Dodecafluoropentane Emulsion in Acute Ischemic Stroke: A Phase Ib/II Randomized and Controlled Dose-Escalation Trial

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ABSTRACT

Purpose: This randomized, placebo-controlled, double-blind, dose-escalation acute ischemic stroke trial was designed to demonstrate maximum tolerated dose, characterize adverse events (AEs), and explore clinical outcomes when intravenous dodecafluoropentane emulsion (DDFPe) was used as neuroprotection.

Methods: Acute ischemic stroke patients (n = 24) with National Institutes of Health Stroke Scale (NIHSS) score of 2–20 were randomized to either 3 doses of intravenous DDFPe or placebo, 1 every 90 minutes, starting within 12 hours of symptom onset. Doses were given without affecting standard stroke care. Each of the 3 dose cohorts included 8 patients, with 2 receiving placebo and 6 receiving DDFPe. Primary outcomes were serious adverse events (SAEs), AEs, NIHSS score, and modified Rankin Score (mRS).

Results: No dose-limiting toxicities were encountered, and no maximum tolerated dose was defined. One unrelated delayed death occurred in a DDFPe patient, and another occurred in the placebo group. Group SAEs and AEs were similar in incidence and severity. Early initiation of DDFPe treatment resulted in better NIHSS score response than late initiation ($P = .03$). Thirty- and 90-day mRS after high-dose therapy suggested clinical improvement ($P = .01$ and $P = .03$, respectively). However, the significance of differences in clinical outcomes was limited by small patient numbers and differences in stroke severity between cohorts.

Conclusions: Intravenous DDFPe appears to be safe at all doses tested. Clinical improvements in NIHSS score and mRS were significant but compromised by small sample size.

ABBREVIATIONS

AE = adverse event, DDFPe = dodecafluoropentane emulsion, mRS = modified Rankin Score, MT = mechanical thrombectomy, MTD = maximum tolerated dose, NIHSS = National Institutes of Health Stroke Scale, SAE = serious adverse event, tPA = tissue plasminogen activator

Stroke therapies have developed rapidly, with broad use of intravenous tissue plasminogen activator (tPA) and also advanced imaging and mechanical thrombectomy (MT) for some patients, but the effective therapeutic time window remains short for the vast majority of patients (1–4). This limits the number of patients who qualify for acute therapy. Enhanced neuroprotection and a wider therapeutic time window remain important goals, especially where thrombectomy may require prolonged transportation for patients.

Work from several laboratories (5–9) has demonstrated the neuroprotective effects of intravenous dodecafluoropentane emulsion (DDFPe) (NuvOx Pharma, LLC, Tucson, Arizona) in acute ischemic stroke and beneficial outcomes in other ischemic events in various animal models, including swine, rat, and rabbit. The oxygen-

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W.C.C. and R.D.S. have allowed patents and patents pending on dodecafluoropentane emulsion use in stroke. None of the other authors have identified conflicts of interest.

Appendix A and Table E1 can be found by accessing the online version of this article on *www.jvir.org* and clicking on the Supplemental Material tab.

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transporting nanodroplet DDFPe, given intravenously in the first 3 hours, can reduce stroke symptoms and stroke volumes by more than 80% and appears to widen the window for therapy by many hours (8,10). The apparent mechanism is improved oxygen transport by DDFPe nanodroplets (250 nanometers in diameter) into ischemic zones where oxygen transport by erythrocytes (8 microns in diameter) is reduced. Although collaterals seem to provide the bulk of oxygen delivery to tissue distal to an occlusion (11), the droplets may be able to pass through blockages that prevent passage of erythrocytes, which are more than 30 times larger in diameter (12,13). Here, better DDFPe oxygenation may improve metabolism and reduce the edema that disrupts erythrocyte movement in capillaries. Improved blood flow may then add erythrocyte hemoglobin transport to the DDFPe oxygen transport. DDFPe is the first perfluorocarbon and oxygen therapeutic to enter a randomized clinical trial in stroke.

Safety of intravenous DDFPe has been demonstrated in animal studies. It transports 3–7 times more oxygen than other perfluorocarbons and 9–15 times more than blood. It is effective below levels expected to cause adverse events (AEs) (12,14). However, large doses given in quick repetition caused pulmonary edema in 1 study in dogs (15), and if the nanodroplets were heated or otherwise damaged, they may coalesce and cause pulmonary emboli. Prior safe use as an ultrasound contrast agent in over 2200 patients involved an activated form of DDFPe subjected to brief negative pressure in a syringe before injection to promote microbubbles and increase visibility by ultrasound (16–18). Here, DDFPe was used in an unactivated form and in larger amounts to evaluate safety in humans with acute stroke. DDFPe is almost completely eliminated through the lungs as a gas, with a therapeutic span of about 2 hours per dose (12,16,19).

The current study was a phase Ib/II dose-escalation trial designed to demonstrate safety in acute stroke patients receiving standard care. It was designed to demonstrate the maximum tolerated dose (MTD), characterize AEs, and also explore the possible effect of DDFPe on acute National Institutes of Health Stroke Scale (NIHSS) score immediately after administration and its effect on longer-term clinical outcomes.

MATERIALS AND METHODS

Study Design

This was a single-center, double-blind, randomized controlled trial of 3 cohorts treated with increasing doses. Each cohort consisted of 6 DDFPe patients and 2 placebo patients, as the U.S. Food and Drug Administration required. Two independent physician safety monitors evaluated each case after 12 hours and after each cohort before allowing progress to the next patient. A data safety monitoring board was not required.

Briefly, inclusion criteria were acute ischemic stroke less than 12 hours from onset with an NIHSS score of 2–20 and age over 18 years. Exclusion criteria were severe hemorrhage, prior stroke, head trauma or intracranial surgery within 3 months, severe chronic obstructive pulmonary disease, or a pre-stroke modified Rankin Score (mRS) higher than 2. Full inclusion and exclusion criteria are listed in **Table E1**(available online on the article's Supplemental Material page at *www.jvir.org*).

The study was approved by the U.S. Food and Drug Administration (ClinicalTrials.gov ID: NCT02963376) with an approved investigational new drug application (IND#131594). It also received institutional review board approval (IRB#205529). Informed consent was obtained from all participants included in the study or from their legal authorized representative. NuvOx had the right to review the manuscript before publication.

Procedure

The Research Pharmacy at the University of Arkansas for Medical Sciences prepared all test drugs and provided randomization. The placebo was initially clear sterile saline and was changed for the last cohort to a sterile 1:101 dilution of Intralipid 20% intravenous fat emulsion in aqueous sodium chloride (0.9% w/v) for injection prepared by pharmacy staff according to instructions in the Pharmacy Manual. This placebo had an identical milky appearance to DDFPe. The clinical team was blinded as to the drug that was actually delivered, using separate physician injectors when required during the first cases. The 3 dose levels were determined using a modified Fibonacci bioequivalence series. Therapeutic reduction in stroke damage was previously observed at dose levels of as little as 0.1 ml/kg in rabbits. Since drug effects tend to correlate with body surface area/weight, one would predict that a dose of approximately 0.03 ml/kg should be as effective in humans compared to rabbits. Doses for the 3 cohorts were 0.05 ml/kg, 0.10 ml/kg, and 0.17ml/kg of 2% weight/volume DDFPe. Patients received the specified initial dose for the cohort given slow push intravenous over 5–10 minutes, with the expected effect lasting 2 hours before elimination via respiration. The same dose was administered again at 90 minutes and 180 minutes to maintain continuous therapeutic effect. Before, during, and after injections, patients were monitored for potential complications; vital signs, oxygen saturation, and laboratory tests were recorded. The full workup and clinical therapy were not allowed to stop if symptoms disappeared with the drug. Once selected, standard care continued in all cases. This included intravenous tPA and MT when appropriate. All MT used Solitaire Platinum retrievers (Micro Therapeutics, Inc, Irvine, California) or Trevo Retrievers (Stryker Neurovascular, Freemont, California) (**Fig 1**). Supplemental oxygen was provided to maintain oxygen saturation of more than 94%, as guidelines require. NIHSS scores were recorded at outside hospitals when appropriate and also at the study center as inside baseline NIHSS score. Repeat NIHSS scores were

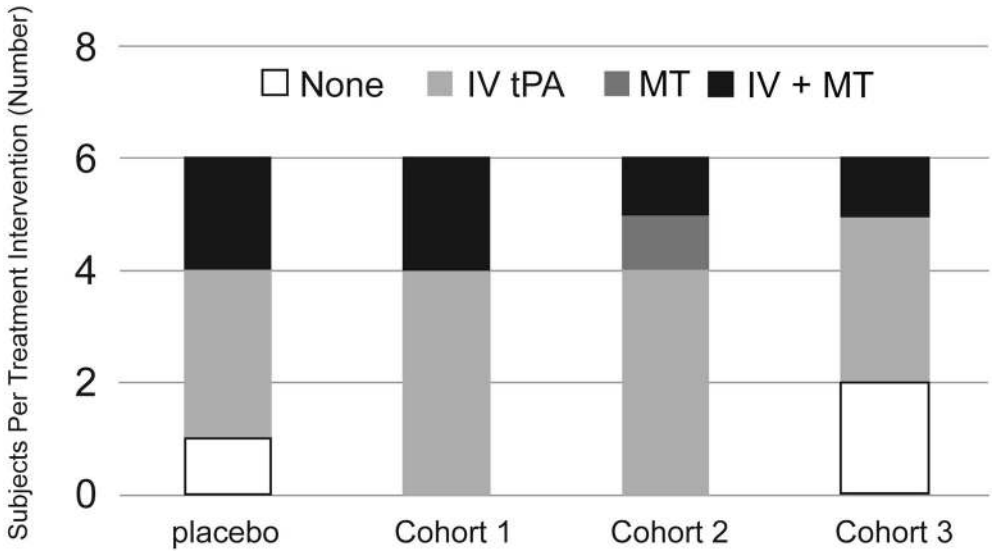

Figure 1. Bar graph of number of stroke intervention therapies with patients in placebo and treatment groups (Cohorts 1–3). Each group had conventional therapies of intravenous tPA and intravenous tPA + MT. Cohort 3 had the most non-therapy (None) stroke interventions. Cohort 2 was the only treatment group that had MT alone.

recorded at 2, 3.5, and 7.5 hours after drug injection and on discharge. "Early DDFPe" was defined as all starting DDFPe injection more less 5 hours since onset of stroke; "Late DDFPe" was defined as the final corresponding number receiving DDFPe. Monitoring continued through 8 hours after the first dose. mRS values were obtained through 90 days. Standard event definitions included dose-limiting toxicity; Common Terminology Criteria for Adverse Events, version 4, grade 3; certain electrocardiogram changes; significantly increased hypertension; and any rise in NIHSS score of 4 points or more.

Statistics

Cohort sizes were based on a double-blind, dose-escalation design with 6 DDFPe patients and 2 placebo patients. All patients were assessed for safety during drug dosage and after 12 hours by medical monitors prior to enrolling subsequent patients. Baseline characteristics of age and gender, as well as stroke treatment delivered prior to or during DDFPe administration, were summarized (Table 1, Fig 1). Safety results of serious adverse events (SAEs), deaths, and AEs were reported (Table 1). Differences were reported as significant when P was $\leq$ .05. Tests for significant differences of baseline characteristics, safety results, and individual AEs were calculated using the Mann-Whitney test for continuous data and Fisher's exact test for numbers of events. Medians and ranges of NIHSS score and mRS values with Mann-Whitney P values for group comparisons are presented in tables and graphs. The initial trial design pre-specified to examine differences in NIHSS scores for patients who received treatment early versus late (Table 2). The analysis used Fisher's exact test and the Mann-Whitney (non-parametric) test. The relationship between the NIHSS scores in the placebo group and the 3 DDFPe cohorts was assessed by time, although the power was limited by the small sample sizes. Data analysis was performed using SAS (version 9.3) and StatXact-11 (Cytel Inc, 2015) software.

RESULTS

During the study period, 26 patients or their legal authorized representatives were contacted and agreed to participate. Of these, 24 gave written informed consent (Table 2, Fig 1) and were included in the study. Two were excluded on the basis of exclusion criteria for prolonged QT intervals. All standard stroke therapy elements continued without interruption. Stroke therapeutic elements took precedence over research elements but never caused delays of drug injection or other major research elements. Vital signs were missed at 35 of 775 scheduled points due to transportation issues.

All patients completed the study to hospital discharge, and all but 2 completed follow up to 90 days (Table 2; Figs 2, 3). One patient died at 39 days of pneumonia (DDFPe Cohort 1), and 1 placebo patient died at 25 days of a presumed second stroke. Patients with SAEs were more common in the control group than in the DDFPe groups (P = .04), but this was thought to be spurious due to the small sample number. No other SAE or AE distribution was significant. Hypertension, cough, headache, and musculoskeletal pain were the most common AEs but occurred in both the DDFPe and placebo groups (Table 1). Only 1 asymptomatic bleed was encountered. It was a parenchymal hematoma type 1 in a placebo patient who had both intravenous tPA and thrombectomy and improved clinically from NIHSS score 18 to 8. No related laboratory abnormalities were identified, and oxygen was used in 5 of 24 patients, which was consistent with standard care. One was in a placebo patient. For details of

Table 1. Baseline Characteristics, Safety Results, and AEs

Baseline characteristics	**Controls 6 (100)**	**DDFPe 18 (100)**
Age (years), mean ± SEM	55.8 ± 7.1	56.9 ± 2.6
Male, n (%)	5 (83)	11 (61)
LKW to Drug (min) [median low-high]	396.5 (6.61 h, [5.25–11.8])	380 min (6.33 h, [4.35–10.25])
Treatment Procedures[†]		
IV tPA only	3 (50)	11 (61)
IA Rx only	0 (0)	1 (6)
Both IV and IA	2 (33)	4 (22)
Neither IV or IA	1 (17)	2 (11)
Safety Results (Number of Patients)[†]		
	Controls	**DDFPe**
SAE (Including deaths)	3 (50)	1 (6)
Deaths	1 (17)	1 (6)
AE	6 (100)	15 (83)
Individual AEs Occurring Twice or More (Number of Patients) [†]		
	Controls	**DDFPe**
Cough	1 (17)	4 (22)*
HTN	1 (17)	4 (22)‡
Headache	2 (33)	3 (17)
MSK Pain	1 (17)	3 (17)
Cold Feeling	1 (17)	2 (11)
Nausea	1 (17)	2 (11)
Flushing	1 (17)	2 (11)
Falls	0 (0)	2 (11)
Restlessness	1 (17)	1 (6)
Sleep Apnea	1 (17)	1 (6)

Note–Single episodes of the following were encountered but showed no pattern except in SAE patients described below: Abnormal lab, hypoglycemia, EKG abnormality, bradycardia, hyperventilation, agitation, CHF worsening due to fluid overload, one asymptomatic hemorrhage in a placebo patient, blood transfusion due to unrelated anemia on admission, pruritus, pneumonia, renal insufficiency, cardiac pause, leukocytosis, fever, confusion, depression, migraine, hemorrhoids, and myocardial infarction.

CHF = congestive heart failure; DDFPe = dodecafluoropentane emulsion; IA = intra-arterial; IV = intravenous; HTN = hypertension; LKW = last known well; MSK = musculoskeletal; SAE = significant adverse event; SEM = standard error of the mean; tPA = tissue plasminogen activator.

*1 pre-existing.

[†]Values are the number (%).

[‡]All pre-existing.

SAE patients, see **Appendix A** (available online on the article's Supplemental Material page at *www.jvir.org*).

All intravenous tPA patients received standard doses and care. Exact technique details of MT varied widely between the 4 interventional physicians. Four patients met the requirements for "Early" DDFPe administration within 5 hours of onset. The latest 4 receiving DDFPe became the comparison group, all after 9 hours from onset, (**Fig 2**, **Table 2**). "Early" included 4 intravenous tPA, and 2 also received MT. "Late" included 2 with intravenous tPA, 2 with MT, and 1 of these had both. One required neither.

DISCUSSION

In this study, safety of intravenous DDFPe in acute stroke was demonstrated at all 3 dose levels, and few events thought to be related to the drug were encountered. AEs and SAEs were characterized, and their incidence and severity were similar in control and DDFPe patients. No signs of dose-limiting episodes were identified at any dose level, and no MTD was defined. All dose levels appear to be well tolerated and safe as given.

One delayed death occurred in a control patient from a second stroke, and another death occurred in a DDFPe patient from pneumonia. Both are common occurrences in stroke patients. Although minor sensations, such as cough, mild hypertension, headache, muscular discomfort, nausea, a cool sensation in the arm, and other minimal perceptions in a few patients, may be related to the test drug injections, these were uncommon (**Table 1**), and nothing was typically reported by the patients receiving the drug.

The exploratory aim assessing the utility of NIHSS score to judge the immediate effect of DDFPe showed that all cohorts, including controls, improved in a similar pattern. The NIHSS score versus time slopes of all DDFPe cohorts and placebos were similar and progressed toward improvement in the time period studied. The overall improvement was to be expected since 21 of 24 patients received some reperfusion therapy, and all received optimum stroke care by the clinical stroke team.

The 4 patients who received DDFPe early showed a significantly more rapid drop in NIHSS score than those receiving later administration (**Fig 2**, **Table 2**). DDFPe has been shown to be effective in animals when starting treatment as late as 3 hours after occlusion of an artery. However, DDFPe was not as effective in the animal model after 6 hours of delay (9), suggesting that those patients receiving DDFPe early may do better than those receiving it late. For this study, an early/late treatment comparison was pre-specified in the trial design. Still, there was no apparent long-term effect on the mRS after 30 or 90 days in these groups. Here, relationships were more closely tied to reperfusion of blood rather than transient DDFPe oxygen transport. Given the small sample size, one must be cautious in drawing inferences about presence or absence of short- and long-term therapeutic effect.

When only the high-dose group of DDFPe was compared to placebo, there was a significant improvement, in both 30-day and 90-day mRS ($P = .01$ and $P = .03$, respectively) (**Fig 2**). These significant differences were encouraging, but with these small numbers the effect should be confirmed with a larger efficacy study. This was especially true since the initial outside NIHSS score median for the placebo group was 13, similar to all DDFPe cohorts at 9, but somewhat higher than the NIHSS score for the Cohort 3 DDFPe group at 5, with each $P = .19$ or higher. The baseline NIHSS scores for Placebo versus Cohort 3 DDFPe were more similar at 9.5 and 4 ($P = .19$) on arrival at the hub hospital and enrollment into the study.

Table 2. Efficacy

	Controls (n = 6)	DDFPe (n = 18)	DDFPe in Cohort			Mann-Whitney (*P* value Control vs. Cohort 3)
			1 (n = 6)	2 (n = 6)	3 (n = 6)	
			NIHSS Assessment Performed: (median; [min, max])			
Outside (PreRx)	13 [5,18]	9 [2,21]	10 [6,21]	13 [7,16]	5 [2,14]	.12
Baseline (PreRx)	9.5 [3,17]	6.5 [0,14]	6.5 [0,12]	8 [4,13]	4 [2,14]	.19
2 hours,	8 [3,17]	5 [0,15]	6 [0,14]	6.5 [1,15]	2 [0,6]	.03*
3.5 hours,	5.5 [3,14]	4.5 [0,15]	5.5 [0,12]	7 [1,15]	2.5 [0,5]	.05*
7.5 hours,	4.5 [2,14]	2.5 [0,13]	5.5 [0,9]	5.5 [1,13]	2 [0,4]	.02*
Day 7 or DoD	3.5 [1,11]	1 [0,11]	2‡ [0,5]	4 [0,11]	1 [0,2]	.05*
		mRS Assessment Performed: (median; [min, max])				Mann-Whitney (*P* value Control vs. Cohort 3)
Day 7 or DoD	2 [1,4]	2 [0,4]	2 [0,3]	3 [0,4]	1.5 [0,2]	.39
30 day	2.5 [1,6]	1 [0,6]	2 [0,6]	2 [0,4]	0 [0,1]	.01*
90 day	3 [0,6]	1 [0,6]	1.5 [0,6]	2 [0,3]	0 [0,1]	.03*

	NIHSS Measurements by Early, Mid and Late DDFPe Administration (median; [min, max])			
NIHSS	Early DDFPe (<5 hours)† (n = 4)	Mid DDFPe (5–9 hours) (n = 10)	Late DDFPe (>9 hours)† (n = 4)	Mann-Whitney (Early vs. Late) (*P* value)
Baseline	7 [3,14]	5.5 [0,13]	9.5 [2,13]	.99
2 hours,	2.5 [1,6]	5 [0,15]	9.5 [2,14]	.23
3.5 hours,	2 [1,3]	5 [0,15]	9 [4,12]	.03*
7.5 hours,	1.5 [1,2]	3.5 [0,13]	7.5 [2,10]	.09
Day 7 or DoD	0.5 [0,1]	1.5 [0,7]	2 [1,11] ‡	.17
	mRS Measurements by Early, Mid, and Late DDFPe Administration (median; [min, max])			
mRS	Early DDFPe (<5 hours)† (n = 4)	Mid DDFPe (5–9 hours) (n = 10)	Late DDFPe (>9 hours)† (n = 4)	P Mann-Whitney (Early vs. Late) (*P* value)
Day 7 or DoD	1.5 [0,3]	2 [0,4]	2.5 [1,4]	.32
30 day	0.5 [0,6]	1 [0,4]	2 [0,4]	.89
90 day	0.5 [0,6]	1 [0,3]	1.5 [0,3]	.88

Note–PreRX NIHSS of Cohort 3 is < Control even though blinded and randomized.
DDFPe = dodecafluoropentane emulsion; DoD = day of discharge; mRS = modified Rankin Score; NIHSS = National Institutes of Health Stroke Scale; PreRX = period prior to placebo or DDFPe treatment.
*Statistically significant at $P \leq .05$.
†Early DDFPe was defined as the patient received DDFPe at <5 hours from their stroke event. Late was defined as an equal number of the last receiving DDFPe which resulted in >9 hours from stroke event (n = 4). All mRS in the Late and Mid categories were nonsignificant.
‡One patient was missing this assessment.

This suggests that, although properly blinded and randomized, this cohort of high-dose patients had less severe strokes, and this could compromise the comparison. This exploratory aim was not powered to demonstrate efficacy, and these trends might be proven or disproven in a properly powered efficacy study.

When designing a future efficacy study, several critical points that were not part of this safety study must be considered. First, DDFPe should be given early after stroke onset when it is most effective. The best trial that met this requirement was the FAST MAG trial (20). In it, most test drug injections were started in the field by emergency medical technicians in under 1 hour. Second, with embolectomy capable and comprehensive stroke centers often located hours away from referring stroke-ready hospitals, the delivery of neuroprotectant in stroke hospitals before transfer to comprehensive stroke centers has great potential.

Since this neuroprotectant is a bridge to reperfusion by whatever method, speculation that the action of "pausing the clock" will have good clinical effect with early administration and when required delays after administration to reperfusion are maximal is justified. It will have the least positive effect when reperfusion delay is minimal. Routine delays in both intravenous tPA reperfusion and MT meet this requirement very well (21,22).

Potential uses are yet to be fully defined, but all ischemic situations should be considered. For acute strokes, this would include early field use in ambulances, early small hospital "drip and ship" use, and large hub hospital use. Essentially, all acute strokes could profit with immediate bridging oxygenation while working toward reperfusion. Trauma of brain or spinal cord also involves some ischemia, which might be responsive. Surgery with high stroke risk, cardiac arrest, simple blood loss, and acute ischemia in other

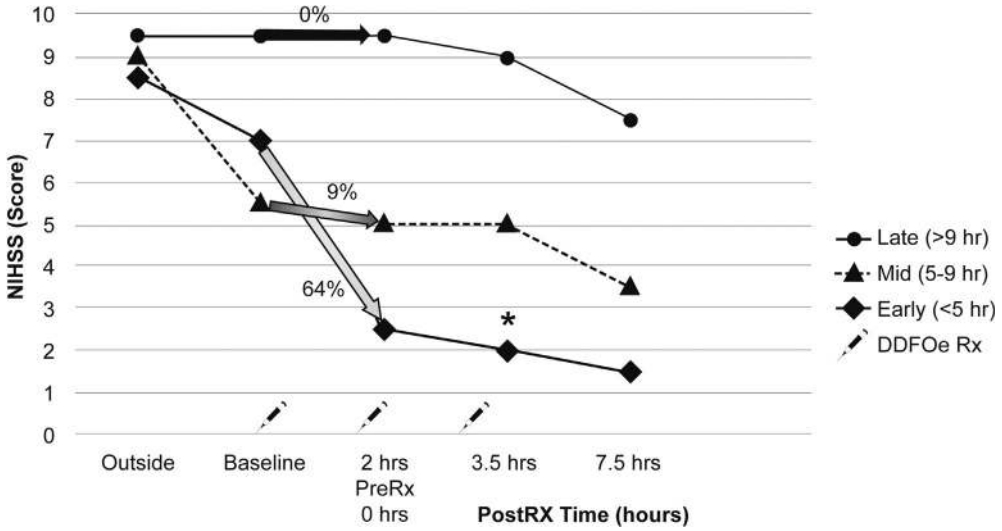

Figure 2. Effect of early versus late DDFPe administration on NIHSS scores. In DDFPe-treated Cohorts 1–3, administration of DDFPe at earlier time points (<5 hours; n = 4) from the initial stroke event was associated with rapidly decreased NIHSS scores (64%) compared to no decrease in the first 2 hours for late time points (>9 hours; n = 4). The mid-time-point group ranked between the early and late groups at 9%. Median NIHSS scores are presented in non-linear time increments (x-axis). PreRX = study point prior to DDFPe administration. *Significance, $P = .03$.

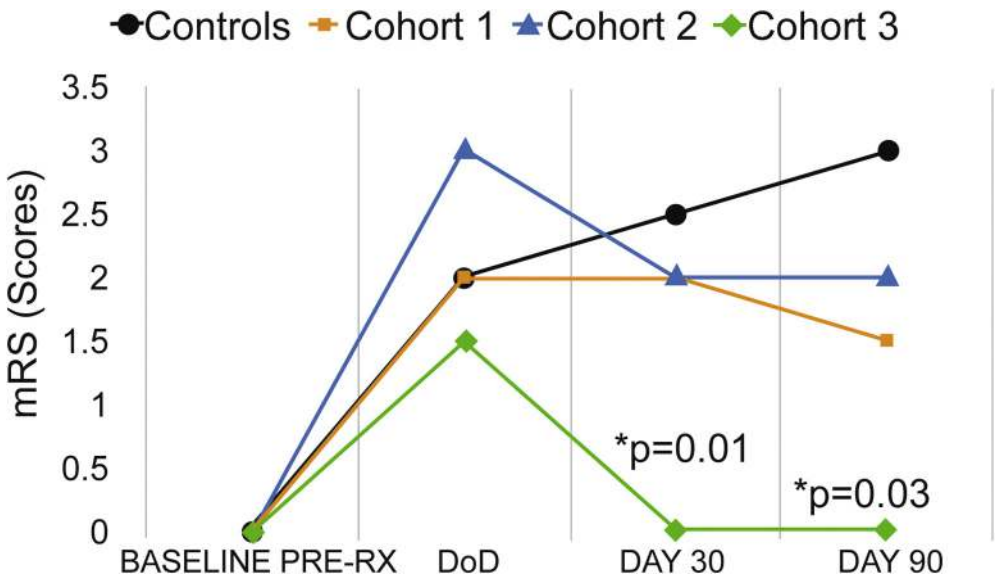

Figure 3. Comparison of Placebo (Control) mRS versus mRS from DDFPe cohorts. The mRS values were significantly decreased (*) at day 30 and day 90 ($P = .01$ and $P = .03$, respectively) in the high-dose DDFPe (Cohort 3) (n = 6) versus Placebo (Controls) (n = 6). Medians are presented. PreRX = study point prior to DDFPe administration; DoD = day of discharge.

organs such as bowel, limbs, and myocardium may also prove responsive to this approach. Many speculative applications have yet to be studied.

Limitations of the current study primarily concern the exploratory aim toward efficacy, which was greatly underpowered for any purpose other than designing the next study. The inclusion of ischemic strokes with mild hemorrhagic transformation was not accomplished, although the protocol was amended after the first 2 cohorts to include mild and moderate bleeds. The failure to define the MTD suggested that higher doses should be tested, but the expected therapeutic range was covered. As with any phase I trial, the small number of cases may only demonstrate side effects and complications that are common, and rare problems must await larger trials to become apparent.

In summary, the safety of DDFPe used with standard stroke therapies was demonstrated with no sign of dose-limiting toxicity. The possible drug-related side effects were mild. The clinical outcome from the high-dose cohort suggested efficacy, and there were trends overall toward long-term efficacy. Earlier treatment seemed more promising here, and findings were consistent with previous animal studies (9) and clinical reviews (4,22).

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APPENDIX A.

SAE Case Detail

A 79-year-old female with atrial fibrillation had an NIHSS score of 12 and received intravenous tPA and placebo study drug. She was discharged on day 4 with an NIHSS score of 4. She had a pacemaker generator changed 6 weeks later and return of stroke symptoms when off anticoagulation. The patient had intermittent expressive aphasia with "word salad" and also depression.

A 67-year-old female with a history of persistent atrial fibrillation could not tolerate anticoagulation, sustained an ischemic stroke, and presented with an NIHSS score of 9. She received intravenous tPA and embolectomy and low-dose DDFPe. She had previous pneumonia and bradycardia but recovered before her stroke. She was discharged with NIHSS sore of 0 and was asymptomatic. On day 14, she was admitted elsewhere with worsening renal function, which resolved, and pneumonia, which worsened. She died of pneumonia on day 39.

A 70-year-old male with borderline renal function, treated hypertension, and treated colon cancer had an NIHSS score of 5 and received placebo study drug. He did not qualify for intravenous tPA or embolectomy. He improved and was discharged on day 3. He was improving on physical therapy when he had an apparent massive stroke on day 25 and died.

A 31-year-old male had an NIHSS score of 14 and received intravenous tPA at an outside hospital. He had a borderline prolonged QT. This was reviewed by the study safety monitor and received a waiver to enter the study. The patient received placebo study drug. NIHSS score rapidly fell to 4, and his echocardiogram was normal. On day 6, he had recurrence of stroke symptoms but was diagnosed as complex migraine, which resolved within 4 hours. He was normal on 30- and 90-day follow-up.

Table E1. Detailed Criteria for Study Inclusion and Exclusion

Inclusion Criteria	
Study Code	**Criteria**
6.2.1	Age 18 years old or older
6.2.2	Diagnosis of AIS
6.2.3	Body weight ≥45 kg
6.2.4	NIHSS score between 2 and 20 inclusive
6.2.5	Patient or legal authorized representative must be willing and able to understand the study and provide written informed consent.
Exclusion Criteria	
Study Code	**Criteria**
6.3.1	Currently pregnant or breastfeeding
6.3.2	History of significantly impaired renal or hepatic function
6.3.3	Severe hemorrhage or severe hemorrhagic stroke on CT scan. Mild or moderate changes of Fisher grade 1 or 2 subarachnoid hemorrhage are allowed, as are hemorrhagic transformation changes of grade HI-1 and HI-2.
6.3.4	Prior stroke, intracranial surgery, or major head trauma within 3 months prior to enrollment
6.3.5	Pre-stroke mRS ≥2
6.3.6	Myocardial infarction within 6 months prior to enrollment
6.3.7	Unstable angina, NYHA Class II or greater congestive heart failure
6.3.8	Uncontrolled hypertension with treatment (SBP >180 mmHg and/or DBP >110 mmHg)
6.3.9	Uncontrolled arrhythmia or history of clinically significant arrhythmia within the past 6 months (except atrial fibrillation)
6.3.10	Clinically significant COPD or other pulmonary condition that is not controlled by medication or requires oxygen frequently or continuously
6.3.11	Pneumonia, bronchitis, or other acute respiratory disease
6.3.12	Current anticoagulant therapy except for antiplatelet therapy (aspirin, NSAIDs) and prophylactic doses of low-molecular-weight heparin to prevent deep vein thrombosis. Note: tPA administered as part of patients' therapy for AIS is allowed. However, thrombolytic therapy is not a study requirement.
6.3.13	History of allergic reaction attributed to compounds of similar chemical composition to DDFPe or soy or egg allergies (see Investigator's Brochure)
6.3.14	Patient has received any investigational drug within 30 days prior to enrollment into the study
6.3.15	Inability to comply with the study procedures
6.3.16	History or evidence of any other clinically significant condition that, in the opinion of the investigator, might pose a safety risk to subjects or interfere with study procedures, evaluation, or completion

Note–For the first 2 cohorts, additional requirements included age less than 81 years, and long QT values and hemorrhages of any sort were not allowed. This was amended as above.

AIS = acute ischemic stroke; COPD = chronic obstructive pulmonary disease; DB = diastolic blood pressure; DDFPe = dodeca-fluoropentane emulsion; HI = hemorrhagic infarction; mRS = modified Rankin Score; NIHSS = National Institutes of Health Stroke Scale; NSAID = nonsteroidal anti-inflammatory drug; NYHA= New York Heart Association; QT = 2 points Q & T on the common electrocardiogram; SBP = systolic blood pressure; tPA = tissue plasminogen activator.