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Lipid-shelled microbubbles for ultrasound-triggered release of bioactive gases to treat stroke and cardiovascular disease

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***Abstract*— Ischemia-reperfusion-induced neurological injury is a primary cause of stroke disability. Xenon (Xe), a bioactive gas, has potential as an effective and nontoxic neuroprotectant for the treatment of ischemic stroke. Nitric oxide (NO) is a potent bioactive gas capable of inducing vasodilatory, anti-inflammatory, neuroprotectant and bactericidal effects. The goal of this work was to develop lipid-shelled microbubbles for site-specific release of Xe or NO upon pulsed ultrasound exposure. Gas-loaded microbubbles were synthesized by high-shear mixing of a lipid dispersion in a vial that contained, Xe or NO, and octafluoropropane (OFP) in combination. Attenuation spectroscopy measurements demonstrate the feasibility of 6-MHz pulsed Doppler ultrasound-triggered release of Xe or NO from microbubbles. The addition of OFP in the lipid-shelled microbubbles increased the number density, size, and stability of the microbubbles, particularly in undersaturated saline. Gas chromatography mass spectrometry was employed to measure Xe dose (127 ± 29 µl Xe/mg lipid). The payload of NO in the microbubbles (97 ± 12 µl NO/mg lipid) was assessed using an amperometric sensor. Intravenous administration of microbubbles carrying a neuroprotective or a vasodilatory gas in combination with ultrasound exposure has potential as a novel noninvasive strategy for local therapeutic delivery to modulate the effects or duration of cerebral ischemia.**

Keywords—Ultrasound-mediated bioactive gas delivery, nitric oxide delivery, xenon delivery, neuroprotection, stroke, cardioprotection

I. Introduction

Stroke is a leading cause of death worldwide [1]. Occlusion of blood flow through diseased arteries is caused by thrombi, blood clots which form in the body. Despite advances in technology and development of lytics to promote rapid recanalization, therapies are needed to arrest neuronal damage caused by excitotoxicity, inflammation, oxidative stress, and edema [2-5] induced during reperfusion. Xenon, a neuroprotectant gas, inhibits glutamatergic effects of N-methyl-D-aspartate (NMDA) receptors and promotes the transcription of prosurvival genes [6, 7]. Additionally, Xe alleviates ischemic injury by altering the cellular pathways involved in neuronal ischemic tolerance and protecting against oxygen and glucose deprivation [6, 8-11]. The small size and low blood–gas partition coefficient of Xe enables this gas to cross the blood–brain barrier rapidly [6]. Nitric oxide (NO) is another potent bioactive gas that plays important roles in physiology, including the regulation of vasodilation, platelet activation, and neurotransmission [12]. NO is downregulated in pathological conditions, such as hypertension, atherosclerosis, and chronic kidney disease [13]. Additionally, recent studies suggest that NO inhalation can prevent chronic lung disease in premature infants and alleviate ischemia-reperfusion injury [14, 15]. However, inhalation of bioactive gases is expensive, cumbersome and suitable for a limited number of applications [16]. Therefore, the development of site-specific and triggered delivery of bioactive gases is an active area of research [12] Loading of Xe or NO in micron-sized microbubbles is another strategy for delivery of bioactive gas [17, 18].

II. Materials and Methods

A. Microbubble preparation and characterization

Gas-loaded microbubbles were synthesized by high-shear mixing of a dispersion of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (18:0 PEG2000 PE) in a gas-tight vial that contained Xe, or NO, and octafluoropropane (OFP) in a 9:1 v/v ratio (Figure 1)[17, 19]. The size distribution of the gas-loaded microbubbles was measured using a Coulter counter (Multisizer 4, Beckman Coulter, Brea, CA, USA). Acoustic attenuation was measured from 2 to 25 MHz using through-transmission attenuation spectroscopy [20]. The Xenon payload in the Xenon-loaded microbubbles (Xe-OFP-MB) was measured by gas chromatography–mass spectrometry (GC/MS) [21]. The NO payload in NO-loaded microbubbles (NO-OFP-MB) was measured using an amperometric micro-electrode sensor (Apollo 4000 with ISO-NOP electrode; World Precision Instruments, Sarasota, FL, USA).

B. In vivo assessment of echogenicity in mice

In vivo imaging was performed in an inbred strain of male C57 mouse (28 g weight). B-mode imaging was performed using a Vevo 2100 imaging system (VisualSonics, Toronto, ON, Canada) equipped with a MS250 probe (13-24 MHz bandwidth) operating at an 18-MHz center frequency according to a protocol described in detail by Shekhar et al. [17]. Briefly, acoustic

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parameters were set to 10% output power and 25 dB gain. Definity® or Xe-OFP-MB were infused into the mouse, and cine loops were recorded. Definity® was diluted 1:10-fold in sterile, air-saturated saline (0.9%), and Xe-OFP-MB and Xe-MB were diluted 1:13.33-fold in xenon-saturated saline to obtain equivalent lipid concentration

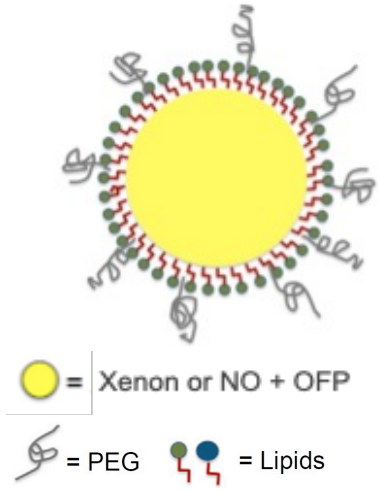

Fig. 1. Schematic of microbubbles manufactured using the DSPC and 18:0 PEG2000 PE lipids , xenon or nitric oxide (NO) and octafluoropropane (OFP) gas.

III. Results and Discussion

Attenuation spectroscopy measurements demonstrate the feasibility of 6-MHz pulsed Doppler ultrasound-triggered release of Xe or NO from microbubbles. The addition of OFP in the lipid-shelled microbubbles increased the number density, size, and stability of the microbubbles, particularly in undersaturated saline. Gas chromatography mass spectrometry was employed to measure Xe dose (127 ± 29 µl Xe/mg lipid). The payload of NO in the microbubbles (117 ± 25 µl NO/mg lipid) was assessed using an amperometric sensor. Intravenous administration of microbubbles carrying a neuroprotective and cardioprotective (Figure 2) or a vasodilatory gas in combination with ultrasound exposure has potential as a novel noninvasive strategy for local therapeutic delivery to modulate the effects or duration of cerebral ischemia.

IV. Conclusions

The findings of this study demonstrate that Xe can be loaded within lipid-shelled microbubbles for ultrasound-triggered release. Co-encapsulation of octafluoropropane gas enhanced the Xe and NO dose, stability, and volume of Xe-OFP-MB and NO-OFP-MB. However, only the retention of Xe in microbubbles was improved by co-encapsulation with OFP. These results suggest that lipid-shelled microbubbles could serve as bioactive gas delivery agents for applications such as neuroprotection or local vasodilation in stroke.

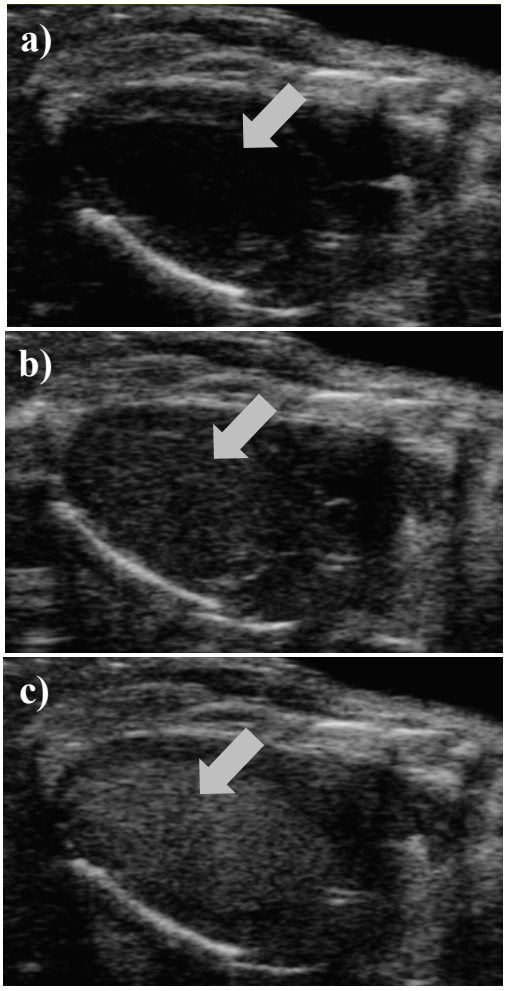

Fig. 2. Parasternal long axis view of the left ventricle (arrows) of a mouse: (a) baseline image without a contrast agent, (b) with Xenon + OFP microbubbles, and (c) with Definity®. *In vivo* 18 MHz B-mode imaging performed with a VisualSonics Vevo 2100 in an inbred strain of male C57 mouse (28 g weight). Microbubbles were injected retrograde in the internal jugular vein.

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