Comparing Resuscitative Measures for Bupivacaine Toxicity Utilizing Lipid Emulsions in a swine model (Sus scrofa)
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Abstract
Introduction: A toxic dose of bupivacaine causes cardiac arrhythmias and ultimately asystole. Resuscitation is difficult and almost always unsuccessful. Until recently, cardiopulmonary bypass was the only effective treatment. Anecdotal evidence suggests that infusion of lipid emulsion may be an effective treatment. No studies have determined the optimal combination of lipid rescue and traditional Advanced Cardiac Life Support (ACLS) therapy for a toxic dose of bupivacaine. The purpose was to determine the optimal combination of lipid rescue and traditional ACLS therapy for treatment overdose of bupivacaine.

Methods: This study was a prospective, experimental, between group research design. Seven swine were randomly assigned to eight ACLS or BLS protocol resuscitation groups: Vasopressin/Lipid; Epinephrine/Lipid; Lipid; Epinephrine; Vasopressin; Epinephrine/Vasopressin; Epinephrine/Lipid/Vasopressin; and CPR. Each subject was administered a toxic dose of bupivacaine (10 mg/kg) until there was a non-perfusing arrhythmia. Each resuscitation protocol was implemented. Survival was defined as return of spontaneous circulation to a systolic blood pressure ≥ 60 mm/Hg. A chi-square and odds ratio was used to analyze the data.

Results: Seventy one percent of the epinephrine/lipid group survived compared to 19% of all the groups without lipid therapy. The chances of survival for the epinephrine with lipids group was 6.2 fold greater than the groups with standard ACLS protocol. Epinephrine with lipid group had a 2.5 times greater chances for survival compared to epinephrine with vasopressin. Epinephrine with lipid offered a 15 times greater chance of survival when compared to the group that received lipid alone. No swine in the CPR or vasopressin group survived.

Conclusions: The combination of epinephrine with lipid emulsion infusion was the best resuscitation method to restore spontaneous circulation with bupivacaine toxicity in this swine model. Epinephrine alone was 43% effective, and lipid only was only 14% effective. The synergistic effect of the two combined drugs may warrant changes in ACLS protocol when there is bupivacaine toxicity.

Introduction
Toxic doses of highly lipophilic drugs, specifically local anesthetics, are usually deadly. The most devastating complication of such drugs is a non-perfusing cardiac arrhythmia, ultimately asystole. An accidental overdose of local anesthetics (or other lipid soluble drugs) have been treated with 20% lipid emulsion infusions [1-6]. Most of these anecdotal letters have stated that the last ditch effort to save the patients by starting lipid infusion was effective. These patients were not responding effectively to standard ACLS resuscitation protocols but recovered shortly after lipid infusions [7]. However, a comparison of different resuscitation techniques with and without lipid emulsion infusion have not been performed on an adult size model to determine if lipid emulsion or ACLS protocol or possibly a combination of the two is the most effective treatment [8]. However, Mauch and associates recently published a similar study using piglets in their model [9]. Another study had used lipid emulsion and Advanced Cardiac Life Support (ACLS) protocols had used hypoxia to induce cardiovascular collapse as well as toxic dose of bupivacaine [10]. When cardiac toxicity occurs, resuscitation is difficult, prolonged, and almost always fatal [11]. Utilizing newer technologies for peripheral regional anesthesia (ultrasound) does not prevent intravascular injection [12]. With widespread use of local anesthetics in the military or civilian for acute and chronic pain control, it is of paramount importance to find methods for effective resuscitation.

Until recently, cardiopulmonary bypass was the only method to effectively treat cardiac arrest from these drugs [13,14]. Infusion of lipid emulsion may be effective in treating an otherwise fatal complication. The proposed mechanism of action of lipid therapy is not known but is thought to be a combination of reduced tissue binding by re-established equilibrium in the plasma lipid phase and possibly a beneficial energetic-metabolic effect [15]. Previous research stresses that future areas of investigation should focus on improved treatment regimes and better understanding of the mechanism of lipid therapy [16].

The optimal combination of lipid rescue and traditional ACLS therapy for treatment of bupivacaine toxicity has not been performed. The hypothesis of this study is as follows: Is there a significant statistical difference in survival when combining lipid emulsion infusion with standard ACLS protocols with bupivacaine toxicity? (Figure 1 is a graphical depiction of the theoretical framework) Current research into the effectiveness of lipid emulsion in animal models has had mixed results. Figure 2 depicts graphically the mixed results that have been obtained regarding optimal treatment of bupivacaine drug toxicity. Moreover, studies are lacking in determining the optimal combination of lipid rescue and traditional ACLS therapy for treatment of overdose of bupivacaine. This study examined the comparative effectiveness of eight resuscitation strategies given a toxic dose of bupivacaine. In accordance with other studies investigating local anesthetic toxicity and resuscitation, our sample size was determined to be 7 in each group [17-19]. The eight groups tested (with 7 in each group) were CPR only and CPR with the following drug combinations: epinephrine alone; vasopressin alone; lipid alone;
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epinephrine and vasopressin; epinephrine and lipid; vasopressin and lipid; and epinephrine, vasopressin, and lipid.

Materials and Methods

Study design

This study used a prospective, experimental, between subjects design with a swine model. The study was approved by our institutional IACUC committee. The animals received care in compliance with the Animal Welfare Act and the Guide for the Use of Laboratory animals.

Setting

Fifty-four Male Yorkshire swine weighing approximately 55-75 kgs were acclimated to the research facility for 4-7 days to ensure good state of health, fed a standard diet and remained NPO after midnight the day of the experiment but were allowed water ad lib. The weight and size of the swine approximates the average weight of an adult male. Male swine were used to decrease the possible confounding variables related to female hormones.

Procedure

Intramuscular sedation was provided with Telezol (4-8 mg/kg) and Glycopyrrolate (0.2 mg) 30 minutes prior to instrumentation by a veterinary technologist or qualified technician. Anesthesia was induced by snout mask with oxygen and isoflurane (2-4%). An ear vein was cannulated and infused with lactated ringers at 10 ml/kg/hr for the remainder of the procedure. Standard ECG pads were applied. Body temperature was monitored with a rectal temperature probe to maintain euthermia with a heating blanket. The trachea was intubated with a cuffed endotracheal tube and the animals were ventilated (tidal volume 8-10 cc/kg, respiratory rate 8-14 breaths per minute) with a standard Narkomed anesthesia machine (Drager, Telford, PA) and continuously monitored with the following standard monitors: HR, SBP, MAP, DBP, ECG, SpO₂, CO, ETCO₂. Isoflurane end tidal concentration. Tidal volume was adjusted (10 ml/kg) to ensure normocapnia (32-36 mmHg end tidal carbon dioxide). A large bore venous catheter was inserted into the left jugular vein and another catheter was inserted into the left carotid artery. These two catheters were used for blood sampling, fluid/ lipid emulsion infusions and cardiac output monitoring via the Vigileo cardiac output monitor (Edwards Lifesciences). Another arterial catheter was inserted into the femoral artery and attached to the Marquette Solar 800 system for continuous BP pressure monitoring. The animals were allowed to stabilize for 10 minutes following instrumentation prior to beginning the procedure.

Methods

Swine were allocated to each group as assigned by a computer random number generator. Following completion of preparatory procedures, the investigators administered a toxic dose of bupivacaine (10 mg/kg) via the jugular catheter followed by 20 ml normal saline flush. Upon confirmation of a non-perfusing rhythm, CPR was initiated, isoflurane terminated and an injection of 0.1mg/kg of Midazolam and 0.6mg of Buprenex was given IV. The external cardiac compressions were delivered by a pneumatic compression device (Life-Stat Cardiopulmonary Resuscitator, Model 1008) we have called the “Thumper”. The Thumper was set at a 30:2 compression ventilation ratio with a rate of 100 compressions per minute and a sternum depth of 20% of the measured thoracic anterior to posterior diameter and a 50:50 compression relaxation ratio. Compressions were stopped every 2 minutes for cardiac rhythm analysis. At the first 2 minute mark, intravenous resuscitation drugs were given to each animal according to its group designation via peripheral IV followed by a 20 ml NS flush. Table 1 displays the drug doses given according to group designation. Ventilation was maintained with 100% oxygen. Blood pressure, HR, pulse oximetry, end-tidal carbon dioxide and cardiac output were recorded every two minutes.

After every two minutes of cardiopulmonary resuscitation, pulse and cardiac rhythm were checked. Ventricular fibrillation or tachycardia was defibrillated with 200J for the initial shock and 360J for subsequent shocks. Treatment ended after 30 minutes with an additional 10 min for those swine that had a return of spontaneous circulation (total 40 min). If a sustainable, perfusing rhythm occurred anytime during the experiment (systolic BP over 60 mm/hg), CPR ended but monitoring continued. Survival for this experiment is defined as a perfusing rhythm that maintains a SBP over 60 mm/hg [20].
technician, or qualified technician without emergence from general anesthesia.

A multivariate analyses of variance was used to determine if the groups were equally distributed with regards to pre-intervention (weight, temperature, vital signs) data. The post-intervention data was analyzed using an odds ratio (chance of survival) to determine odds of survivability between the groups. Additionally, a Chi-Square test was used to test for statistical significance differences among groups. Means and standard deviations from a previous study were used to calculate an effect size \[16\]. The investigators also used G-Power 3.0.10 to determine the sample sizes needed in the bupivacaine experiment. Using an alpha of 0.05, power of .80, and a large effect size, 0.6, the number needed was determined to be 7 per group.

**Results**

A total of 54 animals were included in the research. A MANOVA was used to analyze the pretest variables of all laboratory values, weight, vital signs and NPO deficit replacement. There were no statistically differences between the groups (p>.05) indicating all groups were equivalent relative to these stated parameters. The CPR only group was purposely reduced to 5 swine (instead of 7) because of the lethality of the model and zero probability that any swine in that group would recover in order to reduce the number of swine used.

The epinephrine and lipid emulsion group had the greatest number of survivors with five of the seven swine surviving. The Epinephrine only group yielded three survivors and the Lipid emulsion only group yielded one survivor. No swine in the CPR only or Vasopressin only groups survived. Figure 4 outlines the results of survivability between intervention groups.

The impact of Epinephrine and Lipid emulsion, both alone and in combination, with regard to treatment of bupivacaine toxicity is depicted in Figure 5. Seventy one percent of the animals in the Epinephrine/Lipid emulsion group survived compared to 19% of all the groups without Lipid emulsion therapy (p = 0.008). The chances of survival for the Epinephrine/Lipid emulsion group was 3.7 fold greater than all the groups without Lipid emulsion therapy. Furthermore, there was a statistically significant difference relevant to survival between the Epinephrine/Lipid emulsion group and the CPR only group (p=0.028); and when compared with the Vasopressin group (p=0.05).

Comparison of the chances of survival of different ACLS regimes given a toxic dose of bupivacaine is listed in Table 2. In comparing

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**Table 1: Drugs used for Resuscitation Protocol.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
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<tbody>
<tr>
<td>Epinephrine</td>
<td>1 mg, repeated every 3 minutes</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>40 units given once</td>
</tr>
<tr>
<td>Lipid emulsion</td>
<td>2 ml/kg bolus over 2-3 minutes followed by 0.25 ml/kg/min infusion for 10 minutes</td>
</tr>
</tbody>
</table>

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**Table 2: Comparison of chances of survival of different ACLS regimes given a toxic dose of bupivacaine.**

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**Figure 3: Overview of the Experimental Procedure.**

**Figure 4: Results by Interventions.**

**Figure 5: The Impact of Epinephrine and Lipid Emulsion.**

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the group that represents group 1 to the group the represents group 2, group 1 has a greater likelihood of survival, which is represented by the chances of survival number. For example, there is a 15 times greater odds of survival of bupivacaine toxicity if one used epinephrine and lipid emulsion vs. using lipid emulsion alone. Chi-square results of comparisons of selected groups are listed in the last column of Table 2.

One other notable result of the study was the time of return to spontaneous circulation for the swine that did survive. As shown in Table 3 it also appears that not only did the Epinephrine/ Lipid group have the greatest number of swine that returned to spontaneous circulation, but they returned faster. The mean time for return of spontaneous circulation for that group was 4 minutes. Although this rapid response is two-seven times faster than the other survivors in the other groups, the small number of survivors overall (small n) may be an issue. However, the trend is apparent that this drug combination not only offers increased survivability, but may also deliver quicker onset to return of spontaneous circulation.

**Discussion**

This study investigated eight different rescue methods incorporating ACLS as the basis to survival in the face of bupivacaine toxicity. Our major finding was that epinephrine with lipid emulsion had the highest survivability (71%) and possibly the quickest return to spontaneous circulation in a very lethal swine model.

Based on the anecdotal evidence regarding the benefit of incorporating lipid emulsion for treatment of bupivacaine toxicity [3,21-23], and animal studies [14-16], it is currently recommended by Dr Weinberg at Lipid Rescue [24] that lipid emulsion be available during any regional anesthetic block with local anesthetic. This treatment protocol is widely accepted in many hospitals and countries [25].

The results of this study showed that lipid emulsion and Epinephrine in combination was the single best treatment as far as survival was concerned, being effective 71% of the time when treating a fixed (mg/kg) bupivacaine overdose. A recent study by Mauch et al. [9] utilizing piglets had similar results with the epinephrine / lipid group having the best survivability (86%) although they used a variable dose of bupivacaine. (Questions arise because of the variable bupivacaine dose as the epinephrine / lipid group had the highest survivability but also had the lowest bupivacaine blood concentration levels.) Without the inclusion of lipid emulsion, traditional ACLS therapy with Epinephrine alone was the second most effective treatment group, which was 43% of the time. However, evidence has indicated that epinephrine in high doses offers no or little benefit when combined with lipid infusion in local anesthetic induced cardiac arrest [26].

In this model, lipid emulsion alone was not as effective as expected. Anecdotal evidence available prior to beginning this study indicated that lipid emulsion infusions in the face of bupivacaine overdose was very effective. Lipid emulsion alone was effective only 14% of the time. As similar to the study done by Mauch, lipid alone rarely resulted in return of spontaneous circulation (ROSC). However, in that study, those swine with lipid infusion infusion with adrenergic support required much less epinephrine to maintain ROSC than the epinephrine alone group. Epinephrine has been known to cause significant rhythm disturbances especially in higher doses. Combining lower dose epinephrine with lipid emulsion may lead to less post-code sequelae than frequent doses of epinephrine [27].

In our study it was expected that the best response to the lethal dose of bupivacaine would be lipid emulsion with epinephrine with vasopressin. Surprisingly, that group had a decreased survival rate as compared to lipid emulsion/ epinephrine group and none of the swine that received vasopressin alone survived. Di Gregorio et al. had similar results in a rat model as vasopressin was associated with adverse outcomes [28]. In a study by Hicks, lipid with epinephrine with vasopressin likewise did not improve survival in a swine model [17]. In the study by Mauch, the lipid only and lipid/vasopressin groups had the lowest survivability [9]. However in another swine study by Mayr et al., found epinephrine and vasopressin group had 100% survival, but they used a lower dose bupivacaine toxicity model, no lipid infusion, and had a hypoxic variable [18]. It is suggested by this author that vasopressin may be avoided in patients exposed to a toxic dose of bupivacaine until further definitive studies can be done.

Swine are physically and anatomically very similar to humans and their ease of use has made them used widely in resuscitation protocols. With that stated, it should be noted that recently there has been some issues related to using swine in lipid resuscitation models. In our study, as well as the study by Niiya et al., some of our swine exhibited a transient red mottling rash after administration of the lipid

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**Table 2: Odds Ratio of Survival, Selected Comparisons of Groups.**

<table>
<thead>
<tr>
<th>Group 1: (all groups with epinephrine)</th>
<th>Group 2: (all groups with no epinephrine)</th>
<th>Chances of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>No epinephrine</td>
<td>3.7X times greater survival</td>
<td></td>
</tr>
<tr>
<td>No lipid emulsion groups</td>
<td>1.85X</td>
<td></td>
</tr>
<tr>
<td>Lipid emulsion alone</td>
<td>3X</td>
<td></td>
</tr>
<tr>
<td>Lipid emulsion, and Vasopressin</td>
<td>2.5X</td>
<td></td>
</tr>
<tr>
<td>Lipid emulsion alone</td>
<td>5X</td>
<td></td>
</tr>
<tr>
<td>No lipid emulsion groups</td>
<td>3.7X</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Comparison of Survivors Mean Time to Return of Spontaneous Circulation.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Survivors</th>
<th>Mean Time to Return of Spontaneous Circulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasopressin/ Lipid</td>
<td>2</td>
<td>28 minutes after injection of bupivacaine</td>
</tr>
<tr>
<td>Epinephrine/ Lipid</td>
<td>5</td>
<td>4 minutes after injection of bupivacaine</td>
</tr>
<tr>
<td>Lipid only</td>
<td>1</td>
<td>14 minutes</td>
</tr>
<tr>
<td>Epinephrine/ Vasopressin/Lipid</td>
<td>2</td>
<td>13 minutes</td>
</tr>
<tr>
<td>Epinephrine/ Vasopressin</td>
<td>2</td>
<td>24 minutes</td>
</tr>
<tr>
<td>Epinephrine only</td>
<td>3</td>
<td>9.3 minutes</td>
</tr>
<tr>
<td>Vasopressin only</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>CPR only</td>
<td>0</td>
<td>n/a</td>
</tr>
</tbody>
</table>

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infusion [29]. Therefore, before initiating this study, we performed a small study examining IgE, IgG, c-reactive protein, blood gas analysis and cardiovascular parameters to determine if there are any changes in these parameters before and after lipid infusions. We found that there were no significant changes in any of our measured parameters before and after lipid emulsion infusions [30]. Although these tests were not ‘CARPA’ specific [31], there was no CARPA- like reactions with major cardiovascular changes. Swine have been shown to have consistent major cardiovascular depressive changes after liposome / nanoparticle infusions. Liposome and nanoparticle solutions have been tested as vehicles with other drugs (narcotics, antibiotics and chemotherapeutics) to increase their half-life and effectiveness. It has been hypothesized that swine may also have CARPA like reactions (major cardiovascular depression) after lipid infusions. A transient increase in pulmonary hypertension has occurred with all swine exposed to liposomes/ nanoparticles with significant decreases in systemic arterial pressures [32]. This decrease in SBP did not occur with our infusion of lipids, therefore, offering the possibility that this swine model may be suitable for lipid infusion experiments.

Further research is necessary to validate the findings of this study. Larger study groups and a larger number of subjects may also be helpful to further demonstrate significance of the results, especially to confirm if time to onset of ROSC is indeed quicker with the lipid emulsion/ epinephrine group. Additionally, it is not known if these results are generalizable to other animal species; hence, study replication using another species of animal, such as sheep or rabbits, could potentially confirm the findings of this study. Likewise, it is not known if these results are applicable to other local anesthetics; therefore, research pertaining to toxicity of other local anesthetics could prove invaluable for treatment of local anesthetic toxicity.

Conclusion

Based on the results of this study, the synergistic effect of the two combined drugs (epinephrine and lipid emulsion) may warrant changes in ACLS protocol when there is local anesthetic toxicity. Also the use of vasopressin in this scenario should be used cautiously, if at all, until further research is completed. This finding could have significant implications for improving survival rates following overdose of lipophilic drugs, specifically the local anesthetic bupivacaine.

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References


