Original Article
Effects of intrathecal anesthesia with different concentrations and doses on spinal cord, nerve roots and cerebrospinal fluid in dogs

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Abstract: Objective: To investigate the effects of intrathecal anesthesia with bupivacaine, levobupivacaine and ropivacaine hydrochloride at different doses on the spinal cord, nerve roots and cerebrospinal fluid (CSF) in dogs. Methods: Forty-two mongrel dogs were randomly divided into normal saline group (C; 2 ml), 0.5% (B₁) and 0.75% (B₂) bupivacaine hydrochloride groups (2 ml), 0.5% (L₁) and 0.75% (L₂) levobupivacaine hydrochloride group (2 ml), 0.5% (R₁) and 0.75% (R₂) ropivacaine hydrochloride group (2 ml), and drugs were intrathecally injected. Results: The contents of Ca²⁺ and MDA and SOD activity of the spinal cord were comparable among groups (P > 0.05). In Groups B₁, L₁ and R₁, the neuronal cytoplasm of spinal tissues was basically normal, the majority of mitochondria and endoplasmic reticulum had complete structure, and the lamellar structure of modulated fibers was nearly normal. In Groups B₂, L₂ and R₂, a small amount of mitochondrial vacuolar degeneration was found in the neuronal cytoplasm of spinal cord, but their structures were basically normal; the neural tissues exhibited focal mild edema, and most of the lamellar structure of modulated fibers and Schwann cells were nearly normal except for loose structure in several fibers and cells. Conclusion: When compared with 0.75% anesthetics for local anesthesia, the early adverse effects on the ultrastructure of the spinal cord and nerve root reduce after focal anesthesia with 0.5% anesthetics. Keywords: Amides, intrathecal anesthesia, spinal cord, nerve root, ultrastructure, free radical, Ca²⁺

Introduction

Intrathecal injection of local anesthetics has been widely used in the clinical anesthesia, analgesia and treatment of post-operative acute and chronic pain. In recent years, with the increased use of combined spinal epidural anesthesia and regional nerve block, the complications of intrathecal anesthesia is also increasing, and thus the spinal neurotoxicity of local anesthesia has attracted increasing attention. A lot of studies show that subarachnoid block may occasionally cause transient neurological syndrome (TNS) and caudal equina syndrome (CES), but permanent nerve motor and sensory dysfunction causes life-long suffering to patients. Meta-analysis and evidence-based medicine show more than half of neurological symptoms are caused by the local anesthetic toxicity [1-5]. Therefore, the systematic study of local anesthetic neurotoxicity on the spinal cord has great clinical importance.

Bupivacaine is the most commonly used amide class of local anesthetics, and has been widely used in the spinal anesthesia and nerve block. It has a long half-life, and can block the sensory or motor nerves depending on its concentration. However, it has toxicity to the heart and nervous system, and intravenous administration or overdose of bupivacaine may lead to local anesthetic poisoning, manifested as cardiac toxicity, such as arrhythmias, conduction block, and serious or even sudden cardiac arrest. Ropivacaine and levobupivacaine is a new generation of new long-acting amide local anesthetics. Studies have shown that when compared with racemic bupivacaine, ropivacaine and levobupivacaine not only retain the effective time and intensity and achieves a better sensory and motor block separation, but have little central nervous and cardiac toxicity. Thus, in clinical practice, their application increases and there is a tendency of replacing bupivacaine by ropivacaine and levo-bupivacaine [6, 7].
The advocacy of safe anesthesia practice has raised great concern regarding the local-anesthetic-related neurotoxicity, which is a common issue for all of local anesthetics currently used in clinical practice. With the development and innovation in the field of local anesthetics, the understanding of local-anesthetic-related neurotoxicity becomes in-depth. Available studies suggest that the local-anesthetic-induced neurotoxicity to the spinal cord is associated with the direct/indirect damages exerted by local anesthetics, as well as the subsequent variation in the intra-cellular Ca\(^{2+}\) concentration; in addition, the anesthetic concentration, dose, drug type and other factors are also associated with the neurotoxicity [8, 9]. In the present study, bupivacaine hydrochloride, levobupivacaine hydrochloride and ropivacaine hydrochloride at different concentrations or doses were independently injected into the subarachnoid spaces in dogs, and the effects of local anesthetics on the ultrastructure of the spinal cord and nerve roots and on the biochemistry of canine cerebrospinal fluid were investigated, aiming to evaluate the local-anesthetic-induced neurotoxicity to the spinal cord. Our findings may provide theoretical evidence for the rational application of anesthetics in clinical practice.

Materials and methods

Animals and grouping

A total of 42 healthy mongrel dogs of either sex (age: 4-8 months; weight: 12-15 kg) were provided by the Laboratory Animal Center of Ningbo University (Batch No: H200500221). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol had been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Gongli Hospital in Pudong New District of Shanghai. Dogs were randomly divided into 7 groups (n = 6 per group). In control group (C), animals were received injection of 2 ml of normal saline into the cavum subarachnoidale. In Group B, 2 ml of 0.5% bupivacaine-HCl (10 mg) was injected into the cavum subarachnoidale. In Group B, 2 ml of 0.75% bupivacaine-HCl (15 mg) was injected into the cavum subarachnoidale. In Group R, 2 ml of 0.5% ropivacaine-HCl (10 mg) was injected into the cavum subarachnoidale. In Group R, 2 ml of 0.75% ropivacaine-HCl (15 mg) was injected into the cavum subarachnoidale.

Establishment of animal models

Animals were fasted for 12 h before anesthesia. Basal anesthesia was induced with intramuscular injection of ketamine (20 mg kg\(^{-1}\)) and atropine (0.05 mg kg\(^{-1}\)). An access to the vein of a forelimb was made, and Ringer’s lactate solution was infused at 20 ml kg\(^{-1}\) h\(^{-1}\). Endotracheal intubation was performed for mechanical ventilation via a Dräger Julia anesthesia machine (Germany) with dynamic regulation of the respiratory parameters and the \(P_{\text{ET}}\text{CO}_2\) was maintained between 30 and 40 mmHg. The anesthesia was maintained via continuous infusion of fentanyl and vecuronium bromide combined with intermittent inhalation of isoflurane throughout the experiment. After a neck incision was made, the right arteria carotis interna was exposed and annulated by using a 20 G needle connected the Philips MP60 monitor (America), followed by monitoring of hemodynamic parameters. Animals lied in a lateral position, and after routine removal of the back hair and sterilization, an incision was made at the supra-spinous ligaments at L3-L4. A 5 G nylon needle was used for subarachnoid puncture via a flexed position. When CSF was observed, normal saline (NS) or local anesthetics were injected within 20 s.

Parameters monitoring

1) The hemodynamic parameters (SBP, DBP, MAP and HR) in different groups were monitored and recorded at 7 time points (including before and at 10 min, 20 min, 30 min, 60 min, 120 min and 180 min after intrathecal administration). 2) The arterial blood gases (pH, \(\text{PaO}_2\) and \(\text{PaCO}_2\)), blood electrolytes (\(K^+\), \(Na^+\) and \(Cl^-\)), CSF partial pressure of oxygen (\(\text{PO}_2\)) and CSF lactic acid were also detected in different groups at 4 time points (before and at 1 h, 2 h and 3 h after intrathecal administration). 3) At 3 h after intrathecal administration, animals were sacrificed by air injection. After exposure of canalis spinalis, the spinal cord at L1–L2 was col-
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Table 1. Change in Lac of CSF (X ± s, n = 6, mmol·L⁻¹)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before intrathecal administration</th>
<th>1 h after intrathecal administration</th>
<th>2 h after intrathecal administration</th>
<th>3 h after intrathecal administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.33 ± 0.14</td>
<td>1.34 ± 0.16</td>
<td>1.35 ± 0.18</td>
<td>1.44 ± 0.28</td>
</tr>
<tr>
<td>B₁</td>
<td>1.36 ± 0.17</td>
<td>1.40 ± 0.21</td>
<td>1.43 ± 0.23</td>
<td>1.59 ± 0.26</td>
</tr>
<tr>
<td>B₂</td>
<td>1.34 ± 0.12</td>
<td>1.38 ± 0.18</td>
<td>1.41 ± 0.24</td>
<td>1.67 ± 0.25</td>
</tr>
<tr>
<td>L₁</td>
<td>1.35 ± 0.15</td>
<td>1.39 ± 0.17</td>
<td>1.43 ± 0.21</td>
<td>1.64 ± 0.27</td>
</tr>
<tr>
<td>L₂</td>
<td>1.37 ± 0.19</td>
<td>1.42 ± 0.20</td>
<td>1.44 ± 0.24</td>
<td>1.57 ± 0.29</td>
</tr>
<tr>
<td>R₁</td>
<td>1.34 ± 0.13</td>
<td>1.36 ± 0.22</td>
<td>1.39 ± 0.19</td>
<td>1.43 ± 0.21</td>
</tr>
<tr>
<td>R₂</td>
<td>1.36 ± 0.14</td>
<td>1.39 ± 0.17</td>
<td>1.41 ± 0.23</td>
<td>1.44 ± 0.18</td>
</tr>
</tbody>
</table>

Table 2. Change in PO₂ of CSF (X ± s, n = 6, mmHg)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before intrathecal administration</th>
<th>1 h after intrathecal administration</th>
<th>2 h after intrathecal administration</th>
<th>3 h after intrathecal administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>98.63 ± 5.23</td>
<td>97.38 ± 4.26</td>
<td>97.53 ± 4.47</td>
<td>98.47 ± 0.44</td>
</tr>
<tr>
<td>B₁</td>
<td>97.52 ± 4.35</td>
<td>95.64 ± 3.68</td>
<td>95.89 ± 4.12</td>
<td>96.74 ± 0.49</td>
</tr>
<tr>
<td>B₂</td>
<td>98.78 ± 5.86</td>
<td>97.62 ± 4.26</td>
<td>97.78 ± 4.35</td>
<td>98.34 ± 4.56</td>
</tr>
<tr>
<td>L₁</td>
<td>96.83 ± 5.75</td>
<td>95.78 ± 4.87</td>
<td>96.45 ± 4.21</td>
<td>96.55 ± 4.78</td>
</tr>
<tr>
<td>L₂</td>
<td>97.82 ± 5.63</td>
<td>96.13 ± 4.52</td>
<td>96.47 ± 4.13</td>
<td>97.45 ± 4.35</td>
</tr>
<tr>
<td>R₁</td>
<td>97.43 ± 5.78</td>
<td>96.32 ± 4.78</td>
<td>95.67 ± 3.99</td>
<td>97.12 ± 4.24</td>
</tr>
<tr>
<td>R₂</td>
<td>98.45 ± 6.37</td>
<td>95.48 ± 5.45</td>
<td>95.23 ± 4.87</td>
<td>96.57 ± 5.32</td>
</tr>
</tbody>
</table>

lected. These tissues were washed with deionized water three times, dried with filters and stored at -70°C (America Forma) for use. The Ca²⁺ content of the spinal cord was measured by atomic absorption spectrometry (aas); after homogenation of the spinal cord, SOD activity and MDA content were determined with xanthine oxidase method and thiobarbituric acid (TBA) method. 4) The spinal dorsal horn at L₁-L₂ and nerve root (cauda equina) were collected randomly and fixed in 3% glutaraldehyde phosphate buffer, followed by embedding. Then transmission electron microscopy (Japan) was done to evaluate the change in the ultrastructure of neurons in the spinal cord and nerve root at early stage. The scoring of these ultrastructures was done in a blind manner. The criteria for scoring were as follows [10]: 0, 1, 2, 3 and 4 represent the absence of change, very mild change, minor change, moderate change and significant change. The vacuolization or degeneration of nerve cells was evaluated in 10 randomly selected nerve cells, and the score of 0 represents basically normal or mildly degenerated mitochondria and endoplasmic reticulum; 1 represents cristae loss in about 1/5 mitochondria and/or mild swelling and expansion of endoplasmic reticulum as well as vacuolation or degeneration in some cells; 2 represents cristae loss in about 2/5 mitochondria and/or moderate swelling and expansion of endoplasmic reticulum as well as vacuolation or degeneration in 1/5~2/5 mitochondria; 3 represents cristae loss in about 50% mitochondria and/or expansion of endoplasmic reticulum as well as vacuolation or degeneration in 3/5 mitochondria; 4 represents cristae loss and vacuolation or degeneration in 50%-100% mitochondria as well as moderate to severe expansion of endoplasmic reticulum.

Statistical analysis

Data were expressed as mean ± standard deviation (X ± s), and SPSS version 13.0 was used for statistical analysis; rank sum test was employed for inter-group comparisons of scores among groups; ANOVA and t test were adopted for comparisons of quantitative data, and a value of P < 0.05 was considered statistically significant.

Results

Characteristics at baseline

There were no significant differences in the body weight, gender, age, anesthesia time,
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Table 3. Change in contents of Ca\textsuperscript{2+} and MDA and SOD activity of spinal tissue (\(X \pm s, n = 6\))

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ca\textsuperscript{2+} ((\mu g\cdot g^{-1}))</th>
<th>MDA (nmol\cdot mg\textsuperscript{-1})</th>
<th>SOD (NU\cdot mg\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>48.36 ± 6.25</td>
<td>5.59 ± 0.34</td>
<td>143.83 ± 6.87</td>
</tr>
<tr>
<td>B\textsubscript{1}</td>
<td>50.32 ± 5.67</td>
<td>6.25 ± 0.78</td>
<td>138.45 ± 5.43</td>
</tr>
<tr>
<td>B\textsubscript{2}</td>
<td>52.45 ± 5.28</td>
<td>6.78 ± 0.86</td>
<td>135.74 ± 6.78</td>
</tr>
<tr>
<td>L\textsubscript{1}</td>
<td>51.47 ± 6.78</td>
<td>6.27 ± 0.59</td>
<td>139.36 ± 5.87</td>
</tr>
<tr>
<td>L\textsubscript{2}</td>
<td>53.78 ± 7.14</td>
<td>6.72 ± 0.53</td>
<td>136.89 ± 6.64</td>
</tr>
<tr>
<td>R\textsubscript{1}</td>
<td>50.21 ± 5.78</td>
<td>6.34 ± 0.46</td>
<td>140.35 ± 4.98</td>
</tr>
<tr>
<td>R\textsubscript{2}</td>
<td>52.21 ± 4.89</td>
<td>6.54 ± 0.73</td>
<td>138.46 ± 5.45</td>
</tr>
</tbody>
</table>

intraoperative fluid volume and urine volume among groups (\(P > 0.05\)).

Hemodynamic changes

No significant differences were observed found in the SBP, DBP, MAP and HR after anesthesia as compared to those before anesthesia (\(P > 0.05\)). In Group C, hemodynamics was stable after intrathecal anesthesia (\(P > 0.05\)). In a few animals, a decrease in BP and an increase in HR were observed at 5-10 min after intrathecal anesthesia, but BP returned to baseline level after rapid transfusion and/or intravenous injection of 6-10 mg of ephedrine.

Arterial blood gases (ABG) and electrolytes

The \(\text{PaO}_{2}\), \(\text{PaCO}_{2}\) and pH were comparable in among groups before anesthesia (\(P > 0.05\)). At different time points after intrathecal anesthesia, \(\text{PaO}_{2}\), \(\text{PaCO}_{2}\) and pH remained similar among groups (\(P > 0.05\)). Furthermore, no significant differences were found in plasma \(K^+\), \(Na^+\) and \(Cl^-\) among groups before and after administration (\(P > 0.05\)).

\(\text{PO}_{2}\) and Lac of cerebrospinal fluid

The \(\text{PO}_{2}\) and Lac of the cerebrospinal fluid remained stable after intrathecal anesthesia in each group (\(P > 0.05\)), and were comparable among groups at different time points after intrathecal anesthesia (\(P > 0.05\)) (Tables 1 and 2).

\(\text{Ca}^{2+}\) and MDA contents and SOD activity

No significant differences were found in the \(\text{Ca}^{2+}\) and MDA contents and SOD activity of the spinal cord among groups (\(P > 0.05\)) at 3 h after intrathecal administration (Table 3).

Discussions

As three of the most commonly used long-acting amide local anesthetics, bupivacaine hydrochloride, levobupivacaine and ropivacaine are widely used for nerve block, intrathecal block, labor analgesia and so on. However, the safety of spinal anesthesia (especially spinal neurotoxicity) has been of great concern for a long time. The neurological and cardiac toxicity of bupivacaine is mainly found in dextroisomer; but the toxicity of levobupivacaine, a levo form of bupivacaine, is lower than that of bupivacaine, and thus the safety of anesthesia improves greatly. Ropivacaine, a pure S-type enantiomorph, has certain advantages in clinical application due to weaker neurotoxicity [5], lower nervous centralis and cardiac toxicity, better separation and block of sensory and motor nerve as compared to above two anesthetics [4, 11].

Local anesthetics enter into the Obersteiner-Redlich zone (OR zone) of the spinal cord along the dorsal roots of spinal nerves in the subarachnoid space and diffuse to the distal spinal cord. There is no myelin sheath in the nerve

Early ultrastructure

In Group C, the ultrastructure of the spinal cord was normal, and multiple structurally normal mitochondria and rough endoplasmic reticulum were found in the neuronal cytoplasm with complete envelope of mitochondria and clearly visible ridge, as well as integrated and clear medullated fibers myelin sheath. In Groups B\textsubscript{2}, L\textsubscript{1} and R\textsubscript{1}, the neuronal cytoplasm of spinal cord was basically normal, the vast majority of mitochondria and endoplasmic reticulum had complete structure, and the lamellar structure of medullated fibers was nearly normal; in Group B\textsubscript{1}, L\textsubscript{2} and R\textsubscript{2}, a small amount of mitochondrial vacuolar degeneration were found in the neuronal cytoplasm of spinal cord, and their structures were basically normal; the neural tissues exhibited focal mild edema, and most of the lamellar structure of medullated fibers and Schwann cells were nearly normal, but loose structure was observed in several cells (Figures 1, 2). The scores of ultrastructures of neuronal cytoplasm, gray matter, myelin sheath, inner and outer axolemma and nerve root are shown in Table 4.
fibers of this zone. The neurotoxicity of local anesthetics occurs here first, followed by the dorsal white matter and grey matter. The features of local anesthetics are as follows: 1) Injury is restricted to OR zone, but the dorsal white matter may be also involved; 2) The ventral roots or anterior angle of the spinal nerves are not affected; 3) Most of the injuries are reversible damages to the sensory function. If the concentration of local anesthetics is high enough, the motor function may be affected [12, 13]. The specific mechanism of local-anesthetics-related neurotoxicity is still unclear, which is resulted from multipath or multichannel-mediated effects. The local-anesthetics-related neurotoxicity may be explained as follows: a. The concentration of Glu in CSF increases after intrathecal injection of local anesthetics, which causes damage to nerves [14]; b. Local anesthetics have the features similar to detergent with the capability of molecular aggregation to cause damage to nerve cell membrane [15]; c. Long-term exposure to local anesthetics at a high concentration can lead to reduction in the blood supply to the spinal nerve and spinal cord, and neurotoxicity may be related to the ischaemia of spinal nerves [16]. Some studies have shown that the local-anesthetic-related neurotoxicity is associated with the hypoxic damage to neurons [17]. d. Mitogen-activated protein kinases (MAPKs) play an important role in the signal transduction, and the activation of MAPKpathway is associated with the local-anesthetic-related neurotoxicity. It has been proven that the members of MAPK subfamily (Serine/threonine protein kinase B [Akt], extracellular signal regulated kinases [ERKs] and p38MAPK, etc) are related to the neurotoxicity...
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Table 4. Scores of ultrastructures of spinal cord and nerve root (n = 6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vacuolar degeneration in neuronal cytoplasmic (score)</th>
<th>Vacuolization in gray matter (score)</th>
<th>Rupture of myelin (score)</th>
<th>Clarity of inner and outer membrane (score)</th>
<th>Vacuolar degeneration in nerve root (score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>B_1</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>B_2</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>L_1</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>L_2</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>R_1</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>R_2</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

Scoring criteria: 0 = Normal; 1 = Very mild change; 2 = Mild changes; 3 = Moderate changes; 4 = Significant changes.

It is associated with the mitochondrial dysfunction due to the activation of mitochondria-mediated apoptosis pathway such as release of cytochrome C, asymmetric structure of phospholipid membrane and activation of Caspase family members [20, 21]. h. Studies [22] have confirmed that the increase in intracellular Ca^{2+} concentration is associated with the local-anesthetic-related neurotoxicity, but it is not the only mechanism. g. AMP-activated protein kinase (AMPK) mediates the production of reactive oxygen species (ROS). Apoptosis of nerve cells under oxidative stress may be one of mechanisms for the local-anesthetic-related neurotoxicity [23]. h. Delayed nerve damage is associated with the deficiency of neurotrophic factors in neurons, and local anesthetics can interfere with the axonal transport of neurotrophic factors, leading to delayed nerve damage [24].

Lac is an end-product of sugar anaerobic glycolysis. The concentration of Lac in CSF mainly reflects the state of sugar anaerobic glycolysis of the central nervous system, and may also indirectly reflect the metabolism of the spinal cord. Lips et al [25] confirmed in animal experiments that PO_{2} of CSF was sensitive to the spinal cord ischemia and could be used to monitor the oxygenation of local spinal cord timely. Therefore, PO_{2} of CSF may serve as an indicator for the monitoring of spinal cord ischemia. In the present study, the metabolization and ischemia level of spinal cord and nerve roots was evaluated on the basis of concentration of Lac in and PO_{2} of CSF. Results showed that three local anesthetics at used concentrations and doses did not cause significant hypoxic-ischemic injury to the spinal cord and nerve roots.

Studies have shown that oxygen free radicals and lipid peroxidation of cell membrane play an important role in the pathophysiologic process of spinal cord injury (SCI) [26, 27]. MDA is the end product of lipid peroxidation. The content of MDA indirectly reflects the amount of free radicals. As an endogenous oxygen free radical scavenger, SOD has an anti-oxidive effect by catalyzing the dismutation of superoxide anion. MDA and SOD are now generally accepted as indexes reflecting the formation of oxygen free radicals and the degree of lipid peroxidation. In this study, the MDA content and SOD activity were compared among groups, but there were no significant differences, suggesting that the oxidation and anti-oxidation were similar among various groups and three local anesthetics at above-mentioned concentrations and doses fail to cause direct oxidative injury to the spinal cord. In addition, Xu et al [28] found that multiple and intermittent intrathecal injections of 1% ropivacaine changed the MDA content and SOD activity, suggesting the apoptosis of neurons, which may be related to the concentration of local anesthetics and the exposure time of nerves to local anesthetics.

It has been proposed that the intracellular calcium overload is also an important cause of local-anesthetic-induced injury to the spinal cord and nerve roots. After spinal cord ischemia, ATP stored in cells is exhaustive, and thereby, the concentration of Ca^{2+} in the mitochondria increases because Ca^{2+} cannot be removed out of the cytoplasm, causing mitochondrial injury and dysfunction; activated Ca^{2+} also promotes the production of a large amount of oxygen-free radicals causing neurotoxicity. In
addition, the increased intracellular Ca\textsuperscript{2+} leads to an increased release of excitatory amino acid (e.g., Glu), aggravating the spinal cord damage. However, SU et al [29] speculated that lidocaine can directly act on microglial cells and inhibit the increase in intracellular Ca\textsuperscript{2+}, indicating that lidocaine does not necessarily or indirectly cause the increase in intracellular Ca\textsuperscript{2+}. No significant change was found in the concentration of Ca\textsuperscript{2+} in the spinal cord of each group in this study, which may involve the types, concentrations and doses of local anesthetics and exposure time of nerves to local anesthetics different from previous studies. The specific mechanism is needed to be studied further.

Study in which ropivacaine at different concentrations and doses was injected into subarachnoid space showed that the specimens in 0.5% ropivacaine group was basically normal under an electron microscope, while abnormalities were found in 1% ropivacaine group: layered nerve fiber myelin sheath, rupture of partial myelin sheath, swollen mitochondria in spinal tissues and even vacuolation and degeneration of some mitochondria as well as swollen endoplasmic reticulum, suggesting that ropivacaine at low concentrations and doses for subarachnoid space block is safe, while intrathecal injection of 1% ropivacaine may cause irreversible spinal cord damage [30]. Li et al [31] found that intrathecal injection of 0.5% ropivacaine had no significant impact on the spinal cord of rats, while intrathecal injection of 1% ropivacaine caused neurotoxicity. Repeated intrathecal injection of 0.75% and 1% ropivacaine may also lead to nerve damage and degeneration of the spinal cord and axons as well as paramorphia of spinal cord neurons and apoptosis of nerve cells [2]. As shown in electron microscopy, the neuronal cytoplasm of the spinal cord and the lamellar structure of the medullated nerve fibers were mostly normal, suggesting that the intrathecal injection of bupivacaine, levobupivacaine and ropivacaine at commonly used concentrations and doses had no significant impact on the early ultrastructure of the spinal cord and nerve roots of dogs. However, with the increase in the concentration and dose of local anesthetics, the lamellar structure of mitochondria and endoplasmic reticulum in the neuronal cytoplasm and the lamellar structure of medullated fibers myelin sheath were damaged to different extents, indicating that the toxicity of local anesthetics to spinal nervous system shows an increased trend with the increase in the concentration of anesthetics, which was consistent with previously reported [32-34].

In conclusion, intrathecal injection of bupivacaine, levobupivacaine and ropivacaine at commonly used concentrations and doses has no significant impact on the spinal cord, nerve roots and CSF of dogs. However, with the increase in the concentrations and doses of anesthetics, significant neurotoxicity may be observed.

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Disclosure of conflict of interest

None.

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