

Hypersensitivity Reactions to Intravenous Lipid Emulsion in Swine: Relevance for Lipid Resuscitation Studies

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BACKGROUND: Reports in the recent experimental literature have provided contradicting results in different animal species regarding the efficacy of IV lipid emulsion (ILE) in the reversal of cardiovascular and central nervous system symptoms of local anesthetic and other lipophilic drug overdoses. In particular, ILE seemed to be effective in rats, rabbits, dogs, and humans, but not in swine, for which it not only failed to reverse the adverse effects of anesthetics, but the animals also developed a generalized cutaneous mottling or a dusky appearance immediately after ILE, suggestive of another type of toxicity. The latter symptoms arise in complement (C) activation–related pseudoallergy, a hypersensitivity reaction to particulate drugs and agents.

METHODS: Ten Yorkshire swine (15–20 kg) were sedated with ketamine and anesthetized with isoflurane. ILE 1.5 and 5 mL/kg 20% was administered via the ear vein while pulmonary arterial pressure, systemic arterial blood pressure, electrocardiogram, and end-tidal CO₂ were recorded continuously. Thromboxane was measured in blood collected at baseline and 2 and 10 minutes after injections. Complement activation by lipid emulsion was also assessed in vitro with soluble terminal complement complex (SC5b-9) and sheep red blood cell assays.

RESULTS: Significant increases were observed in the pulmonary pressure (median [interquartile range]) within minutes after the administration of ILE, both at doses 1.5 and 5 mL/kg (15 [12–16.5] to 18.5 [16–20] mm Hg, $P = 0.0058$ and 15.5 [13–17.25] to 39.5 [30.5–48.5], respectively). The systemic arterial blood pressure increased, and the heart rate decreased after both injections. Thromboxane B₂ concentration (median [interquartile range]) in the blood plasma increased from a baseline of 617.3 [412.4–920] to 1132 [597.9–1417] pg/mL ($P = 0.0055$) and from 1276 [1200–2581] to 4046 [2946–8442] pg/mL ($P = 0.0017$) after the administration of 1.5 and 5 mL/kg ILE, respectively. Intralipid did not cause in vitro complement activation in human serum.

CONCLUSIONS: ILE causes clinically significant hemodynamic changes in pigs, in concert with significant increases in the plasma thromboxane concentration. However, the in vitro tests did not confirm involvement of the complement system in human sera, leaving the underlying mechanism of these findings in doubt. Nonetheless, the observed hemodynamic and biochemical effects of ILE serve as a caveat that the pig is not an ideal model for the study of interventions involving ILE. (Anesth Analg 2014;119:1094–101)

The increased use of local anesthetics for surgical and postsurgical analgesia, as well as pain management after trauma, draws attention to the clinically relevant potential for toxic side effects of local anesthetics. Local anesthetic systemic toxicity is one of the most feared complications of regional anesthesia because it can impair function of the central

nervous system and cause cardiovascular collapse.^{1,2} Several case reports in the recent literature suggest the effectiveness of IV lipid emulsion (ILE) in the reversal of cardiovascular and central nervous system symptoms of local anesthetic and other lipophilic drug overdoses.^{3–6} ILE has been included in practice advisories of the American Society of Regional Anesthesia and the Association of Anesthetists of Great Britain and is also recommended in the Advanced Cardiac Life Support guidelines by the American Heart Association for cardiac arrest secondary to lipophilic β and calcium channel blockers, when conventional resuscitative therapies have failed.⁷

Since potentially lethal toxicity studies do not lend themselves to randomized controlled human trials, animal models must be applied to study the usefulness of ILE in reversing the systemic toxicity of local anesthetics and other lipophilic drugs. However, the relevant experimental literature is somewhat ambiguous regarding the efficacy of ILE. Some studies identify a clearly antidotal effect and thereby support the use of lipid as a rescue drug,^{8–10} whereas in other animal models, the results are not as clear. The difference in results appears to be species specific. In some species (rats,

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rabbits, cats, dogs), ILE seems to improve reversal of local anesthetic intoxication, whereas in several studies using a swine model, chosen for its similarity to human cardiovascular physiology, administration of ILE did not change or even worsen the outcome of resuscitation compared with conventional Advanced Cardiac Life Support protocols.^{11–14}

These reports raise concerns that differences in the methodology and interspecific variation in response to ILE might account for differences in outcomes. In particular, ILE seemed to be effective in rats, rabbits, dogs, and humans, but not in swine, for which animals were also observed to develop generalized cutaneous mottling or a dusky appearance immediately after ILE.

Previous studies in our laboratory and others have shown that swine develop a complement activation–related pseudoanaphylactic reaction (CARPA) to certain liposomes, lipid emulsions, and radio contrast media that causes cardiovascular instability and an appearance very similar to the response described in experimental swine receiving ILE.^{15–18} Based on the similarity of the 2 phenomena, we hypothesized that CARPA counteracts or masks the beneficial effect of ILE in local anesthetic intoxications and could be one of the underlying causes for interspecific differences in experimental outcomes after ILE, thereby accounting for the apparent lack of efficacy in swine. In this study, we sought to characterize the potential cardiovascular and immunological reactions to ILE in swine by examining the in vivo hemodynamic responses and in vitro complement activation in human and animal serum. These results would test the hypothesis that swine exhibit adverse reactions to lipid emulsion.

METHODS

This study was reviewed and approved by the Institutional Animal Care and Use Committee of the Uniformed Services University of the Health Sciences, Bethesda, MD.

Animal Study

Ten Yorkshire swine (15–20 kg) were sedated with ketamine (15–25 mg/kg IM, 18–19 gauge needle, dorsolateral neck muscles), and anesthesia was induced with cone-mask inhalation of 3% to 4% isoflurane. After tracheal intubation (6.5–7.0 mm endotracheal tube), the animals were allowed to breath spontaneously and anesthesia was maintained with 1% to 4% inhaled isoflurane as needed. Waste gas from the anesthesia machine was scavenged via carbon filter. A catheter (20–22 gauge) was placed in the right ear vein for IV maintenance fluids (0.9% NaCl at 1–2 mL/kg/h) and infusion of lipid emulsion (Intralipid 20%, Baxter, Deerfield, IL). A 20- to 22-gauge catheter was inserted percutaneously into the superficial femoral artery to provide access for serial blood sampling and systemic arterial blood pressure (SAP) and heart rate (HR) monitoring. A 9-Fr Cordis percutaneous introducer was placed into the right external jugular vein, and a pulmonary artery catheter was passed through the vena cava superior, right atrium, and right ventricle into the pulmonary artery by monitoring the change of pressure for continuous pulmonary arterial pressure (PAP) measurement. Continual respiratory rate, end-tidal CO₂ (ETCO₂), electrocardiogram, and rectal temperature were monitored (M1026A Gas Analyzer and Model 68 clinical monitor, Hewlett-Packard, Andover, MD). Normal body temperature

(37°C–38°C) was maintained by an external warming device. SAP, PAP, ETCO₂, electrocardiogram, and HR were continuously recorded at 100 Hz throughout the experiment.

On completion of instrumentation and a 15- to 20-minute stabilization period, baseline blood samples were taken for biochemical analysis. All blood samples taken during the experiment were drawn into 10 mL tubes containing anticoagulant and supplemented with indomethacin at a final concentration of 10 µM to prevent ex vivo formation of thromboxane B₂. The tubes were centrifuged and the plasma was separated and frozen for later thromboxane B₂ concentration measurements.

After the baseline blood draw, 1.5 mL/kg ILE was injected via the ear vein catheter as a bolus over approximately 1 minute. Blood samples were collected at 2 and 10 minutes after the injection. After a 20-minute stabilization period, an additional 5 mL/kg ILE bolus was injected over approximately 2 minutes, followed by blood sampling at 2 and 10 minutes to confirm or exclude tachyphylaxis (the decrease or complete lack of response on repeated injections). After a 20-minute recovery period following the ILE injections, a 0.1-mg/kg bolus of zymosan (Sigma-Aldrich, St. Louis, MO), a well-characterized complement activator, was injected as positive control. At the end of the experiments, the animals were euthanized.

In Vitro Assays of Complement Activation

For the in vitro soluble terminal complement complex (SC5b-9) assay, 15-µL aliquots of human serum were mixed with 5 µL of phosphate-buffered saline (PBS) or zymosan or undiluted lipid emulsion in Eppendorf tubes, which were then incubated at 37°C for 45 minutes. Samples were placed on ice, and complement reactions were stopped by 980 µL of specimen diluents, provided in the enzyme-linked immunosorbent assay (ELISA) kits, completed with 10 mM EDTA. SC5b-9 concentrations were measured by human SC5b-9 ELISA kit (Quidel Corporation, San Diego, CA) according to kit protocol. Absorbance values at 450 nm were measured by a FluoStar Omega microplate reader (BMG Labtech, Ortenberg, Germany).

Statistical Analysis

A sample of 10 animals was requested based on investigator experience with the model and to have 80% power to detect a 1-SD (paired) difference in outcomes, controlling the probability of a type I error at $\alpha = 0.05$. Maximal absolute changes (both negative and positive) from baseline were measured for PAP, SAP, and HR and compared with baseline values by Wilcoxon signed rank test using matched pairs. Changes in plasma thromboxane B₂ levels were analyzed, and baseline and 2- and 10-minute postinjection blood levels were compared with 1-way repeated-measures analysis of variance (Friedman test) with post hoc pairwise comparisons made using the Dunn test. Data are presented as median (interquartile range).

RESULTS

Physiological Effects of Lipid Administration

Ten animals were instrumented for monitoring hemodynamic variables, and after a rest period of about 15 to 20 minutes, 20% ILE was administered. The first injection was a 1.5 mL/kg bolus of 20% ILE, the standard initial recommended dose for the treatment of local anesthetic systemic toxicity.

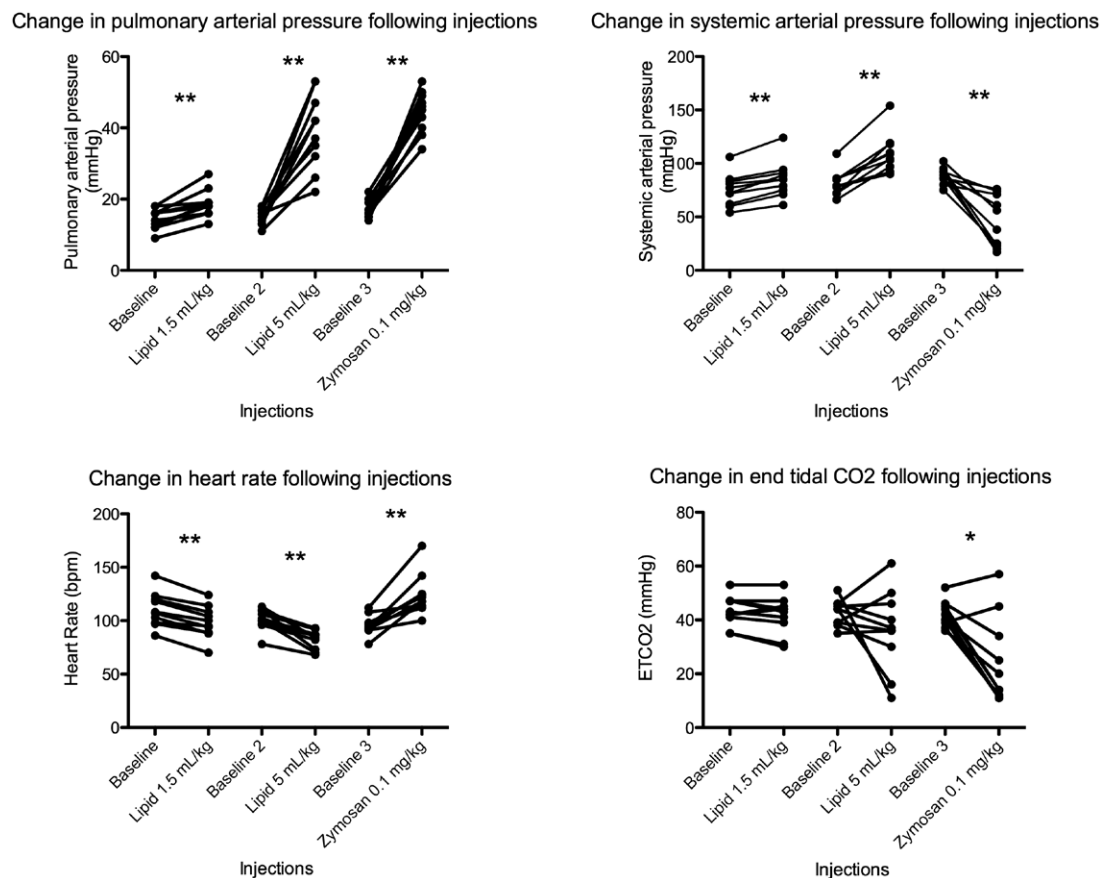


Figure 1. Changes in physiological variables after administration of test substances. ** $P < 0.01$, * $P < 0.05$. Pairwise comparison of baselines and maximum/minimum values after the start of administration of test substances.

Immediately after injecting ILE 1.5 mL/kg, the PAP increased from 15 (12–16.5) to 18.5 (16–20) mm Hg ($P = 0.0058$). The SAP also increased from 74.5 mm Hg (61.5–83.5) to 85 mm Hg (74–91.75) ($P = 0.0059$), and the HR decreased from 107.5 bpm (97.75–120.8) to 97.5 bpm (88.75–109.5) ($P = 0.0059$; Fig. 1).

After a 20-minute rest period, 5 mL/kg of 20% ILE was administered over 2 to 3 minutes. This corresponds to the estimated maximal dose of ILE administered over the course of lipid resuscitation, although in a relatively short time period. The PAP increased from 15.5 (13–17.25) to 39.5 (30.5–48.5) mm Hg ($P = 0.002$), the SAP increased from 82 mm Hg (72–85.25) to 106.5 mm Hg (95.75–118.3) ($P = 0.002$), and the HR decreased from 101 bpm (96.75–109.3) to 85 bpm (72.25–88.25) ($P = 0.0059$). Four of the 10 animals developed apnea 30 to 120 seconds after the start of the ILE injection, as also evidenced by the interruption of spontaneous ventilation on the ET CO₂ trace for at least 20 seconds. In 2 cases, the breathing became gradually shallow and irregular and <2 minutes after the start of the ILE injection, progressed to persistent apnea requiring controlled ventilation. In 2 other animals, about 30 seconds after the start of the ILE injection, the respiration paused for about 20 seconds. In 1 of these 2 animals, the short apneic periods recurred for another 17, 45, 12, and 10 seconds during the next 4 to 5 minutes.

After another 20-minute rest period and the administration of the positive control zymosan (0.1 mg/kg), the PAP increased from 16 (15–19.25) to 45.5 (39.5–49.25) mm Hg ($P = 0.0059$),

whereas the SAP decreased from 87.5 mm Hg (84.5–92.75) to 47 mm Hg (22.25–71.75) ($P = 0.002$) and the HR increased from 95 bpm (91.75–100.5) to 120 bpm (113.5–129.3) ($P = 0.002$).

Observable Changes

Within 6 minutes after beginning the infusion of ILE, all animals presented various degrees of skin rash in the form of red macules and patches, which were most pronounced on the abdominal region, and developed and resolved in parallel with the hemodynamic reactions (Fig. 2). Typically, the skin mottling was more generalized and deeper red after the higher dose of ILE.

Thromboxane

Examining thromboxane B₂ concentration over the entire study period, levels in the blood plasma increased from a baseline of 617.3 (412.4–920) to 654 (501.8–958.8) and 1132 (597.9–1417) pg/mL at 2 and 10 minutes after the administration of 1.5 mL/kg ILE, respectively ($P = 0.0055$ with Friedman test; Fig. 3). After the second 5 mL/kg ILE dose, the thromboxane B₂ concentration further changed from 1276 (1200–2581) to 3808 (1768–10741) and 4046 (2946–8442) pg/mL at 2 and 10 minutes, respectively ($P = 0.0017$ with Friedman test). For each ILE dose, only the thromboxane B₂ concentrations at 10 minutes were significantly higher than baseline when analyzed with the Dunn multiple comparison tests. After administration of the positive control

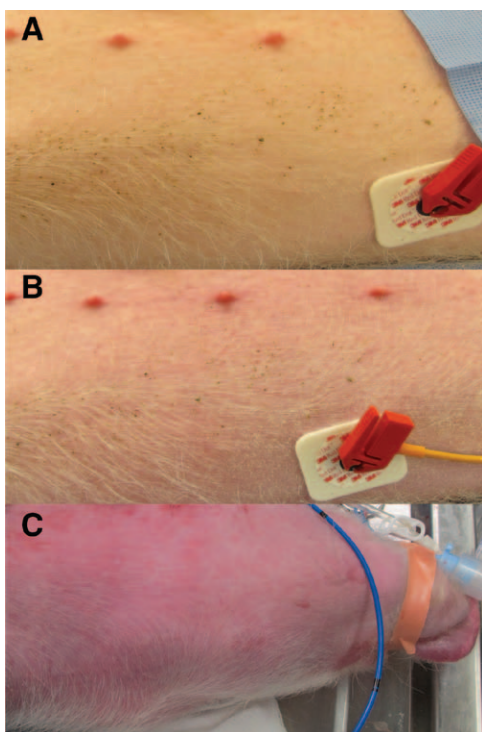


Figure 2. Skin rash after the administration of lipid emulsion. A, Before lipid. B, After 1.5 mL/kg lipid. C, After 5 mL/kg lipid.

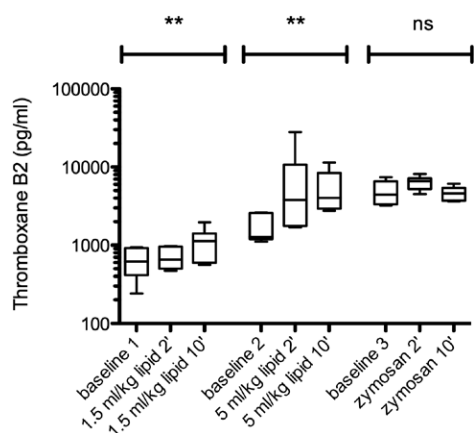


Figure 3. Thromboxane concentration in the blood plasma. ** $P < 0.01$, ns = not significant—analyzed with Friedman test.

zymosan, a yeast extract that activates the classical and alternative pathway of complement, there was no significant change in the plasma thromboxane B_2 levels, which was 4420 (3338–6584) pg/mL at baseline and 6609 (5219–7200) and 4601 (3697–5385) pg/mL at 2 and 10 minutes after injection, respectively ($P = 0.4297$ with Friedman test).

In Vitro C Activation

In the human SC5b-9 assay, the ILE did not activate complement. The SC5b-9 concentrations in the untreated sera and sera treated with PBS and ILE were 1.776 (1.526–2.026), 2.07 (1.991–3.348), and 2.002 (1.991–2.419) $\mu\text{g/mL}$, respectively (Fig. 4). The PBS ($P = 0.25$) and ILE ($P = 0.15$) treatment did not cause a significant difference compared with the

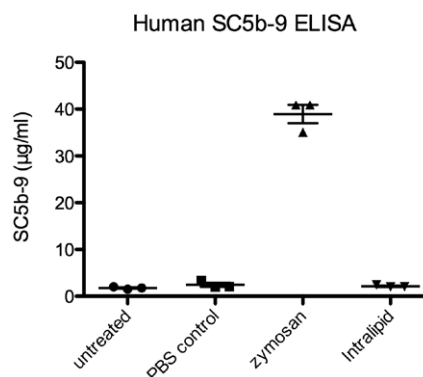


Figure 4. Human SC5b-9 enzyme-linked immunosorbent assay (ELISA). Only zymosan caused complement activation in human serum. PBS = phosphate-buffered saline.

untreated serum, whereas treatment with the positive control zymosan generated an SC5b-9 concentration of 40.90 (35.05–40.9) $\mu\text{g/mL}$ ($P = 0.003$, compared with untreated serum; Fig. 4)

DISCUSSION

The key finding of this study is that swine exhibit major adverse hemodynamic responses to ILE, accompanied by an increase in plasma thromboxane concentration. Reports in the literature suggesting reduced efficacy of ILE resuscitation in swine-based laboratory models for lipophilic drug overdose, together with the repeated occurrence of skin symptoms characteristic of CARPA, raised the possibility of complement activation after ILE administration in pigs. To test the hypothesis that ILE induces a CARPA-like syndrome in pigs, we injected swine with ILE and monitored hemodynamic variables, paying close attention to indicators of CARPA. We also collected blood samples for thromboxane measurements, a mediator that has a central role in the pathogenesis of CARPA.

We observed significant increases in the PAP within minutes after the administration of ILE, both at doses 1.5 and 5 mL/kg. Although the increase after the 1.5-mL/kg dose was relatively small, it was accompanied by a decrease in SAP and increased HR. After the 5-mL/kg dose, the change in PAP was much greater and comparable with the PAP increase caused by the positive control zymosan. It must be noted that this 5-mL/kg ILE dose was injected over a short time (1–2.5 minutes). However, the signs often occurred within seconds of the start of the injection and were usually obvious before the ILE infusion was half completed, suggesting that a lower dose would have provoked similar reaction (Fig. 5). Five of the 10 animals became apneic and required controlled ventilation. The SAP also increased slightly, coupled with a decrease in HR. The time course of these events is consistent with the typical CARPA reaction because the changes in PAP occurred rapidly (within minutes) after the start of administration of the test substances. The preceding negative control with 5 mL/kg saline caused only a minimal change in PAP, which was not significant, excluding the possibility of a volume effect.

The thromboxane B_2 concentrations in the blood samples of ILE-treated animals also align with the CARPA hypothesis because we could observe a 75% increase from baseline to the 10-minute sample after the first injection, and an additional 50% increase by the next baseline, 20 minutes after

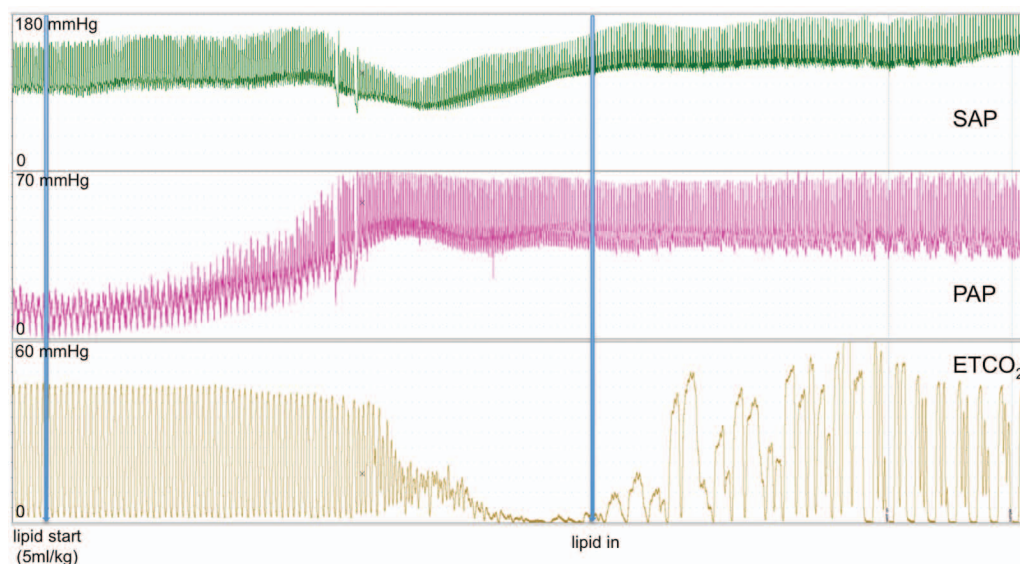


Figure 5. Typical time course of hemodynamic changes after 5 mL/kg lipid emulsion injection in one of the animals. The time span of the events pictured is 5 minutes. Halfway through the injection, the increase in pulmonary arterial pressure (PAP) is apparent, followed by slight decrease in systemic arterial blood pressure (SAP) and complete apnea. ETCO₂ = end-tidal CO₂.

the first injection. After the second injection, we observed a more than 4-fold increase, which proved to be the maximal response; thromboxane concentrations plateaued after this. Concurrently with the hemodynamic changes, we also observed the typical red macular skin rash on the abdomen and neck of every animal (Fig. 2).

CARPA is a relatively new class of hypersensitivity reactions.¹⁸ Unlike immunoglobulin E (IgE)-mediated allergy, these reactions arise without previous sensitization and are mediated by the complement system. The outcomes include activation of mast cells, polymorphonuclear cells and platelets, and the release of vasoactive mediators, such as thromboxane and histamine, with cardiovascular and other effects. Several drugs and chemicals trigger CARPA in pigs, including particulate radio contrast media, drug delivery systems, carbon nanotubes, liposomes (Doxil [Janssen Products, LP, Titusville, NJ], AmBisome [Gilead Sciences, Inc., San Dimas, CA]), and micellar solvents, such as Cremophor EL in Taxol. The best in vivo test is the porcine model because these animals are especially sensitive to complement activation.¹⁵ The underlying cause of the high sensitivity among pigs has not been clarified. However, the predominance of pulmonary symptoms suggests that the reactions may be related to the high number of pulmonary intravascular macrophages (pulmonary intravascular macrophage cells) in the microcirculation of porcine lungs. The major hemodynamic changes of CARPA are most likely due to the release of thromboxane, other eicosanoids and leukotrienes, histamine, and an additional range of potent vasoactive substances from mast cells and basophil leukocytes on binding of C3a and C5a to their respective receptors on these cells.

The typical physiological changes characteristic of CARPA and CARPA-like reactions include increase of PAP, increase or decrease of SAP, increase or decrease of HR, compromised cardiac output, dyspnea, apnea, increased or decreased ETCO₂, decreased arterial oxygen saturation measured by pulse oximetry, skin mottling, rash, etc.

(Fig. 6). These symptoms vary depending on the severity of the reaction. The most consistent sign in the pig model is acute pulmonary hypertension. In mild cases of CARPA, the SAP increases or slightly decreases. However, during severe reactions, due to compromised cardiac output and/or peripheral vasodilation, the mean arterial blood pressure can decrease to very low levels, occasionally even below the minimum required for the perfusion of vital organs. This is also reflected in the unpredictability of ETCO₂. On one hand, high ETCO₂ can result from hypoventilation. However, if the cardiac output is insufficient to maintain proper perfusion of the lungs to enable gas exchange, the ETCO₂ decreases. HR can also change unpredictably. During a minor challenge, HR slightly increases. However, during serious CARPA events, paradoxical bradycardia can be observed.¹⁹

Skin mottling is a nonspecific sign that might hint at the presence of CARPA even when PAP and other physiologic variables are not closely monitored. Similar symptoms have been described by authors of previous studies investigating the effectiveness of lipid resuscitation in swine. Litonius et al.¹⁴ reported that all pigs receiving either ClinOleic or Intralipid (Baxter, Deerfield, IL) exhibited a variable degree of reddish, partly mottled skin coloring, which was not accompanied by any changes in airway pressure or any decrease in arterial blood pressure. The authors did not monitor PAP, and in CARPA, the SAP may change in either direction.¹⁴ In another article by the same group investigating entrapment of bupivacaine in lipid dispersions, pigs experienced cardiovascular collapse after 80 mol% (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) / 20 mol% (1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (POPC/POPG) lipid infusion.²⁰ The SAP, PAP, and pulmonary capillary wedge pressure increased and oxygen saturation decreased, demonstrating typical signs of CARPA. The authors report that they have tested the commercial lipid emulsion Intralipid as well, concluding that it did not have any significant circulatory effect, except for a slight increase in SAP; however, the

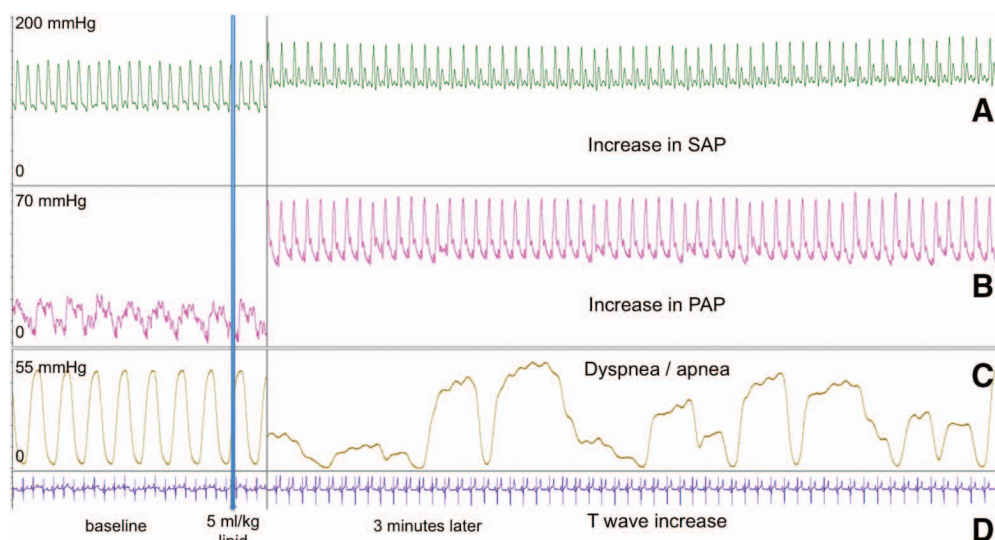


Figure 6. Typical sign of complement activation–related pseudoanaphylactic reaction observed in one of the animals treated with IV lipid emulsion. A, Increase in systemic arterial blood pressure (SAP). B, Increase in pulmonary arterial pressure (PAP). C, Dyspnea and decrease in end-tidal CO_2 . D, T-wave changes.

findings are not described in detail. In another article from the same group, 2 of the 10 pigs in the lipid group became severely hypotensive (<25 mm Hg) and slightly bradycardic and were given epinephrine.²¹ These potentially lethal hemodynamic changes with paradoxical bradycardia may also be typical manifestations of CARPA. As the authors note, the 2 pigs would probably have suffered cardiac arrest, had they not administered IV epinephrine. In a study of ILE sequestration of amiodarone in pigs, Niiya et al.²² reported that all pigs in the lipid group exhibited red skin mottling, whereas none of the pigs in the control group showed such skin reaction when studying whether ILE sequesters amiodarone and prevents its hypotensive effects. Two of these animals showed deep red skin mottling with severe hypoxemia and hypercapnia. Hudson et al.²³ also observed transient red skin rash after the administration of ILE in their resuscitative experiments for bupivacaine toxicity. They noted that “recently there have been some issues related to using swine in lipid resuscitation models,” therefore a group from their institution conducted a study to determine whether there was any change in hemodynamic parameters, blood gas, and IgE, IgG, and C-reactive protein before and after lipid infusions.²³ They found no significant changes and excluded the possibility of CARPA.²⁴ However, the end points and outcome measures in the study are not appropriate for the detection of CARPA, and the data do not support their conclusions. During their experiments, 4 of 5 pigs exhibited a red, blotchy, transient cutaneous mottling immediately after the lipid infusion that disappeared after 10 to 15 minutes. The authors also report a substantial increase of SAP (systolic, diastolic, and mean arterial blood pressure) at 15 minutes. Unfortunately, the PAP, the most sensitive and reliable variable for the detection of CARPA, was not monitored during the experiments. Furthermore, CARPA is mediated by the complement system (hence the name) and IgG, IgM, and C-reactive protein are not involved in CARPA; and the measurement of these markers within 1 hour after a stimulus is impractical nonetheless. Likewise, the time points in the study (baseline, 15, 30, and 45 minutes after lipid administration) are

not well aligned with the time course of the CARPA reaction, which typically appears within several minutes after administration of the triggering substance. Although the authors recorded the hemodynamic variables every 2 minutes, they do not show or mention the initial changes within the critical 10 minutes, the most dynamic period of CARPA.

Limitations of the Study

To further clarify complement activation by lipid emulsion, we performed *in vitro* tests with human and animal sera. There are numerous *in vitro* assays to test the complement activating properties of the various substances, but most of these are limited to human sera, and there are no reliable *in vitro* complement tests for swine, except for the CH50 assay. Furthermore, the fact that some agents with no apparent *in vitro* C activation still caused reactions in pigs suggests that the porcine CARPA test end points provide a more sensitive biomarker for anaphylactic reactions than the *in vitro* C tests. It must also be noted that the porcine CARPA model is more sensitive than the *in vitro* ELISA assays. A possible explanation is that immune responses are augmented by various cascades in the living individual but only to a certain extent in the serum *ex vivo*.²⁵

Due to the lack of suitable porcine complement assays, we were limited to the use of the human *in vitro* SC5b-9 ELISA test with human sera. In this test, the lipid emulsion did not activate to complement, but this does not exclude the possibility of complement activation in pigs, or even in humans, because there is great individual variability in the sensitivity of human sera, and it has been shown to produce negative results even when the *in vivo* model or other human sera showed complement activation.²⁶ Nonetheless, the absence of *in vitro* complement activation by ILE in the tested human sera is in keeping with the lack of reports of CARPA-like response to ILE in clinical practice.

Moreover, the sheep red blood cell assay we performed yielded false results due to interference of the ILE with the assay. The optical density (OD) of the samples treated with ILE was even higher than the OD of the samples treated

with distilled water. Distilled water causes complete hemolysis representing maximal response, and any result higher than that is considered artifact. The extremely high OD of the lipid samples was likely due to the opacity of the emulsion and not a result of complement activation. For these reasons, the *in vitro* tests were inconclusive.

CONCLUSIONS

We found that ILE causes crucial hemodynamic changes in pigs, in concert with significant increases in the plasma thromboxane concentration. The *in vitro* tests did not suggest complement activation by ILE in human sera. The lack of direct complement activation test results in pig sera leave the underlying mechanism of these findings in doubt. Nonetheless, the observed hemodynamic and biochemical effects of ILE serve as a caveat that the pig is not an ideal model for the study of interventions involving ILE. ■■

DISCLOSURES

Name: Peter Bedocs, MD.

Contribution: This author helped design and conduct the study, collect and analyze data, and prepare the manuscript.

Attestation: Peter Bedocs approved the final manuscript, attests to the integrity of the original data and the analysis reported in this manuscript, and is the archival author.

Name: John Capacchione, MD.

Contribution: This author helped design and conduct the study, collect and analyze data, and prepare the manuscript.

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Attestation: Chester C. Buckenmaier approved the final manuscript.

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