Feature Article

Nanoparticle administration across the blood-brain barrier using MRI-guided focused ultrasound

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Summary: This article explores a novel approach to central nervous system pharmacotherapy using focused ultrasound and nanoparticles to bypass the Blood-Brain-Barrier.

Keywords: central nervous system, blood-brain barrier, focused ultrasound, nanoparticles, drug therapy

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Abstract

A vast array of medical conditions affects the central nervous system (CNS), implying a tremendous scope of therapeutic interventions that must target the brain. However, all medical therapy to the brain faces the inherent physiological obstacle of the blood-brain barrier (BBB). Furthermore, after the BBB, drugs must navigate the additional barrier of the brain extracellular space (ECS), which presents its own unique biochemical obstacles. Both the BBB and brain ECS present considerable difficulties for drug therapy to treat diseases affecting the brain. With advancing technology, there has been significant progress towards the goal of overcoming these barriers. An exciting development is the use of MRI-guided focused ultrasound (MRIgFUS) to deliver drug-loaded nanoparticles (NP).

This article describes and explores the use of MRIgFUS and NPs, together as a novel method in CNS drug therapy. First, the basic scientific principles underlying the approach are described. Then, studies that demonstrate key concepts, advancements, strengths, and limitations are discussed to outline directions that have been pursued towards the goal of implementing MRIgFUS NP delivery in practice.

Introduction

The gamut of conditions affecting the central nervous system is vast. Whether they are infectious, inflammatory, neoplastic, degenerative, or psychiatric, the diversity of these diseases' aetiologies implies a tremendous scope of therapeutic interventions targeting the brain, but all drug therapy to the brain faces the inherent structural obstacle of the blood-brain barrier (BBB) [1]. A healthy BBB is important to brain homeostasis as it prevents circulating toxins and pathogens reaching the brain while also controlling the transport of important nutrients and solutes. However, in the pharmacological sense, it hampers the efficacy of drug treatment because most medications administered systemically are often unable to penetrate the BBB in therapeutically relevant doses [2]. Doses must, therefore, be increased to achieve adequate effects, raising the potential for side effects and limiting therapeutic potential. Once a drug passes the BBB, it must then navigate the extracellular space (ECS) of the brain; this presents its own obstacles, such as the tiny intercellular spaces, ionic composition of extracellular fluid and the meshwork of molecules comprising the extracellular matrix [3]. Both BBB and brain ECS present considerable difficulties in using drug therapy to treat the brain. With advancing technology and scientific effort, there have been significant strides towards the goal of overcoming these barriers, such as the use of MRI-guided focused ultrasound (MRIgFUS) to deliver drug-loaded nanoparticles (NP).

Circumventing the barriers

In developing methods to overcome the BBB, multiple approaches have been previously attempted. For example, drugs were developed with more lipophilic properties to better pass through the BBB, or they were bound to carrier molecules to exploit endogenous transport mechanisms [4,5]. A less conservative method involved intra-arterial catheterisation and administration of a hypertonic solution that caused widespread disruption of the BBB to allow molecules through [6]. A more invasive approach was the direct injection of the brain following a craniotomy and visualisation of the brain [7]. A less invasive and promising development is the use of focused ultrasound (FUS) to induce localised BBB disruption (Figure 1) [8,9]. The molecular basis of this can be explained by two main pathways: 1) the absorption of acoustic energy by tissue and 2) the propagation of the acoustic wave through fluid [10]. Firstly, when acoustic waves interact with tissue, there is absorption of the acoustic energy leading to a temperature increase, resulting in local injury. Secondly, when the wave propagates through a fluid medium, "cavitation" occurs; this is the formation of one or many bubbles. These "microbubbles" (MBs) pulsate and collapse or "pop", resulting in mechanical stresses and temperature changes to the surrounding tissue. Significant improvements to this technique have included using agents containing preformed MBs and establishing safe ultrasound delivery parameters so as to avoid *in vivo* cavitation that can inadvertently damage brain tissue or non-target BBB sites [8,9]. A recent addition has been the introduction of MRI into the process. This presents multiple advantages, such as providing a guide for FUS exposure, visualising the BBB opening and monitoring MB distribution [11,12].

In terms of overcoming the brain ECS, the research focus was initially to characterise the size of the spaces a therapeutic agent would have to pass in order to reach the parenchyma. Studies have been done in both animal and human brains to better understand the interstitial dimensions and, unsurprisingly, the spaces are exceedingly small [13]. Therapeutics must, therefore, be small enough and have compatible chemical properties to pass through the brain ECS. In the pursuit of this, nanoparticles have become relevant. Nanoparticles are structures which can range in size between 1 to 100 nm, though the term is often used to describe

particles up to several hundred nanometres in size [14]. One of the significant ways NPs have become relevant in medicine is in the ability to load them with a therapeutic agent and thereby allow them to serve as a "nanocarrier" for medication delivery [15]. Advancements in this area have included synthesizing NPs that are able to pass the BBB and traverse the ECS, improving their characteristics to make delivery and distribution more effective, and successfully attaching various therapeutic agents.

Key studies demonstrating the concept

The techniques involved in BBB disruption and NP drug delivery must encapsulate multiple key elements: 1) the NP must penetrate and move through the brain ECS, 2) the NP must avoid rapid clearance, and 3) there must be a non-invasive approach to circumvent the BBB [16]. Many studies, which explore various aspects of the topic while outlining the applicability of FUS and NP delivery systems to medical treatment, have been conducted (Table 1).

Hynynen et al. were the first to describe the use of MRI to guide the FUS procedure, now known as MRI-guided FUS [9]. Ultrasound contrast agent containing MBs was injected into rabbits, then FUS was administered while MRI scans were done to monitor temperature and tissue changes. Afterwards, relaxation time shortening MRI contrast was administered and further MRIs were performed. The contrast was visualised through signal intensity changes at target sites, and this confirmed BBB disruption. This study was pivotal in improving FUS-mediated BBB disruption by making it more targeted. Furthermore, it contributed to increasing the safety of the procedure. By detecting tissue changes during sonication, ultrasound power may be monitored to prevent brain tissue damage.

In terms of modifying the structure of the NP to improve brain delivery, a study by Nance et al. provides evidence for this [17]. Both fresh human and mice brain tissues were used to determine whether NP diffusion could occur through the pores of the experimental brain ECS, thereby characterising the NP sizes that could move through. Thorne and Nicholson gave the pivotal first direct estimates of ECS width in living brains, suggesting that nanoparticles up to 64 nm could effectively move through the brain ECS [13]. By coating NPs with low molecular weight polyethylene glycol (PEG), Nance et al. showed that NPs with a hydrodynamic diameter up to 114 nm could diffuse through the experimental tissue. This observation was partly explained by the presence of the PEG coating, which inhibited the processes that endogenously eliminate the NPs. This way, the PEGylated NPs had more time to distribute and accumulate. In a follow-up study, the use of PEGylated NPs was combined with MRIgFUS and MBs in a rat model to achieve the safe delivery of 60 and 75 nm NPs [16]. A recent paper by Hersh et al. demonstrated that ultrasound could be used to enlarge the interstitial spaces in living rat brains, opening up the possibility of using larger NPs and improving NP dispersion throughout the brain [18]. Finding an accord between NP size characteristics and FUS delivery is challenging but important, as the value of larger NPs lies in reducing the restrictions on the possible drug and payload sizes.

In terms of improving the delivery of NPs from circulation to the brain, a technique called localised convection enhanced delivery (CED) has been demonstrated as a promising approach [19]. This involves a therapeutic agent being continuously injected in a fluid medium under positive pressure (convection) by a pump via a catheter directly inserted into the brain. This creates a significant continuous pressure gradient *in vivo* across which drugs can move into tissue. Drug delivery via CED has been tested with multiple different NPs. In a

study by Perlstein, CED was used to administer NPs into the striatum of a rat model [20]. The authors employed an MRI-guided technique and also used coated NPs. The NPs were coated in dextran, rather than PEG and worked by similar clearance-evading mechanisms to improve distribution. The value of CED is that it augments the simple diffusion of NPs into the brain, achieving larger volumes of distribution and reaching drug concentrations markedly greater compared to regular means of systemic administration. Challenges remain before CED could be considered in clinical practice. Key limitations include the invasiveness and heterogeneity in the formation of convection which is influenced by many factors such as the NP structure, nature of the infusate, size of delivery catheters and rate of infusion [19].

Advancing the concept

There are many aspects of the research of MRIgFUS and NP drug delivery that deal with the advancement of the technique. While it possesses great breadth, the technique also has significant depth that has been experimentally explored, such as better NP design, improvements to MRI or FUS delivery and loading NPs with active therapeutics for delivery to the brain.

Pivotal to the success of MRIgFUS is the design of an NP that can effectively reach the brain parenchyma. As mentioned previously, molecular coatings have proven to be effective in avoiding premature elimination of NPs [17,20,21]. From examinations of NP surface charge, it has been shown that cationic properties allowed NPs to deposit in the brain [22]. Self-assembling NPs that have the hybrid role of acting as both the MB that disrupts the BBB and the carrier that moves drugs across it have also been developed [23,24]. With the introduction of MRI to guide FUS, there have been strides in molecular design that make NPs more visible on imaging [25].

Improving the MRIgFUS technique beyond NP design is also important [26]. The route by which drugs are administered has been explored, with one novel method using an intranasal approach rather than the conventional intravascular method [27]. A better understanding of FUS and MBs in terms of their optimum parameters has also been gained through the quantification of BBB permeability at different FUS doses, MB sizes, and MB concentrations [28]. The pursuit of perfect parameters is complicated by disease states. For example, a mice model of Alzheimer's disease demonstrate that the pathological changes in cerebral vasculature due to amyloid plaques may reduce BBB permeability and thereby require an alteration of FUS parameters [29]. This highlights the importance of progressing to studies that are more clinically relevant and applicable.

The ultimate goal for using NPs with MRIgFUS is to deliver therapeutic agents to the brain, and the approach to this has been varied. A majority of studies in the literature have focused on anti-cancer agents with the intention of targeting tumours in animal models [30-34]. Outside of chemotherapy, docosahexaenoic acid has also been trialled because of its known neuroprotective effects [35]. There is also interest in loading NPs with non-drug molecules. In particular, DNA and other genetic material have been delivered to the brain in a promising approach to gene therapy [36], while nanoparticles containing gold have also demonstrated therapeutic potential when coupled with FUS [37]. With the breadth of CNS diseases requiring medical therapy, there is great scope for further research into other drugs and their potential for delivery via NPs and MRIgFUS.

Considering the risks

These novel techniques and procedures are not without risks. The FUS used to mediate NP delivery may cause a sterile inflammatory response comparable to those elicited during ischemia or mild traumatic brain injury, with unknown downstream effects on neurological function [18]. There may be irreversible damage to the BBB, potentially allowing entry to the brain parenchyma for unintended molecules or microorganisms [16]. While MRI guidance has its advantages, recent evidence suggests that the gadolinium present in many contrast agents can accumulate in the brain [38-40]. The consequences of the latter are unclear, but may yet prove important when considering use of repeated MRIgFUS in patients who would require long-term therapy for chronic conditions. Nevertheless, the safety profile of this approach continues to be established and supported with pre-clinical and animal studies [18,41]. Researchers are optimistic that current and future approaches will remain safe, ensuring that tissue damage is reversible or negligible when balanced against the benefits of treatment.

Conclusion

The BBB and brain ECS are significant obstacles preventing medications moving from the vascular compartment to the brain parenchyma. Using MRIgFUS and NP drug delivery is a way to potentially provide therapeutics noninvasively while also improving treatment efficacy. Recent research demonstrates that this approach is a multifaceted entity with many aspects that must be improved and progressed before implementation into clinical practice. It is a promising and generic technique, and a cause for much excitement because the possibilities for its application are only as limited as the number of conditions treatable with drug therapy.

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Appendix

For the “Key studies demonstrating the concept” and “Advancing the concept” sections, a literature search was performed to identify studies for discussion in this article. The search was performed on the Medline database using the search terms: exp Nanoparticles/ OR *Nanostructures/ OR exp Drug Delivery Systems/ AND exp Brain/ AND exp Ultrasonography/ OR exp Ultrasonics/ OR exp Ultrasonic Waves/ OR exp Ultrasonic Therapy/ OR *Sonication/ OR exp Microbubbles/ OR focused ultrasound.mp. This returned 147 articles. Following limitation to English language and publications within the last five years, 94 articles remained. After screening the literature by the author title, abstract and full-text assessments, 19 articles were included for discussion.

Table 1. Studies examining various aspects of focused ultrasound and nanoparticle administration.

Author	Aim	Key Findings
Hynynen et al. [9]	To determine if focused ultrasound could be used in targeted blood-brain barrier opening by monitoring with MRI	• Blood-brain barrier opening was confirmed with MRI contrast at targeted sites. • Blood-brain barrier opening was achieved with the lowest ultrasound power, avoiding damage to surrounding tissue.
Nance et al. [17]	To examine pore size in the brain extracellular space and analyse the diffusion of nanoparticles with varying size and coatings	• Nanoparticles up to 114 nm in diameter coated with PEG could diffuse through the brain extracellular space. • PEG coating avoided the premature elimination of nanoparticles.
Perlstein et al. [20]	To demonstrate the use of CED for nanoparticles in a rat brain	• CED was successful in the infusion of nanoparticles into the rat brain, and this was confirmed with MRI.
Yao et al. [21]	To use ultrasound and microbubbles in the delivery of PEG-coated nanoparticles across the blood-brain barrier	• Nanoparticles were significantly more distributed (> 250% more as quantified by electron microscopy analysis of tissue) in brains treated with ultrasound compared to untreated controls. • Ultrasound induced the cavitation of microbubbles, and this was a key determinant allowing nanoparticles across the blood-brain barrier.
Burgess et al. [29]	To examine focused ultrasound mediated blood-brain barrier disruption in an animal model of Alzheimer's disease	• Blood-brain barrier permeability was lower in the disease group compared to the control group. • Presence of amyloid plaque reduced blood-brain barrier opening. • Ultrasound delivery parameters may require an adjustment in Alzheimer's disease models.
Mead et al [36]	To use focused ultrasound mediated blood-brain barrier opening in the delivery of DNA-containing nanoparticles and monitor for gene expression	• Gene expression occurred in the ultrasound treated region and lasted for 28 days. • In the ultrasound treated region, 42% of cells were transfected compared to 6% in the untreated region. • No toxicities were observed.

CED, Convection-enhanced drug delivery; PEG, Polyethylene glycol.