Original Article
Application of ropivacaine combined dexmedetomidine is beneficial for rat spinal cord injury

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Abstract: As one of the most serious complications of spinal injury, spinal cord injury shows increasing trend in recent years, leading to severe limb dysfunction under the damaged section. Since the surgical trauma is large, the reasonable choice of anesthetic is particularly important. This article intends to analyze the effect of anesthesia and postoperative analgesia of ropivacaine combined dexmedetomidine in rat spinal cord injury model. Modified ALLEN striking method was applied to establish rat spinal cord injury model. The rats were randomly divided into three groups, including ropivacaine group, dexmedetomidine group, and ropivacaine combined dexmedetomidine group. Intraoperative heart rate, breathing, blood oxygen saturation, temperature, onset time of anesthesia, duration, postoperative analgesia time, and adverse reactions were compared. BBB score was used to evaluate motor function. Reuter score was adopted to assess spinal cord injury feeling function. Caspase 3 activity was detected. ELISA was performed to test TNF-α and IL-2 expressions. Intraoperative heart rate, breathing, blood oxygen saturation, and temperature showed no statistical difference among each groups. Ropivacaine combined dexmedetomidine resulted in significantly shorter onset time of anesthesia, longer duration and postoperative analgesia time, higher BBB score, and lower Reuter score compared with other groups. It obviously inhibited Caspase 3 activity, declined TNF-α and IL-2 secretions, and reduced adverse reactions compared with single application group (P < 0.05). In summary, Ropivacaine combined dexmedetomidine can promote rat spinal cord injury recover through regulating apoptosis and inhibiting inflammation to improve intra- and postoperative anesthetic and analgesic effect.

Keywords: Ropivacaine, dexmedetomidine, spinal cord injury, analgesia, anesthesia

Introduction

The incidence of spinal cord injury (SCI) caused by car accident or falling injury gradually increased following society development [1, 2]. There are about hundreds of thousands of new cases of spinal injury every year worldwide, mainly in patients younger than 40 [3, 4]. SCI is one of the most serious complications of spinal injury, leading to high disability rate [5]. Because of high incidence, morbidity, and cost, SCI may cause severe limb dysfunction under the damaged section and bring huge economic burden to the society [6, 7]. Since the surgical trauma is large for spinal cord injury, the reasonable choice of anesthetic is particularly important along with the increase surgery number and requirements [8]. Appropriate choice of anaesthetics, anesthesia method, and anesthesia time is of great significance to reduce surgical damage and improve anesthetic and postoperative analgesia effect [9, 10].

Anesthetic selection and combination are critical to guarantee the anesthesia effect in spinal surgery. Traditional local anesthetics widely applied in clinic include tetracaine, procaine, ropivacaine and dexmedetomidine [11]. Dexmedetomidine is a relatively selective α2 adrenergic receptor agonist belonging to excited isopyrazole subtype [11, 12]. It has the pharmacological effects of anti-anxiety, sedation, analgesia, hypnosis, sympathetic block. FDA has approved it to be applied in adult mechanical ventilation in ICU, pediatrics, neurosurgery, and obese surgeries, and fiberoptic bronchos-
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Ropivacaine, belonging to long-term local anesthetics of amide derivatives, is a traditional intraspinal anesthesia drug for decades. Ropivacaine inhibits nerve impulses and conduction, improves electrical nerve stimulation threshold, and reduce the rate of action potential mainly by stabilizing the sodium ion channels on the nerve cell membrane. However, both ropivacaine and dexmedetomidine are limited by long onset time, short analgesia time, and more adverse reactions [15, 16]. Therefore, this article intends to analyze the effect of anesthesia and postoperative analgesia of ropivacaine combined dexmedetomidine in rat spinal cord injury model.

Materials and methods

Experimental animals
SPF grade male Wistar rats with 2-month old and weighted 250±20 g were bought and fed in the experimental animal center of Anhui Medical University. The feeding condition maintained constant temperature at 21±1°C and relative humidity at 50-70%. Day/night cycle was 12 h.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of The Affiliated Clinical College Shenzhen Second People Hospital, Anhui Medical University.

Main materials and instruments
Pentobarbital sodium and lidocaine were got from Shanghai Zhaohui Pharmaceutical co., Ltd. TNF-α and IL-2 ELISA kits were purchased from R&D. Ropivacaine and dexmedetomidine were bought from Sigma. Caspase 3 activity detection kit was purchased from Cell Signaling. Surgery microscopic instruments were from Medical Instrument Factory in Suzhou. Multi-Parameter Monitor small animal physiological monitor was from Yuyanbio. Microplate reader was from BD. Other common reagents were purchased from Sangon.

Methods
Animal grouping and treatment: Modified ALL-EN striking method was applied to establish rat SCI model. The rats were equally randomly divided into three groups, including ropivacaine group, dexmedetomidine group, and ropivacaine combined dexmedetomidine group with 30 in each group.

Rat SCI model establishment: Rat SCI model was established by modified ALLEN striking method [17]. After anesthetized by 30 mg/kg pentobarbital intraperitoneal injection, the rat was fixed on the operating floor to resect the vertebral plate and spinous process of T9-11. T10 spinal cord segment was set as central to expose the damage zone with diameter at 4 mm. A plastic buckling gasket at 3×2×1 cm according to the physiological curvature of dorsal rat spinal cord was put to the epidural of T10 spinal cord segment. Next, a sleeve was vertically put on the center of the gasket and the stick directly hit on the gasket at 5 cm high through the sleeve. The marker of modeling success included retraction flapping of body and lower limbs, and myoclonic swing of tail. The incision was closed and the rat received conventional antibiotics to diminish inflammation.

Anesthesia treatment: Rats in ropivacaine group received ropivacaine femoralis injection at 100 mg/kg. Rats in dexmedetomidine group received dexmedetomidine femoralis injection at 50 mg/kg. Rats in combined group received ropivacaine (100 mg/kg) and dexmedetomidine (50 mg/kg) femoralis injection. Duration of anesthesia was 1 h in each group.

Intraoperative index and anesthetic effect observation
Intraoperative heart rate (300-600 bpm), breathing (70-110 bpm), blood oxygen saturation, and temperature (38.5-39.5°C) were recorded by small animal physiological monitor. Adverse reaction including bradycardia (heart rate < 300 bpm), itching, hypotension (systolic blood pressure < 80 mmHg), chills, and respiratory depression was observed and recorded. Onset time of anesthesia, duration, and postoperative analgesic time were evaluated. BBB score was used to evaluate joints and lower limb motion recovery on the 20th day after surgery. The higher BBB score, the better recovery is. Reuter score was adopted to evaluate pain withdrawal reflex, myotatic reflex, muscle strength, muscle tone, and back sense. The
higher Reuter score, the worse of sensory function recover [17, 18].

Specimen collection

On the 20th day after surgery, a total of 5 ml blood was extracted from caudal vein and centrifuged at 3000 rpm for 15 min. The serum was collected in Eppendorf tube and stored at -80°C. Spinal cord tissue was extracted and stored at -80°C.

ELISA

TNF-α and IL-2 expressions were detected by ELISA according to the manual. A total of 50 μl diluted standard substance or sample was added to the 96-well plate with three repeats. After washed for five times, 50 μl enzyme-labelled reagents were added to the well and incubated at 37°C for 30 min. Next, the plate was treated by 50 μl color agent A and 50 μl color agent B at 37°C for 10 min. At last, the plate was added with 50 μl stop buffer and tested on microplate reader at 450 nm to obtain the absorbance. The linear regression equation was made based on the absorbance value of standard substance. The sample concentration was calculated according to the equation.

Caspase 3 activity

Caspase 3 activity in spinal cord tissue was tested by the kit. The cells was digested by enzyme and centrifuged at 600 g and 4°C for 5 min, the sample was added with 2 mM Ac-DEVD-pNA and detected at 405 nm to calculate Caspase 3 activity.

Data analysis

All data analysis was performed on SPSS 19.0 software. The measurement data was presented as mean ± standard deviation and compared by one-way ANOVA. P < 0.05 was depicted as statistical significance.

Results

The impact of anesthesia on intraoperative heart rate, breathing, blood oxygen saturation, and temperature

Intraoperative heart rate (300-600 bpm), breathing (70-110 bpm), blood oxygen saturation, and temperature (38.5-39.5°C) in ropivacaine group, dexmedetomidine group, and ropivacaine combined dexmedetomidine group were analyzed. Though ropivacaine combined dexmedetomidine reduced intraoperative heart rate, breathing, blood oxygen saturation, and temperature compared with single drug group, the difference was lack of statistical significance (Table 1). It suggested that the application of three anesthetic drug groups showed no obvious impact on vital signs.

The influence of different anesthesia methods on anesthesia effects of SCI rat

The influence of ropivacaine, dexmedetomidine, and ropivacaine combined dexmedetomidine on anesthesia effects of SCI rat was ob-

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart rate (bpm)</th>
<th>Breathing (bpm)</th>
<th>Blood oxygen saturation</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ropivacaine</td>
<td>437±68</td>
<td>82±12</td>
<td>89.7±2.5</td>
<td>38.2±1.6</td>
</tr>
<tr>
<td>Dexmedetomidine</td>
<td>461±22</td>
<td>86±17</td>
<td>85.6±1.6</td>
<td>38.8±1.2</td>
</tr>
<tr>
<td>Ropivacaine combined dexmedetomidine</td>
<td>412±32</td>
<td>76±15</td>
<td>81.4±2.1</td>
<td>37.9±1.1</td>
</tr>
</tbody>
</table>

*P < 0.05, vs ropivacaine group or dexmedetomidine group.
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It was revealed that ropivacaine combined dexmedetomidine was presented as significantly shorter onset time of anesthesia, longer duration and postoperative analgesia time, higher BBB score, and lower Reuter score compared with other groups (P < 0.05) (Table 2).

Serum TNF-α and IL-2 expressions in ropivacaine group, dexmedetomidine group, and ropivacaine combined dexmedetomidine group were tested by ELISA. The results showed that ropivacaine combined dexmedetomidine significantly declined TNF-α and IL-2 secretions.

Table 3. The effect of different anesthesia methods on intraoperative adverse reaction of SCI rat (n%)

<table>
<thead>
<tr>
<th>Group</th>
<th>Bradycardia (heart rate &lt; 300 bpm)</th>
<th>Itching</th>
<th>Hypotension</th>
<th>Chills</th>
<th>Respirator depression</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ropivacaine (30)</td>
<td>2 (6.6%)</td>
<td>5 (16.6%)</td>
<td>4 (13.3%)</td>
<td>6 (20.0%)</td>
<td>0</td>
<td>17 (56.6%)</td>
</tr>
<tr>
<td>Dexmedetomidine (30)</td>
<td>2 (6.6%)</td>
<td>4 (13.3%)</td>
<td>6 (20.0%)</td>
<td>3 (10.0%)</td>
<td>0</td>
<td>15 (50.0%)</td>
</tr>
<tr>
<td>Ropivacaine combined dexmedetomidine (30)</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)*</td>
<td>2 (6.6%)*</td>
<td>1 (3.3%)*</td>
<td>0</td>
<td>5 (16.6%)*</td>
</tr>
</tbody>
</table>

*P < 0.05, vs ropivacaine group or dexmedetomidine group.

The effect of different anesthesia methods on intraoperative adverse reaction of SCI rat

No bradycardia or itching was found after basal anesthesia by pheno- barbital sodium. Ropivacaine combined dexmedetomidine obviously reduced intraoperative adverse reaction in SCI rat compared with single drug groups (P < 0.05) (Table 3).

The impact of different anesthesia methods on Caspase 3 activity in SCI rat

Caspase 3 activity in spinal cord tissue was tested by kit. It was demonstrated that ropivacaine combined dexmedetomidine markedly suppressed Caspase 3 activity compared with single drug groups (P < 0.05) (Figure 1). It indicated that ropivacaine combined dexmedetomidine can reduce apoptosis to alleviate spinal tissue damage.

The impact of different anesthesia methods on TNF-α and IL-2 expressions in SCI rat

Figure 1. The impact of different anesthesia methods on Caspase 3 activity in SCI rat. *P < 0.05, vs ropivacaine group or dexmedetomidine group.

Figure 2. The impact of different anesthesia methods on TNF-α and IL-2 expressions in SCI rat. *P < 0.05, vs ropivacaine group or dexmedetomidine group.
compared with single drug groups (P < 0.05) (Figure 2). It suggested that ropivacaine combined dexmedetomidine can decrease inflammatory injury in SCI rat through suppressing inflammatory cytokines secretion.

Discussion

As SCI seriously damages human health, and brings heavy spirit and economic pressure, it is a vital step to timely and effective treat SCI. Surgery can prevent the further injury of the spinal cord by stabilizing the spine and relieving the compression [19]. However, the huge trauma of surgery may cause severe inflammation and trigger further immunosuppression. The patient’s own factors, as well as low temperature, the application of anaesthetics, and mechanical ventilation can aggravate inflammation. Rational choice of anaesthetic drugs can alleviate stress reaction to different level and reduce the immunosuppression caused by surgery [20].

Ropivacaine is the first discovered local anaesthetic in the pure enantiomers form that featured as fewer side effects, lower central nervous system toxicity and heart toxicity, and long-effectiveness [21]. Dexmedetomidine plays its role in antinociceptive effects, hypnosis, antisypathetic activity, and calm by binding with α2A receptor distributed in the brain. It plays a role in promoting vasoconstriction and increasing blood pressure by binding with α2B receptor distributed in vascular smooth muscle. At last, it can induce hypothermia by acting on α2C receptor to regulate dopaminergic nerve. In clinic, dexmedetomidine plays its function by synergistically activating receptors [22]. Dexmedetomidine can be used for clinical analgesia and sedation, which may reduce the drug dosage. Reasonable application of anaesthetics may reduce the occurrence of postoperative complications and accelerate the efficacy of anesthesia, so as to promote SCI improvement and recovery [23]. This study established rat SCI model and applied ropivacaine and dexmedetomidine for intervention. Intraoperative heart rate, breathing, blood oxygen saturation, and temperature exhibited no statistical difference among each groups. Ropivacaine combined dexmedetomidine was presented as significantly shorter onset time of anesthesia, longer duration and postoperative analgesia time, higher BBB score, and lower Reuter score compared with other groups. It suggested that ropivacaine combined dexmedetomidine can enhance anesthetic effect, shorten postoperative pain course, and promote rat SCI recovery.

Complication after anaesthesia is one of the problems in operation and postoperative recovery, mainly including itching, postoperative pain, respiratory depression, chills, and hypotension. This study found that ropivacaine combined dexmedetomidine can reduce the adverse reaction of anesthesia. Inflammatory cytokines IL-2 and TNF-α secretion can trigger inflammation, facilitating leukocytes adhesion to provide the conditions for the further development of inflammation. Caspase is one of the most important proteases during apoptosis process. It can induce apoptosis through decomposing and activating DNA cleavage related proteins. Caspase-3 is the most critical protease in the process of apoptosis, and also the common downstream effect part of different apoptosis pathways [24, 25]. Further analysis revealed that ropivacaine combined dexmedetomidine can decrease TNF-α and IL-2 secretion and suppress Caspase-3 activity. It suggested that their combination can regulate apoptosis and suppress inflammation cytokines secretion to alleviate inflammatory injury to SCI rat.

Conclusion

Ropivacaine combined dexmedetomidine can facilitate rat SCI recover by regulating apoptosis and inhibiting inflammation to improve intra- and postoperative anesthetic and analgesic effects.

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Disclosure of conflict of interest

None.

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