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Focused ultrasound combined with microbubble-mediated intranasal delivery of gold nanoclusters to the brain

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ABSTRACT

Focused ultrasound combined with microbubble-mediated intranasal delivery (FUSIN) is a new brain drug delivery technique. FUSIN utilizes the nasal route for direct nose-to-brain drug administration, thereby bypassing the blood-brain barrier (BBB) and minimizing systemic exposure. It also uses FUS-induced microbubble cavitation to enhance transport of intranasally (IN) administered agents to the FUS-targeted brain location. Previous studies have provided proof-of-concept data showing the feasibility of FUSIN to deliver dextran and the brain-derived neurotrophic factor to the caudate putamen of mouse brains. The objective of this study was to evaluate the biodistribution of IN administered gold nanoclusters (AuNCs) and assess the feasibility and short-term safety of FUSIN for the delivery of AuNCs to the brainstem. Three experiments were performed. First, the whole-body biodistribution of IN administered ^{64}Cu-alloyed AuNCs (^{64}Cu-AuNCs) was assessed using *in vivo* positron emission tomography/computed tomography (PET/CT) and verified with *ex vivo* gamma counting. Control mice were intravenously (IV) injected with the ^{64}Cu-AuNCs. Second, ^{64}Cu-AuNCs and Texas red-labeled AuNCs (TR-AuNCs) were used separately to evaluate FUSIN delivery outcome in the brain. ^{64}Cu-AuNCs or TR-AuNCs were administered to mice through the nasal route, followed by FUS sonication at the brainstem in the presence of systemically injected microbubbles. The spatial distribution of ^{64}Cu-AuNCs and TR-AuNCs were examined by autoradiography and fluorescence microscopy of *ex vivo* brain slices, respectively. Third, histological analysis was performed to evaluate any potential histological damage to the nose and brain after FUSIN treatment. The experimental results revealed that IN administration induced significantly lower ^{64}Cu-AuNCs accumulation in the blood, lungs, liver, spleen, kidney, and heart compared with IV injection. FUSIN enhanced the delivery of ^{64}Cu-AuNCs and TR-AuNCs at the FUS-targeted brain region compared with IN delivery alone. No histological-level tissue damage was detected in the nose, trigeminal nerve, and brain. These results suggest that FUSIN is a promising technique for noninvasive, spatially targeted, and safe delivery of nanoparticles to the brain with minimal systemic exposure.

1. I. Introduction

The development of effective therapies for central neural system (CNS) diseases is often challenged by the blood-brain barrier (BBB), which prevents most therapeutic compounds from reaching the brain at the therapeutic level [1]. Current clinical strategies to circumvent the BBB are either invasive (*e.g.,* Gliadel wafers, intrathecal injection, and convection-enhanced delivery) or lack specific targeting to the diseased site (*e.g.,* hyperosmolar disruption using mannitol and intranasal brain drug delivery) [2]. The use of focused ultrasound (FUS) and

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microbubbles to enhance the BBB permeability is a recently developed technique for noninvasive and localized delivery of intravenously (IV) injected drugs [3, 4]. However, the goals of drug delivery are not only to increase the local drug delivery efficiency but also to reduce systemic toxicity. Despite the great promise of the FUS-induced BBB opening technique, systemic drug toxicity associated with IV injection remains a clinical challenge. Brain drug delivery strategies that can circumvent the BBB for noninvasive and localized drug delivery with minimized systemic exposure are greatly needed to improve the treatment of CNS diseases. FUS in combination with microbubble-mediated intranasal delivery (FUSIN) has the potential to address this need. FUSIN is a novel technique first introduced by Chen and Konofagou in 2014 [5]. It utilizes the nasal route for direct nose-to-brain drug administration, thereby bypassing the BBB and minimizing systemic exposure. It also uses FUS-induced microbubble cavitation to enhance the transport of IN administered agents to the FUS-targeted brain location.

The IN route can deliver therapeutic agents directly from the nose to the brain through the olfactory and trigeminal nerve pathways, bypassing the BBB and minimizing systemic exposure [6]. Direct IN delivery of therapeutics to the brain was first proposed in the 1980s [7]. The exact mechanisms underlying IN brain drug delivery are not entirely understood; however, two pathways have been identified: the olfactory nerve pathway and trigeminal nerve pathway [6]. IN administered therapeutics can be transported from the nasal cavity to the brain along the olfactory nerve and trigeminal nerve, which innervate the epithelium of the nasal passages and enter the brain in the olfactory bulb and pons, respectively. Once inside the brain entry points (*i.e.*, olfactory bulb and brainstem), the IN administered agents are distributed in the whole brain along the cerebral perivascular spaces – thin annular regions surrounding the blood vessels – and may be propelled through the perivascular spaces by heartbeat-driven pulsations of the blood vessel walls, called the “perivascular pump effect” [8]. A wide-range of therapeutics, such as peptides, proteins, gene vectors, and stem cells, have been successfully delivered to the brain through IN administration and have shown efficacy in treating CNS diseases in small animal models [9–12]. IN insulin delivery for the treatment of patients with Alzheimer's disease has been tested in early-phase clinical trials [13, 14]. Recently, new formulations and delivery approaches have been developed to further enhance the nose-to-brain transport efficiency, mainly with the use of nanoparticles as drug carriers [15–17, 36]. However, IN brain drug delivery remains limited to preclinical studies and small early-phase clinical trials [18–21] mainly because the delivery is inefficient and not diseased-site targeted [19].

Unlike the more established FUS-induced BBB opening technique for trans-BBB delivery of therapeutics in the systemic circulation, FUSIN uses FUS to activate microbubbles at a targeted brain location to enhance the local accumulation of IN administered agents that are already beyond the BBB. Previous studies showed that FUSIN enhanced the delivery of IN-administered dextran and a protein drug (brain-derived neurotrophic factor, BDNF) at the FUS-targeted caudate putamen of mouse brains [5, 22]. Based on our previous work on ultra-high-speed photomicrography of microbubble dynamics in *ex vivo* microvessels [23], we observed that microbubble oscillations push and pull on the blood vessel, which leads to expansion and contraction of the vessel and surrounding tissue. Based on the similarity of this phenomenon with the perivascular pump effect, we hypothesized that the “microbubble pump effect” may be the potential mechanism for FUSIN. This mechanism was indirectly verified by comparing the delivery efficiency of FUS sonication before IN administration (without the microbubble pump effect) to FUS after IN administration (with the microbubble pump effect) [22]. It was found that significant enhancement was observed only when FUS sonication was performed after IN, suggesting that the microbubble pump effect contributes to FUSIN.

The objective of this study was to evaluate the biodistribution of IN administered gold nanoclusters (AuNCs) and assess the feasibility and short-term safety of FUSIN for the delivery of AuNCs to the brainstem.

Ultrasmall nanoclusters have drawn significant attention for biomedical applications due to their size-promoted clearance after systematic injection [24–28], and accurate tumor targeting as we demonstrated in previous research [29]. We have previously reported on renal clearable AuNCs integrated with ^{64}Cu (^{64}Cu-AuNCs) which showed minimal nonspecific organ retention, largely reduced mononuclear phagocytosis system accumulation, and precise detection of cancer biological targets in both primary tumor and distant metastasis using positron emission tomography (PET) [29]. Meanwhile, their sizes were close to that of monoclonal antibodies, which are used with increasing success against many tumors [30]. The first-in-human trial is now ongoing to determine the safety of small-size gold nanoparticles (13 nm in diameter) labeled with spherical nuclei acid in treating patients with recurrent glioblastoma or gliosarcoma (ClinicalTrials.gov Identifier: NCT03020017).

We selected the brainstem as the targeted brain location because our long-term goal is to use FUSIN for the treatment of diffuse intrinsic pontine glioma (DIPG). DIPG, a high-grade glioma that spreads throughout the brainstem, has replaced leukemia as the leading cause of cancer death among children. It has a median survival of less than one year, a dismal prognosis that has remained unchanged over the past 40 years [31]. There are two main reasons why treatment of DIPG is challenging. First, in contrast to other high-grade gliomas (*e.g.*, glioblastoma), which often have a compromised BBB, the BBB in DIPG is frequently intact, as suggested by the lack of contrast enhancement on contrast-enhanced magnetic resonance imaging [32]. Second, the brainstem controls basic life functions, such as breathing, hearing, taste, balance, and communication between different parts of the brain. The critical anatomic location of the brainstem precludes surgical intervention and limits the use of other invasive therapeutic techniques. Therefore, techniques that can noninvasively circumvent the BBB can have a significant clinical impact in treating DIPG. FUSIN has the potential to improve DIPG treatment by bypassing the BBB and addressing the critical need, shared by many pediatric brain diseases, for noninvasive and targeted delivery of therapeutics to the diseased brain site, while minimizing injury to healthy regions of the developing brain and other organs. In addition, the brainstem is also unique in that it is directly connected with the nasal cavity through the trigeminal nerve. Our previous studies showed that FUSIN delivered different agents to the caudate putamen [5, 22]. This study explored the potential to expand the application of FUSIN for drug delivery to the brainstem.

2. Materials and methods

2.1. Animals

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Washington University in St. Louis, in accordance with the National Institutes of Health Guidelines for animal research. C57BL/6 female mice (6–8 weeks, ~25 g body weight) were purchased from Charles River Laboratory (Wilmington, MA, USA). The animals were housed in a room maintained at 72 °F and 55% relative humidity, with a 12-h/12-h light/dark cycle, and provided access to standard laboratory chow and tap water. Mice were divided into multiple groups (Table 1).

2.2. Synthesis and characterization of AuNCs

^{64}Cu-AuNCs were synthesized as described previously [28]. In a typical reaction, water (2.0 mL), $HAuCl_4$ (10 mM, 376 μL), and $^{64}CuCl_2$ (74 MBq) were mixed in a glass vial, followed by the dropwise addition of mPEG-lipoamide (MW = 750 Da, 10 mM, 400 μL). After the mixture was stirred overnight, sodium borohydride (40 mM, 400 μL) was added and stirred rapidly for 2 h. The ^{64}Cu-AuNCs were collected by first filtering the solution through an Amicon 50 K centrifuge filter and subsequently purifying the solution with an Amicon 10 K centrifuge filter.