Pilot Trial of Intravenous Lipid Emulsion Treatment for Severe Nifedipine-Induced Shock

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Abstract Animal studies and human case reports show promise in using lipid rescue to treat refractory calcium channel antagonist toxicity. However, the majority of research and clinical experience has focused on non-dihydropyridine agents. Thus, we sought to investigate the value of lipid emulsion (ILE) therapy for dihydropyridine-induced shock. This IACUC-approved study utilized seven swine that were sedated with alpha-chloralose, mechanically ventilated, and instrumented for drug delivery and hemodynamic measures. After stabilization and basal measures, nifedipine (0.01875 mg/kg/min) was infused until imminent cardiac arrest (seizure, end tidal CO₂ < 10 mmHg, bradydysrhythmia, or pulseless electrical activity). Animals then received a 7 mL/kg bolus of 20% lipid emulsion via central catheter. Lipid circulation was visually confirmed by the presence of fat in peripheral arterial

blood. Hemodynamics were continuously monitored until 10 min after lipid bolus. Surviving animals were euthanized. Pre- and post-lipid treatment parameters were analyzed using the Wilcoxon signed rank test ($p < 0.05$ significant). Nifedipine toxicity was characterized by vasodilatory hypotension, impaired vascular contractility, and tachycardia with terminal bradycardia. The median time to imminent cardiac arrest from start of nifedipine infusion was 218 min. Lipid treatment did not improve hemodynamics or restore circulation in any animal. There was no benefit from lipid rescue in this model of nifedipine toxicity. Further study of ILE for dihydropyridine toxicity is warranted but initial animal model results are not promising.

Keywords Lipid emulsion · Nifedipine · Calcium channel blocker

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Introduction

Since the first dramatic report of lipid rescue for local anesthetic-induced cardiac arrest, lipid emulsion therapy has gained momentum as a treatment option for xenobiotic-induced severe cardiovascular and central nervous system toxicity [1]. Lipid emulsions were originally used for parenteral nutrition and then utilized as a vehicle to deliver xenobiotics too lipophilic for conventional, water-soluble intravenous diluents. The ability of lipid emulsion to dissolve lipophilic xenobiotics is the basis for one of the proposed mechanisms for use in reversing drug toxicity: the lipid sink theory. This theory suggests that if drugs can be delivered via fat emulsion, it follows that drugs can also be removed from circulation and tissues by providing an alternate, lipophilic pharmacokinetic compartment. Calcium channel antagonists are lipophilic and thus would presumptively be amenable to sequester in a fatty

medium. There is a second plausible beneficial mechanism of action of lipid emulsion for calcium channel antagonist toxicity. By reports, several long chain fatty acid components of lipid preparations are capable of opening voltage-gated calcium channels, thus competing with the drug at the ion channel [2]. This makes the use of intravenous lipid emulsion (ILE) particularly intriguing for calcium channel antagonist toxicity where shock is often refractory to conventional therapy, and serious toxicity and mortality remains relatively high [3].

Lipid emulsion therapy has been studied for verapamil toxicity with impressive results in several animal models [4–6]. It has also been used to treat calcium channel antagonist toxicity in at least 20 published human cases, primarily involving verapamil and diltiazem [7–16]. However, there is limited pre-clinical and clinical experience with ILE use for dihydropyridine toxicity [7, 16–19]. Swine have been used in numerous toxin and shock models and are one of the dominant animal species used in evaluating calcium channel antagonists and beta-blockers in the toxicology literature [20–23]. For these reasons, we sought to test the efficacy of ILE rescue in a porcine model of severe nifedipine toxicity. The main study hypothesis was that ILE would restore circulation and improve hemodynamics (cardiac output, mean arterial pressure, and systemic vascular resistance) in swine subjected to severe nifedipine-induced shock.

Methods

The study protocol was approved by the Institutional Animal Care and Use Committee and carried out following the American Association for Accreditation of Laboratory Animal Care guidelines. Four female, domestically bred swine weighing 32–38 kg were acclimatized under standard housing, lighting, and feeding conditions for a minimum of 7 days and then fasted for 12 h (except for access to water) before undergoing the experimental protocol. Each animal was weighed on the day of the experiment, and this weight was used to calculate all drug and fluid doses.

Animal Preparation

Animals were sedated with 4.4 mg/kg intramuscular (IM) Telazol® (tiletamine hydrochloride and zolazepam hydrochloride) mixed with 0.04 mg/kg atropine and 1.5 mg/kg xylazine to establish intravenous access via ear vein, perform orotracheal intubation, and initiate mechanical ventilation with 100 % oxygen. Animals were then given a single dose of 0.02 mg/kg IM buprenorphine. The animals were placed on a warming pad with continuous monitoring of rectal temperature to maintain basal temperature.

An oxygen saturation probe was placed on the tongue and end-tidal CO₂ detector used to monitor expired CO₂. Surface electrodes were attached to monitor heart rate and rhythm.

Anesthesia

Anesthesia was maintained for the remainder of the experiment using an alpha-chloralose 55 mg/kg IV bolus followed by 22 mg/kg/h continuous infusion. Animals were monitored throughout the protocol for depth of anesthesia (jaw tightening and limb movement), and additional boluses of alpha-chloralose (28–55 mg/kg) were given as needed, and the continuous infusion rate was maintained at 22 mg/kg/h.

Experimental Protocol

Following induction of anesthesia, each animal was instrumented to allow for invasive cardiovascular monitoring, intravenous fluid administration, drug infusion, and blood sampling. The internal jugular vein, carotid artery, femoral vein, and femoral artery were cannulated after cutdown and direct visualization. Animals were allowed to stabilize for at least 30 min after surgical instrumentation. Normal saline was used for maintenance fluid infusion at a rate of 2 mL/kg/h. Blood glucose was measured after surgery was completed and every 30 min thereafter. Animals with blood glucose measurements <50 mg/dL received bolused dextrose augmentation IV. After stabilization, basal measurements were obtained and then nifedipine was infused at 0.01875 mg/kg/min for the duration of the protocol. The dose of nifedipine was determined in preliminary dose response curves as the best dose to achieve death within our goal of 4 to 5 h. At the point when MAP $\times$ CO decreased by 25 % from baseline, animals were given a 20 mL/kg normal saline bolus. The animals were then monitored until they reached the toxic end point, defined as shock with imminent cardiac arrest denoted by any one of the following: seizure-like activity, end-tidal CO₂ $\leq$ 10 mmHg, bradycardia, or pulseless electrical activity; these end-points were determined in the preliminary dose response trials. Once the toxic end point was reached, a bolus of 7 mL/kg Intralipid®, a 20 % standard commercial lipid emulsion, was given via femoral central venous line. Although higher than the standard recommended human dose for rescue therapy, this dose was selected because (1) it was effective in a previous model of verapamil-induced cardiac arrest and (2) we sought to avoid a negative result due to potential under dosing [5]. Circulation of ILE was confirmed by the presence of fat in femoral arterial blood noted on visual inspection. Animals were then monitored for 10 min following ILE. Any surviving animals were euthanized with IV Euthasol® (390 mg/mL pentobarbital sodium and 50 mg/mL phenytoin sodium).

Three additional animals were used as surgical controls. They underwent the same surgical procedures as outlined above. Laboratory studies and hemodynamic measures were obtained at regular intervals and all animals received normal saline in place of nifedipine following the protocol described. These animals were monitored for 240 min and then euthanized.

Data

Systolic, diastolic, and mean arterial blood pressure were measured via arterial line. Central venous pressure was measured via central venous line. Additional hemodynamic measures (CO, CI, SVR) were obtained using a FloTrac® device and continuously recorded via the EV-1000® monitor (both from Edwards Lifescience, Irvine, CA). The FloTrac® system is a minimally invasive intra-arterial device that uses pulse contour analysis technology to calculate CO, CI, and SVR. Metabolic measures (hemoglobin, lactate, pH, pCO₂, pO₂, glucose, electrolytes) were performed using an ABL 800® Flex Analyzer (Radiometer, Copenhagen, Denmark) at baseline and then every 30 min during the study.

Statistical Analysis

Wilcoxon signed-rank test was used to compare pre- and post-ILE treatment hemodynamic measurements. Descriptive statistics were used to evaluate changes in hemodynamics, time to death, and change in laboratory values.

Results

Nifedipine toxicity in this swine model was characterized by vasodilatory hypotension, impaired vascular contractility, progressive lactate accumulation, initial tachycardia, and terminal bradycardia [Fig. 1]. Lipid infusion did not improve hemodynamics or restore circulation. All animals died or were euthanized according to our protocol [Table 1].

The median time to imminent cardiac arrest from start of nifedipine infusion was 218 min (mean dose 143 ± 43 mg). During nifedipine infusion, animals demonstrated progressive vasodilatory hypotension. Mean arterial pressure (MAP) declined [Fig. 1a]. Systemic

Fig. 1 a Mean arterial pressure over time, b systemic vascular resistance over time, c heart rate over time, d cardiac output over time

Table 1 Time to imminent arrest after the initiation of toxicity and outcome following ILE infusion

	Time to imminent arrest (min)	Outcome after ILE given
Animal 1	258	In asystole after 7 min
Animal 2	129	In asystole when given, no recovery
Animal 3	216	In asystole when given, no recovery
Animal 4	208	In asystole after 1 min, no recovery

vascular resistance (SVR) decreased until near imminent arrest when it returned to near baseline [Fig. 1b]. We surmise that this increased SVR at the end of each experiment represented a terminal catecholamine surge in a dying animal. Heart rate (HR) steadily increased until near arrest at which point all animals abruptly developed bradycardia with asystole in two animals [Fig. 1c]. Cardiodynamic findings included an increased cardiac output (CO) until terminal [Fig. 1d] and decreased stroke volume (SV) throughout the study [Table 2]. Central venous pressure (CVP) increased initially during nifedipine infusion and then decreased prior to death [Table 2]. Despite the apparent increased CO, we believe that the animals developed true myocardial depression based on decreased SV and increased CVP as a result of the nifedipine. Any increase in CO that was seen was reflective of concomitant increases in HR; there were no indications of increased contractility. At the point of imminent arrest when HR fell, CO also rapidly fell below baseline. From a metabolic standpoint, nifedipine toxicity resulted in lactate accumulation (basal mean of 0.63 ± 0.29 mEq/L to pre-arrest mean of 11.5 ± 1.97 mEq/L). There were no overall changes in electrolytes that might have led to tachy- or bradydysrhythmias [Table 3]. Three animals required bolus dextrose augmentation to maintain blood glucose over 50 mg/dL during the protocol.

Analysis of hemodynamic data at the time of imminent arrest and 10 min after ILE given demonstrated no improvement and no statistically significant worsening [Table 2].

Table 2 Mean hemodynamic data $\pm$ standard deviation at multiple time points during pre-treatment period, at point of imminent cardiac arrest (pre-lipid), and 10 min after ILE given. The Wilcoxon signed-rank test

	Basal	60 min	120 min	180 min	Pre-Lipid	Lipid + 10 min	p value
HR (bpm)	64 ± 7	128 ± 10	157 ± 28	118 ± 81	77 ± 89	33 ± 66	$p = 0.5$
MAP (mmHg)	80 ± 6	46 ± 11	48 ± 9	33 ± 11	24 ± 7	6 ± 11	$p = 0.13$
SVR (dynes/cm ⁵)	608 ± 69	351 ± 85	291 ± 216	320 ± 253	619 ± 291	141 ± 244	$p = 0.89$
CVP (mmHg)	9.8 ± 1	15 ± 2	13 ± 3	11 ± 7	16 ± 2	8 ± 9	$p = 0.5$
CO (L/min)	9.3 ± 1.1	8.0 ± 5.2	11.9 ± 7.1	4.3 ± 5.5	1.5 ± 0.9	0	$p = 0.13$
SV (mL)	145 ± 8	60 ± 38	101 ± 87	26 ± 30	11 ± 4	3 ± 5	$p = 0.25$

Discussion

Overall, this study showed no treatment effect for lipid infusion. Our results corroborate the only previously investigating ILE for the treatment of nifedipine toxicity published abstract by Chu et al. [17]. In their report, ILE did not exert any beneficial survival or hemodynamic effect in rodents poisoned with nifedipine. To the best of our knowledge, there are no other published animal studies of nifedipine or other dihydropyridine class calcium channel antagonists.

Our results are somewhat contrary to limited published clinical experience with ILE for calcium channel antagonist toxicity. The investigators identified at least 17 peer-reviewed, published cases that generally reflect good outcome following infused lipids [7–16, 19, 24]. The majority of these cases involve verapamil or diltiazem. Nifedipine was cited in two publications in which both patients survived [7, 24]. There are three published toxic cases of another dihydropyridine, amlodipine, where ILE was included in therapy; two survived [7, 16]. Of course, all of these reports are subject to confounders including publication bias, effects of co-ingested xenobiotics, and multiple treatments in addition to ILE. There are no prospective clinical studies of ILE for calcium channel antagonist shock.

Although we believe that our swine model with continuous infusion of toxin is reproducible compared to prior studies and reasonably reflects the expected clinical course of an oral calcium channel antagonist overdose in humans, significant limitations remain [21].

Limitations

The anesthetic used in the study, alpha-chloralose, was selected because it has no notable hemodynamic effects compared to other anesthetics that might confound the hemodynamic toxicity being studied [25]. It has been successfully used in prior cardiotoxic drug models performed at our institution and hemorrhagic swine models [26–29]. We tested the alpha-chloralose in several animals prior to initiation of the full study and confirmed a lack of direct hemodynamic effect. Despite

was used to compare pre-lipid and post-lipid hemodynamics (10 min after ILE given). $N = 4$ for all data points

Table 3 Mean $\pm$ standard deviation laboratory values for animals prior to the initiation of toxicity and at the point of imminent cardiac arrest. Normal ranges for swine listed at top of each column. Pre-arrest period

Group	pH		Sodium (140–150 mEq/L)		Potassium (4.7–7.1 mEq/L)		Glucose (85–150 mg/dL)	
	Basal	Pre-arrest	Basal	Pre-arrest	Basal	Pre-arrest	Basal	Pre-arrest
Surgical control ($n = 3$)	7.39 $\pm$ 0.085	7.42 $\pm$ 0.023	150 $\pm$ 4.94	154 $\pm$ 1.41	3.6 $\pm$ 0.35	4.0 $\pm$ 0.14	97.5 $\pm$ 3.53	62 $\pm$ 23
Experimental group ($n = 5$)	7.42 $\pm$ 0.016	7.11 $\pm$ 0.073	142 $\pm$ 2.99	139 $\pm$ 0.5	4.0 $\pm$ 0.45	6.3 $\pm$ 1.0	121 $\pm$ 37.8	142 $\pm$ 112

prior experience and preliminary testing in control swine, we cannot exclude an unrecognized, synergistic reaction between alpha-chloralose and nifedipine that may have resulted in refractory toxicity.

The protocol was designed to reflect the current clinical practice of giving ILE as a “rescue” therapy. Thus, ILE was given late in the course of toxicity, at the point of imminent cardiac arrest based on previous models, and in an effort to have a clearly toxic and reproducible endpoint. The goal of this study was not to assess the benefit or detriment of ILE in combination with standard therapies such as vasopressors or calcium. Despite our negative result, there may be benefit if ILE is given earlier in the course of toxicity or in combination with other medications, but this was not the focus of our study.

It is also possible that the dose of ILE was insufficient to produce a positive effect. However, we utilized a dose previously shown to reverse severe verapamil toxicity and a greater dose than that used to resuscitate bupivacaine-induced cardiac arrest [5, 30]. Conversely, the use of a high dose of ILE raises the possibility of detrimental effect from the experimental treatment itself. However, a similar dose was used in a canine verapamil study without obvious harm, and larger doses (8–32 mL/kg) have been employed in other ILE models [4–6, 22, 31].

Finally, we must consider reports of non-dose related reactions to fat emulsions that might confer a negative treatment effect. Anaphylactoid reactions have been observed after infusion of specific liposomes and fat-containing nanoparticles [32–34]. These so-called CARPA reactions (compliment activation-related pseudoallergy) have been reported in various animal models including dogs, rats, and swine and may result in hypotension, hypertension, tachycardia, bradycardia, flushing, and rash. These reactions have been examined in swine exposed to liposomes like doxorubicin hydrochloride liposome injection and liposome-encapsulated hemoglobin as they mimic human reactions to some liposomes and fat-containing nanoparticles [32]. We cannot completely exclude an effect from CARPA contributing to the demise of our animals at a very vulnerable hemodynamic point in the experiment. However, the commercial fat emulsion used in our study does not contain cholesterol, dimyristoyl phosphatidylcholine, or dimyristoyl phosphatidylglycerol that were implicated in the CARPA reports. Additionally, we did not observe

any rash or flushing in the animals following ILE administration that is associated with CARPA. Finally, other work recently published by Varney, et al. using a similar dose of ILE in swine did not identify any adverse effect of the ILE [22]. Ultimately, we realize examining the effects of ILE alone on control swine is necessary, but funding limits prohibited our ability to further investigate this.

Conclusion

Nifedipine toxicity in this porcine model was characterized by vasodilatory shock, lactate accumulation, and terminal bradycardia. ILE provided no treatment benefit in this pilot study. Taken within the context of the setting of experimental nifedipine-induced shock, we cannot recommend lipid infusion as a rescue therapy for imminent or ongoing cardiac arrest from nifedipine toxicity. Use of ILE for this specific calcium channel antagonist requires further scientific scrutiny.

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Compliance with Ethical Standards

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Conflicts of Interest The authors have no conflicts of interest to disclose.

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