REVIEW

Blood–Brain Barrier Opening With Focused Ultrasound in Experimental Models of Parkinson's Disease

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ABSTRACT: Parkinson's disease has many symptomatic treatments, but there is no neuroprotective therapy currently available. The evolution of this disease is inexorably progressive, and halting or stopping the neurodegenerative process is a major unmet need. Parkinson's disease motor features at onset are typically limited to 1 body segment, that is, focal signs, and the nigrostriatal degeneration is highly asymmetrical and mainly present in the caudal putamen. Thus, clinically and neurobiologically the process is fairly limited early in its evolution. Tentatively, this would allow the possibility of intervening to halt neurodegeneration at the most vulnerable site. The recent use of new technologies such as focused ultrasound provides interesting prospects. In particular, the possibility of transiently opening the blood–brain barrier to facilitate penetrance of putative neuroprotective agents is a highly attractive approach that could be readily applied to Parkinson's disease. However, because there are currently effective treatments available (ie, dopaminergic pharmacological therapy), more experimental evidence is needed to construct a feasible and practical therapeutic approach to be tested early in the evolution of Parkinson's disease patients. In this review, we provide the current evidence for the application of blood–brain barrier opening in experimental models of Parkinson's disease and discuss its potential clinical applicability. © 2019 International Parkinson and Movement Disorder Society

Key Words: alpha-synuclein; blood–brain barrier; focused ultrasound; Parkinson's disease; therapy

Early treatment for Parkinson's disease (PD) remains a challenge. PD has several symptomatic treatments, but there is no neuroprotective therapy currently available. We still do not know its etiology, and we cannot stop the progression of the disease.[1] At onset, there are very few regions that have obvious neuronal loss, and motor symptoms are typically highly asymmetrical and limited to 1 limb.[2] In fact, it is clear that PD motor features are closely associated with the degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SNc), especially those in the ventrolateral part, and with the loss of dopaminergic terminals predominantly in the posterior putamen.[2] The process thus seems to be fairly limited clinically and anatomically in the evolution of the disease.

The other pathological hallmark of PD is the presence of proteinaceous inclusions that are rich in fibrillary forms of alpha-synuclein (α-syn), commonly named Lewy pathology (LP).[3] LP is observed more widely in the brain but to variable degrees. However, it is commonly accepted that LP generally occurs in selected nuclei, including the olfactory bulb, dorsal motor nucleus of the vagus nerve, locus coeruleus, SNc, raphe nuclei, amygdala, or cortex, leaving many other brain regions

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unaffected.[2] Therefore, developing therapeutic strategies that aim to delay the clinical onset of PD in the most vulnerable regions of the brain looks reasonable.

Unfortunately, a number of difficulties, particularly the inaccessibility of the degenerating brain, have made this impossible so far. In this regard, new technologies such as focused ultrasound (FUS) provide an interesting opportunity. In particular, the possibility of transiently opening the blood–brain barrier (BBB) to facilitate the penetrance of putative neuroprotective agents is a highly attractive approach that could be applied readily to PD and other neurodegenerative diseases.[4] Gene delivery is a clear example of a promising therapeutic modality that would benefit.[5,6] However, because there are currently effective treatments for PD patients available (ie, dopaminergic pharmacological therapy) more experimental evidence is needed to construct a feasible and practical therapeutic approach to be tested in the early symptomatic phase of PD patients. In this review, we provide current evidence of the application of the BBB opening for the delivery of therapeutic agents to the brain in experimental models of PD and discuss its potential clinical applicability in the future.

The BBB

The BBB is a term used to describe the unique properties of the microvasculature of the central nervous system (CNS). Blood vessels are made up of 2 main cell types: endothelial cells that form the walls of the blood vessels and mural cells that sit on the ablumenal surface of the endothelial cell layer. Interactions with mural cells, immune cells, astrocytes, pericites, and neural cells in the capillary basement membrane maintain the intact properties of the BBB.[7]

Importantly, the BBB prevents the brain from the entry of neurotoxic plasma components, blood cells, and pathogens in normal conditions. The BBB also serves to regulate the transport of different molecules through the CNS, maintaining control of the environment and homeostasis of the brain required for correct neuronal functioning. However, at the same time the BBB represents an obstacle for drug delivery to the CNS, and thus major efforts have been made to generate methods to bypass the BBB for the delivery of therapeutic compounds and drugs.[8]

The BBB Opening With FUS

The treatment of CNS diseases involves the synergistic action of the BBB to transport therapeutic agents. The BBB hinders the transcellular diffusion path, which is confined only to lipid soluble compounds smaller than 400 Da with fewer than 9 hydrogen bonds crossing via lipid-mediated transport. To overcome this obstacle, the current treatment strategies involve transcranial injection or infusion and the employment of medicinal chemistry to chemically alter the nature of the compound so it can cross the BBB through carrier-mediated, receptor-mediated, or active efflux transport.[9] However, all of these methods are either invasive, nontargeted, and/or involve the alteration of the drug composition. Direct injection, convection-enhanced delivery, and osmotic BBB disruption are some examples of targeted but invasive techniques, whereas biological and chemical approaches and intranasal drug delivery are noninvasive but nontargeted methods.[10,11]

FUS technology has emerged as a promising alternative in delivering pharmacological agents into the brain by overcoming the impermeable BBB and concurrently the adverse events associated with excessive drug administration. FUS coupled with the administration of microbubbles has been proposed as the only noninvasive technique to transiently, locally, and reversibly disrupt the BBB, allowing a temporal and spatial window for molecules to cross to the brain parenchyma[12,13] (Fig. 1).

The microbubbles described herein are perfluorocarbon-filled, lipid-coated microspheres on the order of a few microns in diameter and characterized by slow solubility and dissolution kinetics attributed to their shell composition.[14] Their stability in the blood vessels has been improved by increasing the hydrocarbon chain length of the coat-constituent lipids, improving their physicochemical properties and their overall efficiency.[14-17]

Ultrasonic energy focused at the geometrical center can be tightly deposited deeply within the brain tissue while minimizing skull energy absorption.[18] During FUS, application of the transmitted acoustic pulse generates a radiation force that drives the expansion and contraction (or collapse) of the microbubbles,[14] characterized as acoustic cavitation. Controlled oscillation of the microbubbles results in increased vascular permeability, whereas rapture of the bubbles[19] has been associated with the increased risk for damaging the surrounding microenvironment[20,21] (Fig. 1).

Hence, the size-dependent resonance behavior of the microbubbles and their response to the acoustic field varies according primarily to the center frequency and the applied pressure and the pulse length.[14] The relatively low frequencies combined with various pressures have been shown to successfully induce BBB openings of different sizes depending on the weight of the deliverable agent.[22] The effect of the ultrasound parameters on the vascular permeability is linearly dependent on the microbubble concentration, whereas the functional outcomes are more predictable for narrower size distributions of microbubbles.[23]

The closing timeline and the reversibility of the BBB opening have been extensively investigated to assess the safety profile of the intervention. The time necessary for the barrier to be fully restored has been found proportionally

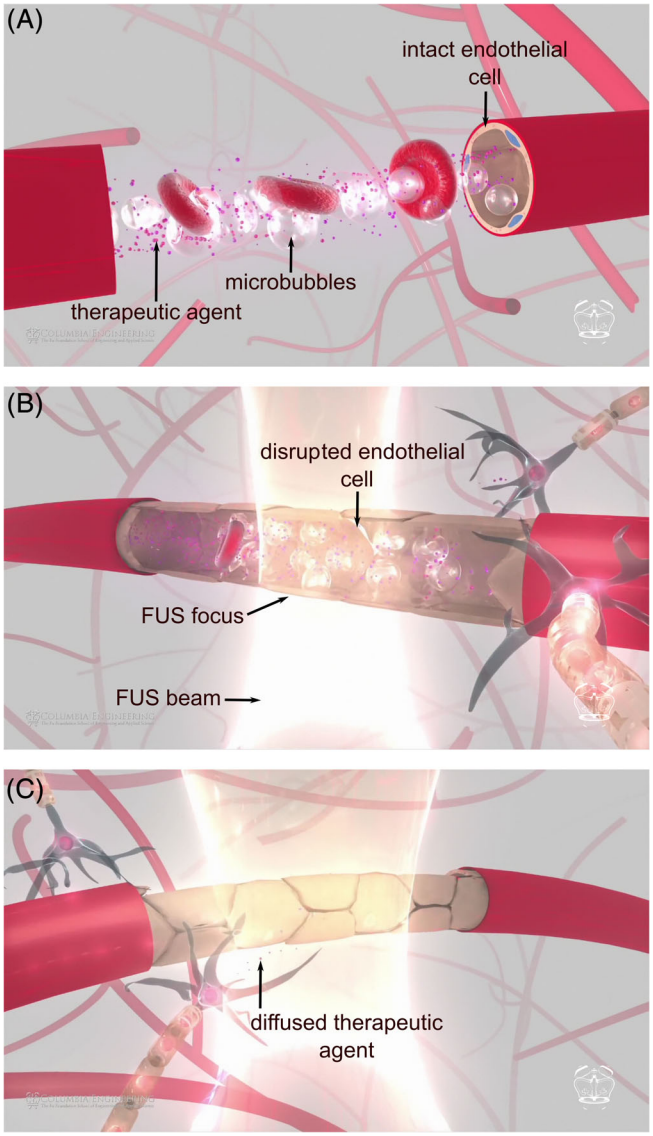

FIG. 1. Mechanism of blood–brain barrier opening disruption. (A) The microbubbles (white) and the therapeutic agent (purple) follow the circulation after intravenous injection. (B) The microbubbles oscillate when reaching the focus of the beam that exerts mechanical forces to the endothelial cells and loosens the tight junctions. (C) The therapeutic agent diffuses through the disrupted barrier into the brain parenchyma. FUS, focused ultrasound. [Color figure can be viewed at wileyonlinelibrary.com]

related to the opening volume assuming the induction of a single opening.[24] The decoupling of this dependence has been achieved by substituting large openings with small multifoci openings decreasing the necessary time for the barrier to be restored.[25,26] Longitudinal studies on rodents and primates have shown that repeated ultrasound-induced BBB opening in the absence of vascular damage is a transient and reversible application.[27,28] Restoration of the barrier was not only macroscopically evaluated by magnetic resonance imaging but also neurologically by visual, cognitive, motivational, and motor function behavioral testing.[27,28]

Although the interaction of systemically administered microbubbles with the capillary walls has been proposed to drive the disruption of the BBB with FUS, the mechanism is not entirely clear as the downstream bioeffects are not fully understood. Disassembling the tight junctional molecular structure has been placed at the beginning of the induced biological cascade, explaining the paracellular passage of molecules that has been reported.[29,30] Moreover, transcriptomic analysis in the acute stages following sonication revealed a transient upregulation in proinflammatory cytokines and chemokines including chemokine (C-C motif) ligand 2, chemokine (C-C motif) ligand 3, and Tumor necrosis factor that have been found to promote the migration, proliferation, differentiation, and survival of neural progenitor cells favoring neurogenesis.[31] The prominent presence of Bromodeoxyuridine-positive cells in animals that survived for a week after the last of 6 sonications has been linked to enhanced neurogenesis attributed to the expression of trophic factors such as brain-derived neurotrophic (BDNF) in the targeted brain.[32] Concurrent with the overexpression of inflammatory markers that mostly resolved within 24 hours was the increase in angiogenetic-related genes and astrocytic activation.[31]

Despite the positive impact of the technique, reports on elevated microtubule-associated protein tau, phosphorylated at the Phospho-Tau (Thr231) Monoclonal Antibody epitope[32] and activated nuclear factor kappa-light-chain-enhancer of activated Bcells (Nf-κB) pathway[33] following repeated sonications, alarmed the ultrasound field and surfaced important unmet needs. Although the uncontrolled phosphorylation of the tau protein has been linked to Alzheimer's disease, phosphorylation has to occur at specific epitopes and be proven to lead to pathological outcomes.[34] Tau phosphorylation at the Thr231 epitope is associated with both physiological and pathological processes,[34] and further experimentation is required to assess the association with Alzheimer's disease as the upregulation alone does not suffice. Regarding the initiation of the Nf-κB pathway, several reports argue on whether it is a byproduct of the sonication regime[33] or it is completely dissociated from the intervention.[31] Contradictory findings and ambiguous interpretations signify the need to fully characterize the biological changes specific to the selected sonication protocol.

So far, FUS has been studied extensively in a multitude of experiments involving the safe disruption of the BBB of various animal species (including rabbits,[35,36] mice,[37] rats,[38] and primates[39,40]) and in different PD animal models (Table 1). The integrity of the BBB is restored within hours, and it remains intact[24] depending on the ultrasound parameters, regardless of the pathological state of the brain at least for the early stages.[41] An FUS-mediated BBB opening has been proven indifferent in terms of the energy requirements to achieve permeability and the closing timeline between transgenic and wild-type mice.[41]

TABLE 1. Summary of studies undertaking blood–brain barrier opening in animal models of Parkinson's disease

Animal model	Deliverable	Delivery vehicle	Delivery method	FUS applications	Staining	Behavioral	Reference
MPTP mice	GDNF	AAV	IV	1	Yes	Yes	Karakatsani et al 2019[47]
MPTP mice	GDNF	Gene-liposome- microbubbles	IV	1	Yes	Yes	Lin et al 2016[48]
6-OHDA rats	GDNF	Brain-penetrating nanoparticles	IV	1	Yes	Yes	Mead et al 2017[49]
6-OHDA rats	GDNF	Cationic microbubbles	IV	1	Yes	Yes	Fan et al 2017[5]
Wild-type mice	NTN	Direct	IV	1	Yes	No	Samiotaki et al 2015[25]
MPTP mice	NTN	Direct	IV	1-3	Yes	No	Karakatsani et al 2019[47]
Wild-type mice	BDNF	Direct	IN	1	Yes	No	Chen et al 2016[43]
MPTP mice	BDNF	Direct	IN	3	Yes	Yes	Ji et al 2018[58]
A53T α-syn mice	Anti α-syn antibody	Direct	IV	3	Yes	No	Zhang et al 2018[65]
Wild-type α-syn mice	α-syn shRNA	AAV	IV	1	Yes	No	Xhima et al 2018[66]

FUS, focused ultrasound; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-hydroxydopamine; A53T, transgenic mice overexpress human α-synuclein with a PD-associated mutation; α-syn, alpha-synuclein; GDNF, glial-derived neurotrophic factor; NTN, neurturin; BDNF, brain-derived neurotrophic factor; shRNA, short hairpin RNA or small hairpin RNA; AAV, adeno-associated virus; IV, intravenous.

This intervention has shown efficacy in delivering various compounds of different molecular weights into the brain parenchyma, including contrast agents,[42] sugars,[43] antibodies,[44] chemotherapeutics,[45] and neurotrophic factors.[6,25,43,46-49] Aside from the direct delivery of the pharmacological agent, FUS-facilitated viral and nonviral vector-based gene delivery has been proven feasible to promote the long-term expression of endogenous proteins.[6,46,49]

An FUS-induced BBB opening is an innovative and noninvasive approach to achieve drug delivery within the CNS by providing significant advantages when compared with other approached in terms of targeting, noninvasiveness, and reversibility. The recent advances in technical optimization and preclinical validation support the immense potential of the intervention as the drug-delivery technique of choice in neurodegeneration models.[18]

FUS-Induced BBB Opening in Experimental Models of PD

When considering opening the BBB in experimental models of PD, we have to consider (1) the animal model of choice and (2) what we are going to deliver. One obvious option is to target the dopaminergic system, preferentially at the level of the SNc or striatum. So far, the toxin-based models (1-methyl-4-phenyl-1,-2,3,6-tetrahydropyridine [MPTP] and 6-OHDA) are the best choices for those studies designed to test neuroprotection because there is clear dopaminergic neuronal loss in the SNc and dopamine loss in the striatum.[50] Looking ahead, the other therapeutic option to consider in PD patients is to modulate α-syn aggregation in the brain. Unfortunately, these models do not recapitulate the α-syn aggregation observed clinically in PD patients.[50] In this case, we have the following different options: (1) intraparenchymal inoculations of exogenous α-syn (eg, synthetic α-syn fibrils), (2) transgenic mice, and (3) animals in which α-syn overexpression is induced by viral vector injections.[51]

Targeting the Dopaminergic System: Trophic Factors

Despite highly positive evidence from preclinical studies,[52] clinical trials of PD testing the delivery of different neurotrophic factors have been largely ineffective for several reasons, including dosage, poor distribution in the brain, poor retrograde transport, and late time points of delivery.[53] FUS can partially address some of these issues primarily by improving the distribution of the deliverable molecule in the targeted location[43,54] (Fig. 2) and thus adjusting the dosage to balance sufficient deposition and saturation, rendering trophic factors a possible alternative for PD treatment.[55]

Different studies have tried to restore the integrity of the dopaminergic system by a combination of FUS with trophic factors in combination with different particles or vectors. Normally, this approach is thought to be either neuroprotective, delaying the death of the dopaminergic neurons in the SNc, or restorative, restoring or improving the capacity of the brain to produce dopamine. Glial-derived neurotrophic factor (GDNF) is usually the first choice, but neurturin (NTN) and BDNF have also been tested.

Following the initial feasibility study, Karakatsani and colleagues[47] delivered adeno-associated virus (AAV)–GDNF (AAV1-CAG-eGFP-GDNF) in the left striatum and midbrain of subacute MPTP-treated mice. Dopaminergic neuronal cell bodies demonstrated a 58.4% upregulation with more than a twofold increase in their projections' density after the administration of AAV-GDNF and its diffusion through the FUS-induced BBB opening, as evidenced by tyrosine hydroxylase (TH) immunostaining. Similar upregulation was observed in the striatum where the evidenced upregulation of the terminal density reached

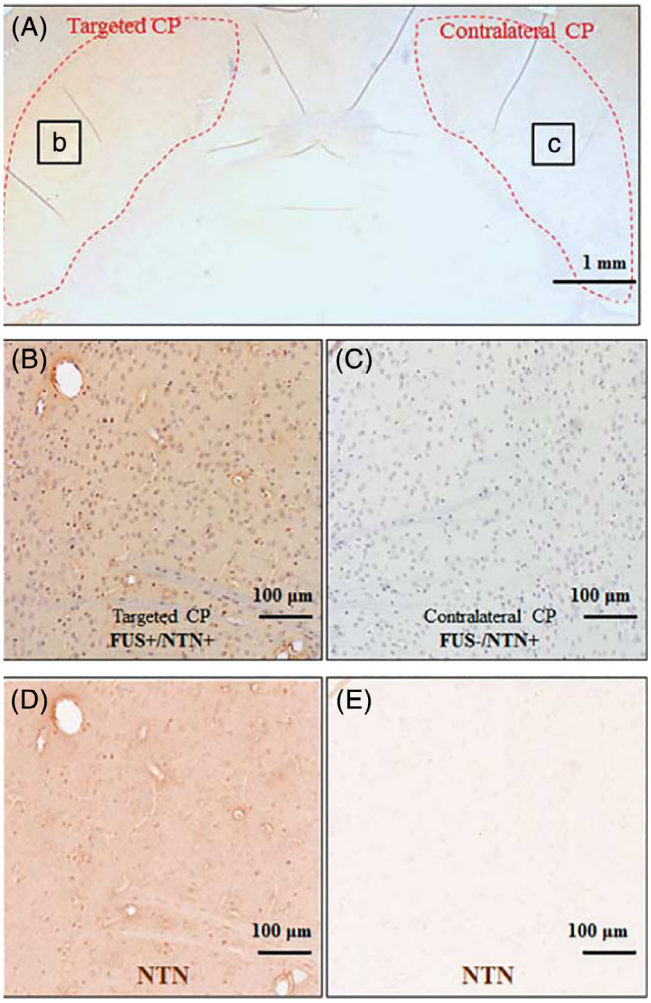

FIG. 2. Figure adjusted from Samiotaki and colleagues.[47] Horizontal section at the striatum, outlined by the red dotted lines, immunostained against neurturin (NTN; brown color), and counterstained with hematoxylin (purple color). (A) The sonicated striatum developed higher intensity of the anti-NTN antibody, suggesting higher concentrations of the trophic factor compared with the contralateral side. (B,C) Higher magnification at the sonicated and contralateral side, respectively. (D,E) Extraction of the brown color only corresponding to NTN for b and c, respectively. CP, Caudate-Putamen; FUS, focused ultrasound. [Color figure can be viewed at wileyonlinelibrary.com]

30%. Dopamine-related behavioral changes demonstrated by amphetamine-elicited unilateral rotations confirmed the physiological advances of the FUS-facilitated viral delivery (Fig. 3).

Similarly, Lin and colleagues[48] delivered gene-carrying liposomes in combination with FUS to improve the GDNF gene-delivery efficiency in the MPTP-mice model.[48] The FUS-induced BBB opening was verified by contrast-enhanced magnetic resonance imaging, and gene expression was verified by in vivo imaging. The focal delivery of gene-liposome complexes successfully served as gene carrier and BBB-opening catalyst. Immunoblotting and histological staining confirmed the expression of reporter genes in neuronal cells leading to reduced expression and progression of motor abnormalities. Postmortem analysis confirmed preserved dopaminergic metabolism associated with the improvement of motor abnormalities.

In another study, Mead and colleagues[49] used FUS in combination with brain-penetrating nanoparticles to induce widespread and focal GDNF transgene expression in the brain following systemic administration in 6-OHDA-treated rats. After only a single treatment, this strategy led to therapeutically relevant levels of GDNF protein content in the striatum that lasted for at least 10 weeks. This strategy restored dopamine levels at the striatum and dopaminergic TH neuron density in the SNc and reversed behavioral abnormalities, with no evidence of local or systemic toxicity.

Fan and colleagues[5] loaded cationic microbubbles with GDNF, and FUS was used to allow transient gene permeation and induce local GDNF expression. In this study, FUS made it possible to achieve higher titer GDNF genes than with intracerebral injections. The combination of GDNF-loaded microbubbles and FUS resulted in restored behavioral motor deficits and ameliorated neuronal death in the SNc and dopamine loss in the striatum of 6-OHDA-trated rats. In a similar approach, the delivery of GDNF alone or in combination with nuclear receptor-related factor1 with polyethylene glycolylated liposomes–coupled microbubbles using FUS alleviated the behavioral deficits and neuron loss in the 6-OHDA rats.[56,57] The multistep process involved in gene transfection requires successful delivery, translation, and release followed by receptor ligation. It is therefore subjected to limited efficacy, shifting the scientific interest toward direct protein delivery.

Samiotaki and colleagues[25] demonstrated enhanced noninvasive local FUS delivery of NTN in wild-type mice at the level of the striatum and midbrain, confirmed by immunostaining (Fig. 2). Briefly, the area of NTN bioavailability was 5.07 ± 0.64 mm^2 in the striatum and 2.25 ± 1.14 mm^2 in the midbrain, with NTN present across the entire ultrasound-treated brain region in contrast to the relatively smaller region reached by direct injection (Fig. 2). Furthermore, NTN bioactivity was evaluated by tracing the local activation of the downstream signaling pathway through the detection of increased phosphorylation of the Rearranged during Transfection receptor, cytoplasmic kinase extracellular signal–regulated kinase 1 and 2, and cAMP response element-binding protein transcription factor in structures associated with their abundance. This finding was particularly significant because the nigrostriatal pathway, which connects the ventral midbrain region with the striatum, is the most severely affected dopaminergic pathway in PD.[43]

To determine the potential value of using FUS to improve the brain penetrance of bioactive molecules of the compromised neurons in the neurodegenerated brain, Karakatsani and colleagues[47] compared TH-based parameters between hemispheres of the MPTP-injected mice that received single or triple systemic NTN injections coupled

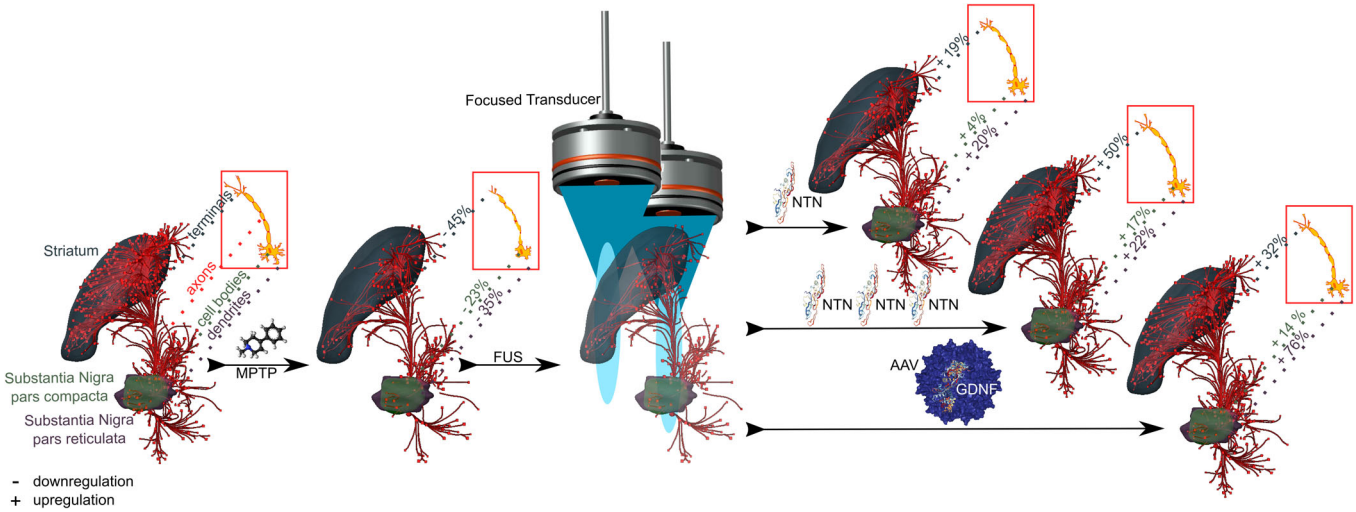

FIG. 3. Figure adjusted from Karakatsani and colleagues.[47] The nigrostriatal pathway includes the substantia nigra (SN), where the dopaminergic neuronal cells (SNc) and dendrites (SNr) lie, and the striatum, where the neuronal terminals can be found. The nigrostriatal pathway is downregulated upon 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induction in a "dying back" regime from the terminals to the cell bodies. The application of focused ultrasound coupled with the administration of microbubbles results in increased blood–brain barrier permeability, allowing the diffusion of neurotrophic factors. Single administration of neurturin (NTN) resulted in a 19% increase in the tyrosine hydroxylase expression at the striatal site and 4% and 20% in the SNc and SNr sites, respectively. Triple administration of NTN with the equivalent number of FUS applications resulted in an increased effect compared to the single treatment on the order of 50%, 17%, and 22% for the striatum, SNc, and SNr, respectively. The diffusion of AAV-GDNF resulted in a similar triple treatment effect on the order of 32%, 14%, and 76% for the corresponding structures. AAV, adeno-associated virus; FUS, focused ultrasound; GDNF, glial-derived neurotrophic factor. [Color figure can be viewed at wileyonlinelibrary.com]

with the equivalent number of FUS applications (Fig. 3). Upregulation of TH expression in the midbrain was initiated by both single and triple administrations, hence the 20% to 22% denser dendritic network, with only multiple exposures achieving restoration of neurotransmission, was evidenced by a 50% increase in the immunoreactivity of the innervating dopaminergic neurons at the striatal level. Significantly increased dopamine levels in the treated ventral midbrain were confirmed with high-performance liquid chromatography analysis, strengthening the relevance of the two pharmacological agents in achieving functional outcomes.[47]

Another promising, noninvasive drug-delivery approach that has been developed and evaluated in animal models and clinical trials is intranasal delivery, which circumvents the impermeable BBB by employing the olfactory epithelium to reach the CNS.[11] Low delivery specificity, the major drawback of this method, was resolved by coupling it with FUS. Chen and colleagues[43] administered BDNF to wild-type mice through the nostrils, before exposing their left striatum to ultrasound.[43] Immunohistochemical findings revealed the increased bioavailability of BDNF in the exposed striatum compared with the contralateral side, showing its immense potential as a surrogate for intravenous delivery especially when systemic exposure to pharmacological agents needs to remain contained.

To explore the translational potential of intranasal BDNF delivery, Ji and colleagues[58] has been investigating the functional outcomes of this delivery approach in subacute MPTP mice employing multiple administrations and exposures, following the rationale of extended dopaminergic upregulation achieved by multiple treatments shown by Karakatsani and colleagues.[47] Expectedly, the fiber density in the ventral midbrain was increased by 13% as evidenced by TH immunopositivity, whereas the projections of the dopaminergic neurons in the striatum were upregulated by 20% after the experimental procedure. Animals tested in the circular open-field task after amphetamine injection showed a significant preference toward ipsilateral rotations, suggesting an amelioration of the pathology in the treated hemisphere.

Similar approaches with molecules other than trophic factors have been adopted with similar results. For example, delivery of nuclear factor E2-related factor 2) with FUS and microbubbles in the SNc of 6-OHDA rats results in reduced reactive oxygen species levels, thereby protecting dopaminergic neurons.[59]

The therapeutic alternatives explored so far have shown a gradual increase in beneficial outcomes, with direct protein delivery proven efficient in upregulating neuronal function. However, translation to clinical practice entails multiple applications that would significantly improve neuronal integrity along the entire nigrostriatal pathway. Gene therapy comes as an alternative to multiple applications, allowing for the constant release of the neurotrophic factor with a single ultrasound session.

Targeting α-Syn Aggregation

Several studies have shown the effectiveness of the use of FUS in animal models of Alzheimer's disease

(AD) targeting both β-amyloid and tau.[4,44,60-62] Indeed, in some of these cases, there was a clear beneficial influence of BBB opening alone (in the absence of any therapeutic agent) on the clearance of β-amyloid plaques[4,44,60] or tau.[61] On the other hand, FUS enhanced antibody delivery, increasing the clearance of proteins.[61] Subsequently to these experiments, BBB opening with FUS was started in 5 AD patients with early to moderate AD in a phase I safety trial.[63] In all of these patients, the BBB within the target volume was safely, reversibly, and repeatedly opened. However, despite the encouraging preliminary results in AD, to date FUS-facilitated therapy against abnormal α-syn accumulation remains uncharted. Only a few studies have targeted this so far, and all of them with positive results.

In a pioneering study in cell culture, Karmacharya and colleagues[64] show that FUS decreases α-syn aggregation by the attenuation of mitochondrial reactive oxygen species in 1-methyl-4-phenylpyridinium MPP(+)-treated pheochromocytoma 12 cells. Regarding in vivo studies, Zhang and colleagues[65] designed a study on A53T transgenic mice that overexpresses human α-syn with prominent inclusions by the presymptomatic age of 9 months. The animals that received three exposures to FUS coupled with the administration of an anti–α-syn antibody experienced a 1.5-fold decrease in the α-syn load in the treated hemisphere compared with the untreated brains one month after delivery completion. These preliminary findings suggest the feasibility of such an approach in clearing accumulated proteins from the degenerated brain.

In a more recent study, transgenic mice expressing wild-type human α-syn were subjected to magnetic resonance imaging–guided FUS focally in different brain regions susceptible to α-syn aggregation (hippocampus, SNc, olfactory bulb, and dorsal motor nucleus) in tandem with intravenous microbubbles and an Adeno-associated Virus9 bearing a shRNA targeting α-syn.[66] One month after treatment, α-syn immunoreactivity was decreased, whereas other neuronal markers such as synaptophysin or TH were unchanged, and cell death and glial activation remained at baseline levels. These results demonstrate that FUS can effectively deliver viral vectors targeting α-syn to multiple brain areas. This approach might be useful to alter the progression of LP in PD patients, particularly in those diagnosed early, thus improving the evolution of the disease.

Limitations and Prospects

The inconsistency in the pathological outcomes stemming from different sonication protocols emphasizes the urgency to establish standardized methods to properly monitor the sonication regime. Aside from the ultrasonic parametric space, microbubble dosing and distribution have to be fully characterized, and brain-structure susceptibility to ultrasound is crucial in understanding the biological effects that occur during drug delivery and to fairly compare the findings.[68] Furthermore, a larger number of molecules should be tested given that so far only GDNF has been widely tested in several independent laboratories. Further studies are needed to assess the effects on the brain of antibody administration and other drug types such as antiinflammatory compounds or drugs regulating genes/enzymes related to PD (ie, glucocerebrosidase) alone or in combination with FUS. In this regard, it is important to remember that none of the available PD animal models recapitulates all of the pathologic abnormalities observed in PD clinical cases (ie, absence of LP in toxin-based models or absence of extensive neurodegeneration in α-syn models).[68] For example, up to now, experiments aimed to reduce α-syn expression with FUS have not addressed if there is neuroprotection. There are a lot of promising candidates, some of which are "old friends" such as GDNF, but others are new, such as glucocerebrosidase modulators, iron chelators, antibodies against α-syn, or antiinflammatory drugs,[69] which can benefit from FUS-facilitated delivery. More gene therapy experiments should be attempted. For example, recent clinical trials involved invasive putaminal injections.[53,70] AAV-AADC (Aromatic L-amino acid decarboxylase) delivery proved to improve motor function,[70] whereas AAV-GDNF did not meet its primary endpoint and did not provide clinical benefits.[53] These approaches could be more effective (and safer) in PD patients if the viral vectors are delivered focally with FUS.

Finally, moving toward a possible clinical application in PD, several important questions remain unresolved. These include defining regions of the brain that should be targeted, how many times this procedure can be performed safely in patients, and the duration of action of the delivered agent(s) to have a significant effect against the neurodegenerative process. Another fundamental underlying issue is the interaction between the described increased permeability of the BBB in the SNc and striatum in PD patients[71-74] and the impact of BBB opening via ultrasound. Indeed, one could question the need for opening the BBB if it is already disrupted. However, we would argue that such abnormalities would not guarantee local and sufficient delivery of putative neuroprotective/restorative agents. In any case, this remains a topic in need of further investigation.

Conclusion

Currently, the use of FUS for ablative purposes shows some promise for the treatment of PD. This includes targeting either the ventral intermediate thalamus, the subthalamic nucleus, or the internal pallidum.[80-83] Phase

I trials exploring both of these indications are currently underway (NCT03608553). However, the use of FUS to disrupt the BBB in a focal and temporary way and facilitate the entry of different compounds such as GDNF, antiinflammatory drugs, or antibodies seems to be an extremely promising therapeutic option for the treatment of PD not only as a symptomatic treatment but also to impact the mechanisms underlying neurodegeneration. This would also hold for other neurodegenerative diseases, including AD or amyotrophic lateral sclerosis.[63,84,85] Here we have reviewed several studies that display encouraging results regarding the possible application of this technology for PD. However, more experimental evidence is needed before clinical applications of these approaches can be developed.

In summary, FUS-facilitated drug delivery is a highly promising therapeutic approach, but it is still in need of additional experimental investigation to further study its range of capabilities as well as optimize the parameters given the intended application. A multitude of studies exploring this range including relevant animal models would significantly assist in accomplishing successful clinical translation and adoption.

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