The therapeutic potential of nerve growth factor combined with blood-brain barrier modulation by focused ultrasound for neurodegenerative disorders

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Nerve growth factor (NGF) is a neurotrophic factor critical for cholinergic neuronal survival, phenotypic maintenance and plasticity in the mammalian brain. NGF has been implicated in the pathogenesis of neurodegenerative disorders, with direct administration of NGF into the brain capable of facilitating neuroprotection and repair. To date, NGF has been proposed as a potential therapy for Alzheimer’s disease (AD) and Parkinson’s disease due to its regenerative effects on cholinergic neurons located in the basal forebrain and striatum, respectively. In other neurodegenerative diseases, including progressive supranuclear palsy, amyotrophic lateral sclerosis (ALS), vascular dementia, Lewy body dementia and frontotemporal lobar dementia, emerging evidence has revealed the impact of cholinergic dysfunction on clinical outcomes, thereby supporting the therapeutic potential of NGF.

In this perspective, we will review the current challenges of NGF-related therapy for clinical use and address the relevance of magnetic resonance imaging (MRI)-guided focused ultrasound (MRgFUS)-induced blood-brain barrier (BBB) permeability enhancement for the delivery of NGF-related compounds and brain repair.

Limitations of current NGF therapeutic strategies: Clinical translation of NGF-related therapies is impeded by fundamental limitations. The key biological challenges that remain to be elucidated include: (1) the impact of the relative shift in expression of the NGF cell surface receptors, tropomyosin receptor kinase A (TrkA) and p75 neurotrophin receptor (p75NTK1) with disease progression, (2) changes in the cellular responses to NGF and its biologically active precursor, proNGF, in target neurons, and (3) optimizing the delivery of NGF-related therapeutics to the central nervous system (CNS).

The nature of the interactions between proNGF/NGF and their receptors must be considered; target engagement could lead to either pro-survival and pro-regenerative effects, or pro-apoptotic and degenerative consequences (Cuello, 2019). For instance, the affinity of proNGF and NGF for TrkA and p75NTK1 and the relative proportion of each receptor bound by ligand can impact downstream signal transduction. It is also important to consider possible alterations in the expression of endogenous ligands and receptors in neurodegenerative states. Further defining ligand-receptor interactions in the context of pathology will be the key to the development of disease-specific therapeutic strategies, so as to restore neuroprotective signaling in target neurons.

The noninvasive delivery of NGF to the CNS in a safe and long-term manner continues to pose a significant challenge. Neurotrophin therapies require a delivery method that achieves sufficient and targeted levels of NGF signaling deep within the brain tissue. Native NGF does not cross the BBB and recombinant NGF protein is an undesirable pharmacological agent due to its poor in vivo stability. The BBB also restricts the entry of an estimated 98% of peripherally administered small molecules and nearly 100% of large-molecule drugs (e.g. proteins, antibodies, gene therapies) into the brain (Pardridge, 2019), including current experimental strategies to achieve NGF bioeffects in the brain; that is, viral vectors or cells for sustained delivery of NGF, NGF-related ligands that modulate NGF signaling and small molecules targeting endogenous NGF production (Cuello, 2019). Thus, a key obstacle for the development of NGF-based therapeutic agents is not only the generation of bioactive substances, but also those that circumvent the BBB.

Intravenous or intraventricular delivery of NGF has been shown to elicit adverse side effects, including pain and weight loss (Cuello, 2019). In AD clinical trials with intraventricular infusion of NGF, pain was alleviated by dextropropoxyphene, a CNS-acting analgesic, suggesting that limiting widespread NGF distribution in the CSF could reduce pain sensitization. By contrast, intraparenchymal infusion of NGF does not induce pain-related behavior. NGF-mediated weight loss is thought to involve the hypothalamus but the underlying mechanisms are not fully understood. As a result of these disabling consequences, alternative administration methods capable of achieving localized brain delivery with a minimal effective dose are needed for clinical utility. Biotherapeutics combined with drug delivery strategies to confer NGF-mediated neuroprotection are still being sought.

To date, clinical applications of NGF to the brain have typically required invasive neurosurgery (Cuello, 2019). In addition to the significant risks that are inherent to brain surgery, challenges remain to provide sufficient NGF to targeted brain regions, without damaging them or their connectivity. Localized drug delivery using intracranial needles or catheters, passing through large areas of the brain to reach the target regions, severs neuronal pathways. Additionally, substance diffusion rapidly drops off from the site of injection (Pardridge, 2019). Drug delivery by intrathecal/intraventricular routes into the CSF compartment is limited beyond the brain surface. Therapeutics can also be delivered to the brain via the olfactory route, from the nasal submucosa into the CSF surrounding olfactory nerves. Although intranasal delivery is noninvasive and easily administered, only small quantities can be transported and there is substantial variability in drug distribution within the brain between individuals (Pardridge, 2019).

MRgLUS in combination with intravenously injected microbubbles offers a minimally invasive technology to supply circulating biologics to the brain, with the advantage of being able to permeabilize the BBB in specific brain regions without widespread drug exposure in the CNS nor the need for multiple, invasive and time-consuming intraparenchymal injections to cover the entire brain region of interest (Xhima et al., 2020). MRI-guidance allows for precise target engagement and provides real-time visualization of drug distribution to the selected brain areas. Here, we will focus our discussion on MRgLUS as an emerging drug delivery platform to facilitate NGF-mediated bioeffects in the brain.

MRgLUS for the delivery of NGF-based therapeutics to the brain: Ultrasound can generate bioeffects via thermal and non-thermal mechanisms, depending on the exposure parameters. FUS is a specific ultrasound mode that can be directed to a target tissue with predictable volume, and is gaining momentum as a promising noninvasive neurosurgical approach. FUS employs spherically curved transducers that have a single geometric focus, at which most of the acoustic energy is delivered during sonication. When encapsulated microbubbles (i.e. an air/perfluorocarbon gas core stabilized by a phospholipid/protein/polymer shell) are introduced into the bloodstream prior to low intensity FUS exposure, non-thermal mechanisms induce BBB permeability in a localized and reversible manner. The acoustic response of microbubbles and their associated bioeffects on their confining vasculature depend on a number of factors, including the FUS exposure parameters (e.g. acoustic pressure, frequency, pulse sequence), microbubble characteristics (e.g. size, concentration, gas composition, shell properties), and the tissue microenvironment (e.g. blood-oxygen level, vascular density, white/gray matter; McMahon et al., 2019). Future studies would benefit from a thorough optimization of
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BBB disruption parameters to consistently prevent tissue damage, and establish the safety and tolerability of repeated dosing regimens in relation to disease state and progression.

Acoustic emissions are used to monitor the in vivo behavior of microbubbles during FUS exposure and to potentially control BBB modulation in the absence of vascular and tissue damage (McMahon et al., 2019). Methods of controlling acoustic emissions in real-time represent a major advance towards clinical applicability, and have been shown to provide safe and well-tolerated acoustic pressures in patients with AD and ALS (Meng et al., 2019; Rezai et al., 2020).

The spatial coordinates of the transducer positioning system can be co-registered to MRI, thereby allowing precise targeting of FUS to specific neuroanatomical locations. In addition, MRI contrast agents, such as gadolinium-based compounds, typically do not cross the BBB when injected into the bloodstream but following FUS, they provide a signal on T1-weighted (T1w) images to visualize BBB permeability in FUS-targeted regions. BBB permeability enhancement occurs within minutes, and is gradually restored to baseline levels within a month (Jordão et al., 2013; Kovacs et al., 2017; Xhima et al., 2018); some macrophage extravasation has also been reported with higher FUS exposure settings (Kovacs et al., 2017). It is important to note that substantial variability between treatment parameters exists, which may influence the extent of FUS-induced inflammation. It is critical that sources of variation are addressed prior to clinical translation; if the brain microenvironment is pushed too far from homeostasis, the inflammatory response can be highly detrimental.

This acute, transient immune response may promote neuroplasticity following FUS-mediated BBB opening (Burgess et al., 2014; Meng et al., 2019). In adult mice, hippocampal FUS exposure increased the number and dendritic complexity newborn neurons in the dentate gyrus (Burgess et al., 2014; Meng et al., 2019). Transient elevations in pAkt along with the downstream signaling effector pGSK3β, detected in neurons following FUS-mediated BBB modulation as early as 5 min post-sonication and declining to baseline by 24 hours (Kovacs et al., 2017), may contribute to neuronal survival post-FUS. Recently, we reported elevated NGF levels by 1.5 hours post-FUS (Xhima et al., 2020). It will be of great interest to identify the cellular source of NGF in response to FUS and its potential impact over time.

It is clear that major biomechanisms related to FUS-induced BBB permeability remain to be investigated: the precise transport routes across the BBB; underlying mechanisms of endothelial, glial and neuronal plasticity; the nature of the regenerative response in the context of neurodegeneration; the spatiotemporal pattern of neural plasticity and repair; how to fine-tune experimental parameters to mediate beneficial responses and limit safety concerns.

**FUS-mediated BBB modulation promotes regenerative processes:** FUS-BBB modulation impacts the local environment beyond endothelial cells; it also influences the plasticity of glia and neurons (Jordão et al., 2013; Burgess et al., 2014; Meng et al., 2019). An acute inflammatory response has been demonstrated following FUS-induced BBB modulation with an early (i.e. within 2 hours post-FUS) upregulation of pro-inflammatory cytokines (e.g. TNFα, IL1α, IL1β, IL6, IL18) and chemotactic factors (e.g. MCP1, G-CSF, GM-CSF, MIP3α, RANTES), lasting 12–24 hours (Kovacs et al., 2017). This response is followed by the delayed (i.e. after 12 hours) expression of anti-inflammatory cytokines (e.g. IL4, IL10, IL13) and trophic factors (e.g. BDNF, SDF1α, VEGF, EPO, GM-CSF; Kovacs et al., 2017) which could promote neuroprotective signaling. Transient astrocyte and microglia activation as well as macrophage infiltration, are thought to represent the cellular origin of these inflammatory mediators (Jordão et al., 2013; Kovacs et al., 2017); the microglial marker Iba1 is upregulated by 1 hour and decreases to baseline by 15 days (Jordão et al., 2013; Kovacs et al., 2017); the astrocytic marker GFAP is increased by 6 hours and normalized within a month (Jordão et al., 2013; Kovacs et al., 2017; Xhima et al., 2018); some macrophage extravasation has also been reported with higher FUS exposure settings (Kovacs et al., 2017).

**Conclusion and future perspective:** Looking forward, FUS-mediated BBB permeability is a promising drug delivery modality for the treatment of neurodegenerative diseases. MRigFUS offers a platform to monitor BBB permeability enhancement in real-time and improve the targeting and spread of NGF-related therapeutics in the brain parenchyma. Neurosurgical risks and complications, that are of concern particularly in elderly patients, may also be avoided using transcranial MRigFUS. Indeed, the feasibility and safety of MRigFUS has been demonstrated in patients affected by AD and ALS (Meng et al., 2019; Rezai et al., 2020) and therefore, may potentially to be combined with NGF-related compounds for brain repair in future clinical trials. Potential NGF-related therapeutic include small molecules, antibodies, as well as cell and gene transfer to the brain for the production of modulators to both ligands and receptors related to NGF (Figure 1).

Future efforts to limit potential adverse effects associated with peripheral administration of NGF-based therapeutics and transient FUS-induced BBB permeability are needed to bridge the translational gap. For instance, to mitigate potential side effects associated with systemic delivery of neurotrophic agents, NGF-related ligands could be delivered intranasally and coupled with MRigFUS Targeted to the basal forebrain or cholinergic projection regions of the cortex and hippocampus. Gene therapy vectors expressing NGF-associated ligands (Castle et al., 2020) with cell-specific promotors could also represent an attractive option. These therapeutic strategies that can benefit from FUS delivery and avoid systemic side effects are proposed for AD and other neurological conditions (Meng et al., 2019). Technological advances have led to FUS applications for brief, reversible BBB permeability enhancement without deleterious complications such as hemorrhage, ischemia, structural abnormalities or behavioral changes (Meng et al., 2019). Nevertheless, the perturbation in BBB integrity following...
Figure 1 | Therapeutic agents to achieve NGF-related bioactivity in the brain, coupled with transcranial FUS for noninvasive, targeted and real-time monitoring of compound delivery.

FUS can be used to enhance bioavailability of NGF and NGF-related agents, administered either intrasurally or intravenously, in the brain. Potential NGF-based therapies include: antibodies or small-molecule mimetics targeting NGF or its cognate receptors, TrKA and/or p75

\*\* nanocarriers loaded with NGF or NGF analogs/receptor ligands; cells genetically modified to secrete NGF or NGF analogs/receptor ligands (ex vivo gene therapy); viral or non-viral vectors carrying the gene for NGF or NGF analogs/receptor ligands (in vivo gene therapy); drugs that augment endogenous NGF expression. FUS-mediated BBB modulation alone, in the absence of drug delivery, can also increase NGF levels in targeted brain areas. FUS: Focused ultrasound; NGF: nerve growth factor; NGF\*: NGF/NGF analog/NGF receptor ligand; p75

\*\*: p75 neurotrophin receptor, TrKA: tropomyosin receptor kinase A.

FUS leads to a transient inflammatory response which is dependent on the exposure parameters (Jordão et al., 2013; Kovacs et al., 2017; McMahon et al., 2019). Thus, investigation into the safety profile of FUS-mediated BBB modulation including the optimal exposure parameters, real-time control of BBB permeation, an appropriate treatment endpoint, patient-specific clinical devices and patient heterogeneity in response to therapy remain important questions for clinical safety and efficacy. For neurodegenerative diseases in particular, BBB dysfunction and cerebrovascular pathology are common and evidence supports their association with poor cognitive outcomes in experimental models and patients (Meng et al., 2019). The extent to which the FUS-induced inflammatory response can resolve in these conditions, and affect long-term BBB integrity and secondary neuronal injury remain open questions. Similarly, it will be essential to study whether repeated FUS-mediated BBB modulation, for chronic drug delivery, is well-tolerated long-term. To this end, non-viral or viral gene vectors or drug-loaded nanocarriers that require infrequent dosage or even a single administration would be particularly advantageous when coupled with FUS for the treatment of neurodegenerative diseases.

It is also possible that secondary mechanisms as a result of BBB permeability enhancement could be harnessed therapeutically, including modulation of the brain’s immune response to promote neuroprotection. Deeper insight into these processes, and their dependence on sonication parameters, will be required to safely develop clinical applications of FUS-induced BBB permeabilization beyond drug delivery. FUS, either as a disease-modifying platform or combined with therapeutics to facilitate their delivery to the CNS, is a powerful technology that can be developed to promote neuroprotective and regenerative mechanisms to repair the diseased brain.

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