

# Effects of Perfluorocarbon Dodecafluoropentane (NVX-108) on Cerebral Microvasculature in the Healthy Rat

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**Abstract:** NVX-108, a dodecafluoropentane-based perfluorocarbon (PFC) emulsion, has therapeutic potential as an oxygen-carrying fluid for emergency medical treatment for traumatic brain injury (TBI) and hemorrhagic shock. Potential cerebral vasoactive properties were assessed by directly measuring pial arteriolar vessel diameters before and after a 30 minute intravenous (IV) infusion of 1.0 ml/kg (high dose [H]) or 0.25 ml/kg (low dose [L]) NVX-108 compared to 2.0 ml/kg Saline (control) in healthy anesthetized rats (N = 6/group). Results showed that post-infusion vessel diameters for small (< 50  $\mu$ m) and medium (50-100  $\mu$ m)-sized pial arterioles were significantly ( $p < 0.05$ ) narrower after only the NVX-108 H infusion although this vasoconstriction was not statistically significant when analyzed as a percentage change in these vessels. Pial arteriolar vessel diameters were not significantly different for mean value or percentage change after either NVX-108 L or Saline infusions. There were no significant post-infusion changes from baseline in systolic, mean or diastolic blood pressures after any of the treatments although post-infusion blood pressure was statistically higher in the NVX-108 L group compared to NVX-108 H and Saline groups. Arterial blood gases, methemoglobin and lactate were not different from baseline or among groups. No adverse events were observed at either dose of NVX-108. In conclusion, neither 0.25 nor 1.0 ml/kg NVX-108 caused vasoconstriction in cerebral pial arterioles of healthy rats nor resulted in blood pressure changes; the compound should be considered for further investigation for TBI therapy.

**Keywords:** Cerebral microcirculation, dodecafluoropentane, intravital microscopy, oxygen therapeutic, perfluorocarbon, perflutren, pial arteriolar vessel diameter, rat, traumatic brain injury, vasoconstriction.

## INTRODUCTION

Tissue hypoxia and ischemia caused by reduced blood flow (e.g., hemorrhage or ischemic stroke) are leading causes of preventable deaths worldwide [1]. Improved oxygen delivery during such an event could prevent tissue damage and decrease mortality. However, current fluid resuscitation therapies such as crystalloids and colloids lack the oxygen-carrying capacity of blood. The administration of blood products is logistically difficult in pre-hospital settings: there are challenges with determining biocompatibility, the cumbersome need for refrigeration and are liable blood supply are not always available or, in the event of a mass casualty, large enough.

Drugs from two classes of products, perfluorocarbons (PFC) and hemoglobin-based oxygen carriers (HBOCs), have been developed, evaluated and abandoned due to safety or efficacy issues for nearly three decades. PFCs are synthetically produced by replacing all of the hydrogen atoms in their hydrocarbon structure with fluorine atoms [2]. The hydrophobic perfluorocarbon is then emulsified with surfactants and salts to make the product miscible with water

and suitable for intravenous (IV) administration. Fluosol-DA (Alpha Therapeutics, California) was FDA-approved in 1989 as an "oxygen therapeutic" to treat myocardial ischemia during balloon angioplasty, but the product was withdrawn because of storage and preparation difficulties [3, 4] and was reported to cause pulmonary hypertension [5, 6]. No PFC is currently approved in the USA as an IV oxygen-carrying fluid, and only one, Perftoran, is clinically-approved for applications outside the USA (Russia and Mexico) [7].

Dodecafluoropentane (DDFP), also known as perfluoropentane, is a relatively new PFC for oxygen therapy and is the main component of NVX-108 (NuvOx Pharma, Tucson, AZ). NVX-108 is an emulsion of 2% w/v DDFP stabilized by surfactant in a buffered sucrose solution. The boiling point of DDFP is  $\sim 29^\circ$  C. When below this temperature (outside the body), NVX-108 consists of nano droplets with a mean particle size of  $\sim 350$  nm diameter and 99% of particles are less than 700 nm diameter. Because of surface tension (i.e., Laplace pressure), DDFP remains in the condensed state when administered IV unless it is pre-activated to form microbubbles prior to IV injection (e.g., by creating negative pressure in the syringe) [8]. When warmed to  $37^\circ$  C *in vitro* (i.e., body temperature), the NVX-108 droplets expand slightly providing them with an enhanced oxygen carrying and transport ability [9]. Ultrasound studies indicate that microbubbles do not form after IV injection of DDFP unless the material is pre-activated to form microbubbles. However,

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enough expansion may occur to provide room for enhanced oxygenation of the blood. Unlike larger bubbles, which can manifest as gas embolism or decompression sickness when in the bloodstream, the DDFP droplets (350 nm diameter) are small enough to pass through systemic and pulmonary capillary beds with no adverse effects. Compared to higher molecular weight PFC with higher boiling points, the doses of DDFP are less than one one-hundredth of the other agents on a weight basis for use as an oxygen therapeutic [2].

At low doses, DDFP is safe as a contrast agent for ultrasound imaging studies in humans [10]. Pre-clinical NVX-108 studies have shown that it can improve tissue oxygenation during hypoxic events. In animal models of stroke, NVX-108 treatment following occlusion of the middle or anterior cerebral artery reduced the infarct volume compared to control animals [11, 12]. Lundgren [12] showed 100% survival after the administration of NVX-108 (0.014 ml/kg) to rats with an otherwise lethal anemia. Lundgren (2006) further showed that NVX-108 microbubbles can exchange respiratory gases *in vitro* with a surrounding aqueous medium. The product has a short *in vivo* half-life (minutes) although its therapeutic effect is more sustained (>90 min) [13]. Metabolism isn't required for drug clearance as it is removed from the body through exhalation from the lungs [14].

These study results and product characteristics suggested that NVX-108 might be suitable for pre-hospital military medical care. On the other hand, much higher doses might be required for Traumatic Brain Injury (TBI) and at least one canine study indicates that a higher dose of DDFP (cumulative dose 4.0 ml/kg) resulted in deteriorating cardiopulmonary parameters [15]. Our laboratory is particularly interested in improving pre-hospital combat casualty care for wounded warriors suffering from TBI. Since regulatory approval of earlier oxygen-carrying therapeutics was hampered by concerns over vaso-active drug characteristics [16], NVX-108 was screened for vasoconstrictive effects using a healthy rat model prior to evaluation in more complex TBI models. Using two different clinically-relevant single doses of NVX-108, this study was designed to directly measure changes in cerebral pial arteriolar vessel diameters using intravital microscopy and secondarily indirectly assessing vasoactivity in the systemic circulation by measuring changes in blood pressure.

## MATERIALS AND METHODS

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research.

Healthy male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were anesthetized with a combination of 72 mg/kg ketamine and 4 mg/kg acepromazine I.P. Rats were intubated and mechanically ventilated (RSP1002, Kent Scientific Corp., Lichtfield, CT) to maintain normocapnia (PaCO<sub>2</sub> between 35 to 45 mm Hg) and prevent hypoxemia (PaO<sub>2</sub>> 60 mm Hg). Inspired gases were 40% medical grade oxygen mixed with 60% nitrogen (Airgas USA, Hyattsville, MD). The femoral artery and vein were

cannulated with PE-50 catheters for arterial blood sampling and blood pressure monitoring (Spectrum Datascope Pressure Monitor; Datascope Corporation, Montvale, New Jersey) and IV infusion of the NVX-108 or control solution. Core body temperature was monitored rectally and maintained at 37 °C with a warming pad (Homeothermic Monitor, Harvard Apparatus, Harvard, MA).

Access to cerebral pial microcirculation was gained through a rectangular craniotomy (~2x4 mm) prepared in the right parietal bone as previously described [17]. The dura was cut and reflected to allow visualization and imaging of pial surface microvasculature. The surface of the brain was superfused with artificial cerebrospinal fluid (Na 150 mM, K 3.0 mM, Ca 1.4 mM, Mg 0.8 mM, P 1.0 mM, Cl 155 mM; Harvard Apparatus, MA) to maintain electrolyte balance. A glass cover slip covered the craniotomy to prevent drying of the brain surface. A stereomicroscope (SZ16, Olympus, Japan) equipped with a DP-73 digital camera was used for direct visualization and imaging of pial vessels at 80X.

After instrumentation and stabilization, baseline (BL) data were collected. Rats (N =25) were then allocated to receive a single 30-min 4 ml/kg/h IV infusion of test fluid: 1) high-dose [H] NVX-108 (1 ml/kg, N =6), 2) low-dose [L] NVX-108 (0.25 ml/kg, N=6), or 3) 0.9% Normal Saline [NS] (Abbott Laboratories, Chicago, IL; n=14). The NVX-108 was diluted with NS as necessary for all groups received an equivalent volume of fluid. As per the manufacturer's recommendations, NVX-108 was stored between experiments at 19° – 25° C (21.8 ± 0.1°C [mean ± SEM]).

Pial arteriolar vessel diameters, hemodynamic parameters (systolic, diastolic and mean arterial pressures), and vital signs (heart rate and body temperature) were monitored and arterial blood samples were collected at the beginning and end of the test infusion. Pial arteriolar diameters were measured at the same locus throughout the experiment using a computer program (CellSens, Olympus, 2010). At the end of the study, a 5% aqueous barium chloride solution was applied topically to the pia to evaluate vessel responsiveness as barium chloride has been shown to be a strong vasoconstrictor in rodent cerebral pial vessels [18]. Arterial blood samples were analyzed (ABL 700, Radiometer, Copenhagen, Denmark) for pH, partial pressures of oxygen (PaO<sub>2</sub>) and carbon dioxide (PaCO<sub>2</sub>), lactate, total hemoglobin, methemoglobin, and bicarbonate (HCO<sub>3</sub><sup>-</sup>).

For statistical analysis, the pial arteriolar vessel diameters were divided into three categories based on size at BL: 1) "small"-sized vessels had diameters less than 50 μm, 2) "medium"-sized vessels had diameters of 50 to 100 μm, and 3) "large"-sized vessels had diameters greater than 100 μm. Grouping the vessels into these categories is the convention in the microvascular literature [19, 20] and is scientifically justified since the amount of smooth muscle increases with vessel size and the degree of contraction correlates with the amount of vascular smooth muscle [21]. Because the vessels analyzed were categorized into three groups through convention, it was conceivable that overall differences in the size categories between the groups might have been due to random differences in the BL sizes of the vessels within the groups. For this reason, data were further analyzed using the absolute and percentage change in vessel size. Data analyses

were performed using IBM SPSS Statistics 21.0 (IBM Corporation, 2012). Mixed general linear models were used for analyzing heart rate, blood pressures, absolute and percent change in vessel diameters, temperature, blood gases, methemoglobin and lactate data, while body weights were analyzed using a one-way analysis of variance. Least square means were used for posthoc t-tests. For all parameters, a  $p$ -value  $\leq 0.05$  was considered statistically significant. All results are reported as mean  $\pm$  SEM.

## RESULTS

Twenty-five (25) of the 26 animals survived the experimental protocol without adverse clinical signs while one animal (NS) was excluded due to a fatal ventilator malfunction. There were no significant differences among the groups in body weight ( $397 \pm 8$  g) or temperature ( $36.7 \pm 0.1^\circ\text{C}$ ). Post-infusion heart rates were not different from baseline for any treatment nor were there differences among the groups (Table 1). Overall, heart rates were  $302 \pm 10$  beats/min pre-infusion and  $301 \pm 9$  beats/min post-infusion.

None of the infusions caused a significant change in blood pressures (Table 1, Fig. 1). Despite a lack of effect within a treatment group, the post-treatment SBP and MAP of NVX-108 [L] group were each  $\sim 15$  mm Hg higher than the NVX-108 [H] measurements and  $\sim 17$  mm Hg higher than the NS control measurements. Although statistically significant, this was thought to be due to the consistently higher blood pressure measurements in the NVX-108 [L] group, even at BL (Fig. 1) as well as the small sample size. For any individual NVX-108 [L] rat, the highest SPB was 158 mm Hg and highest MAP was 129 mm Hg.

Pial vessel diameters are summarized in (Table 2 and Fig. 2). The number of vessels within a treatment group ranging from 62 to 71 for the small-, 40 to 56 for the medium-, and 1 to 6 for the large-sized vessels. Due to the small number of large-sized vessels, these data were not analyzed for statistical differences. Topical administration of barium chloride showed vasoconstriction compared to BL in both small-sized and medium-sized vessels in all three treatment groups. For *within* treatment group differences, the only pre-treatment vs post-treatment difference was a significant decrease in small- ( $P = 0.002$ ) and medium-sized ( $P < 0.001$ ) vessels following

the NVX-108 [H] infusion although there was a visual trend for vessel diameters to decrease in all three treatment groups over time (Fig. 2). There were no changes in small- and medium-sized vessels following either NVX-108 [L] or NS infusions (Table 2, Fig. 2) and there were no differences in percent change of these vessel sizes among the three treatment groups (NVX-108 [H], NVX-108 [L], and NS;  $P \geq 0.40$ ).

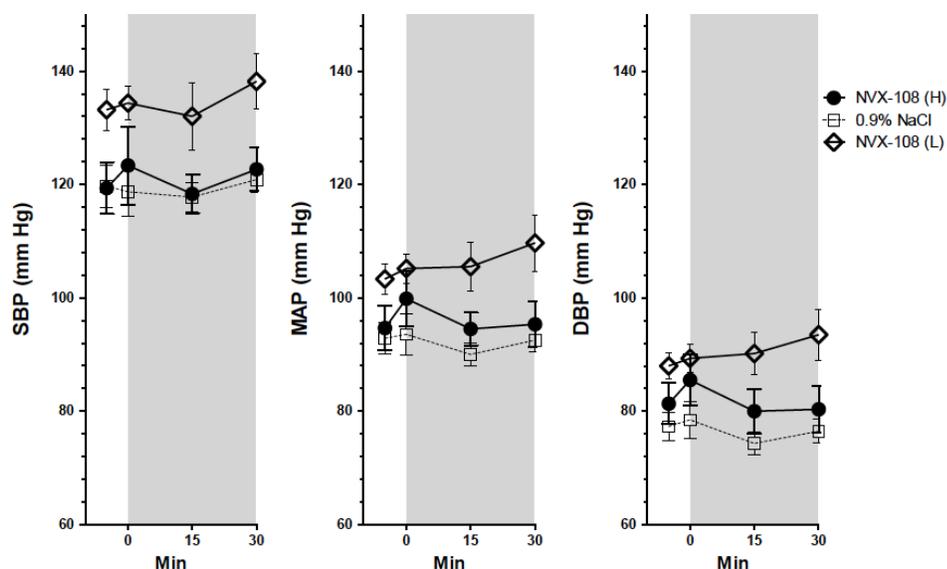
Blood hemoglobin, lactate, and bicarbonate levels did not show any statistically or clinically significant changes over time or among groups (Table 3). Methemoglobin concentrations were too low to be detected in all groups (data not shown). Arterial blood gas analysis showed some minor, clinically insignificant changes in arterial  $\text{PaO}_2$  and  $\text{PaCO}_2$  ( $\text{PaO}_2 > 140$  mm Hg,  $\text{PaCO}_2 < 50$  mm Hg; Table 3).

## DISCUSSION

This study in healthy anesthetized rats demonstrated that the PFC NVX-108 as a single dose at either 1.00 or 0.25 ml/kg IV caused no significant percent change in small- and medium-sized (less than 100 nm) cerebral pial arteriolar vessel diameters as directly measured using intravital microscopy and showed no evidence of increases in systemic vasoconstriction as indirectly assessed by measuring systemic blood pressures. Although the pial arterioles showed a trend towards vasoconstriction over time, this occurred in all treatment groups (including the Control group) and such a trend was similarly reported in a previous study [19]. Therefore this trend toward a decrease in vessel diameters is likely due to changes over time in the experimental system such as local changes in surface brain temperature or other factors influencing the vessels once the dura was cut and the pial vessels exposed to atmospheric pressures. Numerous physiological factors including oxygen levels, blood viscosity, hydrostatic capillary pressures, and vasoactive mediators (i.e., nitric oxide and endothelin-1) also play a role in the regulation of arteriolar diameters and may have contributed to the observed trend. As none of these parameters were measured in this study, the cause(s) for the trend remains uncertain but the lack of a specific effect from NVX-108 on vessel size suggests NVX-108 did not amplify or diminish the impact these effects may have had on vessel diameter.

**Table 1. Systemic blood pressures. Mean (MAP), systolic (SBP), and diastolic (DBP) blood pressures and heart rate before (Time 0) and after 30-min infusion of Normal Saline (NS), 0.25 ml/kg NVX-108 [L] or 1.0 ml/kg NVX-108 [H] (means  $\pm$  SEM). There was no significant ( $p \leq 0.05$ ) difference between pre-infusion and post-infusion within any treatment group. Groups with the same superscripted letters are significantly different from each other.**

Group	Time (min)	SBP (mm Hg)	MAP (mm Hg)	DBP (mm Hg)	Heart Rate (beats/min)
NS	0	119 $\pm$ 4	94 $\pm$ 4	78 $\pm$ 3	305 $\pm$ 17
NVX-108 [L]	0	134 $\pm$ 3	105 $\pm$ 3	89 $\pm$ 2	295 $\pm$ 19
NVX-108 [H]	0	123 $\pm$ 7	100 $\pm$ 5	86 $\pm$ 4	302 $\pm$ 14
NS	30	121 $\pm$ 2 <sup>a</sup>	93 $\pm$ 2 <sup>a</sup>	76 $\pm$ 2 <sup>a</sup>	308 $\pm$ 12
NVX-108 [L]	30	138 $\pm$ 5 <sup>a,b</sup>	110 $\pm$ 5 <sup>a,b</sup>	94 $\pm$ 4 <sup>a,b</sup>	313 $\pm$ 17
NVX-108 [H]	30	123 $\pm$ 4 <sup>b</sup>	95 $\pm$ 4 <sup>b</sup>	80 $\pm$ 4 <sup>b</sup>	294 $\pm$ 14



**Fig. (1).** Mean arterial and systolic blood pressure in healthy rats before and after a 30-min infusion of Normal Saline, 0.25 ml/kg NVX-108 [L] or 1 ml/kg NVX-108 [H] (mean  $\pm$  SEM). See Tables and text for statistical analysis of data.

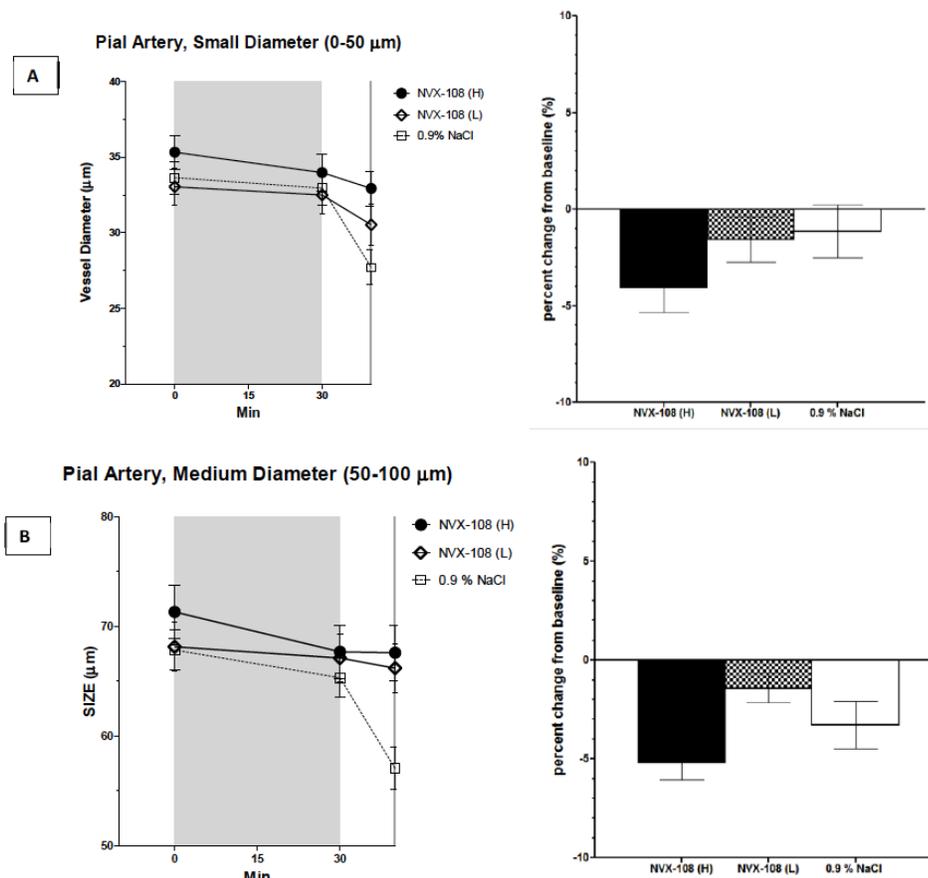
**Table 2.** Cerebral pial arteriolar vessel diameters. Pial vessel diameters before and after a 30-min infusion of Normal Saline (NS) control, 0.25 ml/kg NVX-108 [L] or 1.0 ml/kg NVX-108 [H] (mean  $\pm$  SEM). \*indicates a significant ( $p \leq 0.05$ ) difference from time 0 within a treatment group. Groups were not significantly different from each other for small ( $P = 0.405$ ) or medium ( $P = 0.711$ )-sized vessels. #indicates a significant ( $p \leq 0.05$ ) difference from time 30 within a treatment group.

Vessel Size	Time (min)	NS ( $\mu\text{m}$ )	NVX-108 [L] ( $\mu\text{m}$ )	NVX-108 [H] ( $\mu\text{m}$ )
Small	0	33.7 $\pm$ 1.1	33.0 $\pm$ 1.2	35.2 $\pm$ 1.1
	30	33.0 $\pm$ 1.2	32.5 $\pm$ 1.2	34.0 $\pm$ 1.2*
	BaCl <sub>2</sub>	27.7 $\pm$ 1.3* <sup>#</sup>	30.5 $\pm$ 1.3* <sup>#</sup>	32.9 $\pm$ 1.2*
Medium	0	67.6 $\pm$ 1.8	68.2 $\pm$ 2.2	71.3 $\pm$ 2.4
	30	65.3 $\pm$ 1.8	67.1 $\pm$ 2.2	67.7 $\pm$ 2.4*
	BaCl <sub>2</sub>	57.1 $\pm$ 2.3* <sup>#</sup>	66.2 $\pm$ 2.2*	67.60 $\pm$ 2.5*

While this study is the first to directly measure the vasoactive effects of NVX-108 on the pial cerebral vessel diameters, the microcirculation of other tissues has been previously investigated after administration of other PFCs. The PFC emulsion, Oxycyte<sup>®</sup> (Oxygen Biotherapeutics, Morrisville NC), also showed no evidence of arteriolar vasoconstriction in the skeletal muscle (the dorsal skinfold) of conscious hamsters following isovolemic hemodilution and while breathing enriched oxygen [22, 23]. On the other hand, Wei and Kontos [24] observed pial vessel vasoconstriction in anesthetized cats after topical PFC FC-80 application but vasoactivity varied depending upon local oxygen tensions. They showed that either increased venous pressure alone or in combination with topical FC-80 equilibrated with 100% nitrogen resulted in vasodilation of cerebral pial vessels but, when the FC-80 was equilibrated with 100% oxygen, vasoconstriction of pial vessels occurred. Later studies confirmed that local oxygen tensions exert a powerful effect on myogenic control of the microvasculature. For example, skeletal muscle arterioles in a conscious hamster model will vaso-

constrict to increases in tissue oxygenation [25]. Our study results are restricted to the conclusion that single dose of NVX-108 did not result in vasoconstriction of cerebral pial arterioles when healthy rats were breathing 40% oxygen and PaO<sub>2</sub> was only slightly elevated (~140 mm Hg, Table 3) compared to normal (PaO<sub>2</sub> between 80-100 mm Hg). Since brain tissue oxygenation was not directly measured in this study, conclusions cannot be made on the effect of the product on cerebral vessel diameters of rats breathing 100% oxygen or if NVX-108 will, itself, enhance brain tissue oxygenation during hypoxia.

There were no significant changes in systemic blood pressures or heart rate with either NVX-108 dose, indirectly suggesting a lack of systemic vasoconstriction. An earlier study using anesthetized rats also reported normal circulatory parameters when they were treated with a profoundly lower dose (0.014 ml/kg IV vs. our doses of 0.25 to 1.0 ml/kg) of DDFP, the active component of NVX-108 [12]. Without more invasive monitoring, a more complete hemodynamic assessment of NVX-108 was not possible from the data



**Fig. (2).** (A) Cerebral pial small-sized (<50 μm) arteriolar vessel diameter [left panel] and percent change [right panel] and (B) cerebral pial medium-sized (50-100 μm) arteriole diameter [left panel] and percent change [right panel] following a 30 minute infusion of Normal Saline, 0.25 ml/kg NVX-108 [L] or 1.0 ml/kg NVX-108 [H] (mean ± SEM). See Tables and text for statistical analysis of data. The final data point in the left panels shows vessel diameters after topically applied Barium Chloride (see text for details).

**Table 3. Blood gas analysis. Blood gas and chemistry analysis before and after a 30-min infusion of Normal Saline (NS), 0.25 ml/kg NVX-108 [L] or 1 ml/kg NVX-108 [H] (mean ± SEM). \*indicates a significant (p ≤ 0.05) difference from time 0 within a group. Groups with the same superscripted letters are significantly different from each other.**

Treatment	Time (min)	Hb (g/dL)	pH	PaCO <sub>2</sub> (mmHg)	PaO <sub>2</sub> (mmHg)	Lactate (mmol/L)	HCO <sub>3</sub> <sup>-</sup> (mmol/L)
NS	0	12.5 ± 0.3	7.349 ± 0.022	47 ± 3	110 ± 11 <sup>a, b</sup>	0.7 ± 0.1	23 ± 0
NVX-108 [L]	0	12.3 ± 0.3	7.361 ± 0.031	42 ± 2	169 ± 11 <sup>b</sup>	0.8 ± 0.1	23 ± 2
NVX-108 [H]	0	11.7 ± 0.5	7.392 ± 0.013	41 ± 3	163 ± 6 <sup>a</sup>	0.8 ± 0.1	25 ± 1
NS	30	12.4 ± 0.3	7.352 ± 0.019	46 ± 3	116 ± 10 <sup>a</sup>	0.7 ± 0.1	23 ± 0
NVX-108 [L]	30	12.6 ± 0.4	7.320 ± 0.041	43 ± 3	166 ± 9 <sup>a</sup>	0.5 ± 0.1	21 ± 2
NVX-108 [H]	30	12.5 ± 0.5	7.366 ± 0.010	46 ± 2*	140 ± 8*	0.9 ± 0.1	25 ± 1

collected. However, when 0.1 ml/kg DDFP was administered 1-3 times to anesthetized pigs with pulmonary shunts (providing a cumulative dose of up to 0.3 ml/kg, approximately equal to our 0.25 ml/kg NVX-108 [L]), there were no increases in systemic or pulmonary artery pressures, right ventricular pressures, or CVPs [26]. In addition, spontaneously breathing anesthetized pigs treated with a 30 min infusion of 0.08 ml/kg of DDFP also had no change in blood pressures

or cardiac output [27]. These four preclinical studies collectively show no adverse hemodynamic or cardiac effects from DDFP. On the other hand, an older study assessed hemodynamic effects of repeated injections of DDFP and found that eight doses of 0.5 ml/kg 10 min apart resulted in cardiopulmonary deterioration which included increased pulmonary artery pressures and pulmonary vascular resistance, decreased arterial oxygen saturation, cardiac output and stroke

volume [15]. This study may have shown hemodynamic instability because of the high cumulative dose (4 ml/kg) or the dosing protocol (repeated dosing itself instead of a single dose) or the short time interval between doses because when four doses of 0.5 ml/kg (cumulative 2.0 ml/kg) were administered 30 min apart, adverse cardiopulmonary effects were not observed. It is also possible, per those authors themselves, that the product might have been pre-activated prior to IV administration because a different handling technique (i.e., no negative pressure) after study completion resulted in no adverse hemodynamic effects [15].

No adverse hemodynamic effects of EchoGen (SONUS Pharmaceuticals, Bothell, WA), a 2% DDFP emulsion, were reported in a Phase III, multicenter, single-blind, active controlled trial [10]. Sequential IV injections of active control or 0.05 ml/kg DDFP were given 30 min apart to 254 adult patients with a suboptimal echocardiogram for left ventricular (LV) cavity opacification. There was no change in heart rate, blood pressures, respiratory rate, oxygen saturation, ECG variables or laboratory values and no difference from control drug in the number of patients with adverse events attributed to the test drug [10]. Furthermore, no adverse hemodynamic effects were reported in either of two smaller clinical trials using 0.05 ml/kg IV EchoGen; one study (N = 40) evaluated DDFP as a contrast-enhancing agent during transthoracic echocardiography and the other study as a contrast agent in patients (N = 16) with advanced common carotid artery stenosis [28, 29]. Both EchoGen and NVX-108 contain the same active ingredient although EchoGen was pre-activated by negative pressure to create microbubbles while NVX-108 was not activated in the current study and NVX-108 is in a buffered solution while EchoGen was not buffered. In the only other published clinical trial of 2% DDFP, 24 healthy human volunteers received 0.01 to 0.1 mL/kg IV DDFP emulsion to obtain pharmacokinetic data and although no hemodynamic data were presented, no adverse effects were reported [14]. Thus 2% DDFP shows hemodynamic stability and minimal adverse effects in humans, although these doses are relatively low and these findings may not be applicable to patients with TBI.

In summary, our study showed no cerebral vasoconstriction in the normal brain under normoxic conditions while other studies suggest an acceptable cardiovascular safety profile when administered to hemodynamically stable, normovolemic subjects. This permits NVX-108 to pass to the next stage of the screening process for a pre-hospital TBI indication. In support of the decision to proceed with evaluating NVX-108 for TBI, repeated doses of 0.1, 0.3 or 0.6 ml/kg NVX-108 have been shown to decrease the infarct size in models of ischemic stroke suggesting that it protects neural tissue from ischemia [11, 13]. A single infusion of 0.7 ml/kg NVX-108 has also been shown to reverse the hemodynamic decompensation, tissue hypoxia and mortality in an otherwise 100% lethal model of severe isovolemic exchange hemodilution in rats [12]. Because the most recent stroke study indicated an equivalent degree of protection from the low dose (0.1 ml/kg) as the high dose (0.6 ml/kg) [13], a full characterization of the drug's potency and efficacy is incomplete. The optimal individual dose, dosing interval and duration of infusions will require additional studies and may be different for each clinical indication for which DDFP or NVX-108 might be used.

## CONCLUSION

Collectively, these results suggest that NVX-108 may be able to provide oxygen and enhance brain tissue oxygenation without causing cerebral or systemic vasoactivity. We conclude that NVX-108 is a possible candidate for an in-depth pre-clinical evaluation using a more clinically relevant pre-hospital TBI trauma scenario.

## CONFLICT OF INTEREST

The authors have no declarations of interest to report. The authors alone are responsible for the content and writing of the paper. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. Authors are military service members (or an employee of the US Government). This work was prepared as part of their official duties. Title 17 USC §105 provides that 'copyright protection under this title is not available for any work of the US Government.' Title 17 USC §101 defines a US Government work as a work prepared by a military service member or employee of the US Government as part of that person's official duties. The work was supported by Defense Health Program work unit #602115HP.3720.001.A1011.

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