The Relative Toxicity of Amitriptyline, Bupivacaine, and Levobupivacaine Administered as Rapid Infusions in Rats

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Intravascular injection of local anesthetics carries the risk of cardiovascular (CV) and central nervous system (CNS) toxicity. Amitriptyline, a tricyclic antidepressant, has local anesthetic potency that is more than that of bupivacaine. In this study, we compared the CV and CNS toxicity of the local anesthetics bupivacaine and levobupivacaine with that of amitriptyline. Twenty-nine Sprague-Dawley rats had their right external jugular vein and carotid artery cannulated under general anesthesia. On Day 2, rats were sedated with midazolam (0.375 mg/kg intraperitoneally) and received rapid infusions of either 1) bupivacaine, levobupivacaine, or amitriptyline at 2 mg · kg⁻¹ · min⁻¹ (5 mg/mL concentration) or 2) normal saline (400 μL · kg⁻¹ · min⁻¹) through an external jugular vein cannula. Electrocardiogram and arterial blood pressure were measured until the dose to cause impending death was reached (heart rate 50 bpm/asystole or apnea for >30 s). The mean dose required to cause apnea and impending death was significantly larger for amitriptyline (74.0 ± 21 mg/kg and 74.5 ± 21 mg/kg, respectively) than for levobupivacaine (32.2 ± 20 mg/kg and 33.9 ± 22 mg/kg, respectively) or bupivacaine (21.5 ± 7 mg/kg and 22.7 ± 7 mg/kg, respectively) (P < 0.05). A significantly larger dose of amitriptyline, given by rapid infusion, is required to cause CV and CNS toxicity in rats, when compared with bupivacaine and levobupivacaine.

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room temperature (24°C) and a 12-h (6:00 AM to 6:00 PM) light-dark cycle. The animals had unlimited access to food and water. On Day 1, the rats were anesthetized via intraperitoneal injection with ketamine 45 mg/kg (100 mg/mL; ketamine HCl; Abbott Laboratories, North Chicago, IL) and xylazine 20 mg/kg (Xyla-Ject; Phoenix Pharmaceuticals Inc., St. Joseph, MO) and had right external jugular vein (EJV) and right carotid artery catheters placed.

After the induction of general anesthesia, the right anterior neck area was shaved, and a vertical parametrical incision was made over the clavicle. The EJV was identified, and 2 loose 3-O silk suture ties were placed around the vein. The distal ligature was tied tightly. Next, an incision was made over the vein, and a 12-cm-long polyethylene catheter (PE-50), which was previously flushed with heparin saline 2 U/mL (1000 U/mL; heparin sodium; American Pharmaceutical Partners Inc., Los Angeles, CA), was threaded approximately 2 cm into the vein. The other end of the catheter was attached to a 1-mL syringe filled with heparin saline (2 U/mL) through a tubing adapter. After free aspiration of blood was confirmed, the proximal ligature was tied, securing the catheter to the vein. A hemostat with a soft tip (so as not to damage the catheter) was applied to the catheter 2–3 cm from the incision, and the syringe was removed. The free end of the catheter was then threaded through a 17-gauge needle with the hub removed. The needle and the catheter threaded through it were then tunneled subcutaneously to the midline in the posterior cervical area below the level of the ears, exiting the skin at this point. The needle used for subcutaneous tunneling was removed and the catheter was cut, leaving 4 cm protruding from the skin. The tip of the catheter was sealed by heating it with a match and compressing it with a hemostat.

With the same surgical incision, the right carotid sheath lying posterolateral to the trachea was identified, and the internal carotid artery was dissected. The carotid artery cannulation and tunneling of the catheter were identical to those described with the external jugular vein, except that the catheter exited the skin approximately 5 mm proximal to the jugular venous catheter.

On Day 2, the rats were sedated with midazolam 0.375 mg/kg (1 mg/mL, midazolam HCl; Abbott Laboratories) intraperitoneally. After 10 min and under brief sevoflurane (Ultane; Abbott Laboratories) inhaled anesthesia (2–3 min), electrocardiogram (ECG) leads were placed subcutaneously, and Lead II was measured (Model 90603A; Spacelabs Medical Inc., Redmond, WA). The heart rate (HR) was recorded as beats per minute. The right carotid artery line was connected to a transducer, and arterial blood pressure was monitored. The right EJV was connected to an infusion pump (Harvard Model 22 Infusion Pump; Harvard Apparatus Inc., Holliston, MA) for delivery of the study drugs (5 mg/mL or 0.5%). The animal was placed in a small cage with an open top, which restricted movement to a small area, allowing the lines to reach the animal from the top while preventing the animal from chewing on the lines. The animals were allowed to wake up spontaneously from the inhaled anesthetic for at least 10 min before the drug infusion was started. The ECG and arterial blood pressure tracings were displayed continuously, and hard copies were obtained every 30 s and as needed. The investigators were blinded to the drug under study. The animals received either 1) bupivacaine 0.5% (n = 7) (bupivacaine HCl; Abbott Laboratories), levobupivacaine 0.5% (n = 6) (Chirocaine; Purdue Pharma LP, Stamford, CT), or amitriptyline 5 mg/mL (n = 10) (10 mg/mL; Elavil; Zeneca Pharmaceuticals, Wilmington, DE) at 2 mg · kg⁻¹ · min⁻¹ or 2) normal saline (n = 6) in the volume of 400 µL · kg⁻¹ · min⁻¹ (the same volume given to the animals in the drug group) as infusion. Seizure dose was defined as the time (and, hence, the amount of the drug) when the first convolution occurred, and apnea dose was when there was apnea for 15 s. Apnea was determined during the study by observation of chest movement. The dose to cause impending death was marked by any of the following: a decrease in HR to 50 bpm (from our preliminary studies, this HR indicated impending death, because it was immediately followed by asystole), ventricular tachycardia (VT), ventricular fibrillation (VF), or respiratory arrest for 30 s. The control group received an infusion of normal saline for 30 min. The cumulative doses of drugs required to reach the study end-points were calculated. When the rat reached a study end-point, all data collection was stopped, 1.5 mL of blood was drawn from the arterial line, and the samples were frozen. In the control group, the sample was collected after 30 min of normal saline infusion. The drug concentrations were measured in whole blood by high-performance liquid chromatography at MedTox Laboratories Inc. (St. Paul, MN).

Data are presented as mean ± sd. Intergroup comparisons of the dose to impending death were performed by using unpaired Student’s t-tests. Fisher’s exact probability tests were performed to assess the likelihood of a drug-induced seizure, apnea, or both for amitriptyline versus bupivacaine and amitriptyline versus levobupivacaine. A P value of <0.05 was considered significant.

Results

The baseline readings of HR, mean arterial blood pressure (MAP), and body weight did not differ significantly among the groups (Table 1). The dose to cause
Table 1. Baseline Data and Time to Reach the Dose to Impending Death (Heart Rate of 50 bpm, Ventricular tachycardia, Ventricular Fibrillation, or Respiratory Arrest for 30 seconds) Are Presented as mean ± sd

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bupivacaine</th>
<th>Amitriptyline</th>
<th>Levobupivacaine</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>212 ± 12</td>
<td>219 ± 12</td>
<td>215 ± 15</td>
<td>225 ± 10</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>122 ± 18</td>
<td>162 ± 61</td>
<td>135 ± 18</td>
<td>118 ± 9</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>411 ± 64</td>
<td>426 ± 60</td>
<td>460 ± 72</td>
<td>400 ± 62</td>
</tr>
<tr>
<td>Time to reach dose to cause impending death (min)</td>
<td>11 ± 3</td>
<td>36 ± 10*</td>
<td>16 ± 11</td>
<td>—</td>
</tr>
</tbody>
</table>

There were no significant differences among the groups for these variables. Duration of infusion to reach “impending death” was significantly longer in the amitriptyline group when compared with bupivacaine and levobupivacaine. *P < 0.05 for amitriptyline versus bupivacaine and levobupivacaine.

impending death was larger in the amitriptyline group (74.5 ± 21 mg/kg) than in the levobupivacaine (33.9 ± 22 mg/kg) and bupivacaine (22.7 ± 7 mg/kg) groups (Fig. 1). The difference between the dose for impending death in the amitriptyline group and the other groups was significant (P < 0.05). Seizures, respiratory arrest, and death occurred in rapid succession in the animals in the levobupivacaine and bupivacaine groups when compared with those in the amitriptyline group. In the bupivacaine group 5 (71%) of the 7 animals, in the levobupivacaine group 5 (83%) of the 6 animals, and in the amitriptyline group 5 (71%) of the 7 animals had seizures. The doses required to cause seizures were larger in the amitriptyline group (50.1 ± 5 mg/kg) than in the levobupivacaine (31.3 ± 20 mg/kg) and bupivacaine (23.1 ± 6 mg/kg) groups. In the amitriptyline and levobupivacaine groups, all of the animals developed respiratory arrest, whereas six (85%) of the seven animals in the bupivacaine group had respiratory arrest. The doses required to cause apnea were larger in the amitriptyline group (74.0 ± 21 mg/kg) than in the levobupivacaine (32.2 ± 20 mg/kg) or bupivacaine (21.5 ± 7 mg/kg) groups. In the amitriptyline group 1 (10%) of 10 animals, in the levobupivacaine group 1 (16%) of 6 animals, and in the bupivacaine group 3 (42%) of 7 animals had VT/VF as the terminal rhythm.

In the amitriptyline group, 7 (70%) of 10 animals had myoclonus, which occurred at a dose of 24.9 ± 9 mg/kg. All animals in the amitriptyline group that had seizures also had myoclonus. Myoclonus was not seen in the other groups.

In all study groups, the MAP showed a tendency to increase before CV collapse (Fig. 2). The decline in HR was slower in the amitriptyline group compared with the bupivacaine and levobupivacaine groups, but this was not statistically significant (Fig. 3). The blood concentration of bupivacaine (5.76 ± 3 μg/mL) at the time of impending death was significantly smaller than that of levobupivacaine (13.08 ± 7 μg/mL). The blood concentration of amitriptyline was 3288.6 ± 1139 ng/mL. The blood from the control group tested negative for the study drugs.

Discussion

This study demonstrates that significantly larger doses of amitriptyline given by rapid infusion are required to cause CV and CNS toxicity in rats, when compared with bupivacaine and levobupivacaine. The CV toxicity of bupivacaine, a widely used long-acting local anesthetic, is well known (1). For this reason, 0.75% bupivacaine was withdrawn from use in obstetrical anesthesia (12). The longer-acting amide local...
anesthetics levobupivacaine and ropivacaine, which have less CV toxicity, were later introduced into clinical practice (2,3). Levobupivacaine is equipotent to bupivacaine (13), whereas ropivacaine is at least 40% less potent than bupivacaine (14–16). Variations in potency between ropivacaine and bupivacaine could therefore account for the differences in their side effects and toxicity (17). We did not choose to include ropivacaine in this study because of the continuing controversy regarding its potency. Amitriptyline, a TCA, was found to be 5–10 times more potent than bupivacaine in binding to Na⁺ channels in cultured neuronal cells (8). Further, in experiments in rat sciatic nerve, blockade was approximately two to four times longer at 5 and 10 mM amitriptyline, respectively, versus bupivacaine at 15 mM (8).

Amitriptyline overdose causes cardiac conduction delays, arrhythmias, and hypotension (10,18). Amitriptyline overdose, principally due to Na⁺ channel blockade (19), manifests as sinus tachycardia and QRS prolongation, although ECG changes do not necessarily represent significant cardiac or neurological toxicity (10). On the basis of studies in rats, Knudsen and Abrahamsson (20) concluded that myocardial depression was the likely cause of death in amitriptyline toxicity and that both epinephrine and norepinephrine appeared effective in reversing the hemodynamic alteration caused by amitriptyline.

In this study, MAP increased in all the groups before death. We speculate that this could be because of hypercarbia and hypoxia caused by respiratory depression secondary to CNS toxicity. Of interest, in anesthetized dogs amitriptyline in smaller doses increased HR, contractility, and coronary blood flow. Larger doses caused initial CV depression, followed by a secondary reflex increase in the same variables. This secondary reflex increase was attenuated by propranolol (21).

The advantage of the model we have chosen is that the rats were sedated and spontaneously breathing, a common clinical scenario when nerve blockade is performed on humans. The use of midazolam could have influenced the study in two ways. Midazolam could have increased the doses of the study drugs required to cause seizures, and, conversely, it could have potentiated the respiratory depression caused by the study drugs and hence decreased the doses needed to reach the study end-points. Rapid infusion over a period of time (as in cases of accidental intravascular injection) facilitated reaching the end-points in the study predictably. However, the oxygen saturation and other measures of ventilation (end-tidal CO₂) were neither monitored nor controlled during the study; only the respiratory rate was monitored.

Figure 2. Mean arterial blood pressure (MAP) changes (mm Hg) during infusion of amitriptyline (n = 10), bupivacaine (n = 7), and levobupivacaine (n = 6) at 2 mg·kg⁻¹·min⁻¹ or of normal saline at 400 μL·kg⁻¹·min⁻¹ (control) (n = 6); 0 min is the start of infusion. Infusion was stopped when the dose to cause impending death (heart rate of 50 bpm, ventricular tachycardia, ventricular fibrillation, or respiratory arrest for 30 s) was reached. Data are presented as means ± sd.

Figure 3. Heart rate (HR) changes (bpm) during infusion of amitriptyline (n = 10), bupivacaine (n = 7), and levobupivacaine (n = 6) at 2 mg·kg⁻¹·min⁻¹ or of normal saline at 400 μL·kg⁻¹·min⁻¹ (control) (n = 6); 0 min is the start of infusion. Infusion was stopped when the dose to cause impending death (heart rate of 50 bpm, ventricular tachycardia, ventricular fibrillation, or respiratory arrest for 30 s) was reached. Data are presented as means ± sd.
could explain the smaller doses of drugs required to reach the toxic end-point in this study, when compared with other studies performed under general anesthesia with controlled ventilation (3). Further studies that investigate the success of resuscitation from CV collapse after TCAs such as amitriptyline would be beneficial to establish the role of these drugs in regional anesthesia.

In conclusion, significantly larger doses of amitriptyline, given by rapid infusion, are required to cause CV and CNS toxicity in rats, when compared with bupivacaine and levobupivacaine.

References