



Dodecafluoropentane emulsion delays and reduces MRI markers of infarction in a rat stroke model: a preliminary report



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ABSTRACT

Background: Dodecafluoropentane emulsion (DDFPe), an oxygen transport agent, has been shown to reduce infarct volume in animal models of acute ischemic stroke (AIS). Our study assesses the effect of DDFPe on MRI markers of infarct evolution in the early hours after vascular occlusion in a rat AIS model. We hypothesized that DDFPe will delay the development of MRI markers of AIS and/or reduce the extent of infarction.

Methods: Permanent, unilateral surgical occlusion of the middle cerebral and common carotid arteries was performed in control (n = 4) and treatment (n = 10) rats. The treatment group received 1 IV dose of 2% w/v DDFPe at 0.6 mL/kg at 1 hour post-occlusion versus none. Diffusion-weighted (DWI) and inversion recovery (IR) MRI sequences were obtained over the 4 hours following occlusion. Infarct extent was quantified by number of abnormal MRI slices per sequence for each group and time point. Student's T-test was applied.

Results: DDFPe-treated rats demonstrated reduced infarct extent versus controls over combined time points on IR at 5.43 ± 0.40 (mean \pm standard error) abnormal slices vs. 7.38 ± 0.58 (P = 0.01) and on DWI at 5.21 ± 0.54 vs. 9.00 ± 0.95 (P < 0.01). Development of abnormal MRI signal was delayed in the treatment group.

Conclusions: DDFPe delays and reduces MRI markers of AIS in the early hours following vascular occlusion in a rat stroke model. Further investigation of DDFPe as a neuroprotectant is warranted.

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1. Introduction

Stroke is a major cause of morbidity and the 4th leading cause of mortality in the United States [1]. Current strategies for the treatment of acute ischemic stroke (AIS) focus on revascularization, either via pharmacotherapy or catheter-based interventions. Tissue plasminogen activator (tPA), the most commonly employed thrombolytic agent, is FDA approved for use only within 3 hours of symptom onset. Such time constraints, combined with various contraindications, allow the use of tPA in <5–10% of patients with AIS [2,3]. Although efficacious, tPA therapy is not without potential complications, most importantly post-therapeutic hemorrhage. Given the many challenges of timely revascularization, many groups have investigated the potential of neuroprotective agents to support ischemic but not yet infarcted

parenchyma, thus prolonging the time window for revascularization. Although at least a dozen agents have progressed to phase III clinical trial, none have been shown to improve outcomes [4].

Our group has previously demonstrated that DDFPe (dodecafluoropentane emulsion) (NuvOx Pharma, Tucson, Arizona) reduces infarct volume in a rabbit embolic stroke model [5,6]. DDFPe, a perfluorocarbon (PFC), boasts high oxygen dissolving capacity, hydrophobic and lipophobic properties, and extreme inertness, making it an ideal candidate neuroprotectant [7]. Initially utilized as a sonographic contrast agent, the human *in vivo* half-life of DDFPe ranging from 1.8 to 2.5 min facilitates complete removal of typical doses through normal respiration at approximately 2 hours without long-term retention in the body [8]. Such half-life estimates are congruent with data obtained in a rabbit model demonstrating a blood half life of 1.45 ± 0.17 minutes after a single 0.6 ml/kg dose [6]. Although not currently available for clinical use due to inadequate demand for sonographic contrast agents, no significant adverse effects have been reported in prior human use [8].

Among PFC's, DDFPe emulsion consisting of nano-droplets ranging from 250 to 300 nm in diameter is unique in that this form of the molecule transitions to the gaseous state at 29 °C and exists as a gas at body temperature. Such properties allow DDFPe to carry and deliver many times the oxygen content of liquid PFC's [9]. *In vivo*,

Abbreviations: DDFPe, Dodecafluoropentane emulsion; AIS, acute ischemic stroke; tPA, tissue plasminogen activator; FDA, food and drug administration; PFC, perfluorocarbon; STAIR I, Stroke Therapy Academic Industry Roundtable I; GABA, γ -Aminobutyric acid; MCA, middle cerebral artery; NMDA, N-methyl-D-aspartate; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

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DDFPe nano-droplets expand only slightly due to intravascular pressure, thus facilitating oxygen delivery to tissues that can no longer be supplied by erythrocytes (6.2–8.2 μm) due to macrovascular occlusion. As such, DDFPe may reach penumbral tissue beyond such occlusions through intra-thrombus fissures or defects, microvasculature, collateral vessels, or via diffusion gradients.

In a rabbit embolic anterior circulation infarction model, DDFPe (0.6 mL/kg) decreases final infarct volume at 4 hours whether administered as a pre-treatment, at the time of ictus, or up to 3 hours after embolization [5]. When carried out to 7 hours, animals treated at 1 hour but not at 6 hours showed a decreased infarct volume relative to controls [5]. At 24 hours, animals treated at 60 minutes post-embolization and thereafter at 90-minute intervals with DDFPe doses ranging from 0.1 mg/kg to 0.6 mg/kg all showed statistically significant reductions of final infarct volume [6].

2. Aims and hypothesis

Based on promising prior data from our group and others, we aim to evaluate the *in vivo* effects of DDFPe in a rat stroke model with high-field strength (7 Telsa) MR imaging in the early hours following arterial occlusion. We hypothesize that DDFPe administered 1 hour after occlusion will delay and/or reduce the MR imaging signs of acute infarction.

3. Methods

3.1. Stroke model

Approval for this study was obtained through our Institutional Animal Care of Use Committee. Male Sprague Dawley (SD) ($n = 11$) and spontaneously hypertensive (SHR) ($n = 3$) rats weighing 400–450 g were anesthetized using isoflurane to effect. AIS was induced by surgical occlusion of the common carotid and middle cerebral arteries using an established model [10]. The middle cerebral artery was occluded at the distal M1 segment beyond the lenticulostriate arteries and proximal to the bifurcation. The treatment group ($n = 10$; 7 SD and 3 SHR animals) received 1 intravenous dose of 2% w/v DDFPe at 0.6 mL/kg at 1 hour post-occlusion, and the control group ($n = 4$; all SD animals) received none.

3.2. Imaging methods

Rats were imaged on a Bruker PharmaScan 7Telsa MRI (Bruker, Ettlingen, Germany) housed in the UAMS Molecular Imaging Core at 1-hour intervals beginning 1 hour post-occlusion out to 4 hours. During imaging, anesthesia was provided with isoflurane adjusted to maintain a breathing rate at 40–55 breaths per minute. Parameters of the diffusion-weighted sequence were as follows: b-value 1000 s/mm²; TR 10,000 ms; TE 22 ms; section thickness 0.8 mm; field of view 4 × 4 cm; matrix size 128 × 128. IR images were obtained using a RARE (rapid acquisition with relaxation enhancement) IR sequence as follows: TR 20,567 ms; TE 30 ms; section thickness 0.8 mm; field of view 4 × 4 cm; matrix 256 × 256; flip angle 180°.

3.3. Data analysis

MR images were reviewed by two board-certified, blinded radiologists, and the number of positive image slices per sequence was scored for each animal, image sequence (DWI and IR), and time point as a measure of infarct extent. Image slices showing hyperintense signal within the MCA territory ipsilateral to occlusion were scored as positive. In the analysis, imaging time points were rounded to the nearest hour. Student's *t* test with a two-tailed distribution with two sample unequal variance was used to generate

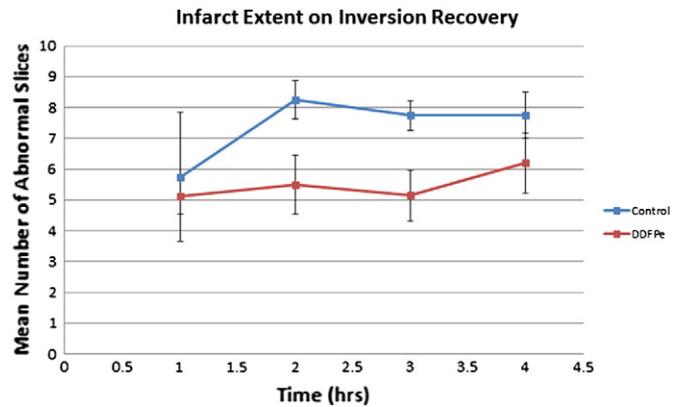


Fig. 1. Extent of infarction (y-axis) assessed by the number of abnormal slices per IR exam at each measured time point (x-axis). Control animals are displayed in blue and treatment animals in red.

standard deviation and P-values for each time point as well as comparison of the treatment and control cohorts.

4. Results

Infarct extent, as determined by the number of DWI or IR-positive image slices per study, was reduced in DDFPe-treated animals versus controls. Over combined time points on IR scans, mean abnormal slices for treated animals totaled 5.43 ± 0.40 standard error versus 7.38 ± 0.58 ($P = 0.01$) for controls. On DWI, the mean of 5.21 ± 0.54 for DDFPe-treated animals was significantly lower than that of controls 9.00 ± 0.95 at ($P < 0.01$). Graphical representation of mean abnormal slices as a measure of infarct extent versus time is displayed in Figs. 1 and 2. Although our primary assessment focused on the number of DWI/IR positive images slices per study, qualitative evaluation of comparable DWI slices from serial scans in individual subjects revealed attenuation of infarct growth over time in DDFPe-treated animals relative to controls, which show a gradual, step-wise expansion of infarction over time (Fig. 3).

5. Discussion

The concept of neuroprotection in AIS entails any therapy that confers increased survivability of ischemic brain tissue. Although the primary goal of AIS treatment has been revascularization, therapies

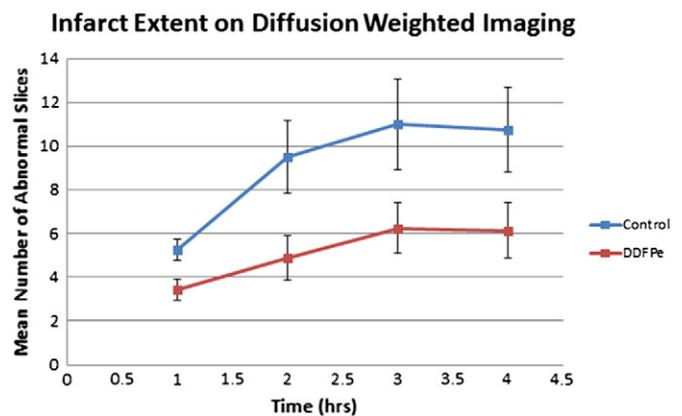


Fig. 2. Extent of infarction (y-axis) assessed by the number of abnormal slices per DWI exam at each measured time point (x-axis). Control animals are displayed in blue and treatment animals in red.

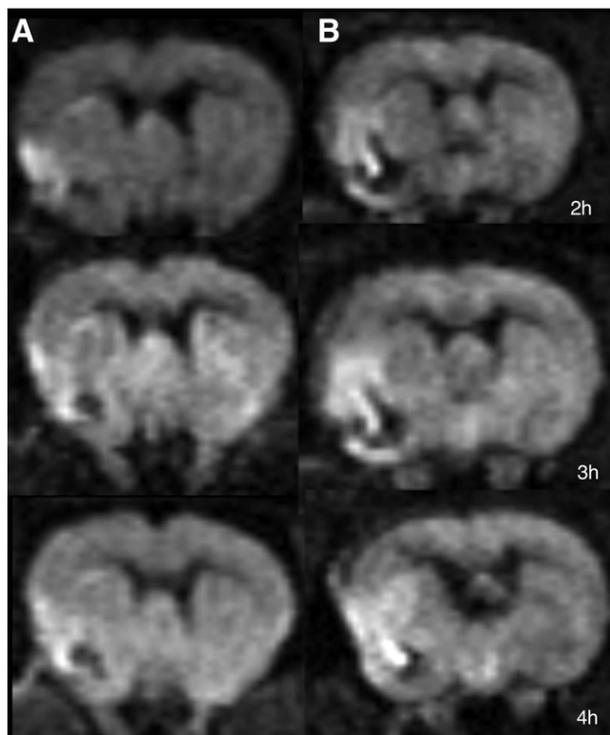


Fig. 3. Diffusion-weighted images at 2-hours (top), 3-hours (middle), and 4-hours (bottom) in a DDFPe-treated animal (column A) versus control (column B) show the relatively stable volume of signal abnormality over time with DDFPe treatment compared to increasing extent of abnormal DWI signal in the control subject over time.

that can prolong the viability of ischemic but not yet infarcted tissue until revascularization can be achieved have the potential to dramatically improve stroke outcomes. Although the field of neuroprotection has received much attention in recent years, with no fewer than a dozen agents advancing to stage III clinical trial, no such treatments have entered standard clinical use to date. In addition to therapies based on hyperbaric oxygen, hypothermia, and hemoglobin-based blood substitutes, various pharmacologic strategies have been examined including agents targeting glutamate-mediated excitotoxicity through antagonism of NMDA and AMPA receptors, calcium channel blockers, anti-inflammatory agents, antioxidants, γ -aminobutyric acid (GABA) agonists, opioid antagonists, and uric acid [3,4,11]. The failure of late stage trials of neuroprotective strategies to provide convincing evidence of benefit is multi-factorial and may involve inadequate or poorly designed pre-clinical and clinical studies, lack of biologically relevant endpoints such as functional and/or behavioral outcomes, and the inherent heterogeneity of study candidates [4,12]. Confirmatory studies employing hypothermia and uric acid in the setting of AIS are ongoing.

Herein, we present pre-clinical data demonstrating the ability of DDFPe to delay and reduce the MR imaging markers of AIS following permanent MCA occlusion in a rat model. Such validation of neuroprotective efficacy in a second animal model of AIS, in addition to the positive findings previously published by our group in a rabbit model, represents an important step on the path toward the initiation of human trials of DDFPe in AIS [5,6]. Our group continues to pursue other aspects of the pre-clinical evaluation as put forth by the Stroke Therapy Academic Industry Roundtable (STAIR) criteria including assessment of DDFPe in a temporary occlusion or reperfusion AIS model and incorporation of functional outcomes [4,13,14].

Several factors speak to the potential of DDFPe as a clinical neuroprotectant—DDFPe's superior oxygen carrying capacity among other PFC's, small particle size *in vivo* allowing delivery of oxygen

into vasculature beyond macrovascular occlusions, previous human use as a sonographic contrast agent during which no adverse events were encountered, and pre-clinical data now in two animal models showing the ability of DDFPe to decrease infarct volumes in AIS. In addition to our study showing efficacy of DDFPe administered 1 hour after occlusion, prior work by our group has shown benefit with administration up to 3 hours after arterial occlusion [5]. Thus, we posit that DDFPe may be utilized as a “bridge” to revascularization, sustaining penumbral tissue until restoration of arterial supply can be achieved. Beyond applications in AIS, the neuroprotective effects of DDFPe could be beneficial on a prophylactic basis prior to certain high risk cardiac or endovascular procedures, in acute blood loss patients, or even in the setting of cardiac arrest, in which case such an agent could be given during the early stages of resuscitation in order to preserve neuronal viability.

Limitations of this study include the use of a surrogate measure of stroke extent (number of abnormal MRI slices) rather than quantitative measurement. Due to the use of an open craniotomy occlusion model with resultant hemorrhage and gas adjacent to the region of infarction combined with the ultra-high field strength 7 T MRI environment, determination of exact infarct volume was impaired in many of the subjects. For this preliminary report, we used the number of abnormal image slices to measure the rostral-caudal extent of MR signal abnormality. Future experiments will use a filament occlusion model to avoid such artifacts and thus allow precise measurement of infarct volume in cubic millimeters. Another important component of future work will include variation in the timing of DDFPe administration including dosing at later time points than the 1-hour post occlusion schedule used in this study as well as repeated dosing. Although prior work in other animals has suggested that a single DDFPe dose may remain effective for up to 2 hours [15], further investigation of the potential benefits and possible risks of repeated dosing is required.

In conclusion, DDFPe delays the development of MRI markers of AIS and reduces eventual infarct extent in the early hours following arterial occlusion in a rat AIS model. Considered in conjunction with prior work demonstrating reduction of infarct volumes in a rabbit model of AIS, demonstration of efficacy in a second animal model represents an important step in the progress of DDFPe toward clinical trial.

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