Blood-brain barrier opening of the default mode network in AD with MR-guided focused ultrasound

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Abstract

The blood-brain barrier (BBB) is an important obstacle for the effective delivery of therapeutics in Alzheimer’s disease (AD) and other neurodegenerative disorders. Transcranial MR-guided focused ultrasound (MRgFUS) has been shown to reversibly disrupt the BBB. However, treatment of diffuse regions across the brain along with the effect on AD relevant pathology need to be better characterized. This study is an open-labeled single-arm trial (NCT04118764) to investigate the feasibility of modulating BBB permeability in the default mode network and the impact on cognition, amyloid and tau pathology as well as BBB integrity. Nine participants (mean age 70.2±7.2 years, mean MMSE 21.9) underwent three biweekly procedures with follow-up visits up to 6 months. The BBB permeability of the bilateral hippocampi, anterior cingulate cortex, and precuneus was transiently increased without grade 3 or higher adverse events. Participants did not experience worsening trajectory of cognitive decline (ADAS-cog11, MMSE). Whole brain vertex-based analysis of the [¹⁸F]-florbetaben PET imaging demonstrated clusters of modest amyloid reduction in the right parahippocampal and inferior temporal lobe. However, CSF and blood biomarkers did not demonstrate any amelioration of AD pathology (P-tau181, Abeta42/40 ratio), nor did it show persistent BBB dysfunction (plasma PDGFRbeta and CSF-to-plasma albumin ratio). This study provides neuroimaging and fluid biomarker data to characterize the safety profile of MRgFUS BBB modulation in neurodegeneration as a potential strategy for enhanced therapeutic delivery.
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Abbreviations: AD: Alzheimer’s disease; BBB: blood-brain barrier; PET: positron-emissions tomography; NFT: neurofibrillary tangle; Ab: beta-amyloid; MRI: magnetic resonance imaging; MRgFUS: MR-guided focused ultrasound; CSF: cerebrospinal fluid; GDS: Depression Scale Short Form (GDS); NPI-Q: Neuropsychiatric Inventory Questionnaire; ADCS-ADL Alzheimer’s Disease Cooperative Study – Activity of Daily Living Scale; ADAS-Cog 11: Alzheimer’s Disease Assessment Scale-Cognitive 11-item Subscale; ADNI: Alzheimer’s Disease Neuroimaging Initiative; MMSE: Mini-Mental Status Exam; FLAIR: fluid-attenuated inversion recovery
Introduction

Alzheimer’s disease (AD) is the most common cause of dementia, with progressive, diffuse neurodegeneration leading to cognitive and functional decline. The disease is defined by the presence of two pathologic hallmarks, amyloid fibrils and neurofibrillary tangles (NFTs) of microtubule-associated tau, as well as a chronic pro-inflammatory milieu. The insoluble amyloid-beta plaque is thought to be an important inciting event in AD pathogenesis, and is distributed to networks such as the default mode network (DMN), which has selective vulnerability to amyloid accumulation. The DMN consists of the bilateral posterior cingulate, inferior parietal, medial prefrontal, ventral anterior cingulate cortices (ACC), and left inferolateral temporal, and emerges during resting state or low cognitive demand tasks on functional magnetic resonance imaging (MRI). Abeta accumulation in the DMN regions affects inter and intra-network connectivity and is associated with decline in cognition.

Targeted therapies for Abeta, such as aducanumab – a high-affinity monoclonal antibody to aggregated forms, clear plaques in a dose-dependent fashion with disease modification only at higher doses. The blood-brain barrier (BBB) prevents therapies like aducanumab from penetrating the brain (< 0.1%). There is a strong rationale to improve drug delivery in a targeted and non-invasive way. Early clinical trials in patients with AD, amyotrophic lateral sclerosis, and brain tumours show low-intensity MR-guided focused ultrasound (MRgFUS) with microbubbles can noninvasively, focally and transiently disrupt the BBB. In transgenic animals modeling AD pathologies, FUS has been combined with various antibody and gene therapies targeting amyloid and tau, resulting in enhanced histochemical and behavioral benefit. Furthermore, BBB opening alone reduced amyloid deposition, increased hippocampal neurogenesis, and improved memory deficits.

While previous studies involving patients with AD have safely targeted the frontal lobe and hippocampus, here we aim to investigate the treatment of brain networks involving multiple, diffuse regions. Furthermore, the biological effects on AD pathology and BBB function elicited by transient BBB opening in a neurodegenerative environment have yet to be elucidated in the clinical setting. We elucidate these changes through MRI, 18F-florbetaben imaging, and fluid biomarkers.
Methods

Study design

This was a single-arm, open-label study of transient MRgFUS BBB modulation in participants with mild-to-moderate AD. Informed consent was obtained from participants and their primary caregivers. Complete inclusion and exclusion criteria are listed in Supplementary Table 1. Briefly, participants were between 50 to 85 years of age with a Mini-Mental Status Exam (MMSE) score between 16 and 28, and visually rated amyloid positivity on $[^{18}\text{F}]$-florbetaben PET scan by an experienced neuroradiologist (NCT04118764).

The study protocol consisted of three biweekly MRgFUS procedures targeting the bilateral precuneus, bilateral ACC, and unilateral/bilateral hippocampi (Supplementary Table 2). Advancement from unilateral to bilateral hippocampi was made after reporting initial safety data for unilateral targeting to Health Canada. Follow-up visits after all three procedures occurred at one-week, one-month, three-months, and six-months, and consisted of clinical history, neurological exam, and psychometric testing. $[^{18}\text{F}]$-florbetaben PET and structural MRI were performed, and cerebrospinal fluid (CSF) and blood were collected, at baseline and one-week after the last procedure. Further, the final procedure for one participant and follow-up testing in five participants were either missing, delayed, or conducted remotely due to COVID-19 related restrictions.

MR-guided focused ultrasound

BBB opening was achieved using transcranial MRgFUS ExAblate Neuro 4000 220kHz system (InSightec, Israel) and preformed microbubble (ultrasound contrast agent Definity®, Lantheus Medical Imaging, USA) as described previously$^{21}$ and in Supplementary methods. Target selection and contouring was guided by anatomical labels generated through automated atlas segmentation of the subject-specific T1-weighted MRI (FreeSurfer, v6.0-Linux, https://surfer.nmr.mgh.harvard.edu)$^{22}$. The acoustic power was automatically modulated by the cavitation feedback controller based on the acoustic activity during sonications, or spectral integration around the subharmonic frequency$^{15}$. 
Clinical safety and feasibility

Safety was assessed through clinical examinations and structural MRIs, including fluid-attenuated inversion recovery (FLAIR), and T2 and T2*-weighted sequences. Adverse events (AEs) were rated as procedure related or unrelated and severity by the Common Terminology Criteria for Adverse Events (CTCAE) terminology. BBB permeability was assessed with gadobutrol contrast-enhanced T1-weighted MRI within 1-2 hours and then 18-20 hours of the sonications.

Imaging acquisition and processing

Simultaneous PET-MR scans were acquired with the $[^{18}F]$-florbetaben tracer (8 mCi ± 20%) at baseline and one week following the last MRgFUS procedure on the Biograph mMR (Siemens, Germany). FreeSurfer (v6.0-Linux, https://surfer.nmr.mgh.harvard.edu)\textsuperscript{22,23} was used to analyze both the structural and molecular imaging, where the standardized uptake value ratios (SUVR) were normalized to the pons for the latter $^{24}_{(p20)}$. Statistical testing of values before and after FUS controlled for age, apolipoprotein E (APOE) status, and PET acquisition interval, and accounted for multiple comparisons by Monte Carlo simulations and Bonferroni corrections. The workflow is illustrated in Supplementary Fig. 1 and Supplementary methods.

Psychometric testing

Cognitive performance was measured using the MMSE and the Alzheimer’s Disease Assessment Scale-Cognitive 11-item Subscale (ADAS-Cog 11). Depressive symptoms were assessed with The Geriatric Depression Scale Short Form (GDS), neuropsychiatric symptoms were assessed with Neuropsychiatric Inventory Questionnaire (NPI-Q), and functional status was assessed with the Alzheimer’s Disease Cooperative Study – Activity of Daily Living Scale (ADCS-ADL). Caregivers of the participants completed the latter two questionnaires. Under COVID-19 related restrictions, MMSE, GDS, and ADCS-ADL were administered over videoconference in one participant at month three, and four participants at month six. It was not possible to administer the ADAS-Cog remotely and therefore these scores were missing in one participant at week one, one participant at month one, and three participants at month six.

Cerebrospinal fluid and plasma measurements
APOE allele status was determined using the Spartan Cube (Spartan) DNA analyzer. Cerebrospinal fluid (CSF) and plasma were collected and stored with strict adherence to protocol detailed in Supplementary methods to prevent protein aggregation or degradation. Biomarker measurements were performed off-site and listed in Supplementary methods.

Statistical analysis

All statistical analyses of psychometric testing and biological markers were carried out in R (version 4.0.2). Outcomes of clinical safety and feasibility were reported descriptively without statistical comparisons. Cognitive and disease-specific scores were analyzed with linear mixed-effect (LME) models with the fixed effect of interest being visit, subject as a random effect to account for between-subjects variance, and covariates of age, sex, education, and APOE status. LME models were conducted using the lme4 package.

Performance on cognitive testing of subjects in the study were further statistically compared to 16 matched Alzheimer’s Disease Neuroimaging Initiative (ADNI) subjects (Supplementary Table 3), with selection criteria described in Supplementary methods. An LME model was constructed with the interaction between group and time as the fixed effect of interest, and subject as a random effect. Covariates included age, sex, years of education, time interval between the two testing sessions (in days), and APOE status.

Wilcoxon signed-rank test was used to test changes in CSF and plasma biomarkers. Further comparison of CSF biomarker changes to positive MRI findings, specifically T2* intensity changes, were reported descriptively.

Results

Study population

26 participants were screened and nine were enrolled for the study, with mean age 70 ± 7 years, five women and four men, and mean MMSE of 21.9 (Table 1). Eight of the nine participants completed all follow-up visits and assessments (up to 6 months, Supplementary Fig. 2). Twenty-six procedures were completed in total with 139 anatomical targets. In the first three
participants, the bilateral precuneus, bilateral ACC, and right hippocampus were targeted, with the addition of the left hippocampus in subsequent participants after safety analysis. Participant 4 returned for a fourth procedure due to technical difficulties with the MRI during the third procedure.

**Feasibility and Safety**

The treatment volumes were planned by the treating surgeon, in consultation with a neuroradiologist, with each contoured to the anatomical segmentation of the ACC, precuneus, and hippocampus based on subject-specific pre-procedure T1-weighted MRIs (Fig. 1A). The cumulative acoustic activity detected during each sonication was mapped and correlates with the level of microbubble response to FUS (Fig. 1B). The treated tissue volume was on average 9.2 (range 2.8 - 15.7) cm³, over 122 (range 45 to 170) minutes (Supplementary Table 2).

Increased parenchymal contrast-enhancement on T1-weighted MRIs demonstrated successfully increased BBB permeability post-sonication within all targets (Fig. 1C). One day post-treatment, resolution of the enhancement indicated restoration of the BBB permeability in all cases, however 21 of the 139 (15%) regions still demonstrated hyperintensities in adjacent sulcal regions on contrast-enhanced FLAIR sequence (Supplementary Fig. 3A). This was previously described and hypothesized to be residual leakage of gadolinium into the perivascular spaces²⁵.

T2- and T2*-weighted MRIs immediately post-sonication did not demonstrate vasogenic edema or large hemorrhage, but subtle hypointensities were detected on T2* in 6 of the 139 (4%) treated regions. These were all demonstrated in participants P4 and P5, and resolved by the next day (Supplementary Table 4, Supplementary Fig. 3B). These patterns have been previously reported and share the same morphology as sonication spots⁹.

Clinical examination did not show any new neurological deficits or SAEs related to the procedure (Table 2). Two procedures (8%) resulted in transiently increased confusion that resolved by the next morning in one participant and by day seven in another (P9). Both situations were managed non-pharmacologically at home, but for this reason, P9 was excluded from further procedures.

**Amyloid PET imaging**
Baseline and one-week following the last procedure [\(^{18}\text{F}\)]-florbetaben amyloid PET scans (2.2 ± 0.7 months interval) were available for seven of nine participants and compared for effect of BBB modulation on amyloid. With vertex-based analysis of the whole brain, [\(^{18}\text{F}\)]-florbetaben uptake was reduced after MRgFUS in two small clusters located in the right parahippocampal and inferior-temporal lobe (Fig. 1D, Supplementary Table 5, corrected \(p < 0.01\)). The parahippocampal cluster corresponded to target volumes in the right mesial temporal lobe that was consistently sonicated in every participant (Supplementary Fig. 4). No cluster showed a significant increase in PET signal from baseline to follow-up.

**Neuropsychological testing**

ADAS-Cog and MMSE scores over time are displayed in Fig. 2A,B. At the last follow-up, 6.2 ± 1.1 months from baseline, MMSE was 20.1 ± 5.3 (from 22.1 ± 3.7), and ADAS-Cog 11 was 23.8 ± 6.1 (from 20.9 ± 5.1). The change was statistically significant (MMSE \(p = 0.0004\), ADAS-Cog 11\(p = 0.0002\)) in a LME model controlling for age, sex, years of education, and \(APOE\ v4\) status (Fig. 2A,B). However, LME analysis did not reveal between-group differences with matched-control ADNI data, suggesting the cognitive measurements did not differ from an anticipated trajectory of decline (Fig. 2C,D). Further analysis showed a change over time in ADCS-ADL score (decrease, \(p = 0.0001\)), but not in GDS (\(p = 0.177\)), NPI-Q severity (\(p = 0.769\)), or NPI-Q distress (\(p = 0.523\)) scores (Supplementary Fig. 5).

**Cerebrospinal fluid and plasma**

CSF and plasma biomarkers from baseline and one week following the last procedure were compared (Supplementary Figure 6). AD pathology specific biomarkers, CSF Abeta42/40 ratio and P-tau181 did not change following MRgFUS procedures. CSF T-tau increased from 696 ± 414 to 924 ± 464 pg/mL (Wilcoxon signed-rank test, \(p < 0.05\)), but plasma Abeta42, Abeta40, and T-tau were stable (Supplementary Figure 7). Furthermore, BBB modulation did not lead to significant changes in BBB integrity biomarkers: plasma PDGFR-beta and CSF-to-plasma albumin ratio (Supplementary Figure 6G,H).

CSF NfL increased from 1509 ± 480 to 4235 ± 2857 pg/mL (Wilcoxon signed-rank test, \(p < 0.05\)), and were noted to be the greatest in the two patients with T2* changes. Plasma NfL also
increased from 17.0 ± 6.2 to 64.7 ± 61.0 pg/mL (Wilcoxon signed-rank test, p < 0.05), but demonstrated a return towards baseline at six-months in three patients: 21.3 ± 3.9 pg/mL.

**Data availability**

Raw data were generated at Sunnybrook Health Sciences Centre. Derived data supporting the findings are available within this article and its supplementary material, and is available from the corresponding author on request.

**Discussion**

In this study, we modulated BBB permeability of the DMN in patients with mild to moderate AD with no serious adverse events or long-term deleterious cognitive effects. We found modest reduction of $[^{18}F]$-florbetaben uptake within right parahippocampal and inferior temporal clusters one-week after BBB opening through an unbiased whole-brain approach, consistent with previous studies that detected modest -5%$^{26}$, -1.6%$^{11}$ reductions in the hippocampus. Finally, CSF and plasma biomarkers revealed stable AD pathology and BBB integrity, but transiently elevated NfL.

We established the feasibility of targeting whole-brain networks, which has not yet been demonstrated in previous literature. While the procedures were generally well-tolerated, a worsening of ADCS-ADL measures might be explained by the burden of multiple medical procedures posed by the study as well as the impact of COVID-19 related restrictions during the study on daily activities. We found that implementing the procedure as day surgery was beneficial through both minimizing perioperative drug administrations and having patients recover in a familiar environment. The use of fentanyl, midazolam, and propofol, common in routine surgical procedures, can provoke delirium$^{27}$. Furthermore, low-intensity MRgFUS does not elicit significant pain.

T2* hypointensities, seen in < 5% of the targeted regions immediately after BBB disruption, might be explained by extravasation of erythrocyte or other substrates$^{28}$. They are also associated with greater increases in CSF T-tau and CSF and plasma NfL concentrations, which may indicate neuroaxonal injury. However, as most of these changes were transient, some resolving within 24 hours, we hypothesize either a transient, self-limited inflammatory process or release
of these proteins in the perivascular and extracellular space as the underlying mechanism. Furthermore, in 15% of the targeted regions, extravascular enhancement on contrast-enhanced FLAIR sequences (ranging from 18 to 24 hours post-procedure) suggested partial recovery of the BBB. Their occurrences were not associated with T2* changes or APOE ε4 allele status. The dynamics of BBB restoration in human subjects appears to be different, possibly slower, than in animal subjects\textsuperscript{29}. Future studies will focus on disentangling the relationship and mechanisms behind these observations and their clinical implications.

\textsuperscript{[18]}F-florbetaben uptake was reduced in the right parahippocampus and inferior temporal lobe after rigorous statistical testing, a result consistent with animal studies and previous human data\textsuperscript{11,26}. Increased clearance of Abeta through glial activation and glymphatic clearance has been hypothesized as the underlying mechanism\textsuperscript{30,31}. However, the effect size is small, and likely accounts for a lack of positive effect on ADAS-cog or MMSE. Furthermore, no other cluster emerged in our analysis. This might be explained by variable target placement in larger brain regions leading to less sonication overlap, or intrinsic anatomical differences such as glymphatic clearance. While the dynamics of amyloid clearance after BBB opening are known through \textit{in vivo} transgenic animal studies, we do not yet know the optimal timing of the measurement in human subjects \textsuperscript{29}, and this remains an area for future investigation.

Our study had several limitations, including the small sample size and lack of control or placebo arm. Specific challenges in the analysis include the impact of tissue atrophy in AD, particularly hippocampal/parahippocampal structures, on PET imaging\textsuperscript{32}, and sensitivity of CSF and plasma biomarker measurements to location specific changes. In addition, SUVR quantification may be influenced by changes in cerebral blood flow or radiotracer clearance, and full kinetic modeling with arterial blood sampling or dual-window analyses are more desired for longitudinal investigations\textsuperscript{33,34}. The cumulative treatment volume is still a relatively small proportion of the whole brain.

Our study showed, for the first time, that large, multi-volume brain regions can be targeted with MRgFUS BBB opening, and that the procedure can be performed safely, with no serious adverse events. These results set the stage for larger trials pairing MRgFUS with promising AD therapeutics for which the BBB is an important obstacle. Intravenous immunoglobulin, for example, may be a promising and ready candidate as it has been shown to synergistically
promote neurogenesis and modulate the inflammatory milieu with FUS in animal models\textsuperscript{35}. Additionally, future studies might select patients with lower baseline amyloid burden for the purpose of disease modification, focus on elucidating the biomarker changes reported in our study, and measure tau deposition, as improved tau clearance from BBB opening has also been reported in pre-clinical models \textsuperscript{36,37}.

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**Competing interests**

NL has received honorarium for serving on an expert steering committee for the Focused Ultrasound Foundation. KH is an inventor of intellectual property related to the brain application of focused ultrasound owned by Brigham and Women’s hospital and Sunnybrook Research
Institute. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

Other authors have no potential conflicts of interest to disclose.

**Supplementary material**

Supplementary material is available at *Brain* online.


Figure legends

Figure 1. Image-guided FUS delivery resulted in transient BBB opening and $[^{18}\text{F}]-$florbetaben uptake in select regions. (A) Contouring of the sonication target volumes (green polygons) consisting of multiple spots (green dots) to the bilateral hippocampus based on anatomical segmentation of subject-specific T1-weighted MRI. (B) The acoustic emission measured during sonications, as indicated by the color maps, in the bilateral hippocampus, anterior cingulate cortex, and precuneus. (C) Increased BBB permeability in the sonicated anatomical regions detected with contrast extravasation on T1-weighted MRI subsequent to MRgFUS in the bilateral hippocampus (solid arrows), anterior cingulate cortex (long dashed arrow), and precuneus (short-dashed arrow). On the right is the demonstration of recovery of parenchymal contrast extravasation in the treated regions the next day. (D) Left: Vertex-wise analysis of the $[^{18}\text{F}]-$florbetaben PET scans obtained pre and post treatment showed two significant clusters (white) of reduced tracer uptake in the right parahippocampal gyrus and right inferior temporal gyrus (after controlled for age, APOE status, and PET acquisition interval, and correcting for multiple comparisons by Monte Carlo simulations and Bonferroni corrections; corrected p <0.01). No cluster showed significantly increased amyloid deposition. Right: Voxel-wise t-statistic results on coronal slices, highlighting the significant PET uptake changes in the right parahippocampal gyrus and inferior temporal clusters (in MNI152 volumetric template space, arrows). The parahippocampal cluster corresponds to sonicated volumes in the right mesial temporal lobe (see Suppl. Fig. 5 for anatomic correlation between MRgFUS targeting of the parahippocampus and cluster of PET uptake reduction.). Color map represent the t-statistics from the longitudinal (pre/post MRgFUS) group analysis.

Figure 2. Results of cognitive testing. Boxplots of (A) ADAS-Cog 11 and (B) MMSE performance over the course of follow-up after the last MRgFUS procedure. In ADAS-Cog 11, higher score indicates worse performance. In MMSE, higher score indicates better performance. LME models show time to be a statistically significant explanatory variable for change in ADAS-Cog 11 (p = 0.0004) and MMSE (p = 0.0002), controlling for age, sex, years of education and APOE ε4 status. (C-D) Further comparison to data from ADNI matched-control subjects do
not show statistically significant worsening in cognitive scores. The lines are the predicted scores from the LME models, with shaded areas indicating 95% confidence interval. The control subjects were matched two-to-one on age, sex, years of education, baseline MMSE scores, baseline ADAS-Cog scores, APOE ε4 status, and amyloid-PET positivity.