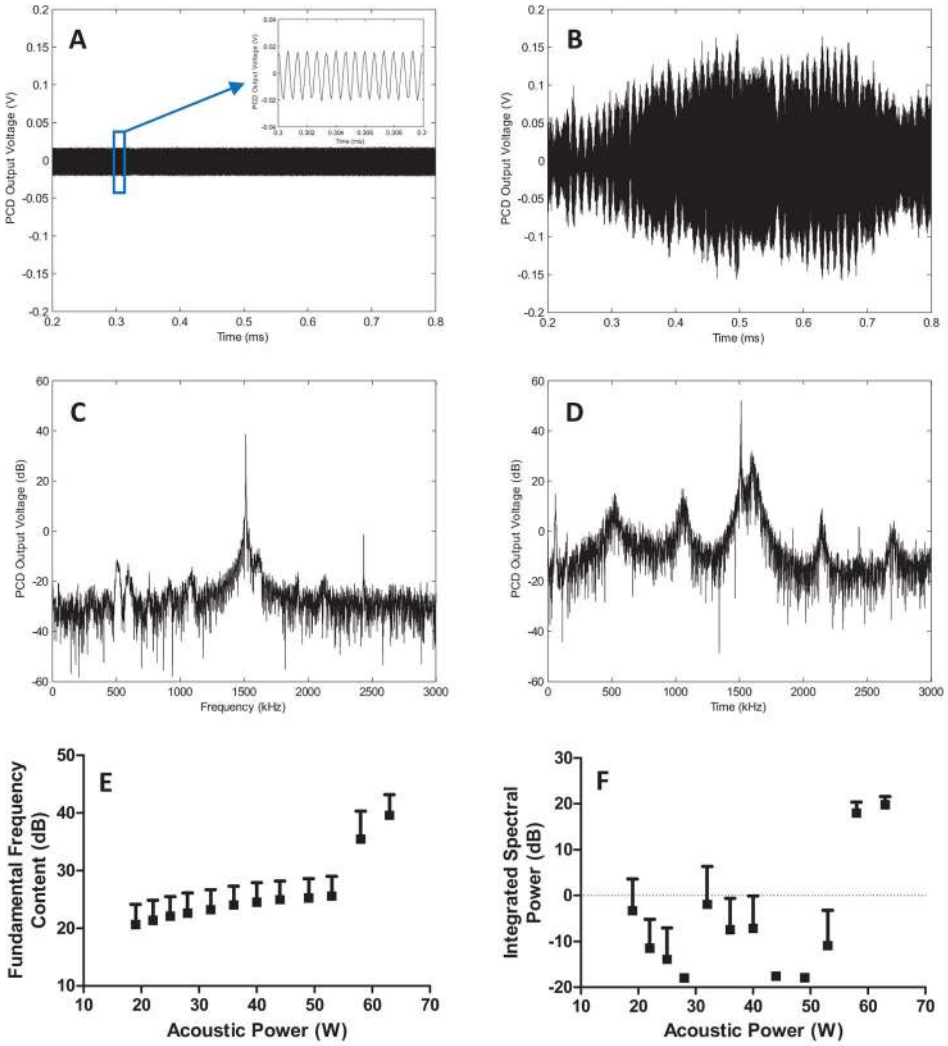

Figure 3.

PCD signals were used to identify whether inertial cavitation was occurring. A) 60W PCD signal without IC; B) 60W PCD signal with IC; C) FFT of no-IC signal from (A); D) FFT of IC signal from (B); IC threshold illustrated with E) increased fundamental and F) increased integrated spectral power (500–550 kHz). Data within this figure was taken from a single sonication and error bars represent standard deviations over multiple pulses. Apart from the fundamental peak, the spectral peaks within panel E are associated with the PCD's frequency response.

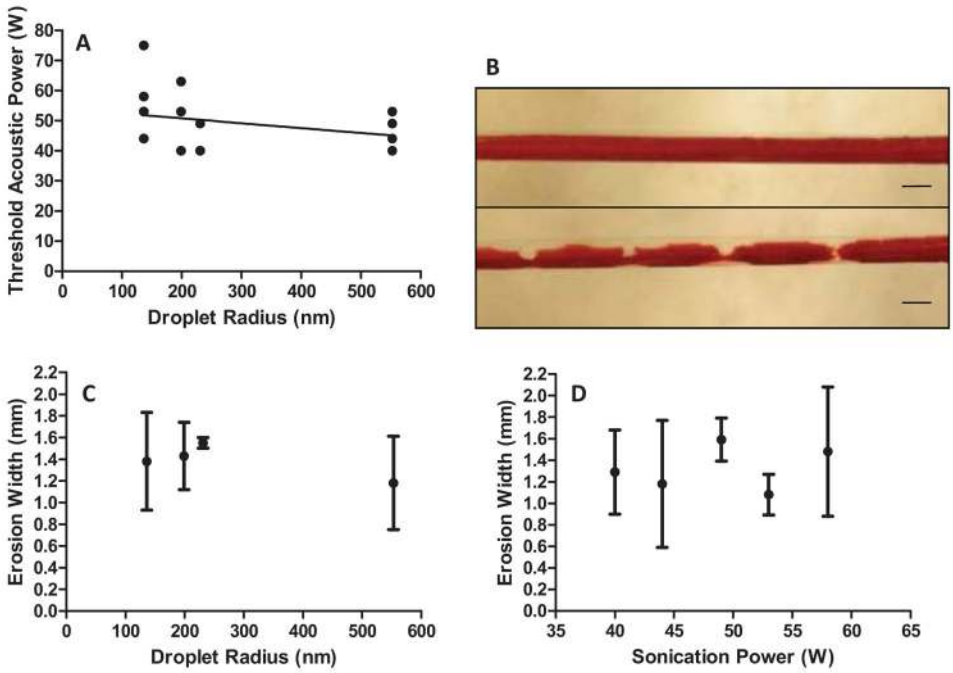

Figure 4.

A) IC-mediated clot lysis thresholds over multiple in vitro sonication experiments. Line is a linear fit between all points. B) Pre-sonication (top) and post-sonication (bottom) clots. C) In vitro post-sonication clot erosion widths vs droplet size and D) sonication power are plotted. Error bars represent standard deviation.

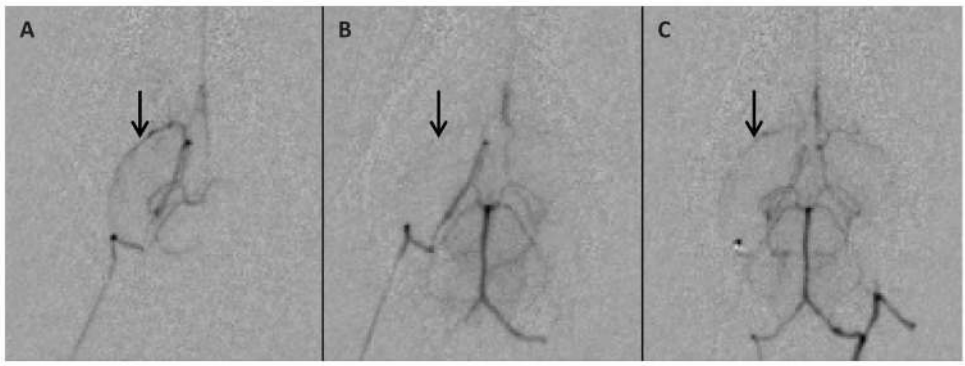

Figure 5.

Three angiograms are shown. The first was taken prior to embolus injection and shows a perfused MCA (A). The second image was taken after injection of an embolus, which blocked blood flow to the MCA (B). The last image was acquired following sonication and demonstrates recanalization (C).

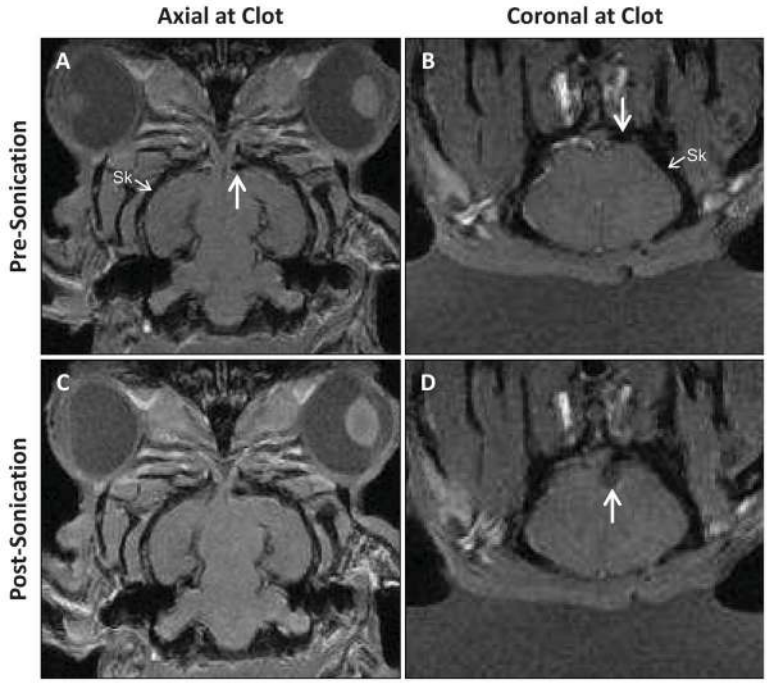

Figure 6.

MR images, from a group A rabbit, were taken A, B) prior to sonication and C, D) after sonication. A clot artifact is visible in the axial image (A) and coronal image (B), which was used to localize the sonication target. A hypo-intense region stretching from the clot into the near field is visible in the coronal image following sonication (D). The location of the skull is indicated (Sk).

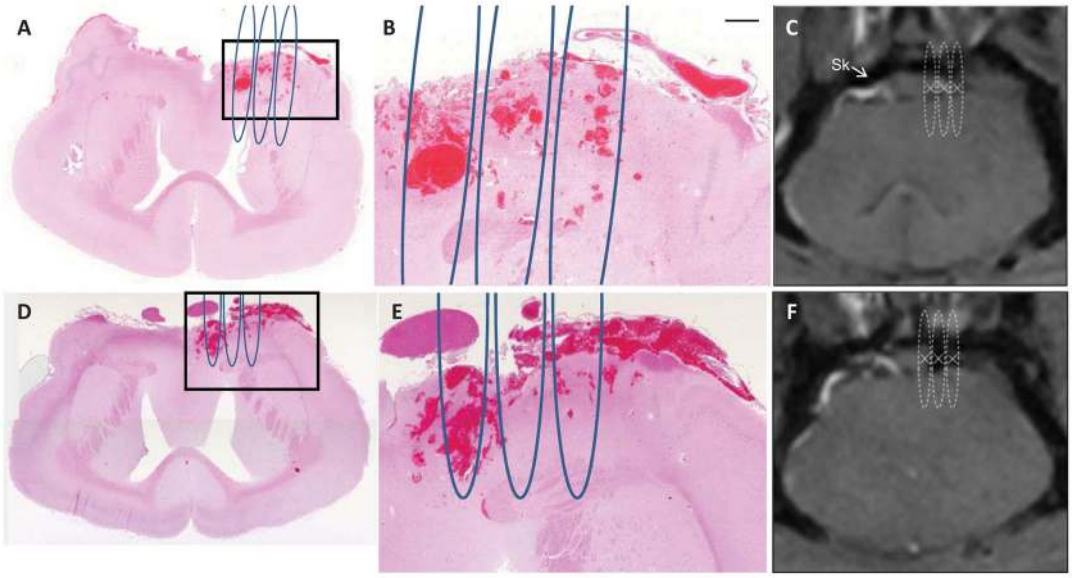

Figure 7.

Coronal views of two separate rabbits are shown, A,B,C) receiving 0.1 ms pulses and sonication farther from the skull, and D, E, F) receiving 1 ms pulses and sonication closer to the skull. A, D) Full brain and B, E) zoomed H&Es are shown. C, F) The images are parallel to the long axis of the ultrasound beam path, which would originate from the bottom of each image. Focal target centres and −6dB contours, that are 7.1 mm long, are overlaid on coronal MR images to illustrate the degree of shift and skull exposure. The location of the skull is indicated in the MR images (Sk). The approximate location of the focal contours are overlaid on the histology images as well. (Scale bar = 500 μm)

Table 1

Summary of *in vivo* experimental parameters for the treatment groups.

Cohort	Wait after droplet injection	Axial Offset of Focus from Skull	Pulse Length	Droplet Solution Dilution	Sonication Duration per Target	Number Treated[a]
A	1 min	0 mm	1 ms	1:1	20 s	2
B	No	1.5–2 mm	1 ms	1:1	20 s	7
C	No	1.5–2 mm	0.1 ms	1:5	20 s	2

[a]Number treated doesn't include 4 subjects that were excluded from the study due to an inability to cause a stroke or early death.

Table 2

Summary of *in vivo* results.

Cohort	Wait after injection	Focused placed away from skull	Pulse Length	Recanalization	P-value vs Controls
A	Yes	No	1 ms	2/2	0.067
B	No	Yes	1 ms	5/7*	0.041
C	No	Yes	0.1 ms	1/2	0.378
Ctrl	N/A	N/A	N/A	1/8	N/A

* denotes statistical significance, $p<0.05$