HHS Public Access

Author manuscript

Metabolism. Author manuscript; available in PMC 2019 May 10.

Published in final edited form as:

Metabolism. 2017 April ; 69 Suppl: S3–S7. doi:10.1016/j.metabol.2017.01.012.

New Horizons for Focused Ultrasound (FUS) – Therapeutic Applications in Neurodegenerative Diseases

Diane B. Miller[1] and **James P. O'Callaghan**[2]

[1]Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Morgantown, WV 26505.

[2]Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Morgantown, WV 26505.

Abstract

Access to the CNS and delivery of therapeutics across the blood brain barrier remains a challenge for most treatments of major neurological diseases such as Alzheimer's disease (AD) or Parkinson's disease (PD). Focused ultrasound represents a potential approach for overcoming these barriers to treating AD and PD and perhaps other neurological diseases. Ultrasound (US) is best known for its imaging capabilities of organs in the periphery, but various arrangements of the transducers producing the acoustic signal allow the energy to be precisely focused (F) within the skull. Using FUS in combination with MRI and contrast agents further enhances accuracy by providing clear information on location. Varying the acoustic power allows FUS to be used in applications ranging from imaging, stimulation of brain circuits, to ablation of tissue. In several transgenic mouse models of AD, the use of FUS with microbubbles reduces plaque load and improves cognition and suggests the need to investigate this technology for plaque removal in AD. In PD, FUS is being explored as a way to non-invasively ablate the brain areas responsible for the tremor and dyskinesia associated with the disease, but has yet to be utilized for noninvasive delivery of putative therapeutics. The FUS approach also greatly increases the range of possible CNS therapeutics as it overcomes the issues of blood brain barrier (BBB) penetration. In this review we discuss how the characteristics and various applications of FUS may advance the therapeutics available for treating or preventing neurodegenerative disorders with an emphasis on treating AD and PD.

Summary -

As we have noted, recent animal studies suggest FUS alone or in conjunction with microbubbles may provide a quick, efficient, and non-invasive way to deliver therapeutics to brain for treatment of AD and PD. Many disease modifying therapies do not effectively cross the BBB, including antibodies, genes, growth factors, stem cells, etc. However, the successful preclinical applications

Corresponding authors. D.B. Miller, Tel.: +1 304 285 5732., J.P. O'Callaghan, Tel.: +1 304 285 6079; fax: +1 304 285 6266, dum6@cdc.gov(D.B. Miller), jdo5@cdc.gov(J.P. O'Callaghan.

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"The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health"

of FUS technology suggest that its introduction in clinical settings may provide new avenues for treatment of neurodegenerative diseases.

Keywords

AD; PD; microbubbles; Aβ antibodies; electroencephalography; β-amyloid plaque; MRI; neurotrophic factors (NTF); obicodilation; sonothrombolysis; transcalvarial FUS; transcranial

Introduction -

The strikingly detailed images produced by diagnostic ultrasound (US) have made this technology and its applications familiar to physicians and the lay public alike. In this brief article we will review a modification/extension of US technology, focused ultrasound (FUS), that may find application in the treatment of Alzheimer's disease (AD) and Parkinson's disease (PD), neurodegenerative diseases that have proven resistant to current therapies. Unfortunately, to date, the available treatments for these devastating diseases are merely palliative and directed towards ameliorating symptoms (e.g., acetylcholinesterase inhibitors in AD, levodopa in PD) but do not prevent, cure, or slow disease progression [1,2]. FUS would allow precise targeting of the diseased brain areas to more effectively deliver potential therapeutics than systemic delivery methods. As most neurodegenerative diseases do not affect the brain in a global fashion, the current means of making a precise delivery to the target site (e.g. direct injection) are highly invasive and can involve a craniotomy or placement of a cannula, etc. Although other techniques used to deliver therapeutics to brain (e.g., intranasal) are not invasive, they are inefficient at precise delivery to the desired target site; high potency agents are required to offset the dilution caused by travel distance to the site and brain wide delivery remains an unwanted outcome. In contrast, FUS may be able to overcome many of the technical issues involved in direct delivery of therapeutics to a target brain area in an efficient and safe fashion [3]. Here, we highlight the relevant properties of FUS that make this technology suitable in a number of ways for treating neurodegenerative diseases such as AD and PD. Of note, others have described the beneficial properties of both US and FUS that make them suitable for neurological applications and treating a variety of CNS diseases [4–7].

US and FUS defined -

US technology may seem "magical" to those awed by the detail of its imaging capability, but in truth it is a mechanically produced pressure/sound wave of 20 kHz or greater with physical properties like that of any other sound wave. In simple terms, a transducer or piezoelectric crystal is electrically stimulated and produces an oscillation/vibration (i.e., sound wave) that moves through the transmission media. The 20 kHz wave is a higher frequency than most humans can hear; hence the label ultra. The US can be move directly through, or be refracted, absorbed or reflected by the media which can be air, water or tissue. Of most importance for therapeutic applications, these waves possess the remarkable ability to penetrate deep into various solid substances, including brain tissue. US produces biological effects in living tissue that are considered to be due to thermal or mechanical

actions and consequently there are low and high power applications of US with heating likely dominating when the power is high [8]. Low power uses include enhanced drug uptake through the skin, i.e., sonophoresis, and momentary disruption of the cell membrane to facilitate delivery of therapeutic agents, i.e., sonoporation. In FUS, an array of transducers is used to produce multiple beams of US which are focused on the target via an acoustic lens, resulting in a therapeutic effect only where the beams intersect, but not where the individual beams pass through tissue. FUS is frequently used in conjunction with real-time imaging by US or MRI to monitor the target site. This technology has been used in the treatment of uterine fibroids, kidney stones, blood clots, excessive bleeding in traumatic injury, and in cosmetic medicine for fat removal [8].

Microbubbles - Application in FUS -

These micron-scale gas bubbles are frequently used in conjunction with FUS and US. They are about the size of a red blood cell (~10 or less μm diameter) and are stabilized by a shell of albumin, lipid, protein or polymer – if not they would quickly disperse into blood once they enter the circulation. Microbubbles have seen wide usage as they can both image and treat the target and for these reasons are considered theranostic agents [9]. FUS activates the microbubbles when they pass through the focal point of the converging beams causing an expansion and contraction of the gas core. In brain, this can affect cellular structure and result in an opening of the tight junctions between the endothelial cells of the BBB at the focus site, allowing delivery of the substances comprising the shell or carried by the shell. There appear to be no long term consequences of the opening and closing of the BBB in this fashion [e.g., 10]. Both imaging and therapeutic agents have been delivered in this manner.

Barriers to effective treatment of the most common neurodegenerative disorders, AD and PD - using FUS to improve treatment -

Although, AD and PD are among the most prevalent neurodegenerative disorders, efforts at developing treatments of these diseases have been hampered by a lack of understanding of their causes, as well as a paucity of biomarkers with which to diagnose and follow disease progression in living patients. Both diseases involve the death of neurons that begins long before the classic symptoms that define each disease are seen. The protracted course of neuronal death long before symptoms occur make it difficult to prevent either disease. So does the lack of suitable biomarkers for prodromal detection and for evaluating the therapeutic efficacy of various treatments, especially in early stage disease. To date, efforts to slow or prevent either of these diseases have been unsuccessful [1, 2, 11–12].

Therapeutic efforts in AD largely have focused on β-amyloid. Aberrant turnover, metabolism and deposition of amyloid precursor protein (APP) and amyloid-β peptide, along with possible aberrant phosphorylation of tau, lead to large aggregations of these proteins and formation of amyloid plaques. Amyloid plaques are considered by many to be a risk factor and an underlying cause in the development of AD. Thus, the amyloid hypothesis [13] drives treatment strategies for AD because elimination or reduction of amyloid plaques may reduce the neurodegeneration that ultimately serves as the basis for this disease. Despite positive results from preclinical studies utilizing various mouse models of AD, effective

therapies have been harder to achieve than first expected [11]. The mouse studies demonstrated the ability of passive and active immune strategies to reduce or eliminate plaque and restore cognitive abilities. However, these immunotherapy strategies have not been as effective in humans and/or clinical trials were halted prematurely due to unexpected side-effects. The human trial failures may be due to the necessity of delivering antibodies systemically, resulting in an insufficient amount of antibody at the appropriate brain target or a failure of the aged AD patient to mount a sufficient antibody response to the administered antigen. Although Aβ antibodies cross the BBB, they do so at a very low level (~ 0.1 – 2.0% of the administered dose) making it difficult to achieve an efficacious amount of the antibody in brain which is crucial [14]. Due to this therapeutic obstacle, cost can be an issue because of the large volume of monoclonal antibodies needed for efficacy with systemic treatment. FUS offers a non-invasive and potentially inexpensive approach to achieving targeted therapeutically effective levels of Aβ immunotherapy. In an AD mouse model, FUS with microbubbles delivered [15] antibodies directly to brain at levels able to rapidly (within 4 days) reduce pathology. Also, it appears FUS with microbubbles may allow endogenous IgG and IgM Aβ antibodies to enter brain through the opened BBB as both types were found bound to plaques in treated cortex [16].

As immunological strategies for AD treatment have not yet been successful, non-immunological or pharmacological approaches to prevent, reduce or eliminate amyloid plaques are being sought. Recent studies with AD mouse models and FUS or Scanning (the focus of the beam is moved in small increments to treat a defined area) FUS, employing a limited number of exposures (~1/week for 5 – 6 weeks) in conjunction with microbubbles, show opening of the BBB allowing an unexpected entry of albumin and immunoglobulins. Significant reductions of amyloid plaques and the activation of microglia involved in plaque clearance were obtained with this non-immune approach. Furthermore, the plaque reduction was accompanied by an improvement in cognition and evidence of neurogenesis, findings suggestive of increased neuronal plasticity [16–18]. It has not been determined if the plaque reduction, microglia activation and clinical improvement can be induced by just FUS without the use of microbubbles or entry of albumin into brain. Albumin is known to activate microglia and almost all circulating Aβ is bound to it [19–20]. AD 3xTg mice develop significant amyloid beta (Aβ) pathology and direct infusion of human serum albumin (HSA) for ~ a month significantly reduced the plaque burden as well as the level of the toxic Aβ oligomers [21]. In a study with a small number of AD patients, exchange of endogenous plasma with plasma containing 5% albumin (prepared from whole human blood and available at different percentages as a therapeutic) to increase the patient's level, over a several month period, resulted in reduction of Aβ40 and Aβ42 in cervical spinal fluid (CSF). Reduced Aβ peptide levels were associated with stability in cognitive scores apparent at 1-year follow-up [22]. It is clear albumin can bind Aβ and aid in its clearance. However, direct delivery of albumin to brain in AD patients has not been tested with FUS-delivered microbubbles containing albumin, an approach that may be an effective treatment strategy for increasing Aβ clearance from brain.

PD, like AD, is characterized by a pathologic hallmark involving a misfolded/aggregated protein – namely, α-synuclein found in the extracellular space, and in Lewy bodies and neurites [14]. Alpha-synuclein also is present in CSF and plasma of PD patients. Thus, a

reduction of extracellular α-synuclein, as well as preventing its aggregation into large fibrillary clusters, are clear therapeutic strategies for various immunotherapies [23]. In animal models of PD, active immunotherapies were effective at reducing the accumulated protein in neurons, they reduce α-synuclein inclusions in substantia nigra, and they reduce the neurodegeneration caused by accumulation of the misfolded protein. Passive immunization studies also have been effective in reducing the α-synuclein pathology and associated behavioral problems. Limited clinical information is available concerning immunotherapy, with little activity being undertaken in implementing this potential therapeutic in PD. However, it would be expected that many of the problems found in developing immunotherapies for AD might be expected to occur in PD applications. Accordingly, FUS applications would be expected to reduce or mitigate the problems of PD immunotherapies in humans.

In PD, unlike AD, where immunotherapies are in the forefront, the main therapeutic focus has been on finding ways to replace or stave off the continuing degeneration of the affected dopamine (DA) neurons of the substantia nigra. Considerable research with various animal models of neurodegeneration has shown that many different neurotrophic factors (NTF)s can protect and repair damaged neurons [24–25]. DA containing neurons, in particular, appear very responsive to and dependent for survival on various NTFs, especially those of the transforming growth factor-β (TGFβ) family. This family includes glial cell-line derived neurotrophic factor (GDNF), neurturin, and growth/differentiation factor 5 (GDF5). Of note, several members of this family have been evaluated in clinical trials. Other NTFs have been identified as well that display beneficial effects in the compromised nigral striatal pathway. Due to promising preclinical work in animal models of PD, there have been a number of human trials to determine if NTFs are efficacious in the treatment of PD. There has been no successful translation of these treatments to humans. The failure in translation may be directly related to technical issues concerning delivery of the factors to the target area in sufficient quantities.

NTFs are too large to cross the BBB and are rapidly metabolized in the periphery which necessitates their direct delivery to brain or a means to generate them locally within the brain. NTFs have been delivered to or produced in selected brain sites by direct infusion, implants of stem cells genetically modified to overexpress a particular NTF, or introduction of recombinant viral vectors able to effect long-term expression in the target cells. Intranasal administration of NTFs can result in their entry into the CNS but not to specific targets. These drawbacks in current therapies with NTFs may be overcome with the application of FUS. Indeed, preclinical work has demonstrated the ability of FUS to deliver sufficient levels of neurturin to the nigral striatal area of mouse brain. FUS delivered a greater amount of the factor than direct injection and engendered the expected bioactivity of this NTF [26]. To get the same coverage of the target structure of caudate-putamen in humans would require placement of multiple cannulas. Interestingly, intranasal administration of substances in conjunction with FUS and microbubbles was able to effect targeted delivery to the caudate putamen and may be another means of delivery of neurotrophic factors [3].

Also of note for potential treatment of AD and PD are the temporary and reversible neuromodulating and neurogenic properties of FUS. These properties may be beneficial in

slowing or repairing neuronal damage in neurodegenerative disorders. Low-intensity FUS without microbubbles can alter neurotransmitter levels and stimulate or suppress brain activity in multiple areas (e.g., visual, motor, etc.), without causing apparent damage to brain. Furthermore, FUS, when applied along with microbubbles, can open the BBB and stimulate cell proliferation and neurogenesis in hippocampus [27–28]. The observed neurogenesis in this brain area may be due to increases in trophic or growth factors (BDNF, VEGF, bFG). That FUS can increase these factors may be of benefit in both AD and PD.

Possible safety issues -

The acoustic energy of FUS can be converted to thermal or mechanical energy and used as a tool for ablating brain tumors, epileptic foci, and brain areas causing essential tremor and the dyskinesia of PD [6]. Despite the potential for FUS at this level to cause damage, at lower energy levels this technology appears relatively safe. At lower acoustic energies in conjunction with microbubbles, long-term repeated exposure (4 – 20 months) causes focused opening of the BBB in the cortex or caudate-putamen area with no apparent signs of tissue damage (e.g., edema, hemorrhage) or deficits in a variety of behavioral tests [29]. It also should be noted that transcranial continuous US has been used for many years at low levels to visualize the human brain for diagnostic purposes in echoencephalography; no safety concerns have arisen despite the potential to cause elevated brain temperatures. Use of MRI for accurate targeting along with imaging of the outcome, as well as use of real-time acoustic feedback controllers, is expected to result in an improved safety profile for FUS [30]. Animal research also shows FUS is able to stimulate brain motor pathways and neurotransmitter levels, induce cortical excitation, and modulate brain region-specific activity without BBB opening [27,28]. These effects do not appear to be due to thermal or mechanical mechanisms and are suggestive of a margin of safety.

Of course, wider transition of the technology to clinical application will require more extensive testing prior to using it routinely. A more thorough examination of the possible safety issues that may occur due to repeated BBB opening, or other aspects of the technology in therapeutic applications, will need to be undertaken. Depending on the acoustic pressure and pulse applied, the BBB can remain open for minutes to hours [31]. Although FUS carries little risk of infection when used to deliver therapeutics, there may be unintended consequences. For example, unsafe levels of cavitation and subsequent heating or ablation of tissue occurring at temperatures above 55°C may lead to damage and prolonged BBB opening. This condition may promote the unwanted brain entry of cells (e.g., immune cells like macrophages, red blood cells, bacteria, viruses, etc.), proteins and peptides (albumin, chemokines, cytokines, etc.) from the periphery. Other unwanted consequences may include failure of the BBB to reclose, injury due to unexpected mechanical effects, unexpected interactions with the anesthesia needed in certain applications, or problematic properties (toxicity, microvascular effects, etc.) of microbubbles [32]. Careful attention to controlling the degree of BBB opening may mitigate some of these concerns [16].

Finally, to move forward with FUS for treatment of AD and PD will require continued development of equipment, as the shape and positioning of the transducers determines the

brain area that can be targeted. Many efficacy and safety studies have been conducted in experimental animals whose skulls are much thinner and differently shaped than that of humans. Even though some studies have involved larger animals [10, 29, 33] with thicker skulls (e.g., non-human primates and pigs), the human skull is quite variable in shape, density, and thickness. Bone is much more effective at mitigating acoustic energy than soft tissue. The characteristics of the human skull makes focusing the multiple beams and delivery of effective US levels more difficult, as the individual beams may get out of phase. Care must be taken to mitigate focal heating and reduce the chance of injury along the beam path. Thus, the thickness and shape of the skull are more of a barrier for the delivery of FUS in humans than in experimental animals. Also, BBB opening duration may depend on individual patient characteristics as well as the degree of anesthesia [29, 31]. Consequently, exposure parameters may need to be adjusted for each individual to insure delivery of the desired amount of acoustic energy.

Acknowledgments

Publication of this article was supported by the College International de Recherche Servier (CIRS)

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