

Original Article

Emulsified halothane produces long-term epidural anesthetic effect: a study in rabbits

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Abstract: Previous studies have demonstrated that volatile anesthetics could produce local anesthesia. Emulsified isoflurane at 8% has been reported to produce epidural anesthetic effect in rabbits. This study was designed to investigate the long-term epidural anesthetic effect of emulsified halothane in rabbits. In this study, 40 healthy adult rabbits (weighting 2.0-2.5 kg) with an epidural catheter were randomly divided into 4 groups (n=10/group), receiving epidural administration of 1% lidocaine (lido group), 8% emulsified isoflurane 1ml (8% E-iso group), 8% emulsified halothane (8% E-Halo group) and 12% emulsified halothane (12% E-Halo group). After administration, sensory and motor functions as well as consciousness state were assessed until 60 minutes after sensory and motor function returned to its baseline or at least for 180 min. After epidural anesthesia, all the rabbits were continuously observed for 7 days and sacrificed for pathological evaluations. As a result, all the four study solutions produced typical epidural anesthesia. Onset times of sensory and motor function blockade were similar among the four groups ($P>0.05$). Duration of sensory blockade in 12% E-Halo group (83 ± 13 min) was significantly longer than other groups: 51 ± 12 min in 8% E-Halo group ($P<0.01$), 57 ± 8 min in 8% E-iso group ($P<0.01$) and 47 ± 9 min in lido group ($P<0.01$). Duration of sensory blockade in 8% E-iso group is longer than lido group ($P<0.05$). Duration of motor blockade in 12% E-Halo group (81 ± 12 min) was also significantly longer than other groups: 40 ± 8 min in 8% E-Halo group ($P<0.01$), 37 ± 3 min in 8% E-iso group ($P<0.01$), 37 ± 6 min in lido group ($P<0.01$). Normal consciousness was found in the rabbits from 8% E-Halo, 8% E-iso and lido groups while there were four rabbits in 12% E-Halo group (4/10) showed a light sedation. For all the rabbits, no pathological injury was found. The present study demonstrates that emulsified halothane produces reversible concentration-dependent epidural anesthesia and at 12% (v/v), emulsified halothane could produce long-term anesthesia without pathological injury.

Keywords: Emulsified halothane, emulsified isoflurane, epidural anesthesia, rabbits

Introduction

Local injection of volatile anesthetics at relatively high concentrations produces regional anesthesia, including infiltration anesthesia (in rats and humans) [1], cutaneous anesthesia (in rats and humans) [2, 3], spinal anesthesia (in dogs) [4], epidural anesthesia (in rabbits) [5] and intravenous regional anesthesia (in rats) [6]. Previous studies demonstrate that halothane (1 mM) and methoxyflurane (2.5 mM) could decrease potential threshold in peripheral nerve [7, 8]. Our previous studies have demonstrated that 8% emulsified isoflurane produced a reversible epidural anesthesia in

rabbits [5]. Therefore, we hypothesized that emulsified halothane could produce similar epidural anesthetic effect. Among commonly used volatile anesthetics, oil/gas partition coefficient of halothane is 224.0 (37°C), which is much higher than that of isoflurane (94.0 at 37°C) [9]. Dissolved in 30% Intralipid, saturated concentration of isoflurane is 8.24% (20°C) while the saturated concentration of halothane is higher than 12% [10].

Long-term regional anesthesia is obvious useful in clinical settings such as post-operative analgesia. If a local anesthetic could produce long-term regional anesthetic effect without

significant neural toxicity, it would be widely used in clinic. Under inhaled administration, MAC (minimal alveolar concentration) of halothane is lower than that of isoflurane because of its high hydrophobicity, which represents the higher anesthetic potency of halothane. Therefore, in the present study, we hypothesized that dissolved in 30% Intralipid at a relatively high concentration (12%); emulsified halothane could produce long-term epidural anesthesia without pathological injury.

Materials and methods

Animals

With the approval of the Institutional Animal Experimental Ethics Committee of Sichuan University (Chengdu, China), 40 adult male New Zealand rabbits weighting 2.0-2.5 kg were used in the present study. All the rabbits were housed in an individual cage and free access to food and tap water and were kept in a 12-hour light-dark cycle under 25°C.

Chemicals

Eight percent emulsified isoflurane (v/v) used in the present study was prepared according to the established methods developed by our laboratory [10]. The 8% emulsified isoflurane (20 ml) contained pure liquid isoflurane 1.6 ml and 30% intralipid (lipid emulsion) 18.4 ml. Halothane used in this study was manufactured by Halocarbon Laboratories (River Edge, NJ, US). Emulsified halothane was prepared according to the same methods as 8% emulsified isoflurane [10]. For instance, 8% emulsified halothane (20 ml) contained pure liquid halothane 1.6 ml and 30% intralipid (lipid emulsion) 18.4 ml; 12% emulsified halothane contained pure liquid halothane 2.4 ml and 30% intralipid (lipid emulsion) 17.6 ml. Lidocaine solution (w/v) was prepared by diluting 2% lidocaine (Shanghai Fortune Zhaohui Pharmaceutical Co. Ltd., Shanghai, China) with normal saline. The 30% intralipid (w/v), the solvent of emulsified volatile anesthetics, was provided by the Sino-Swed Pharmaceutical Co. Ltd. (Wuxi, Jiangsu, China).

Epidural anesthetic model in rabbits

Epidural anesthetic model in rabbits were used in the present study as described by Chai et al. [5]. In brief, under general anesthesia of isoflu-

rane, all the rabbits were epidural catheterized before administration. A polyethylene catheter was catheterized between the sixth and fifth lumbar spinous and was inserted 6-7 cm in cephalic direction. Negative pressure of the lacuna was test to ensure the position of catheter. After completely recovery from general anesthesia, the rabbits with normal sensory and motor functions were selected. For all the rabbits, 1 ml lidocaine (1%) was injected to further ensure the catheter in right location. Twenty-four hours later, all the 40 rabbits were randomly divided into 4 groups (n=10/group): respectively receiving 1 ml of 1% lidocaine (lido group), 8% emulsified isoflurane (8% E-iso group), 8% emulsified halothane (8% E-halo group) and 12% emulsified halothane (12% E-halo group). The epidural catheter was flushed with 0.2 ml normal saline. The rabbit behavioral observers were blinded to drug injection.

Sensory functions of the rabbits were evaluated at baseline and at 1, 2, 3, 5, 10 min after injection, then again at 5-min intervals for at least 3 hours (if no blockade observed), or until 1 hour after full recovery. Sensory blockade was evaluated by observing an aversive response (jerk, flinch, or other evasive movements) to pin-prick stimulus with a 23-gauge needle [11]. No aversive responses to pin-prick stimulus were considered as sensory blockade. Onset time of sensory blockade was defined as the time between the drug administration and start of any degree of blockade; duration of sensory blockade was the time from onset and recovery of any degree of blockade. In addition, sensory blockade level was recorded according to following scale [12]: foot =-2, knee =-1, epigastrium =+1, chest =+2 and hand =+3.

Motor function of rabbits was scored based on following scale [12]: 0= optional movements of hind limbs of the rabbits without any limitation or loss of balance; 1= restricted or asymmetrical movements of the hind limbs but ability to support the body and walk; 2= disability to support the body on the hind limbs, but no complete sensory blockade and existing ability to respond to needle stimulus; and 3= total paralysis of the hind limbs, complete blockade both to sensory and motor function. Onset time of motor blockade was the time between drug administration and the start of any degree of blockade; duration of motor blockade was

Table 1. Body weight, onset time and duration of blockade

| Groups | 8% E-halo | 12% E-halo | 8% E-iso | 1% lido |
|------------------------------------|-----------|------------|-----------|-----------|
| Body weight (kg) | 2.14±0.13 | 2.15±0.11 | 2.22±0.12 | 2.19±0.14 |
| Onset of sensory blockade (min) | 1.4±0.8 | 1.2±0.6 | 1.8±1.0 | 1.6±0.9 |
| Duration of sensory blockade (min) | 51±12 | 83±13** | 57±8 * | 47±9 |
| Onset of motor blockade (min) | 1.8±1.0 | 1.4±0.8 | 1.4±0.8 | 1.8±1.0 |
| Duration of motor blockade (min) | 40±8 | 81±12** | 37±3 | 37±6 |

Values are expressed as means ±SD (n=10/group). *: $P<0.05$ vs. Lidocaine group; **: $P<0.01$ vs. Lidocaine group.

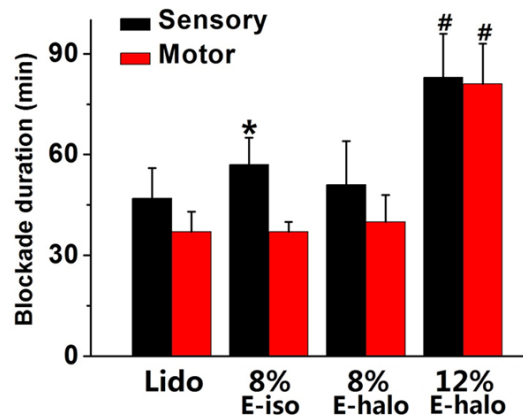


Figure 1. Comparisons of durations of sensory and motor blockade among the four groups. Values are expressed as means ±SD (n=10/group). The drug solutions were injected: 8% emulsified halothane in the 8% E-halo group, 12% emulsified halothane in the 12% E-halo group, and 8% emulsified isoflurane in the 8% E-iso group and 1% lidocaine in the Lido group. Duration was defined as the time from onset (appearance of any sensory and/or motor blockade) to complete recovery (absence of any sensory or motor blockade). * $P<0.05$ versus Lidocaine group; #: $P<0.01$ versus Lidocaine group.

defined the time from onset and recovery of any degree of blockade.

Under epidural anesthesia, consciousness state of all the rabbits were scored as following scale [13]: 1= spontaneous eye-opening without stimulus; 2= rabbit tended to close its eyes spontaneously, but would open eyes when called or patted on the head; 3= rabbit tended to close eyes spontaneously, but would open eyes if there was a painful stimulus; 4= no eyes open even if there was a painful stimulus (general anesthesia). The score <2 was considered as normal consciousness.

Histopathological evaluation

All the 40 rabbits were observed after epidural anesthesia to ensure no obvious sensory and/

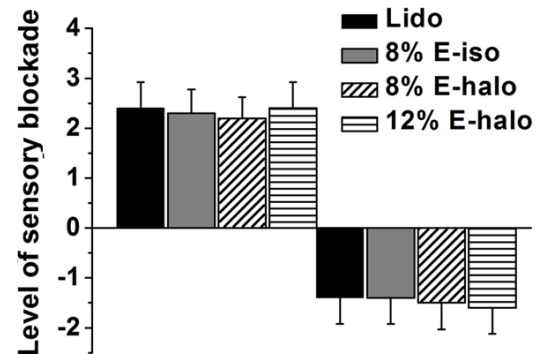


Figure 2. Comparisons of sensory blockade range (upper level and lower level of the blocked) among the four groups. The drug solutions were injected: 8% emulsified halothane in the 8% E-halo group, 12% emulsified halothane in the 12% E-halo group, and 8% emulsified isoflurane in the E-iso group and 1% lidocaine in the Lido group. Each score was defined as: hand =+3, chest =+2, epigastrium =+1, knee =-1, foot =-2. Values were expressed as means ± SD (n=10/group). No significant difference was found in neither upper level nor lower level among the four groups ($P>0.05$).

or motor function impairments. In addition, 24 hours (n=5) and/or 7 days (n=5) after epidural anesthesia, the rabbits were perfused with formaldehyde under general anesthesia. Their spinal cord (at the section of administration) and dorsal root ganglia were removed for hematoxylin-eosin staining.

Statistical analysis

The data were analyzed using SPSS 16.0 for windows. Values of onset time and duration of epidural anesthesia were expressed as mean ± SD. Comparisons of the onset times and durations among the four groups were performed by one-way ANOVA with *post hoc* of Bonferroni test. Kruskal-Wallis test was applied for comparisons in sensory blockade levels and maximum motor blockade degrees. In all cases, $P<0.05$ was considered as statistical significant.

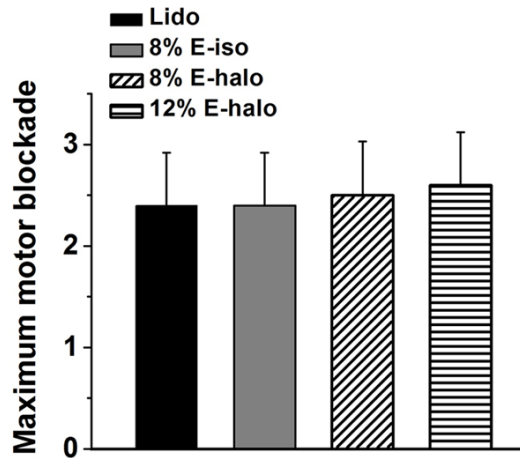


Figure 3. Comparisons of the maximum degree of motor blockade among the four groups. The drug solutions were injected: 8% emulsified halothane in the 8% E-halo group, 12% emulsified halothane in the 12% E-halo group, and 8% emulsified isoflurane in the E-iso group and 1% lidocaine in the Lido group. The degree of motor function blockade was evaluated as: 0= optional movements of hind limbs of the rabbits without any limitation or loss of balance; 1= restricted or asymmetrical movements of the hind limbs but ability to support the body and walk; 2= disability to support the body on the hind limbs, but no complete sensory blockade and existing ability to respond to needle stimulus; and 3= total paralysis of the hind limbs, complete blockade both to sensory and motor function. Values were expressed as means \pm SD ($n=10$ /group). No significant difference was found among the four groups ($P>0.05$).

Results

There was no difference in weight of rabbits among the 4 groups (**Table 1**). After drug solution injection, typical epidural anesthesia was found in all the four groups. Onset time of sensory and motor function blockade were similar among the four groups ($P>0.05$, **Table 1**). The duration of sensory blockade in 12% E-halo group (83 ± 13 min) was significantly longer than other three groups: 47 ± 9 min in 1% lido group ($P<0.01$), 57 ± 8 min in 8% E-iso group ($P<0.01$), and 51 ± 12 min in 8% E-halo group ($P<0.01$). The duration of sensory blockade in 8% E-iso group was significantly longer than that of 1% lido group ($P<0.05$). Duration of sensory blockade in 8% E-halo group and 1% lido group were similar ($P>0.05$). For motor blockade, as shown in **Figure 1**, duration of motor blockade in 12% E-halo group (81 ± 12 min) was also significantly longer than other three groups: 37 ± 6 min in 1% lido group ($P<0.01$), 37 ± 3 min in 8% E-iso group

($P<0.01$) and 40 ± 8 min in 8% E-halo group ($P<0.01$).

No difference was found in sensory blockade level (upper level and lower level of sensory blocked, **Figure 2**) and the degree of maximum motor blockade (**Figure 3**) among the four groups ($P>0.05$).

For consciousness observation, all the rabbits were with normal consciousness state in the groups of 8% E-halo group, 8% E-iso group and 1% lido group. However, there were 4 out of 10 rabbits in the 12% E-halo group showed a light sedation (score 2).

All the rabbits were completely recovered after epidural anesthesia and no pathological injury (edema, necrosis et al.) were found in all the rabbits from the 4 groups. As shown in **Figures 4-6**, all the neurons in spinal anterior and posterior horns and dorsal root ganglia were normal in nuclear or cytoplasmic area. There were no abnormalities in spinal white and grey matters.

Discussion

The present study demonstrates that emulsified halothane could produce a concentration-dependent epidural anesthetic effect. The duration of epidural anesthesia is prolonged with higher concentration. Although the exact mechanisms of local anesthetic effects of volatile anesthetics are still unclear [14-20], many previous studies have demonstrated that volatile anesthetics could produce typical regional anesthesia [1-6].

Long-term regional anesthesia is useful in clinic settings such as post-operative analgesia. The results of the present study indicate that long-term regional anesthesia could be induced by dissolving volatile anesthetics in intralipid. However, pharmacokinetic of emulsified volatile anesthetics after local administration is complicated because characters of water solution, Intralipid and volatile anesthetics are different [21-23]. Among the commonly used volatile anesthetics, oil/gas partition coefficient of halothane is 224 (at 37°C), which is much higher than that of isoflurane (94 at 37°C). Dissolved in 30% intralipid, saturated concentration of isoflurane is 8.24% (at 20°C) and saturated concentration of halothane is higher than

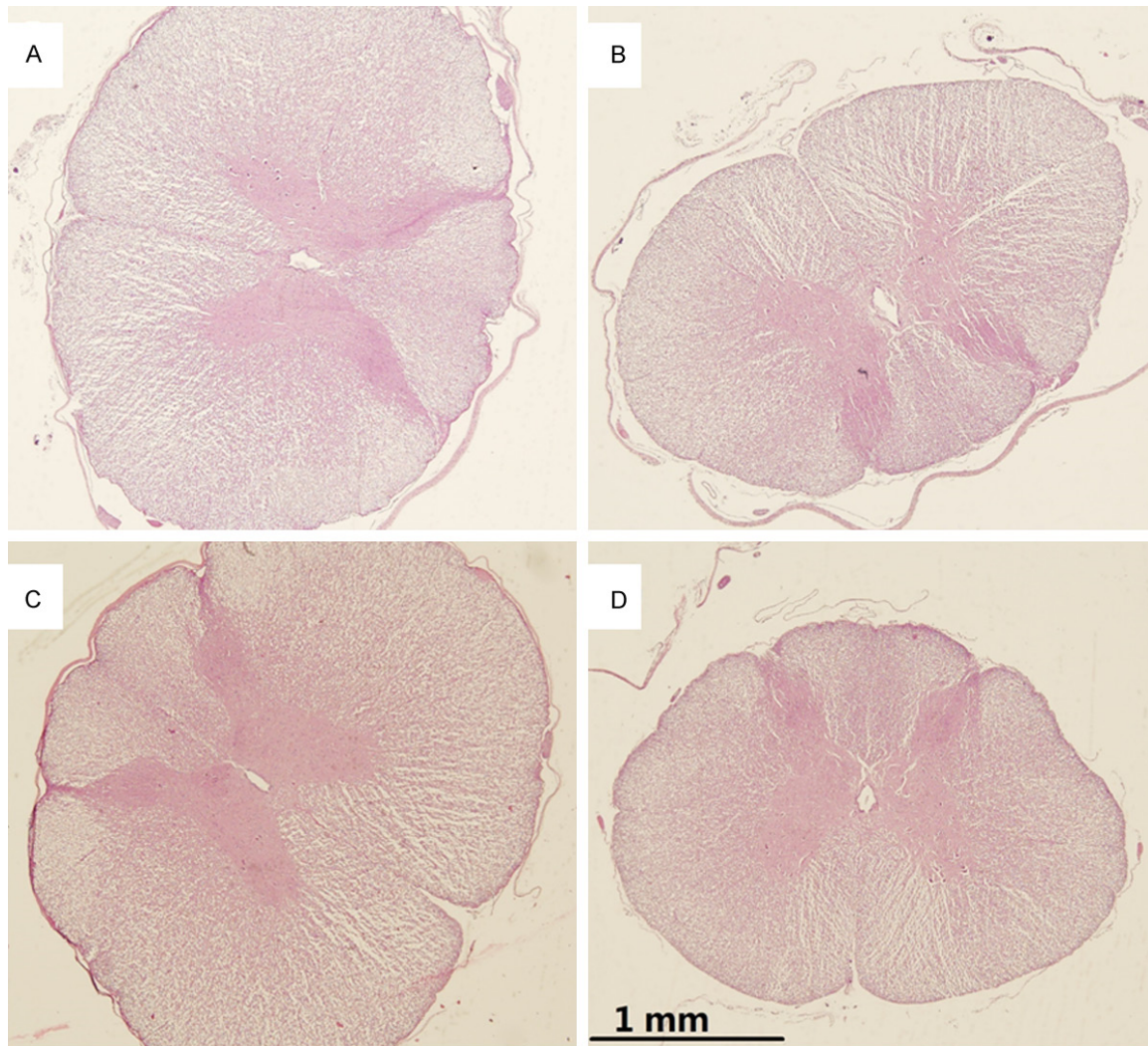


Figure 4. Transverse sections of spinal cord at injected area were stained. No pathological injury was found in all the four groups 24 hours after recovery from epidural anesthesia. A: 1% lidocaine group; B: 8% emulsified isoflurane group; C: 8% emulsified halothane group; D: 12% emulsified halothane group.

12% [10]. Epidural anesthetic effects of 8% emulsified halothane are similar to that of 8% emulsified isoflurane in onset time, duration and blockade level. For both sensory and motor blockade, emulsified halothane at 12% produces a significantly longer duration of epidural anesthesia than 8% emulsified isoflurane (83 ± 13 min vs. 57 ± 8 min in sensory blockade, 81 ± 12 min vs. 37 ± 3 min in motor blockade). There are two main causes for long-term epidural anesthetic effects of emulsified halothane: firstly, the solvent of emulsified volatile anesthetics, 30% intralipid, could accumulate at local area after injection; therefore, high concentration of halothane (12%) in intralipid might be continuously released to produce per-

sistent epidural anesthetic effect; secondly, MAC (minimum alveolar concentration) of halothane is much lower than that of isoflurane because of its high hydrophobicity, indicating a higher anesthetic potency of halothane. The higher anesthetic potency in general anesthesia of halothane might result in higher potency when locally injected. Thus, both preparation type and anesthetic potency of emulsified halothane induce long-term epidural anesthesia. It is reasonable to expect that volatile anesthetics with even higher oil/gas partition coefficient such as methoxyflurane [9] could produce even longer epidural anesthesia when dissolve into 30% intralipid at higher concentrations. The method of the present study provides an under-

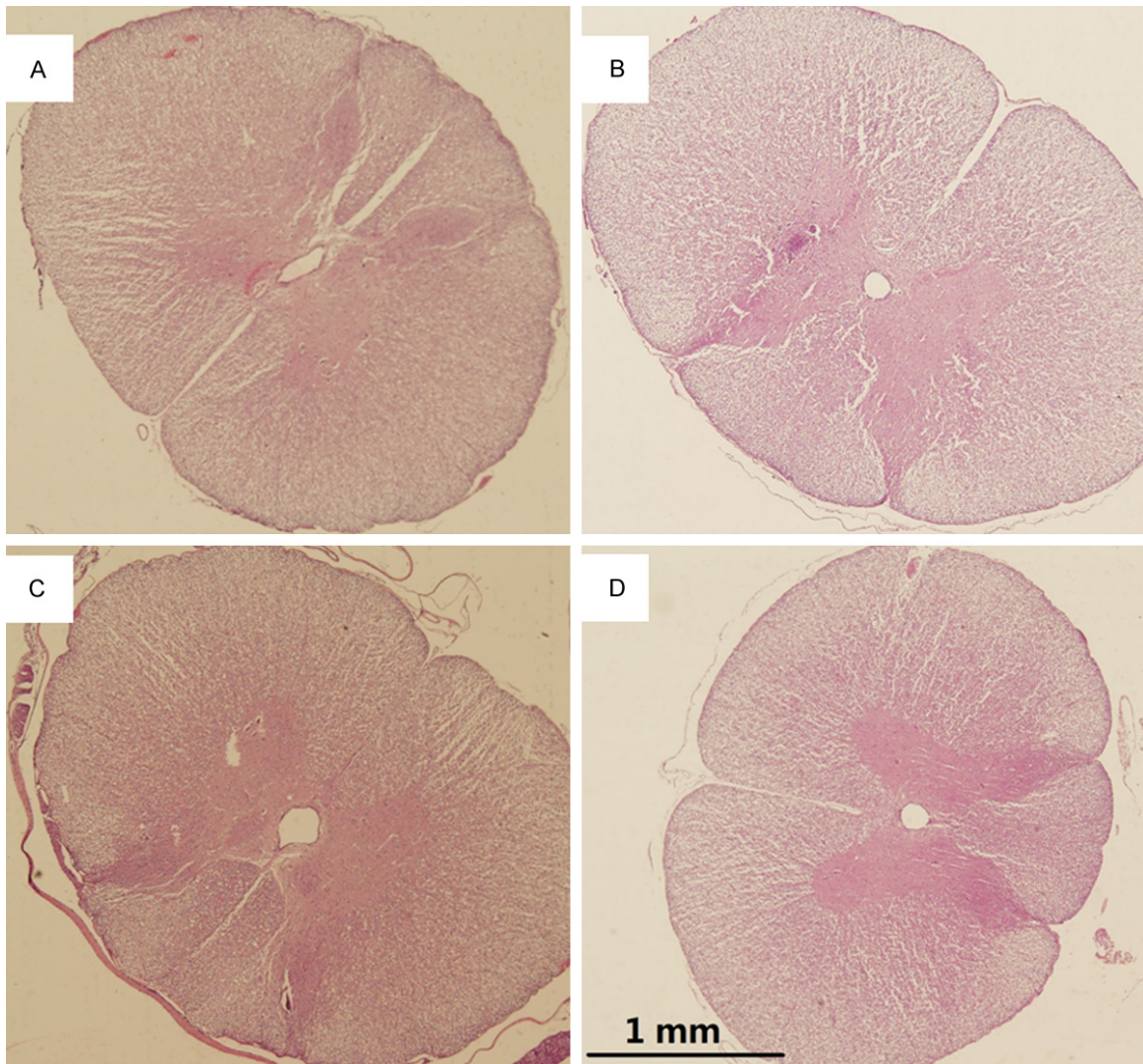


Figure 5. Transverse sections of spinal cord at injected area were stained. No pathological injury was found in all the four groups 7 days after recovery from epidural anesthesia. A: 1% lidocaine group; B: 8% emulsified isoflurane group; C: 8% emulsified halothane group; D: 12% emulsified halothane group.

lying technique to produce long-term regional anesthesia in clinic.

Although many previous studies [24-29] have demonstrated that volatile anesthetics might induce toxic side effects to some main organs such as liver toxicity by halothane and renal toxicity by methoxyflurane, we demonstrated that the emulsified volatile anesthetics are safe in regional anesthesia. Comparing to systemic inhalation, doses of volatile anesthetic is relatively low, which are far lower than their toxic doses. Emulsified halothane at 12% is lower than its saturated concentration in 30% intralipid; therefore, there is no pure liquid halothane in 30% intralipid and 12% emulsified halothane might be safe. In the present study,

12% emulsified halothane produced completely reversible epidural anesthesia and no obvious pathological injury was found. The only observed side effect of 12% emulsified halothane is light sedation (consciousness score =2) in 4 out of 10 rabbits. The sedative effect of 12% emulsified halothane might result from systemic effects of halothane because some halothane is absorbed to systemic circulation. However, this side effect is acceptable and incidence rate is lower than 50%. Therefore, local administration of 12% emulsified halothane is feasible.

In summary, emulsified halothane could produce a concentration-dependent epidural anesthesia. Dissolved in 30% intralipid at rela-

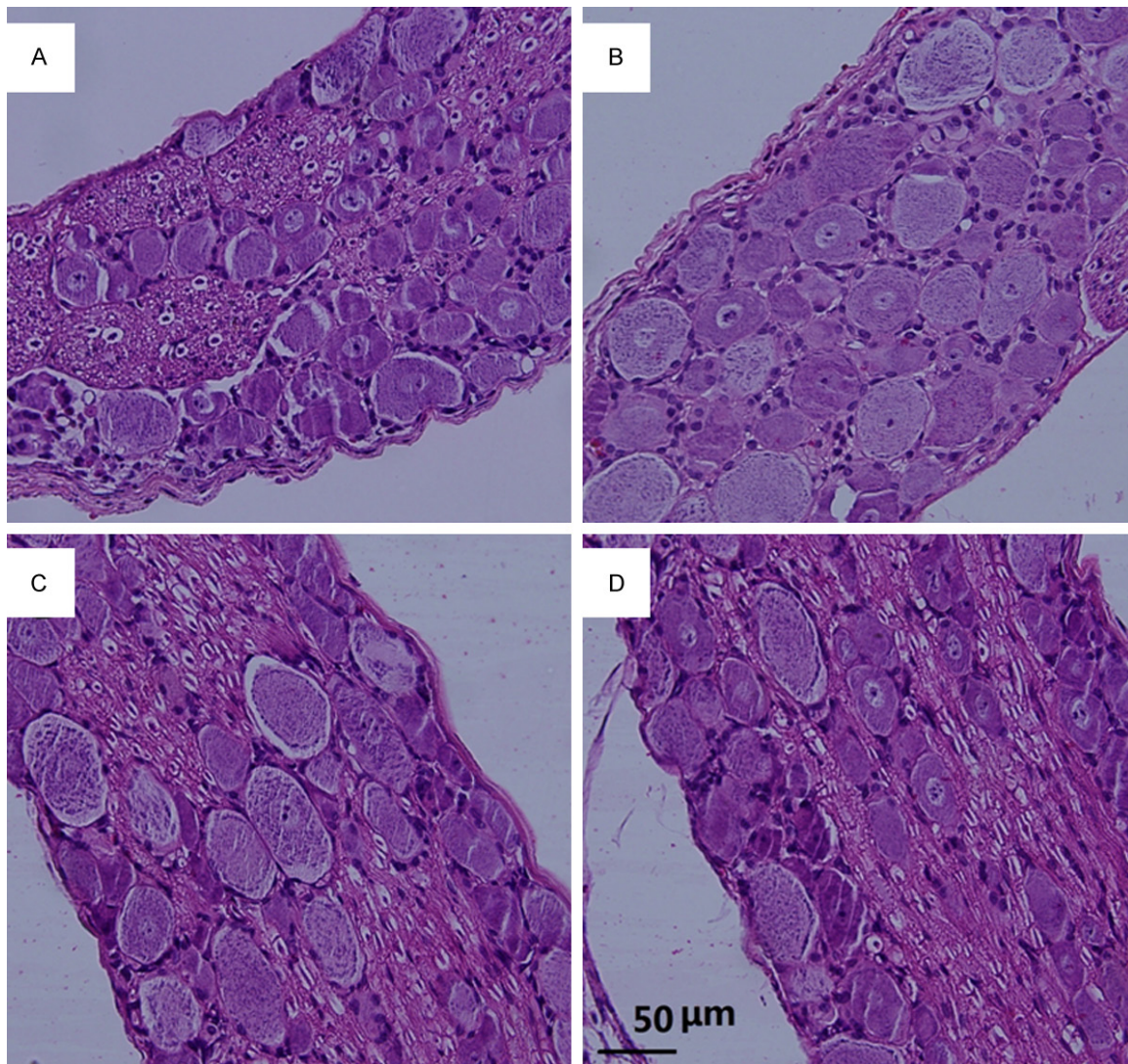


Figure 6. DRG of injected area were stained. No pathological injury was found in all the four groups 7 days after recovery from epidural anesthesia. A: 1% lidocaine group; B: 8% emulsified isoflurane group; C: 8% emulsified halothane group; D: 12% emulsified halothane group.

tively high concentrations, emulsified volatile anesthetics could produce long-term regional anesthesia.

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Disclosure of conflict of interest

None.

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