Epinephrine Deteriorates Pulmonary Gas Exchange in a Rat Model of Bupivacaine-Induced Cardiotoxicity

A Threshold Dose of Epinephrine

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Background and Objective: The study goal was to compare the effect of epinephrine in different doses on pulmonary gas exchange in a rat model of bupivacaine-induced cardiac depression.

Methods: Twenty-four adult male Sprague-Dawley rats were divided into 4 groups (n = 6), and each group received a bupivacaine infusion (2.5 mg/kg per minute, 6 minutes) via the left femoral vein to induce cardiac depression. At the end of the bupivacaine infusion, each group was immediately given either isotonic sodium chloride solution (normal saline; NS group), 5-μg/kg epinephrine (Epi5 group), 10-μg/kg epinephrine (Epi10 group), or 20-μg/kg epinephrine (Epi20 group). Left atrial pressures were monitored for 20 minutes after epinephrine was administered (as was the NS group). Arterial blood gas analyses were performed before bupivacaine infusion and at the end of the 20-minute monitoring period.

Results: The Epi10 and Epi20 groups had lower pH (P < 0.02 and P < 0.001, respectively) and PaCO2 (P = 0.049 and P < 0.001, respectively), and a higher PaO2 (P < 0.001 and P = 0.001, respectively) compared with the NS group. There were no statistical differences between the Epi5 and NS groups in pH, PaCO2, or PaO2. Left atrial systolic pressure was higher in the Epi10 group (P = 0.002) and the Epi20 group (P < 0.001) within 2 minutes of epinephrine administration. There was no statistical difference between the Epi5 and NS groups in left atrial systolic pressure.

Conclusion: A single injection of 10 μg/kg epinephrine or greater was associated with deterioration of pulmonary gas exchange in our rat model of bupivacaine-induced cardiac depression.

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rats had an ad libitum diet of stock laboratory nutrition (Beijing Keao-Xieli Feedstuff Co, Ltd, Beijing, China) and tap water. After the animals had exhibited adequate weight gain, 24 rats age 8 to 9 weeks, weighing 320 to 350 g, were included entered into the study.

The rats were fasted for 12 hours before the experiments, and they had free access to water. On the day of the experiment, the rats were anesthetized with an intraperitoneal injection of chloral hydrate (350 mL/kg). Tracheal intubation was performed via tracheotomy, and the rats were mechanically ventilated with 1% to 2% sevoflurane and 100% oxygen using a rodent volume-controlled ventilator (tidal volume, 6 mL/kg; rate, 63–65 breaths-per minute; inspiratory/expiratory ratio, 2:3; HX-100E, TME Technology Co, Ltd, Chengdu, China). Body temperature was maintained at 38°C to 39°C using a thermal insulation blanket underneath the body and a heating lamp kept at a safe distance. The left femoral artery was cannulated for arterial blood pressure monitoring and sample collection. The left femoral vein was cannulated for administration of medications. The left internal jugular vein was cannulated for central venous pressure monitoring. The skin of the left chest wall was shaved, and the third and fourth ribs were exposed. The third and fourth ribs and intercostal muscles between them were incised, followed by separation of the left pleura from the left lung and the pericardium from the heart. A catheter for left atrial pressure monitoring was inserted into the left atrium through an incision on the left atrium appendage and ligated with silk thread. The surgical exposure was sealed with a rubber dam to protect the thoracic structures. Proper seating of the seal was confirmed by observation of the rhythmic swing of the rubber dam with ventilation (Figs. 1–3).

Sufficient ventilation with a 100% fraction of inspired oxygen was achieved with a tidal volume of 8.5 mL/kg.

**FIGURE 1.** Exposure of the third and fourth ribs.

**FIGURE 2.** Opening of the left chest wall and separation of the left pleura from the left lung and the pericardium from the heart.

**FIGURE 3.** Catheter for left atrial pressure monitoring and exposed thoracic contents sealed with a rubber dam.
Hemodynamic parameters, left atrial pressure, and central venous pressure were recorded by a data archiving and retrieval system (RM6240, TME Technology Co, Ltd). After completion of the invasive procedures, all animals were allowed to stabilize physiologically for 20 minutes (to recover from induction of anesthesia and invasive monitor/device insertion). An arterial blood gas analysis was then performed for measurement of baseline parameters.

Experimental Protocol

Twenty-four adult male Sprague-Dawley rats were divided into 4 groups: NS, Epi5, Epi10, and Epi20 groups according to random number table (n = 6). At the end of stabilization period (baseline time, designated T0), bupivacaine hydrochloride (Sigma-Aldrich Co, St. Louis, Missouri) was infused at 2.5 mg/kg per minute for 6 minutes via the left femoral vein. Once the infusion of bupivacaine was completed (designated 0 minute), each group of 6 rats immediately received their assigned administration of isotonic sodium chloride solution bolus of 0.1 mL (NS group), or an epinephrine (Jinyao Amino Acid Co, Ltd, Tianjin, China) bolus of 0.1 mL: consisting of 5 μg/kg (Epi5 group), 10 μg/kg (Epi10 group), or 20 μg/kg (Epi20 group). Hemodynamic parameters, left atrial pressure, and central venous pressure were monitored for 20 minutes after completion of the bolus. All intravenous fluids were aspirated into syringes of the same type marked A, B, C, and D and preheated to 37°C before administration by personnel who did not otherwise participate in the conduct of the study. Investigators were blinded as to the contents of syringes A, B, C, and D. Arterial blood gas analyses (2 mL of arterial blood taken from the left femoral artery) were performed just before the study. Investigators were blinded as to the contents of syringes A, B, C, and D and preheated to 37°C before administration by personnel who did not otherwise participate in the conduct of the study. Investigators were blinded as to the contents of syringes A, B, C, and D. Arterial blood gas analyses (2 mL of arterial blood taken from the left femoral artery) were performed just before bupivacaine infusion and just before killing the animals at the termination of the 20-minute observation period.

Lung Permeability Index

The blood sample taken at the end of the observation period was centrifuged (3000 revolutions per minute [rpm], 10 rpm, 10 minutes), and the separated plasma was centrifuged (3000 rpm, 10 minutes), and the supernatant was removed. After thoracotomy, the left lung lobe was intubated and lavaged 3 times using 10 mL of isotonic sodium chloride solution. The bronchoalveolar lavage fluid (BALF) was centrifuged at 1000 rpm for 10 minutes (at 4°C), and the supernatant was removed. All the samples were stored at −80°C for future analysis. Protein concentrations of BALF in the supernatant and serum were determined by the bicinchoninic acid protein assay, using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc, Rockford, Illinois) according to the manufacturer’s instructions. The protein concentration in BALF/serum ratio was defined as lung permeability index.12

Observation of Specimens for Light Microscopy

The second lobe of the right lung was removed and placed in 10% formalin fixative. The slices were stained with hematoxylin and cosin, embedded into paraffin, observed under light microscopy (TS100-F, Nikon Co, Tokyo, Japan) (×400 power) and photographed. We randomly selected 50 views in each sample and counted the total number of the pulmonary alveoli and the number that were injured in each view. When more than 2 inflammatory cells or 2 red blood cells were evident in one pulmonary alveolus, we determined the alveolus was injured. We then calculated the ratio of the injured alveoli in each view: the number of injured alveoli / the total number.13

Observation of Specimens for Electron Microscopy

The third lobe of the right lung was removed, resected into small sections (1 mm³), and fixed in 2.5% glutaraldehyde at 4°C. Tissues were washed 3 times in phosphate buffer and then postfixed with 2% osmic acid, followed by 3 more washes in phosphate buffer, dehydrated by passage through graded alcohol concentrations, embedded with epoxy resin, and then sectioned into ultrathin slices. The slices were stained with 1% uranyl acetate and lead citrate and photographed for ultrastructure under a transmission electron microscope (H-7500, Hitachi Co, Tokyo, Japan) (×30,000 power).

Wet-to-Dry Ratio

The fourth lobe of the right lung was weighed immediately after removal (wet weight) and again after drying in an oven at 60°C for 72 hours (dry weight). The lung wet-to-dry weight ratio was calculated as the ratio of wet weight to dry weight.

Statistical Analysis

The number of animals in the NS, Epi5, Epi10, and Epi20 groups was determined based on our preliminary study (n = 3) in which the oxygen partial pressures at the end of the observation period were 336 ± 58 mm Hg, 310 ± 46 mm Hg, 216 ± 46 mm Hg, and 175 ± 22 mm Hg, respectively. A sample size of 5 per group was obtained by PASS 11.0, with α = 0.05 and β = 0.1. We enrolled 6 rats per group to account for attrition.

Statistical analysis was performed using SPSS Statistics for Windows 17.0 (SPSS Inc, Chicago, Illinois). Normal distribution measurement data were presented as mean ± SD. Differences in baseline parameters, arterial blood gas, wet-to-dry ratio, lung permeability index, and systolic left atrial peak pressure in the 4 groups were examined by one-way analysis of variance, and Bonferroni posttests for data that had homogeneity of variance, or Dunnett T3 posttests for data that exhibited heterogeneity of variance when indicated by significance of difference. Differences in mean arterial blood pressure (MAP), heart rate (HR), HR-blood pressure product (RPP), systolic left atrial pressure, and coronary perfusion pressure (CPP) in 2 groups were analyzed by two-way repeated-measures analysis of variance and Bonferroni posttests when significance was achieved. Values of P < 0.05 were considered significant. Curve fitting and bar charts were performed by GraphPad Prism version 5.0 (GraphPad Software Inc, San Diego, California).

RESULTS

Baseline Values

No differences were observed among the groups in weight, baseline hemodynamic metrics, or baseline arterial blood gas values (Table 1).

Blood Gas Analysis

Arterial blood gas values at 20 minutes are shown in Table 2. Compared with the NS and Epi5 groups, the Epi10 and Epi20 groups had lower pH (NS group vs Epi10 group, P = 0.022; NS group vs Epi20 group, P < 0.001; Epi5 group vs Epi10 group, P = 0.032; Epi5 group vs Epi20 group, P < 0.001) and PaO2 (NS group vs Epi10 group, P < 0.001; NS group vs Epi20 group, P < 0.001; Epi5 group vs Epi10 group, P < 0.001; Epi5 group vs Epi20 group, P < 0.001). While exhibiting higher PaCO2 (NS group vs Epi10 group, P = 0.007; NS group vs Epi20 group, P < 0.001; Epi5 group vs Epi10 group, P = 0.016; Epi5 group vs
The comparison of the HCO$_3^-$ between the Epi20 group, $P < 0.001$). The comparison of the HCO$_3^-$ between the groups demonstrated no statistical significance.

### Hemodynamic Measures

Once the infusion of bupivacaine was completed (T$_0$), RPP of all animals was decreased to less than 30% of baseline values. Hemodynamic parameters are represented as RPP, MAP, and HR in the 4 groups and are presented graphically in Figure 4. The MAP of the NS group increased gently after completion of the bupivacaine infusion. Whereas the MAP of the Epi5, Epi10, and Epi20 groups increased dramatically, the curve then became flat after reaching a peak at approximately 20 seconds. Within 6 minutes after epinephrine administration, the MAPs of the Epi5, Epi10, and Epi20 groups were higher than that of the NS group (NS group vs Epi5 group, $P = 0.016$; NS group vs Epi10 group, $P = 0.005$; NS group vs Epi20 group, $P = 0.001$). However, there was no significant difference among the Epi5, Epi10, and Epi20 groups in MAP within 6 minutes. The comparison of RPP, HR, and MAP during the entire 20-minute observation period between the 4 groups demonstrated no statistical significance.

### Systolic Left Atrial Pressure

Systolic left atrial pressure of the observation period is demonstrated in Figure 5. The systolic left atrial pressure of the NS group changed more slowly after epinephrine (isotonic sodium chloride solution) injection than that of the Epi5, Epi10, and Epi20 groups, which increased dramatically, then decreased slowly after reaching a peak at approximately 30 seconds, with flattening of the curve after 90 seconds. Within 6 minutes after epinephrine injection, compared with the NS and Epi5 groups, the Epi10 and Epi20 groups had a higher systolic left atrial pressure (NS group vs Epi10 group, $P = 0.002$; NS group vs Epi20 group, $P < 0.001$; Epi5 group vs Epi10 group, $P = 0.001$; Epi5 group vs Epi20 group, $P < 0.001$). The Epi5, Epi10, and Epi20 groups produced superior maximum systolic left atrial pressure compared with the NS group (NS group vs Epi5 group, $P = 0.049$; NS group vs Epi10 group, $P = 0.01$; NS group vs Epi20 group, $P < 0.001$) as did the Epi20 group compared with the Epi5 group (Epi5 group vs Epi20 group, $P < 0.001$). There was no statistical significance when comparing systolic left atrial pressure between the groups during the entire 20-minute observation period.

### Coronary Perfusion Pressure

Coronary perfusion pressure (CPP), defined as simultaneously recorded diastolic arterial pressure minus central venous pressure, after epinephrine administration is demonstrated in Figure 6. During the first 6 minutes after epinephrine administration, the Epi5, Epi10, and Epi20 groups had a higher CPP compared with the NS group (NS group vs Epi5 group, $P = 0.009$; NS group vs Epi10 group, $P = 0.006$; NS group vs Epi20 group, $P < 0.001$). There was no statistical significance between the groups when CPPs during the entire 20-minute observation period were compared.

### Table 1. Baseline Values of Weight, Hemodynamic Metrics, and Blood Gas Values for NS, Epi5, Epi10, and Epi20

<table>
<thead>
<tr>
<th></th>
<th>NS (n = 6)</th>
<th>Epi5 (n = 6)</th>
<th>Epi10 (n = 6)</th>
<th>Epi20 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>337 ± 8</td>
<td>334 ± 10</td>
<td>330 ± 8</td>
<td>333 ± 9</td>
</tr>
<tr>
<td>HR, beat/min</td>
<td>414 ± 41</td>
<td>417 ± 37</td>
<td>409 ± 58</td>
<td>384 ± 40</td>
</tr>
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<td>MAP, mm Hg</td>
<td>85 ± 11</td>
<td>85 ± 6</td>
<td>84 ± 11</td>
<td>83 ± 8</td>
</tr>
<tr>
<td>RPP, mm Hg × beat/min</td>
<td>49056 ± 1043</td>
<td>48949 ± 5953</td>
<td>46758 ± 9782</td>
<td>42147 ± 6695</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.02</td>
<td>7.39 ± 0.03</td>
<td>7.38 ± 0.02</td>
<td>7.39 ± 0.03</td>
</tr>
<tr>
<td>PO$_2$, mm Hg</td>
<td>324 ± 34</td>
<td>326 ± 44</td>
<td>291 ± 27</td>
<td>333 ± 62</td>
</tr>
<tr>
<td>PCO$_2$, mm Hg</td>
<td>37.3 ± 3.4</td>
<td>38.5 ± 2.7</td>
<td>38.4 ± 2.3</td>
<td>36.6 ± 2.6</td>
</tr>
<tr>
<td>HCO$_3^-$, mmol/L</td>
<td>22.3 ± 1.4</td>
<td>23.3 ± 1.3</td>
<td>22.8 ± 1.1</td>
<td>22.0 ± 0.7</td>
</tr>
<tr>
<td>BE, mmol/L</td>
<td>−2.7 ± 1.2</td>
<td>−1.8 ± 1.6</td>
<td>−2.7 ± 1.0</td>
<td>−2.7 ± 0.8</td>
</tr>
</tbody>
</table>

All values are mean ± SD. Baseline values showed no difference among the 4 groups.

BE indicates base excess.

### Table 2. Arterial Blood Gas Parameters at 20 Minutes

<table>
<thead>
<tr>
<th></th>
<th>NS (n = 6)</th>
<th>Epi5 (n = 6)</th>
<th>Epi10 (n = 6)</th>
<th>Epi20 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.39 ± 0.02</td>
<td>7.38 ± 0.02</td>
<td>7.32 ± 0.05*; †</td>
<td>7.27 ± 0.03†††, +++</td>
</tr>
<tr>
<td>PaO$_2$, mm Hg</td>
<td>323 ± 24</td>
<td>312 ± 43</td>
<td>174 ± 23***; ††††</td>
<td>146 ± 36†††, +++</td>
</tr>
<tr>
<td>PaCO$_2$, mm Hg</td>
<td>37.3 ± 1.7</td>
<td>38.1 ± 3.7</td>
<td>45.8 ± 5.4**; †</td>
<td>51.4 ± 3.8†††, +++</td>
</tr>
<tr>
<td>HCO$_3^-$, mmol/L</td>
<td>22.3 ± 0.6</td>
<td>22.6 ± 1.7</td>
<td>23.7 ± 2.1</td>
<td>23.7 ± 0.6</td>
</tr>
</tbody>
</table>

All values are mean ± SD. The NS, Epi5, Epi10, and Epi20 groups displayed differences in pH ($P < 0.001$); NS group vs Epi10 group, $P = 0.022$; NS group vs Epi20 group, $P < 0.001$; Epi5 group vs Epi10 group, $P = 0.032$; Epi5 group vs Epi20 group, $P < 0.001$. The NS, Epi5, Epi10, and Epi20 groups displayed differences in PaO$_2$ ($P < 0.001$); NS group vs Epi10 group, $P < 0.001$; NS group vs Epi20 group, $P < 0.001$; Epi5 group vs Epi10 group, $P < 0.001$; Epi5 group vs Epi20 group, $P < 0.001$. The NS, Epi5, Epi10, and Epi20 groups displayed differences in PaCO$_2$ ($P < 0.001$); NS group vs Epi10 group, $P = 0.016$; Epi5 group vs Epi20 group, $P < 0.001$. No significant differences were demonstrated among the 4 groups in HCO$_3^-$. *,**,***NS group vs Epi10 group, ††††NS group vs Epi20 group, †††††Epi5 group vs Epi10 group, and †††††††Epi5 group vs Epi20 group. *$P < 0.05$, ‡$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ††$P < 0.001$, †††$P < 0.001$, ††††$P < 0.001$, †††††$P < 0.001$. **P < 0.001**.
FIGURE 4. Hemodynamic parameters for all rats. Data are presented as mean ± SD. A-C, Heart rate pressure product (RPP), heart rate (HR), and mean arterial pressure (MAP) versus time for rats during 20 minutes of observation. No significant differences were demonstrated among the 4 groups in RPP, HR, or MAP during the 20-minute observation period. D, Mean arterial pressure versus time for rats within 6 minutes. Within 6 minutes after epinephrine (isotonic sodium chloride solution/NS) administration, significant differences were demonstrated between the NS, Epi5, Epi10, and Epi20 groups. The Epi5, Epi10, and Epi20 groups produced superior MAPs compared with the NS group (NS group vs Epi5 group, \( P = 0.016 \); NS group vs Epi10 group, \( P = 0.005 \); NS group vs Epi20 group, \( P = 0.001 \)). No significant differences were demonstrated among the Epi5, Epi10, and Epi20 groups in MAP within 6 minutes.

FIGURE 5. Systolic left atrial pressure for all rats. The data are presented as mean ± SD. A, Systolic left atrial pressure (sLAP) versus time for rats during 20 minutes. No significant differences were demonstrated among the 4 groups in sLAP during 20-minute observation period. B, Systolic left atrial pressure versus time for rats within 2 minutes. Within 2 minutes after epinephrine administration, significant differences were demonstrated among the NS, Epi5, Epi10, and Epi20 groups. The Epi10 and Epi20 groups produced superior sLAPs compared with the NS and Epi5 groups (NS group vs Epi10 group, \( P = 0.002 \); NS group vs Epi20 group, \( P < 0.001 \); Epi5 group vs Epi10 group, \( P = 0.001 \); Epi5 group vs Epi20 group, \( P < 0.001 \)). C, Maximum sLAP during 20-minute observation period. Significant differences were demonstrated among the NS, Epi5, Epi10, and Epi20 groups. The Epi5, Epi10, and Epi20 groups produced superior sLAP\(_{\text{max}}\) compared to the NS group (NS group vs Epi5 group, \( P = 0.049 \); NS group vs Epi10 group, \( P = 0.01 \); NS group vs Epi20 group, \( P < 0.001 \)), and the Epi20 group produced superior sLAP\(_{\text{max}}\) compared to the Epi5 group (Epi5 group vs Epi20 group, \( P < 0.001 \)). sLAP, systolic left atrial pressure; sLAP\(_{\text{max}}\), maximum of systolic left atrial pressure.
Lung Permeability Index and Wet-to-Dry Ratio

The Epi20 group had an increased lung permeability index (NS group vs Epi20 group, \( P = 0.030 \); Epi5 group vs Epi20 group, \( P = 0.019 \); Epi10 group vs Epi20 group, \( P = 0.025 \)), and wet-to-dry ratio (NS group vs Epi20 group, \( P < 0.001 \); Epi5 group vs Epi20 group, \( P < 0.001 \); Epi10 group vs Epi20 group, \( P = 0.044 \)) when compared with the NS, Epi5, and Epi10 groups (Fig. 7).

Light Microscopic Examination of Lung

Light microscopic examination of the NS (Fig. 8A), Epi5 (Fig. 8B), and Epi10 (Fig. 8C) groups showed that there was no evidence of alveolar structural change, edema, or hemorrhage. In the Epi20 group, there were numerous erythrocytes in alveoli accompanied by damaged alveolar framework (Fig. 8D). The ratio of the injured alveoli of the Epi20 group was higher than that of the NS, Epi5, and Epi10 groups (NS group vs Epi20 group, \( P = 0.035 \); Epi5 group vs Epi20 group, \( P = 0.035 \); Epi10 group vs Epi20 group, \( P = 0.041 \)). However, there was no significant difference between the NS, Epi5, and Epi10 groups in the ratio of the injured alveoli (Fig. 9).

Electron Microscopic Examination of Lung

The electron microscopic examination of the NS group (Fig. 10A) and the Epi5 group (Fig. 10B) revealed that there was no abnormal change found in ultrastructures. In the Epi10 group (Fig. 10C), the tight junctions were widened intermittent between adjacent capillary endothelial cells. In the Epi20 group (Fig. 10D), the tight junctions between adjacent capillary endothelial cells were open, the basement membrane was ruptured, and a large number of plasmalike exudates were in the alveolar cavity.

DISCUSSION

We found that a bolus of 10 μg/kg epinephrine or higher would cause a deterioration in the pulmonary gas exchange in our rat model of bupivacaine-induced cardiac depression, manifested as decreased \( \text{PaO}_2 \), increased \( \text{PaCO}_2 \), differences in lung permeability indices, wet-to-dry ratios, and damage to the alveolar ultrastructure.

As in our previous study, we used a model of bupivacaine toxicity without cardiac arrest and infused bupivacaine at 2.5 mg/kg per minute for 6 minutes (totaling 15 mg/kg), which has the potential to induce cardiovascular complications such as...
arrhythmia or hypotension. Using this model allows a more prompt recovery when such an infusion is discontinued, or when a bolus of epinephrine is introduced.

Lipid emulsion has been regarded as effective and essential drug for treating local anesthetic–induced cardiotoxicity owing to its binding capacity for bupivacaine in plasma and cardiac tissue.\textsuperscript{15,16} However, the role of epinephrine in lipid resuscitation of bupivacaine-induced cardiac arrest is controversial. Liu et al\textsuperscript{17} found that the combination of lipid and epinephrine was superior to lipid alone in the resuscitation of bupivacaine-induced cardiac asystole in an in vitro model. Nonetheless, the adverse effects produced by epinephrine raises concern. Weinberg et al\textsuperscript{9} revealed that epinephrine was ineffective when given without lipids and caused severe metabolic acidosis and pulmonary edema when administered at a dose of 30 $\mu$g/kg after local anesthetic overdose. Di Gregorio et al\textsuperscript{18} demonstrated that the administration of epinephrine after local anesthetic–induced cardiac arrest would increase the blood concentration of lactate and produce acidosis and hypoxemia. Additionally, Hiller et al\textsuperscript{11} concluded that a “threshold effect” existed when administering epinephrine in the lipid resuscitation of local anesthetic cardiotoxicity; epinephrine would lead metabolic acidosis and unsuccessful resuscitation when given at a dose of 10 $\mu$g/kg or greater. All these studies indicated that the dose of epinephrine was closely related with resuscitation results in the treatment of local anesthetic–induced cardiotoxicity.

In our current study, a bolus of epinephrine at a dose of 10 and 20 $\mu$g/kg resulted in a decreased PaO\textsubscript{2}, increased PaCO\textsubscript{2}, and severe damage of alveolar ultrastructure; whereas a bolus of 5-$\mu$g/kg epinephrine had no impact on pulmonary oxygen exchange, indicating that the threshold effect of epinephrine was associated with deterioration of pulmonary gas exchange. These results are consistent with the finding of Hiller et al.\textsuperscript{11}

We also found that compared with a normal saline bolus, administration of epinephrine led to a dramatic elevation of systolic left atrial pressure that gradually declined after reaching peak at approximately 90 seconds. We speculate that $\alpha$-adrenoceptor–mediated peripheral vasoconstriction\textsuperscript{10} (bupivacaine enhanced the $\alpha$-adrenergic effect as well\textsuperscript{19}) elevated the afterload of the left ventricle (elevation of left ventricular wall stress), whereas $\beta$-adrenoceptor stimulation increased cardiac output\textsuperscript{10} (increasing pulmonary inflow) and HR (shortening of ventricular diastolic period). Thus, epinephrine increased pulmonary blood flow while impairing left ventricular diastolic function (elevation of left

![FIGURE 8. Light microscopy view of the second right lobe in the NS (A), Epi5 (B), Epi10 (C), and Epi20 groups (D) (original magnification $\times 200$). A-C, Structure of alveoli is normal. There is no accumulation of erythrocytes observed in alveoli, or exudation in pulmonary interstitium. D, Numerous erythrocytes are evident, and accompanied by a damaged alveolar framework.](image)

![FIGURE 9. Ratio of the injured alveoli for all rats. Significant differences were demonstrated among the NS, Epi5, Epi10, and Epi20 groups. The Epi20 group had a higher ratio of the injured alveoli compared with the NS, Epi5, and Epi10 groups (NS group vs Epi20 group, $P = 0.035$; Epi5 group vs Epi20 group, $P = 0.035$; Epi10 group vs Epi20 group, $P = 0.041$).](image)
ventricular wall stress and shortening of the ventricular diastolic period), leading to elevation of left atrial pressure, pulmonary transudation, and deterioration of pulmonary gas exchange. Furthermore, we observed damage of alveolar ultrastructure in the Epi10 and Epi20 groups, a severe accumulation of erythrocytes in alveoli, and a higher ratio of injured alveoli in Epi20. This might be explained by acute pulmonary congestion caused by the elevated systolic left atrial pressure, as demonstrated by the Epi20 group, when compared with that of the NS group and Epi5 groups. These structural alterations were associated with pulmonary gas exchange dysfunction: decreased PaO$_2$ and increased PaCO$_2$. When injured, lungs seem to have functional deterioration, changes in ultrastructure, along with evidence of microscopic and macroscopic injury as the level of severity increases. The pulmonary gas exchange might have been deteriorated before the destruction of alveolar structure as the dose of epinephrine increased. Krishnamoorthy et al.$^{10}$ reported that a bolus of 25-μg/kg epinephrine or higher in an intact, anesthetized rat impaired pulmonary gas exchange, and they attributed this to the potent activation of α- and β-adrenergic receptors. However, we found that even the lower dose of 10 μg/kg could exert a harmful effect on pulmonary gas exchange.

Harvey et al.$^2$ demonstrated that epinephrine was essential in the resuscitation of local anesthetic–induced cardiac arrest in a rabbit model. Their study revealed that epinephrine improved resuscitation results through its increase of CPP. This conclusion is similar to the findings of Li et al, where the combination of epinephrine and lipid was superior to epinephrine alone in improving cardiac function and CPP after resuscitation.$^{15}$ We also found that epinephrine (5, 10, and 20 μg/kg) resulted in a higher CPP compared with the NS group within the first 5 minutes after epinephrine administration. After 5 minutes, the CPP between the 4 groups showed no differences. This may indicate that a bolus of epinephrine could continuously improve CPP for 5 minutes, which may be instructive in determining an appropriate administration interval of epinephrine. Furthermore, we found that there were no differences in CCP between Epi5, Epi10, and Epi20 groups during the initial 6 minutes, which may imply that epinephrine was beneficial for resuscitation of local anesthetic–induced cardiotoxicity, as it could elevate the CPP. However, the CPP showed no further improvement as the dose of epinephrine was increased; an epinephrine bolus of 10 μg/kg or greater demonstrated disadvantages.

Our study has several shortcomings. (1) While monitoring the left atrial pressure, the chest remained open (during the observation time). The physiology of the open chest may have caused a ventilation/perfusion defect, thereby limiting the application of this model to real-world clinical settings. (2) Whereas repeated administration of epinephrine would normally occur in real clinical scenarios, here, we focused on a single bolus dose of epinephrine and its experimental effects. (3) We focused purely on epinephrine administration and its effect on pulmonary gas exchange in the

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**FIGURE 10.** Electron microscopy view of the third right lobe in the NS (A), Epi5 (B), Epi10 (C), and Epi20 groups (D) (original magnification ×30,000). A, Tight junctions between adjacent endothelial cells were extremely tight. B, No widening of the tight junction, basement membrane rupture, or exudate in the alveolar cavity was evident. C, Tight junctions were widened intermittently between adjacent capillary endothelial cells. D, Tight junctions between adjacent capillary endothelial cells were open, the basement membrane was ruptured, and there was a plasmalike exudate in the alveolar cavity.
resuscitation of local anesthetic–induced cardiotoxicity and excluded groups that received lipid emulsion (use of lipid emulsion is recommended by guidelines in such settings). (4) General anesthesia may have an effect on the pharmacokinetics of local anesthetic and the responses of the central nervous and cardiovascular systems in the setting of local anesthetic toxicity.20 Finally, it should be noted that we had to maintain the animals under general anesthesia during the experimental period for both ethical and practical considerations.

In conclusion, a bolus of 10-μg/kg epinephrine or greater in our study caused deterioration of the pulmonary gas exchange in the setting of local anesthetic–induced cardiotoxicity in a rat model, and this may be caused by increased pulmonary blood flow, acute left ventricle dysfunction, and pulmonary edema/ alveolar structural damage. Further studies are needed to investigate the optimal dose of epinephrine and the impact of repeated administration of epinephrine on the pulmonary gas exchange in the setting of local anesthetic–induced cardiotoxicity.

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