

Original Research Article

Local anesthetic effect and safety of ropivacaine oily solution

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Abstract

Purpose: To investigate the effectiveness and safety of ropivacaine oily solution (ROPOS).

Methods: After effecting nerve block in postoperative rats by injection of ROPOS near the sciatic nerve, the expression levels of sodium-potassium-chloride co-transporter 1 (NKCC1) and potassium-chloride co-transporter 2 (KCC 2) in the spinal dorsal horn, were assayed. The safety of ROPOS in beagle dogs was evaluated through subcutaneous injection.

Results: The duration of anesthetic effect of ROPOS was 5 times longer than that of ropivacaine hydrochloride injection. The analgesic effect occurred via downregulation of the expression of NKCC1. Dogs administered ROPOS had acute inflammatory reactions at the injection sites within 3 days, but they recovered after 14 days. No other toxic reactions were observed. The T_{max} values for dogs subcutaneously injected with ROPOS at doses of 15, 30 and 60 mg/kg were 2.15 ± 3.09 , 6.25 ± 9.62 and 14.85 ± 11.82 h; with the C_{max} values of 0.78 ± 0.51 , 1.35 ± 0.38 and 2.09 ± 0.53 $\mu\text{g/mL}$, while the $AUC_{(0-48h)}$ values were 15.88 ± 5.41 , 31.46 ± 11.45 and 66.10 ± 17.81 h· $\mu\text{g/mL}$, respectively. The values of T_{max} , C_{max} and $AUC_{(0-48h)}$ were positively correlated with ROPOS dose.

Conclusion: Ropivacaine oily solution has a longer duration of local anesthetic effect than ropivacaine hydrochloride injection and it also possesses a good safety profile. The acute inflammatory reactions are noteworthy and will be further investigated in subsequent studies.

Keywords: Ropivacaine oily solution, Local anesthesia, Rats, Safety evaluation, Beagle dogs

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INTRODUCTION

Postoperative pain is a strong and complex physiological response to surgical wound with duration longer than 24 h after the operation. Postoperative pain affects the body function and also causes mental stress. Therefore, it is important to use postoperative analgesia so as to

avoid possible physiological disorders [1,2]. Ropivacaine is a single enantiomer (S-configuration) amide local anesthetic that blocks nerve excitation and conduction by inhibiting sodium ion channels. Ropivacaine hydrochloride injection (RHI) is used for postoperative analgesia. However, the duration of RHI analgesia in single local injection is about 6 – 8

h, which can hardly satisfy the demands of postoperative analgesia [3].

Oily solution, which uses organic liquid as solvent and vegetable oil or phospholipid as dispersants, is considered a sustained-release drug delivery system [4,5]. Several studies have shown low clearance of the bioactive substance from the delivery depot at the injection site. Oily solution is cleared slowly by capillary absorption and lymphatic capillary phagocytosis. Thus, the clearance time of oily solution significantly affects its drug release behavior. This means that the time taken for the gradual entry of drug molecules from oily solution to the interstitial fluid at injection site, and into the blood and systemic circulation, varies with the clearance time of oily solution [6,7]. This type of solution is commonly used as the delivery system for reproductive or psychiatric drugs which include haloperidol decanoate, flupentixol decanoate, fluperazine decanoate, testosterone isohexanoate, testosterone enanthate, and testosterone undecanoate [7,8]. Ropivacaine oily solution (ROPOS) is designed for injection around surgical incisions. It is composed of benzyl alcohol, benzyl benzoate and soybean oil, with the solubility of ropivacaine being about 30 mg/mL. The present study aimed to investigate the local anesthetic effect of ROPOS and its safety (toxicity and toxicokinetics) profile.

EXPERIMENTAL

Drugs and animals

Ropivacaine, ROPOS and related solvents were provided by Libang Pharmaceutical Co., Ltd. (Xi'an, China). Bupivacaine hydrochloride (99.9 % purity) was purchased from the National Institute for Food and Drug Control (Beijing, China).

Fifty Sprague Dawley (SD) rats (about 4 – 5 months old; 25 males and 25 females) free from specific pathogens, and weighing about 250 – 300 g, were purchased from Laboratory Animal Center, Xi'an Jiaotong University, China. The protocol was approved by the Animal Ethics Committee of Xi'an Jiaotong University, China (approval no. 19-1148). Fifty Beagle dogs (25 males and 25 females), aged 8 – 11 months and weighing between 6 – 10 kg, were purchased from Beijing Marshall Biotechnology Co., Ltd, China. The protocol was approved by the JOINN Institutional Animal Care and Use Committee, (approval no. ACU16-728). The animals were allowed ad libitum access to clean water and feed in a well-ventilated animal house at temperature range of 20 – 26 °C, and humidity of

40 – 70 %.

Determination of pain threshold in the postoperative rats

Forty-eight (40) SD rats were anesthetized via intravenous injection of propofol at a dose of 25 mg/kg. A 1 cm incision was made on the plantar of the right rear foot of each rat after disinfecting it with 75 % alcohol. The muscle was separated and picked up from the fascia. Then, the skin was stitched and sterilized with 75 % alcohol. On the next day, the initial pressure pain threshold was determined. Finally, the rats were intraperitoneally injected with 10,000 units of penicillin once a day for 3 consecutive days [9]. Thereafter, the rats were randomly divided into 6 groups (8 rats per group) viz: normal saline (NS) group, solvent (excipients in formulation) group, three ROPOS dose groups (9, 16 and 30 mg/mL), and ropivacaine hydrochloride injection (RHI; 9 mg/mL) group. Rats in the six groups were injected with 0.4 mL NS, solvent, ROPOS and RHI, as applicable, near the sciatic nerve in the middle of the right popliteal fossa. The pain threshold values were determined before drug administration and at 2, 4, 6, 8, 10 and 12 h after drug administration.

Rats were placed on the glass pane of a hot algometer (PL-200, Taimeng Technology, China). When they were motionless, the wound was irradiated with an infrared heat source from the bottom of the pane. The time taken for the rat to lift the foot was recorded. The time from irradiation to foot lift was the reaction time, i.e., pain threshold. If the reaction time exceeded 15 sec, the pain threshold was recorded as 15 sec. The pain threshold of each rat was determined twice and the average value was recorded. Also, the threshold of pressure pain was determined using an ALMEO electronic anesthesia algometer (Anesthesiometer 2391, IITC Life Science). The rats were placed on the steel net, the probe was placed near the wound when each rat was still, and pressure was then applied to the wound. The value of the applied pressure at which the rat lifted its foot was recorded. Measurement on each rat was made three times and the average value was recorded.

Determination of expressions of NKCC1 and KCC2 in the postoperative rats at different times

Twenty SD (20) rats were randomly divided into 4 groups namely: normal (0 h), 2-h, 2-day and 6-day postoperative pain models, with 5 rats in each group. The rats were operated on at different times. Following intravenous anesthesia

with propofol (25 mg/kg), the rat's abdomen was opened to expose the heart. Then, 200 mL of PBS was transfused from the left ventricle into the ascending aorta, and 200 mL of 4 % paraformaldehyde was re-perfused twice. Then, 1 h later, the rats were dissected, and the 4th and 5th lumbar spinal cords were removed and placed in 4 % paraformaldehyde solution for 24 h. The membrane of the lumbar spinal cord was stripped and soaked in 30 % sucrose solution for 48 h [10]. The lumbar spinal cords were rinsed 3 – 5 times with PBS and the spinal cords were immersed in tissue-freezing medium, frozen and sliced into 20- μ m sections. The tissue sections were soaked in PBS in the perforated plates and oscillated on a shaker for 15 minutes, re-soaked in PBS for 1 h, washed with PBS for 15 minutes, and thereafter immersed in PBS for 30 minutes. Finally, the tissue sections were immersed in PBS containing antibody-1 solution (rabbit anti-NKCC1, rabbit anti-KCC2, 1:200 dilution, Proteintech, China) for 48 h at 4 °C, followed by incubation at 37 °C for 45 minutes. After washing with PBS for 15 min, the tissue sections were incubated in PBS containing antibody 2 (FITC-labeled goat anti-rabbit IgG, 1:600 dilution, Zhuangzhi Biotechnology, China) for 4 – 6 h. After rinsing with PBS for 15 min, the processed sections were analyzed with ImageJ Plus 6.0 software under a fluorescence microscope. The absorbance and the highest fluorescence intensity were measured as indicators of the expressions of NKCC1 and KCC2.

The effect of ROPOS on expressions of NKCC1 and KCC2 in postoperative rats

Eighteen male (18) rats were divided randomly into three groups: normal group, model group, and ROPOS group, with 6 rats in each group. The rats in the normal and model groups were injected with solvent, while rats in ROPOS group were injected with ropivacaine oily solution. All injections were given near the popliteal sciatic nerve. After two days (which was the optimal time for expression of NKCC1 and KCC2 as confirmed in the previous experiment), all rats were anesthetized intravenously with propofol at a dose of 25 mg/kg, and the lumbar spinal cords were excised and processed into tissue sections which were analyzed using immunohistochemistry. The fluorescence intensities of NKCC1 and KCC2 were determined and fluorescence density values were calculated.

Toxicology of ROPOS in dogs

Fifty (50) Beagle dogs were randomly divided into saline group, solvent group, and three ROPOS dose groups, with 10 dogs (5 males and

5 females) per group. The back of the neck of each dog was subcutaneously injected with the respective drug at multiple time points. The volume of administration was no more than 2 mL per point. Physiological signal telemetry system (Data Science, USA) was implanted in each dog and consisted of data acquisition card, environmental pressure reference device, data exchange matrix, implant-element, acceptor, data acquisition and analysis system. The signal telemetry systems collected blood pressure, electrocardiogram, respiration, body temperature and other indicators before drug administration and at 1, 2, 3, 4, 5, 6, 10, 14, 18, 24 and 48 h after drug administration.

Daily behaviors and injection sites of dogs were examined after drug administration, and body weights were measured before injection and at 1, 4, 7, 11 and 14 days after injection. In addition, the feed consumption by each dog was noted within the first 24 h after drug administration. Blood was collected from the forelimb vein before drug administration and on the 3rd and 14th days following drug administration. The following parameters were determined in the blood: blood counts, coagulation function, biochemical parameters and electrolytes. In addition, urine samples were collected from dogs before and on the 14th day after drug administration and the samples were analyzed using the Clinitek Advantus TM analyzer.

Anatomical and pathological examinations were carried out on the dogs under anesthesia on the 3rd day (6 dogs from each group) and on the 14th day (4 dogs from each group). All the tissues and organs, including the nerves, blood, digestive system, respiratory system, urogenital system, and reproductive system, were excised and evaluated. The body and major visceral organs were weighed, and the organ: body weight ratios were calculated. The tissues were fixed in 10 % neutral formalin solution, embedded in paraffin, sliced, stained, and examined under a light microscope.

Toxicokinetics of ROPOS in dogs

Thirty (30) dogs were divided into three ROPOS groups, with 10 dogs per group (5 males and 5 females). Blood (1 mL) was obtained from the forelimb veins before and at 0.25, 0.5, 1, 2, 4, 8, 24, 48 and 72 h after injection of ROPOS. The blood samples were collected in centrifuge tubes containing 20 μ L of heparin sodium (1,000 IU/mL). The plasma was obtained after centrifugation of the blood samples at 8,000 rpm for 5 min. Then, the plasma was mixed with internal standard (bupivacaine acetonitrile

solution of concentration 20 ng/mL) at a volume ratio of 5:95. The mixture was vortexed and centrifuged at 12,000 rpm for 10 min. The supernatant was mixed with sterile water at a volume ratio of 5:95, and the mixture was analyzed using LC-MS/MS (HPLC, LC-30A, SHIMADZU; MS/MS, 4000 QTRAP (Applied Biosystems); Ultimate XB-C18 Chromatographic Column of dimensions 4.6 x 150 mm, 5 μ m (Welch Materials). The mobile phase used was a 5:95 (v/v) ratio of aqueous solution of 5 mM ammonium acetate and acetonitrile, at flow rate of 1.0 mL/min, injection volume of 10 μ L, and run time of 4 min. The detector used was ion trap mass spectrometry in positive ion mode at capillary voltage of 0.5 kV, ion source temperature of 120 $^{\circ}$ C, atomization temperature of 350 $^{\circ}$ C, conical hole gas velocity of 150 L/h and atomized gas velocity of 650 L/h. The method was validated with respect to specificity, accuracy, limit of quantitation, precision and recovery [11].

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 7.0 (Graph-Pad Software, La Jolla, CA, USA). The results are expressed as mean \pm standard deviation (SD). Plasma concentrations of parameters were analyzed using WinNonlin (V6.2), while C_{max} , T_{max} and AUC were calculated with non-atrioventricular modeling. Values of $p < 0.05$ were considered indicative of statistical significance.

RESULTS

Local anesthetic effect of ROPOS

The results from measurements of pressure pain thresholds are shown in Figure 1 A. The pressure pain threshold value in postoperative rats was 16.4 – 17.4 g before drug administration. There were no significant differences in pressure pain threshold values among the groups. There was no significant difference in pressure pain threshold value in model group before and after dosing ($p > 0.05$). However, the threshold values due to ROPOS at doses of 16 and 30 mg/mL were increased at 2 – 8 h and 2 – 10 h, respectively, when compared with the model group. The pressure pain threshold value in RHI group increased at 2 h. The results of heat pain threshold were similar to those of pressure pain threshold, as shown in Figure 1 B.

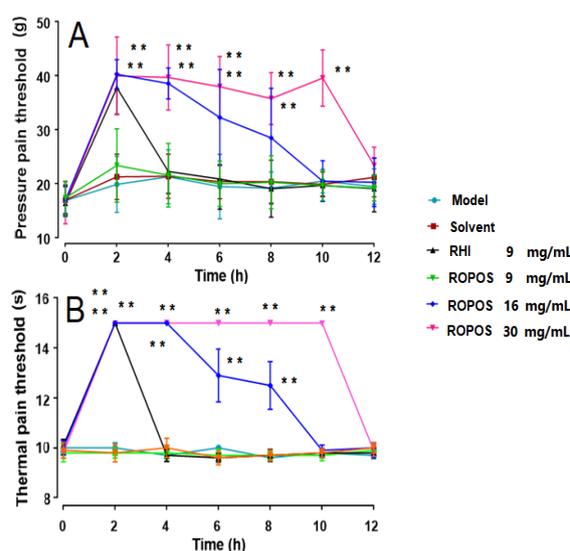


Figure 1: Effect of ROPOS on postoperative pain in rats. (A) Pressure pain threshold. (B) Heat pain threshold $**P < 0.05$, vs model group

Expressions of NKCC1 and KCC2

The expression of NKCC1 in spinal dorsal horn produced a small amount of green fluorescence, indicating low expression of NKCC1 in normal group. However, the expression of NKCC1 in the spinal dorsal horn in the model group was slightly increased at 2 h, further enhanced on day 2 and decreased on day 6 after surgery. The absorbance of NKCC1 in postoperative model rats were 0.0313 ± 0.0062 , 0.0521 ± 0.0195 and 0.0291 ± 0.0051 at 2 h, 2 days, and 6 days after surgery, respectively, which were significantly higher than that in normal group (0.0226 ± 0.0045 , $p < 0.01$; Figure 2 A). Similarly, in the spinal dorsal horn of normal rats, KCC2 showed a strong green fluorescence which indicated high expression levels of the protein in normal group. However, KCC2 protein expression in the spinal dorsal horn of postoperative rats was decreased at 2 h, day 2 and day 6 after surgery. The absorbance presented in Figure 2 B show that, compared with normal group, KCC2 protein expression in the spinal dorsal horn of postoperative rats was decreased at day 2 and day 6 after surgery (0.0642 ± 0.0044 and 0.0713 ± 0.0092 , vs 0.0910 ± 0.0220 ; $p < 0.05$). The expressions of NKCC1 and KCC2 in the spinal dorsal horn of the model rats were most significantly altered at day 2 after surgery.

Effect of ROPOS on CNKK1 and KCC2 expression

Compared with normal group, the expression of NKCC1 was increased significantly in the model group. The expression of NKCC1 in the ROPOS

group was lower than that in model group (Figure 3 B - D). The absorbance values were 0.0360 ± 0.0063 , 0.0544 ± 0.0103 and 0.0313 ± 0.0114 in the normal, model and ROPOS group, respectively (Figure 4 A). Moreover, the expression of KCC2 was lower in model and ROPOS groups than in normal group (Figure 3 F - H), with absorbance of 0.1830 ± 0.0266 , 0.1277 ± 0.0169 and 0.1283 ± 0.0122 , respectively (Figure 4 B). These results indicate that ROPOS downregulated the expression of NKCC1, but did not affect the expression of KCC2 in the spinal dorsal horn of postoperative rats.

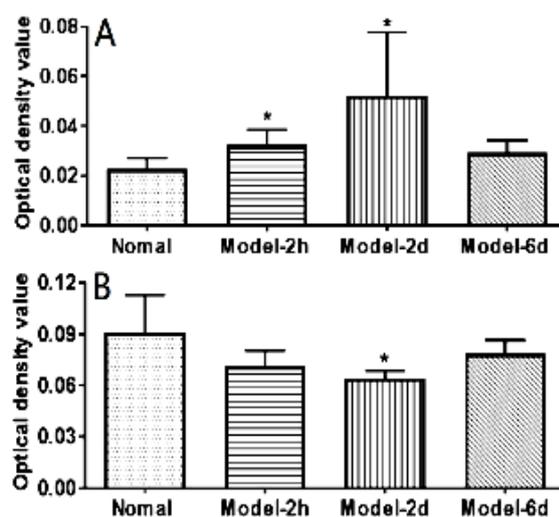


Figure 2: The expression levels of NKCC1 (A) and KCC2 (B) in spinal dorsal horn of model rats at different times, relative to normal group. * $P < 0.05$, vs normal group

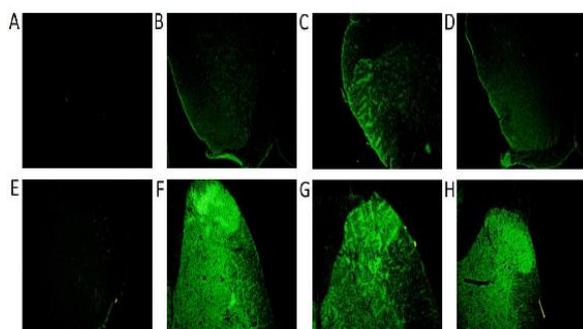


Figure 3: Stained slices showing the expressions of NKCC1 (A-D) and KCC2 (E-H) in spinal dorsal horn of rats (x10). A and E: PBS group; B and F: normal group; C and G: model group; D and H: ROPOS group

Acute toxicity of ROPOS

Subcutaneous administration of ROPOS at doses of 15, 30 and 60 mg/kg did not result in the death of any dog during the experiment. Ten percent (10 %) of dogs in the solvent and

ROPOS groups suffered swelling, erythema, scleroma, rupture and scab at the injection sites from the 2nd to 14th day after ROPOS administration. There were no significant differences in values of the major cardiovascular indices such as heart rate, blood pressure and electrocardiogram among the ROPOS, solvent and saline groups. The Q-T interval of the 60 mg/kg ROPOS group was significantly increased in the first 2 – 4 h ($p < 0.05$). However, the increase was within the normal range, without any toxicological significance (Figure 5A-5D). In addition, there were no significant differences in respiratory functions and body temperature among the saline group, solvent group and ROPOS groups (Figure 5E and 5F).

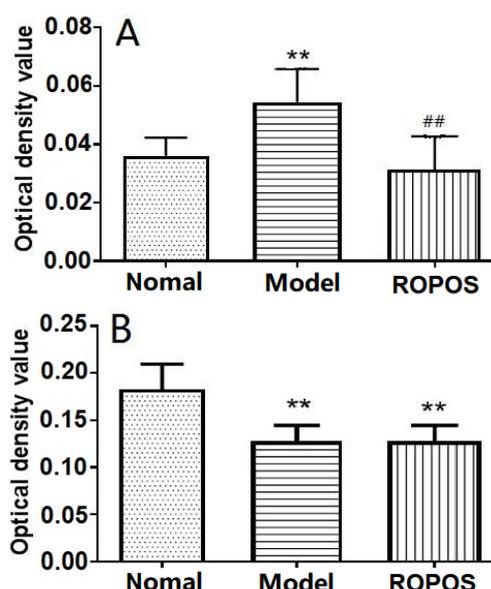


Figure 4: Effect of ROPOS on expressions of NKCC1 (A) and KCC2 (B) in the spinal dorsal horn of rats after 2 days of drug administration. ** $P < 0.01$, vs. normal; ## $p < 0.01$, vs. model

Compared with the saline group, there were no significant changes in blood cell count and coagulation function in the solvent and ROPOS groups, and the urine in the ROPOS groups showed no obvious abnormalities either. The adrenal weights of the solvent and ROPOS (15 and 60 mg/kg) groups were decreased on the 3rd day after administration (Table 1). There was swelling with local blood stasis in some injection sites. On 14th day, the weights and the ratios of organ weight-to-body weight for the thyroid and parathyroid glands were increased in the 60 mg/kg ROPOS group, when compared with the saline group ($p < 0.05$). Nodules were visible at the injection sites in some dogs in the solvent and ROPOS groups.

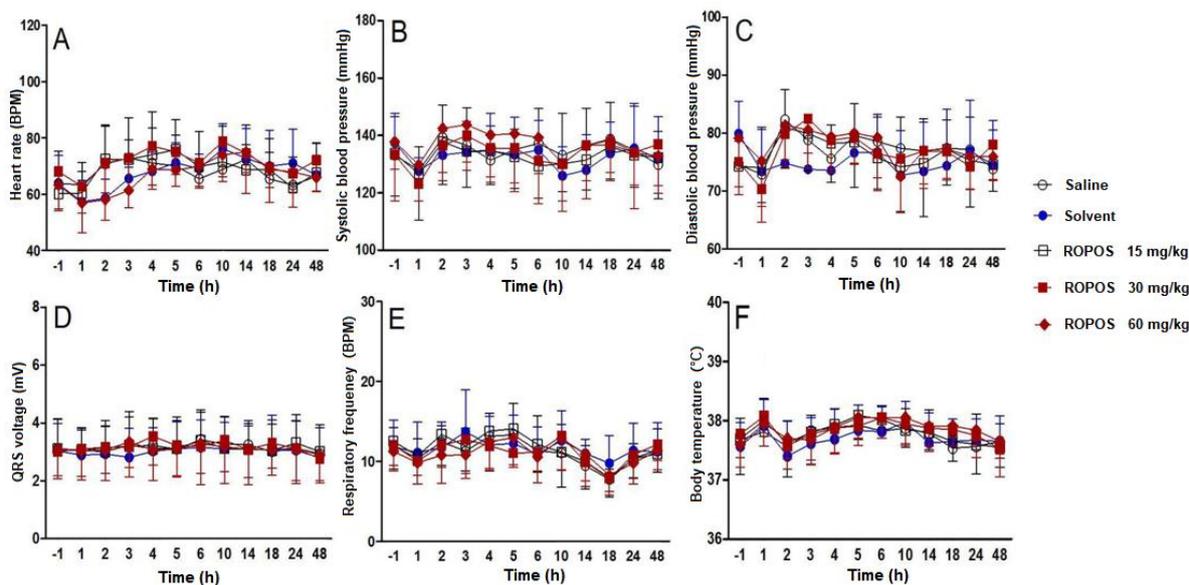


Figure 5: Effect of ROPOS on heart rate (A), systolic blood pressure (B), diastolic blood pressure (C), QRS voltage (D), respiratory frequency (E) and body temperature (F)

Table 1: Effect of subcutaneous injection of ROPOS on organ weight (g) in Beagle dogs

Group	saline	solvent	ROPOS doses		
			15 mg/kg	30 mg/kg	60 mg/kg
Organ					
Day 3 after ROPOS injection					
Brain	77.68±6.13	75.36±5.24	76.47±6.46	74.62±3.02	74.07±3.18
Thyroid	0.63±0.12	0.56±0.16	0.68±0.11	0.54±0.06	0.66±0.17
Spleen	29.42±6.02	23.38±5.44	23.24±5.01	23.28±3.19	21.19±4.19
Liver	211.31±16.45	192.84±16.89	200.01±40.73	200.69±26.68	195.96±23.26
Adrenal gland	1.44±0.10	1.01±0.08*	0.97±0.09*	1.16±0.24	1.03±0.13*
Kidney	38.83±10.27	36.52±3.11	37.41±5.42	36.59±6.28	36.01±4.74
Thymus	7.10±1.49	6.82±1.53	7.07±2.08	6.02±2.24	6.26±1.81
Heart	65.46±7.09	60.68±7.23	64.70±8.73	67.50±16.94	57.36±13.47
Lungs	69.61±4.76	62.12±1.98	70.25±7.34	67.76±8.49	61.19±10.62
Organ					
Day 14 after ROPOS injection					
Brain	73.26±3.43	71.29±8.37	77.68±5.36	73.20±6.04	72.27±4.39
Thyroid	0.57±0.10	0.63±0.11	0.64±0.11	0.61±0.08	0.80±0.15*
Spleen	25.12±5.42	26.02±3.93	25.07±4.00	28.04±4.09	22.07±2.99
Liver	198.7±26.4	199.9±21.7	210.8±31.9	227.7±40.5	223.8±33.6
Adrenal gland	1.25±0.12	1.29±0.20	1.19±0.21	1.15±0.11	1.35±0.10
Kidney	35.83±6.18	36.60±6.92	38.31±5.36	42.39±5.83	40.17±4.33
Thymus	7.50±2.56	7.52±2.41	5.92±1.66	8.90±3.53	9.46±4.58
Heart	60.91±11.05	61.49±6.81	63.26±6.11	64.67±7.54	60.77±5.72
Lungs	65.36±7.19	64.82±9.31	68.32±5.06	73.57±5.36	68.48±9.16

* $P < 0.05$, vs. normal saline group

On the 3rd day after drug administration, microscopic examination revealed mild-to-moderate acute subcutaneous necrosis, hemorrhage and inflammatory cell infiltration at the injection sites of dogs in the solvent and ROPOS groups. Also, mild-to-moderate chronic subcutaneous inflammation was revealed on the 14th day, with granulation tissue or fibrosis and necrotic tissue, suggesting that the acute inflammation was reversible (Figure 6). In addition, the degree of inflammation was comparable in the solvent and ROPOS groups, indicating that the inflammation was related to

the solvent, and not to ropivacaine *per se*.

Verification of detection methods of ropivacaine in dog plasma

The LC-MS/MS method used for analyzing ropivacaine was specific and accurate. Within the concentration range used in the calibration curve (4 – 2000 ng/mL), the accuracies were within 92.1 – 111.6 %. The correlation coefficients were more than 0.998. The mean accuracies of equality control samples were within 99.6 – 111.5 %. The inter-batch and intra-batch precisions in

terms of coefficient variation (CV) were less than 10 %. The lower limit of quantification (LLOQ) was 4 ng/mL and the accuracies of LLOQ samples were within 96.2 – 104.4 %. The CV of extraction recovery was within 2.98 – 3.72 % while The CV of matrix effect was within 1.10 – 1.78 %. The recoveries of 10-fold and 100-fold dilution were 100.9 – 102.4 % and 104.3 – 106.4 %, respectively. The recoveries of samples kept at room temperature for 4 h were 101.1 – 105.6 %, 95.8 – 101.3 % for samples kept below -15 °C for 4 weeks and 99.9 – 103.4 % for samples subjected to three freeze-thaw cycles. The recoveries of processed samples kept below -15 °C for 3 days ranged from 96.6 % to 107.6 %. Therefore, the LC-MS/MS method adequately met the requirements for bioanalysis of ropivacaine in the biological samples.

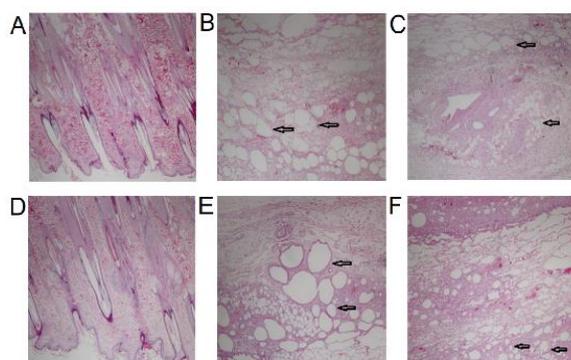


Figure 6: The effect of subcutaneous injection of ROPOS on the injection sites in Beagle dogs. A and D: were injected with normal saline on day 3 and day 14, respectively; B and E: were injected with solvent on day 3 and day 14, respectively; C and F: were injected with 60 mg/kg ROPOS on day 3 and day 14, respectively. Arrows show vacuoles formed by inflammatory cell infiltration

Toxicokinetics of ROPOS

The toxicokinetic parameters of ropivacaine in dogs are shown in Table 2. In the 15 mg/kg ROPOS group, the values of T_{max} , C_{max} and $AUC_{(0-48h)}$ were 2.15 ± 3.09 h, 0.78 ± 0.51 $\mu\text{g/mL}$

and 15.88 ± 5.41 h· $\mu\text{g/mL}$, respectively. In the 30 mg/kg ROPOS group, the values of T_{max} , C_{max} and $AUC_{(0-48h)}$ were 6.25 ± 9.62 h, 1.35 ± 0.38 $\mu\text{g/mL}$ and 31.5 ± 11.5 h· $\mu\text{g/mL}$, respectively, while the corresponding values in the 60 mg/kg ROPOS group were 14.9 ± 11.8 h, 2.09 ± 0.53 $\mu\text{g/mL}$ and 66.1 ± 17.8 h· $\mu\text{g/mL}$, respectively. These data indicate that the T_{max} was prolonged, while C_{max} and $AUC_{(0-48h)}$ rose with increase in ROPOS dose. There were no significant differences in $t_{1/2}$ among the ROPOS (15, 30 and 60 mg/kg) groups. As shown in Figure 7, the plasma concentrations of ropivacaine in 15, 30 and 60 mg/kg groups at 48 h after administration of ROPOS were 26.8 ± 14.8 , 35.1 ± 28.5 and 254.5 ± 179.9 ng/mL, respectively, but the corresponding concentrations of ropivacaine after 72 h were 0, 7.38 ± 4.05 and 32.1 ± 29.6 ng/mL, respectively. The release time of ropivacaine was lengthened with an increase in dose and the changes were positively correlated.

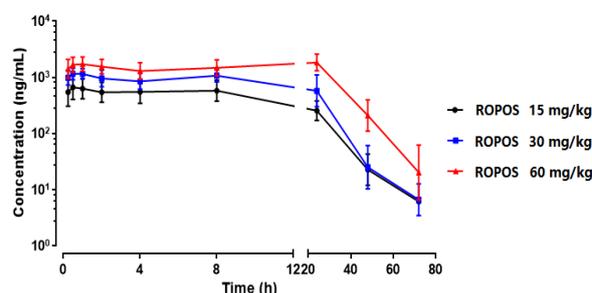


Figure 7: Plasma ropivacaine concentration-time curve after subcutaneous injection of ROPOS to Beagle dogs at doses of 15, 30 and 60 mg/kg, respectively

DISCUSSION

At present, ropivacaine hydrochloride injection (RHI) is commonly used for local nerve block and it is administered via local infiltration, subcutaneous injection or ganglion injection. The anesthetic effect usually lasts 6 – 8 h.

Table 2: Toxicokinetic parameters of ropivacaine in the plasma of beagle dogs

Dose (mg/kg)	Sex	$t_{1/2}$ (h)	T_{max} (h)	C_{max} ($\mu\text{g/mL}$)	$AUC_{(0-48h)}$ (h· $\mu\text{g/mL}$)	AUC_{inf} (h· $\mu\text{g/mL}$)
15	♂	7.67 ± 1.68	2.00 ± 3.35	0.89 ± 0.72	16.51 ± 6.88	16.88 ± 6.68
	♀	7.13 ± 0.62	2.30 ± 3.19	0.66 ± 0.22	15.25 ± 4.19	15.47 ± 4.23
	Total	7.40 ± 1.23	2.15 ± 3.09	0.78 ± 0.51	15.88 ± 5.41	16.17 ± 5.32
30	♂	6.82 ± 1.87	5.80 ± 10.18	1.49 ± 0.46	35.18 ± 10.82	35.63 ± 10.99
	♀	6.81 ± 1.06	6.70 ± 10.20	1.20 ± 0.24	27.75 ± 11.98	28.18 ± 12.47
	Total	6.82 ± 1.43	6.25 ± 9.62	1.35 ± 0.38	31.46 ± 11.45	31.90 ± 11.76
60	♂	9.57 ± 2.32	10.30 ± 12.52	2.12 ± 0.66	67.48 ± 20.26	71.31 ± 19.78
	♀	8.04 ± 3.12	19.40 ± 10.29	2.05 ± 0.45	64.71 ± 17.28	68.93 ± 20.71
	Total	8.81 ± 2.72	14.85 ± 11.82	2.09 ± 0.53	66.10 ± 17.81	70.12 ± 19.13

When the dose of RHI is increased, the duration of nerve block is correspondingly prolonged and the potential toxicity of ropivacaine also increases. Excessive doses of ropivacaine may inhibit cardiac conduction and myocardial contractility, leading to arrhythmias or blurred consciousness in patients. *Al-Alami et al* [12] reported that a patient injected with 400 mg of ropivacaine developed confusion, muscle fibrillation, breathing acceleration and tachycardia. Interestingly, the oily solution has a slow-release effect. Ropivacaine oily solution (ROPOS) is an oil drug delivery drug system in which ropivacaine is the main drug, while benzyl benzoate, benzyl benzoate and soybean oil serve as excipients. Experimental results in rats showed that the nerve block duration of RHI was about 2 h, while the nerve block time of ROPOS exceeded 10 h. These results suggest that the duration of ROPOS-induced painlessness may be up to 30 – 48 h. This has important clinical significance. Sodium-potassium-chloride co-transporter 1 (NKCC1) and KCC2 are two primary regulatory proteins for chloride ions in the central nervous system. These proteins are involved in the conduction of pain signals in the spinal cord. Sodium-potassium-chloride co-transporter 1 regulates chloride ions influx, while KCC2 adjusts efflux of chloride ions [13-15]. The present study showed that, compared with normal rats, NKCC1 expression was higher in spinal dorsal horn of model rats, while the expression of KCC2 was lower in the spinal dorsal horn, which is similar to data from other studies [16-17]. The expression of NKCC1 in spinal dorsal horn of rats in ROPOS group was significantly decreased on the second day. In contrast, the expression of KCC2 did not change. This suggests that the mechanisms underlying the inhibitory effect of ropivacaine on the sensitivity to postoperative pain may be related to reduction of the expression of NKCC1 in the spinal dorsal horn.

Pharmacokinetic parameters of ROPOS in dogs showed that the mean C_{max} and $AUC_{(0-48h)}$ values of ROPOS were increased with an increase in ROPOS dose within the range of 15 – 60 mg/kg. The dose ratio of ROPOS in the 3 groups was 1:2:4, the average C_{max} ratio of ropivacaine was 1:1.73:2.68, and the average $AUC_{(0-48h)}$ ratio was 1:1.98:4.16. These data indicate that the exposure doses of ropivacaine were positively correlated with the doses of ROPOS given to the dogs. Furthermore, the average C_{max} ratio of ropivacaine between male and female dogs ranged from 1.03:1.05 in the dose range of 15 – 60 mg/kg, and the $AUC_{(0-48h)}$ ratio was 1.04:1.27. These results reveal that different genders of dogs do not differ significantly in the effect of

ropivacaine exposure. In this toxicology research, the maximum dose of ROPOS in dogs was 60 mg/kg, and neurotoxicity and cardiovascular toxicity were not manifested. However, Griffiths *et al* studied 30 patients who were injected with RHI at a dose of 2.5 mg/kg in the abdominal muscles after surgery and observed that 3 of the patients suffered nervous system toxicity [18]. Therefore, the safe dose of ROPOS may be more than 24 times that of RHI, which suggests that the clinical dose of ROPOS in humans will be higher. This further suggests that the release time of ropivacaine from ROPOS will be significantly extended, i.e. the local nerve-blocking effect will be prolonged. The results from toxicology studies indicate that the three doses of ROPOS used did not produce obvious toxicity on the nerves, blood and the cardiovascular, urinary and respiratory systems of the dogs. All the ROPOS and solvent groups had some irritations at the injection sites, suggesting that the observed irritation may be from the solvent.

CONCLUSION

Ropivacaine oily solution (ROPOS) slowly releases ropivacaine, thereby producing a long duration of anesthetic effect after subcutaneous injection. The drug did not produce any obvious toxic effect. In addition, the mechanism of action of ropivacaine is related to the inhibition of sensitivity of postoperative pain through reduction of the expression of NKCC1 in spinal dorsal horn. Future studies will focus on the inflammatory reactions associated with ROPOS.

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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