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Original Article

Intravenous Administration of Ropivacaine Reduces Minimal Alveolar Concentration of Sevoflurane in Rats

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ABSTRACT

Background: Whether intravenous infusion of ropivacaine at a constant rate can affect the minimum alveolar concentration (MAC) of sevoflurane in rats and different concentration can produce different effects.

Methods: We used the Dixon "up-and-down" method to determine the MAC of sevoflurane in three groups (20 subjects per group). After a 30-minute equilibration, the medications (saline, 0.5% ropivacaine or 1.0% ropivacaine) were continuously infused at a rate of 0.5 mL/h/kg for 30 minutes, and the response to the supramaximal noxious stimulus was recorded (movement versus no movement).

Results: The MAC was 2.34 ± 0.26% (95% confidence interval [CI], 2.22-2.46) in the saline group, 1.22 ± 0.31% (95%CI, 1.07-1.37) in the 0.5% ropivacaine group, and 1.06 ± 0.42% (95%CI, 0.86-1.26) in the 1.0% ropivacaine group. The MAC of the 0.5% ropivacaine (mean difference of 1.12% sevoflurane [95% adjusted CI, 0.86-1.38]; $P < 0.01$) and 1.0% ropivacaine groups (mean difference of 1.28% sevoflurane [95% adjusted CI, 1.02-1.54]; $P < 0.01$) were significantly lower than that of the saline group.

Conclusion: Intravenous administration of 0.5% and 1.0% ropivacaine both decreased the MAC of sevoflurane in rats, and 1.0% ropivacaine showed more decline. (Funded by the Distinguished Professorships Awards from the China Medical Board.)

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With increasing surgical volume (266.2 to 359.5 million operations in 2012, China) and popularization of painless examinations, more varieties of anesthetics have been developed, which can fulfill different requirements for better analgesia and sedation. Inhaled anesthetic agents are the most broadly accepted anesthetics for the provision of general anesthesia owing to their convenience of administration, observable end-tidal concentration, and foreseeable intraoperative and postoperative state (1). As a fluorinated ether inhalational agent with low blood / gas partition coefficient (0.6), sevoflurane offers fast wash in and out, leading to early postoperative recovery (2). A combination of sevoflurane with the intravenous sedative, analgesic, and muscle relaxant, keeps dosage of sevoflurane low and avoids an adverse hemodynamic effect at high concentrations, the underlying mechanisms, may involve inhibition of vascular smooth muscle contraction through the intracellular KCl-induced Class II phosphoinositide 3-kinase α (KCl / PI3K-C2α) pathway (3). With more clinical applications of regional anesthetics, lidocaine began to be intravenously used during general anesthesia with sevoflurane.

Numerous studies demonstrated that intravenous lidocaine along with or without ketamine/dexmedetomidine provided a significant reduction in MAC of the inhaled anesthetics sevoflurane, isoflurane and halothane during anesthesia in animals (4-7). Among them, researchers found that the intravenous bolus of 1.5 mg/kg lidocaine intraoperatively decreased the MAC by at least 0.23% sevoflurane, and the underlying mechanism is probably related to the modification of signal transduction in dorsal horn neurons (8-10). As a long-acting regional anesthetic, ropivacaine has a longer analgesic effect than lidocaine and lower potential toxicity than bupivacaine, with applications for surgical anesthesia, for labor pain and postoperative pain in adults and children (11). The use of ropivacaine in the management of chronic pain has also been evaluated in various modalities (12, 13). Despite cardiotoxicity and central nervous system (CNS) toxicity resulting from unintended intravascular injection of ropivacaine, the threshold for toxicity is higher compared with general clinical dose (up to 115 mg in healthy volunteers), and the incidence of probable cardiovascular events appears to be low (14). Whether the ropivacaine will demonstrate similar effects as lidocaine is unknown. There is little evidence about the effect of intravenous ropivacaine with sevoflurane. However Zhang J et al. found that epidural ropivacaine reduced sevoflurane requirements, and the effect was ropivacaine concentration-dependent (15).

The objective of this study was to determine the effects of a constant-rate infusion of ropivacaine on the MAC of sevoflurane in rats, and whether different concentrations can produce different results.

METHODS

Animal Preparation

A total of 60 healthy male Sprague Dawley rats were enrolled, weight from 260 to 320 g, following approval of the Sichuan University Animal Ethics Committee (No. 2017011A). All subjects fasted for at least 8 hours prior to anesthesia, but were allowed access to water. Rats were randomly divided into three groups. Randomization was performed with a predefined list containing the medication data (0.9% saline as control, 0.5% ropivacaine in saline and 1.0% ropivacaine in saline).

Surgical Procedures

We determine the MAC of sevoflurane with an anesthesia chamber which consisted of two tubes for gas in and out, a sealed glove for operation, and a heating pad. All subjects measured body temperature by rectal probe before entering the anesthesia chamber. The MAC of sevoflurane was determined with the tail-clamp technique in the chamber. In this techniques, fresh gas flowed to the chamber with 0.5-1.0 L/min of pure oxygen and maintained the end-tidal sevoflurane concentration (SEVO%) for 30 minutes to allow equilibration of alveolar. The SEVO% of the first subject in each group was 2.0%. If the first subject showed a positive response to the supramaximal noxious stimulus, the SEVO% in the subsequent subject of this group was increased by 0.2%. Similarly, when a negative response was seen, the SEVO% was decreased by

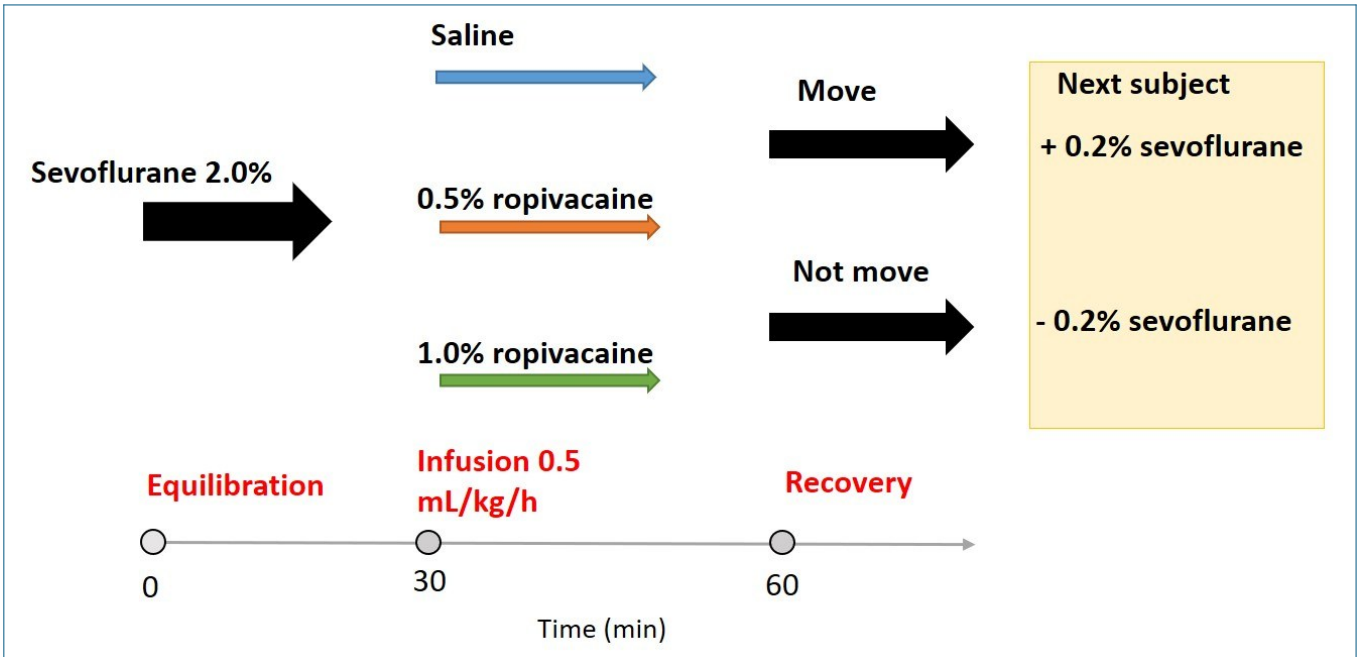

Figure 1. Study Design.
The measure of minimum alveolar concentration (MAC) and administration of ropivacaine for each group is shown in this figure. The black thick arrow means sevoflurane anesthetic, the color arrows means administration of ropivacaine in three groups, and the grey arrow shows the duration of the anesthetic progress.

0.2%, as shown in Figure1. A supramaximal noxious stimulus was applied with a small alligator clip to the distal third tail for one minute or until positive movement appeared, which did not cause injury to the tail. The gross purposeful movements of the head, body or extremities were considered positive responses, while grimacing, chewing, swallowing, tail flacking, or lack of movements were considered as negative responses. The "up-and-down" adjustment of the SEVO% was continued in all subjects in every group. This procedure was referred as the Dixon up-and-down method (8). The ropivacaine was administered following the equilibration of sevoflurane and continuously infused at a rate of 0.5 mL/kg/h for 30 minutes. Throughout the process, adverse effects such as seizures, muscle trembling, or other CNS toxicity symptoms, and the time of total recovery were recorded properly. All subjects were observed more than an hour then sent back to the cages.

Statistical Analysis

These estimates of the MAC of sevoflurane (mean and standard deviation of each group) were calculated by the analysis of variance (ANOVA) statistics and showed with confidence intervals (CIs) and P-values. P-values were adjusted using the three tests for multiplicity with Bonferroni correction. All adjusted P-values < 0.05 were considered statistically significant. Analyses of the calculations and comparisons of the MAC of sevoflurane were performed with SPSS software, version 22.0 (IBM).

RESULTS

There was no difference of age, weight and rectal temperature between the saline, 0.5% ropivacaine, and 1.0% ropivacaine group ($P = 0.423$), and the basic characteristics were comparable among the groups (Table 1) except for the time of recovery. The time of recovery in the 0.5% ropivacaine group and 1.0% ropivacaine group were significantly longer than the saline group (adjusted $P < 0.01$). No adverse effects were observed during the progression of the experiment and recovery.

Table 1. Rat Characteristics and Potential Confounding Factors.

Characteristics Parameters	Saline Group	0.5% Ropivacaine Group	1.0% Ropivacaine Group	P
Age (weeks)	9.3 ± 1.2	9.2 ± 1.2	9.0 ± 1.1	0.704
Weight (g)	301.2 ± 16.2	307.4 ± 21.6	301.6 ± 17.2	0.423
Time of recovery (min)	1.0 ± 0.18	2.15 ± 0.29	2.59 ± 0.23	0.000*
Rectal temperature (℃)	36.9 ± 0.5	36.8 ± 0.5	36.9 ± 0.5	0.912

All data are showed as mean ± SD. All values were obtained from ANOVA and adjusted with Bonferroni correction, the rectal temperature was measured at the time entering the anesthesia box. *P < 0.05, significant statistical difference.

The time from the entrance of the chamber to the clip stimulus was at least 55 minutes in all subjects. The MAC of sevoflurane was 2.34 ± 0.26% (95% CI, 2.22-2.46) in the saline group, 1.22 ± 0.31% (95% CI, 1.07-1.37) in the 0.5% ropivacaine group, and 1.06 ± 0.42% (95% CI, 0.86-1.26) in the 1.0% ropivacaine group (Figure 2).

The mean difference between the MAC of sevoflurane in the saline group and in the 0.5% ropivacaine group was 1.12% sevoflurane (95% adjusted CI, 0.86-1.38). The mean difference between the MAC of sevoflurane in the saline group and in the 1.0% ropivacaine group was 1.28% sevoflurane (95% adjusted CI, 1.02-1.54). No significant difference in the MAC was noted between the 0.5% ropivacaine group and 1.0% ropivacaine group (adjusted P = 0.42).

DISCUSSION

We found that intravenous infusion of 0.5% ropivacaine significantly decreased the MAC of sevoflurane by 1.2%, and 1.0% ropivacaine also decreased by 1.28%. Meanwhile, we did not observe any sign of CNS toxicity, and the recovery time was significantly longer in 0.5% and 1.0% ropivacaine group than in the saline group (adjusted P < 0.01).

The intravenous administration of ropivacaine could reduce the MAC of sevoflurane was never reported before. While, the lidocaine infusion reduced the MAC of isoflurane 26.1-27.3% in dogs (4, 16), 57% in alpacas (6), and 10.5-21.7% in New Zealand white rabbits (7). Intravenous administration regional anesthetics is a widely used technique for brief surgical interventions, primarily on the upper limbs and less frequently, on the lower limbs. The technique to accomplish anesthesia is particularly combined with long-term anesthetics, such as ropivacaine in low concentration. Corresponding experiments reveal that MAC as a convenient and reproducible metric keep constant in both animals and humans, and that MAC did not increase with stimulus intensity beyond a certain point (supramaximal stimulation) (17). When a factor, especially some drugs, is present to decrease MAC in an inhalational anesthetics will increase potency, while the patient requires a lower concentration of the volatile agent. Notwithstanding, it is necessary to emphasize that drugs may alter MAC, but not similarly affect other MAC derivatives, for instance, might not decrease the concentration to achieve amnesia. A study shows that most of the capacity of volatile anesthetics may produce immobility was mediated by the spinal cord, as precollicular decerebration did not show the difference on MAC of isoflurane (18). Also, the blockade of gamma-aminobutyric acid (GABA) and glycine inhibitory chloride channels descend the depressant effect of anesthetics in the intact spinal cord (19, 20). The GABA receptor and glycine receptor function could be potentiated with most of intravenous anesthetics (21). When local anesthetics show depressant effect, the GABA receptor complex conducts disinhibition of nervous conduction because of the blockade of sodium channels. Therefore, the mechanism of reduction in inhalant MAC may elucidate because systemic administration ropivacaine exerts analgesia at the spinal level, which is expected to decrease inhalant MAC. Also, both ropivacaine and volatile anesthetics act on voltage-gated sodium channels in the spinal and produce the additive effect (22), which can explain why higher concentration ropivacaine showed more reduction of

MAC. Meanwhile, this may also explain that the recovery time of every subject and found that the time of 0.5% and 1.0% ropivacaine group were significantly longer than the control group, prolonged more than one minute. The phenomena are not complicated to understand, ropivacaine can play the analgesia and sedative role during the anesthesia.

While, Ropivacaine show less cardiovascular and CNS toxicity with less enantioselective actions on coronary resistance vessels through modulating coronary vascular tone on smooth muscle cells (23). Laser Doppler by Kopacz et al. demonstrated the local skin blanching, suggesting that ropivacaine performed as a vasoconstrictor of cutaneous vessels, unlike other local anesthetics generally produced vasodilation (24). When ropivacaine was administered intravenously, its pharmacokinetics were linear and dose proportional up to 80 mg with a terminal half-life of 1.8 ± 0.7 hours, and 86% was excreted in urine (25). The mean half-life of the initial phase is approximately 14 minutes, followed by a slower phase with a mean absorption t1/2 of approximately 4.2 hours. While through several reviews (26-28), ropivacaine was proved well tolerated in the clinical trials, that large doses could be administered in peripheral nerve blocks particularly and the cardiovascular and CNS advert incidence appeared to be low. And ropivacaine-reduced cardiovascular symptoms are age-related, patients aged ⩾ 60 years showed higher incidence of bradycardia (58% vs 15% patients aged 41-60 years; P = 0.005) and hypotension (74% vs 20% patients aged 18-40 years; P = 0.002), and massive absorption of the drug (29). Besides, ropivacaine seems to less neurotoxic and cardiotoxic than bupivacaine according to the basis of animal and volunteer studies. The cumulative dose of ropivacaine required to produce toxicity to heart cell is significantly lower than bupivacaine (30). As for healthy volunteers, the maximum tolerated dose of ropivacaine was 115 mg. Our cumulative dose of ropivacaine was 2.5 mg/kg, far away from the dose required to produce seizure (ropivacaine 13.2 ± 3.0 mg/kg) in healthy volunteers (31). Nevertheless, a reasonable dose of intravenous ropivacaine could decrease MAC of sevoflurane without producing severe local anesthetics reduced effects.

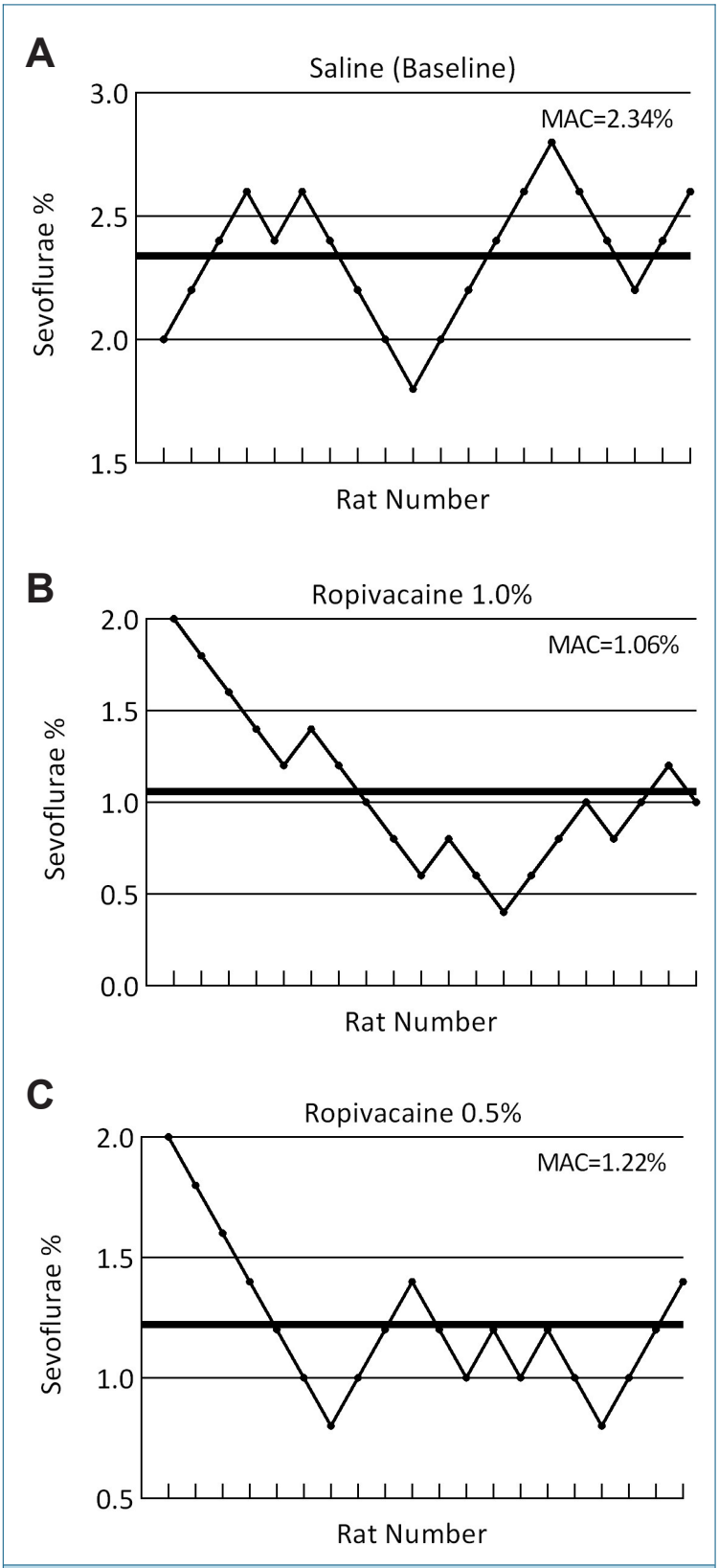

Figure 2. The Minimum Alveolar Concentration (MAC) of Sevoflurane in the Study Groups.

There are potential limitations to the study. The same investigator who record data and administered the drugs evaluated the MAC determination, the blinding was neglected. Also, the detection of general vital signs of subjects as heart rate, arterial blood pressure, and SpO2 were not monitoring continually. There were several reasons: the process of place the equipment on rodents was not as easy as human, which could cause the different duration between each subject in one group; the determination of arterial blood pressure is invasive and inappropriate position may cause arterial rupture or shock, and may alter the accuracy of MAC; the anesthesia chamber limited the control of many wires. We only choose the male rats other than two genders, neglected the effect of sex. This study only described the effect of ropivacaine on the MAC of sevoflurane, but the underlying mechanism not involved. We hope that further investigation may answer this question.

In conclusion, our results demonstrate a wider therapeutic use of ropivacaine, by administrating intravenously to decrease the MAC of sevoflurane. Someday, the clinical research referred to the effects of local anesthetics on inhaled anesthetics will be carried out in depth, which has more practical significance.

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The authors have no other potential conflicts of interest for this work.

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