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Research Article

The Pharmacokinetics and Analgesic Effectof Ropivacaine Oil Solution after Subcutaneous Injection to Mini-Pig Operative Incision

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2. Keywords

Ropivacaine oil solution; Pharmacokinetics;Analgesic; Benzyl alcohol; Mini-pig

1. Abstract

1.1. Objective

Ropivacaine is an anesthetic blocker used for local wound infiltration after operation. In the present study, we examined the pharmacokinetics and analgesic effect of ropivacaine oil solution after local infiltration. The concentration of ropivacaine in myocardial tissue and injection site, and the benzyl alcohol residue in injection site were detected.

1.2. Methods

Mini-pigs were injected with ropivacaine oil solution or ropivacaine hydrochloride injection. The concentration of ropivacaine in the plasma, myocardial tissue and local subcutaneous tissue were measured by liquid chromatography coupled with tandem mass spectrometry method. The pharmacokinetics of ropivacaine were investigated. Ropivacaine residues in myocardial tissue and injection site were investigated 24 h after administration. Benzyl alcohol at injection site 24 h after administration was investigated by a high performance liquid chromatography method.

1.3. Results

Ropivacaine oil solution (3.5mg/kg) showed an analgesic effect for a duration of 14 h. The C_{max} is 1122±405 ng/ml, the T_{max} is 2.6±2.7 h, and the MRT is 6.0±2.0 h. Compared with ropivacaine hydrochloride injection at 2.3 mg/kg, the analgesic duration of ropivacaine oil solution is increased by 3.5 times, MRT is increased by 3 times, the C_{max} is was significantly reduced by 65%, and the T_{max} is was delayed by 2.5 h. After drug administration for 24 h, there is no significant difference of ropivacaine residues in myocardial tissue of mini-pigs between the two groups. The ropivacaine oil solution has lower drug residues in the injection site compared with the ropivacaine hydrochloride injection. There is no obvious adverse reaction.

1.4. Conclusions

Compared with the ropivacaine hydrochloride injection, ropivacaine oil solution showed a longer effective analgesic duration.

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3. Introduction

Ropivacaine, a sodium ion channel inhibitor of neurocyte, could block the excitation and conduction of nerves and therefore possesses anesthetic and pain relief properties. Ropivacaine has the characteristics of separation of sensory-motor nerve block. Local infiltration of anesthesia, such as ropivacaine, bupivacaine, mepivacaine, etc., to postoperative wound is the main administration way in clinic. However, a single dose of ropivacaine could only relief pain in a short duration. Wulf, Hinnerk [1] reported that among 21 patients who had been given anesthesia via local infiltration after inguinal hernia repair, the pain relief time of most patients was 6-12 h. Postoperative pain mainly happens in the first 48 h after surgery, some may last for more than 72 h with more intense pain. It is difficult to prolong the anesthetic duration of local infiltrated anesthesia after surgery. F Duarte et al. [2]. found that infusion of ropivacaine on cesarean section (10 mg/h for 48 h) could better relieve pain than epidural injection of morphine (2 mg/12h). Moreover, infusion of ropivacaine on cesarean section could significantly reduce the incidence rate of nausea, vomiting, pruritus and urinary retention and decrease the time needed for the recovery of intestinal functions. After studying the post-operative pain relief of patients with thoracic muscle retention-lung cancer resection, Alfonso Fiorellia and Anna Cecilia Izzo et al. [3]. found that continuous ropivacaine dripping on the incision sites for 48 h could significantly reduce the expression of IL-6, IL-10 and TNF-α, and decrease the need of additional morphine intake in the whole operation.

Local catheterization and continuous dripping of ropivacaine or bupivacaine on open wounds is an effective method of analgesia. However, it still also has some problems such as wound infections, wrapping difficulties, inconvenient for patient to move, complex equipment operations, etc. Therefore, it is important and meaningful to develop a drug delivery system in which ropivacaine and other local anesthesia could exert extended analgesia effect. In 2010, liposome injection of bupivacaine hydrochloride (Exparel) from Pacira Pharmaceuticals was approved by FDA. It is the first marketed analgesic injection with long-acting effects in the world, which is mainly used to relieve the postoperative pain. When compared with the placebo, it could significantly reduce the cumulative scores of pain in 72 h after hemorrhoidectomy and reduce the amount of opium demand [4]. Davidson EM, Haroutounian S, et al. [5], reported that when placed in water-based medium (normal saline or plasma), a drug delivery system of ropivacaine with ethanol, phospholipid and castor oil as the carrier, would form liposomes with particle sizes of 1-4 um. In phase I clinical trials, the drug administration dosage is 100 mg, which is compared with the drug effect of adopting aqueous solution of 25 mg. The results showed that the preparation pain relief (thermal stimulus) duration is 36 h, namely three times of aqueous solution. However, due to its high viscosity, it was difficult to prepare [5].

Oily solution is commonly used in clinic, such as progesterone injection, testosterone tetrad crate injection and fulvestrant injection. The excipients used in oily solution were also proved to be safe. After being injected into human body, the vegetable oil or synthetic oil in the system act as a reservoir for drugs and thereafter, the drug-oil solution enter blood circulation via capillary absorption, lymphatic absorption, phagocytosis, *et al*. [6] It is also believed that the elimination of oil is associated with inflammation reaction caused by the solvent, or in situ metabolism after absorption. Its slow-release mechanism is clear [6].

In ropivacaine oil solution, 30 mg/ml ropivacaine freebase is the active substance, soybean oil is the dispersing agent, benzyl alcohol and benzyl benzoate act as solvent. In our study, ropivacaine oil solution was infiltrated in the incision site in an animal model-pig hind limb model of incisional pain, the pharmacokinetics and pharmacodynamics (PK/PD) behavior of ropivacaine such as the concentration of roivacaine in the blood, injection site and myocardial tissues, the analgesic effect of the drug were examined. Besides, the benzyl alcohol concentration in the injection site was also evaluated.

4. Materials and Methods

4.1. Reagents and Chemicals

Ropivacaine oil solution (specification: 300 mg/10 ml, batch number: R20150801) and solvent (benzyl alcohol, specification: 10ml/bottle, batch number: 20150801) was provided by Xi'an Libang Zhaoxin Biotechnology Co., Ltd. Ropivacaine hydrochloride injection (specification: 100mg/10ml, batch number: NAPL) was bought from AstraZeneca AB, Sweden. USP reference standard of ropivacaine hydrochloride(specification: 200 mg, purity: 99.8%, batch number: F0J017) and reference standard of bupivacaine hydrochloride (specification: 5ml purity: 99.6%, batch number: BOGO-OZNY) are purchased from National Institutes for Food and Drug Control. Benzyl alcohol (specification: 5ml, batch number: K44737087, content 98.0% to 100.5%) is bought from Merck & Co. Inc. Propofol injection (specification: 200mg: 20ml, batch number: 1404193) is acquired form Xi'an Libang Pharmaceutical Co. Ltd. Chromatographically pure methanol and acetonitrile are purchased from Merck, Germany; methanoic acid is chromatographically pure (Fluka, Germany).

4.2. Animals

Ba-ma mini-pigs (15 male and 15 female) with weight of 5-8 kg are bought from Shanghai Jiagan Biotechnology Co., Ltd. with the production license number of SCXK (Hu) 2015-0005. The pigs were raised in common mini-pig hoggery (room temperature of 23+2°C and humidity of 60-70%). Artificial lighting with diurnal variations and free access to water and food were also provided.

4.3. Instruments

Waters ultra-high performance liquid chromatography-tandem mass spectrum coupled system (LC-MS/MS, including online degasser, ultrahigh pressure gradient pump, column oven, automatic sampler, Waters I-CLASS UPLC liquid phase system, Xevo TQ-S mass spectrum, UNIFI workstation); Ultimate 3000 liquid chromatography system (HPLC, including QuatPump, automatic sampler, DAD detector, ChromeLeon workstation, DIONEX Limited); mechanical hyperalgesia stimulator , Electronic von Frey Anesthesiometer, manufacturer: IITC life science; Eppendorf 5810 R full-automatic high-speed refrigerated centrifuger; Eppendorf MixMate vortex; METTLER TOLEDO XP105DR electronic balance; HF Super NW30VF ultrapure water system, Scient refiner.

4.4. Chromatography Conditions

Chromatographic condition for ropivacaine concentration determination: Waters ACQUITY UPLC BEH C18 column (50×2.1 mm, grain size 1.7 μm); column temperature was set at 30°C; mobile phase was distilled water with 0.1% formic acid: methanol =45:55; the flow rate was 0.4 mL/min; the injection volume was 1 μL.

4.5. Mass Spectrum Condition

Ion source was electrospray ionization source (ESI), detection with positive ion mode was implemented, capillary voltage was 0.5 kV, ion source temperature was 120°C, atomized gas temperature was 350°C, cone gas flow was 150 L/h, atomized gas flow velocity was 650 L/h, detection mode was multi-ion reaction detection (MRM), bupivacaine was the internal standard, charge-to-mass ratio of ropivacaine and bupivacaine was respectively: 275.12 and 289.1, bore-hole voltage was 5v, and collision pressure was respectively 20 and 34.

4.6. Phenylcarbinol Detection Chromatographic Condition

Kromasil® C18 chromatographic column (250×4 mm,grain size 4.6 μm); column temperature was set as 30°C; detection wavelength was 204 nm; The aqueous phase of the mobile phase was water, and the organic phase was acetonitrile (gradient elution, please see **Table 1**); flow rate was 1.0 mL/min; and the injection volume was 50 μL.

Table 1: Benzyl alcohol liquid phase gradient method.

Time (min)	Flow Rate (mL/min)	Acetonitrile (%)	Water (%)	Curve
0	1	15	85	Initial
1	1	15	85	5
9	1	80	20	5
13	1	80	20	5
13.2	1	15	85	5
15	1	15	85	5

4.7. Biological Sample Treatment Method

4.7.1. Plasma Sample Treatment

20 μL plasma was placed into a 1.5 mL centrifuge tube. Next, 80 μL bupivacaine hydrochloride (internal standard, 2 ng/mL) methanol solution was added , and the sample was centrifuged at 12000 rpm for 5 min after being vortexed for 30 sec, 20 μL supernatant was placed into another 1.5 mL centrifuge tube, 180 μL water-methanol solution (water : methanol = 45:55) was added for dilution. The diluted supernatant was then injected into LC-MS/MS for analysis.

4.7.2. Myocardial Tissue Treatment

One hundred mg right atrium and right ventricle of pig was collected and placed into a 2 mL centrifuge tube. Next, 10 μL bupivacaine hydrochloride (40 ng/ml, internal standard) methanol solution and 400 μL methanol solution, and small magnetic bead were added and the sample was homogenized for 3 times (60 Hz for 30 sec per time). Later, the sample was centrifuged at 12000 rpm for 5 min, and the supernatant was collected and filtrated through a 0.2 μM microfiltration membrane before test.

4.7.3. Skin Tissue Treatment for Ropivacaine Concentration Determination

The pig skin of wound site was cut into pieces and 100 mg was collected and placed into a 2 mL centrifuge tube Next, 10 μL bupivacaine hydrochloride methanol solution (40 ng/ml, internal standard) and 400 μL methanol solution, a small magnetic bead was added and the sample was homogenized for 3 times (60 Hz for 30 sec per time). The homogenization was centrifuged at 12000 rpm for 5 min, and the supernatant was collected and filtrated through a 0.2 μM microfiltration membrane before test.

4.7.4. Skin Tissue Treatment for Benzyle Alcohol Determination

The pig skin of wound site was cut into pieces and 100 mg was collected and placed into a 2 mL centrifuge tube. Next, 400 μL methanol solution, a small magnetic bead was added and the sample was homogenized for 3 times (60 Hz for 30 sec per time). The homogenization was centrifuged at 12000 rpm for 5 min, and the supernatant was collected and filtrated through a 0.2 μM

microfiltration membrane before test.

4.8. Verification of Ropivacaine Concentration Determination Method in Pig Blood

4.8.1. Specificity

Three different sources of plasma was respectively added into ropivacaine working solution and bupivacaine internal standard, blank plasma (control). The plasma was treated with the above mentioned method and then injected into LC-MS/MS for analysis. The retention time of different determination materials is respectively shown as follows: ropivacaine 0.67 min, internal standard bupivacaine 0.86 min (**Figure 1 and 2**), and the specificity is consistent with requirements.

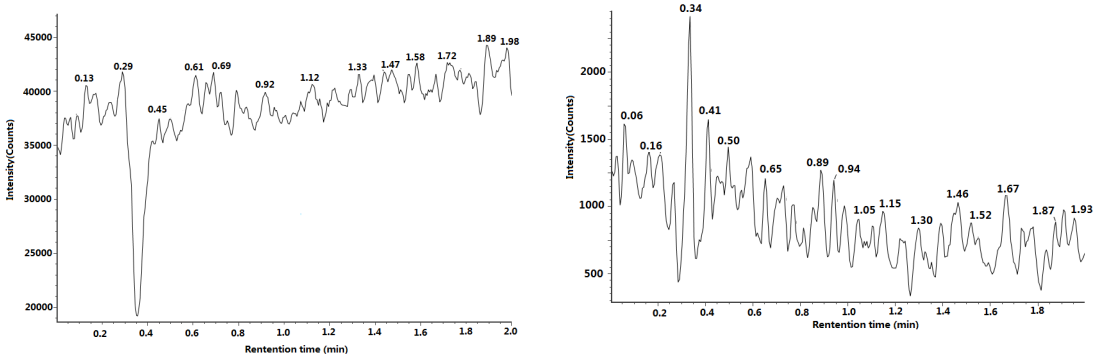

Figure 1: Typical specificity graph for the validation of ropivacaine determination in mini-pigs. Plasma levels were determined quantitatively by LC-MS/MS. blank sample atlas of ropivacaine (left) and bupivacaine (right).

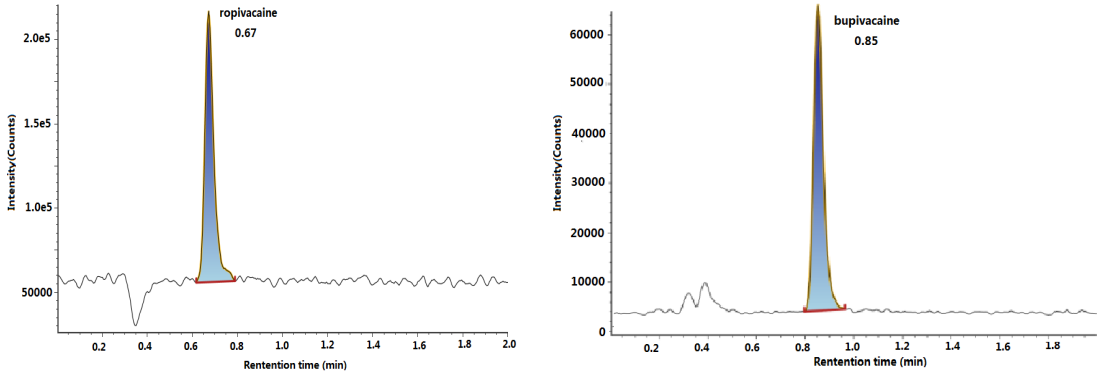

Figure 2: Typical specificity graph for the validation of ropivacaine determination in mini-pigs. Plasma levels were determined quantitatively by LC-MS/MS. ropivacaine (left) and bupivacaine (right) quality control samples.

4.8.2. Standard Curve

90 μL blank plasmas was added into 10 μL ropivacaine solution with concentration of 0, 10, 50, 200, 500, 1000, 2000, 3000 and 5000 ng/ml respectively, which make the plasma drug concentration to be 1, 5, 20, 50, 100, 200, 300 and 500 ng/ml respectively. The sample was treated according to the above mentioned method before being injected into LC-MS/MS. standard curve is shown as follows: Y=1.26X+9.87, r=0.99, RSD=5.40%.

4.8.3. Precision and Accuracy

Five ropivacaine plasma samples of 1, 2, 150 and 375 ng/ml were prepared respectively and were treated according to the above mentioned method before analysis. Then 3 analysis batches were determined continuously, the within-run precision of the method is respectively 6.73%, 6.16%, 1.99% and 2.25%, the accuracy is 99.5%-109.5%, batch-to-batch precision is respectively 7.07%, 4.58%, 3.09% and 4.14%, and the accuracy is 102.0%-110.45%.

4.8.4. Recovery Rate

Five ropivacaine plasmas samples of 2 and 375 ng/ml were prepared respectively and were treated according to the above mentioned method to make the quality control sample. 800 μL methanol was added into 200 μL blank pig plasma, the sample was centrifuged at 12000 rpm for 5 min after being vortexed for 30 sec. Next, 49 μL supernatant was transferred into a 0.5 mL centrifuge tube, 1 μL mixed working solution (ropivacaine 20 ng/ml, bupivacaine 80 ng/ml or ropivacaine 3750 ng/ml, bupivacaine 80 ng/ml) were added to make the concentration of ropivacaine and bupivacaine consistent with the theoretical concentration in quality control samples. For each concentration, 5 samples were prepared, wherein extraction recovery rate is respectively 102.0±4.6% and 100.0±7.1%.

4.8.5. Matrix Effect

Two-hundred micro liter water and 800 μL methanol were added into a 1.5 mL centrifuge tube, and were vortexed for 30 sec. 49 μL of the vortexed solution was transferred into a 0.5 mL centrifuge tube, in which 1 μL mixed working solution (ropivacaine 20 ng/ml, bupivacaine 80 ng/ml or ropivacaine 3750 ng/ml, bupivacaine 80 ng/ml) were also added to make the concentration of ropivacaine and bupivacaine consistent with the theoretical concentration in quality control samples. Five samples per concentration were prepared, wherein the matrix effect is respectively 99.0±3.1% and 98.2±2.9%.

4.8.6. Dilution Effect

Plasma samples with ropivacaine concentration of 1 μg/mL were prepared. 10 μL of the blood sample were diluted with 90 μL blank plasma, and 5 diluted samples were prepared for analysis after sample treatment, wherein the diluted accuracy respectively is 90.78±7.28%.

4.8.7. Stability of the Plasma Sample

Six groups of ropivacaine (2 ng/ml and 375 ng/ml) in pig plasma samples in pig plasma were prepared (for each group, 5 samples per concentration is needed) for stability test. The results showed that the concentration is respectively 2.05±0.20 ng/ml and 359.5±9.5 ng/ml after samples are repeatedly frozen thawed at -80 for 3 times, the concentration is 2.01±0.08 ng/ml and 345.4±8.6 ng/ml after storage at room temperature for 4 h, the concentration is 1.89±0.08 ng/ml and 351.7±9.0 ng/ml after cold storage at -80 for 5 days, the concentration is 2.05±0.10 ng/ml and 355.6±14.0 ng/ml after cold storage at -80 for 2 weeks, obtain supernatant after sample treatment, store it at room temperature for 4 h, the concentration is respectively 1.79±0.07 ng/ml and 339.6±18.8 ng/ml, the concentration is respectively 2.25±0.07 ng/ml and 389.3±10.4 ng/ml after storage for 48 h in the sampler, and analysis shows that ropivacaine has excellent stability in plasma, the method is shown as follows: feed blank pig whole blood 90 μL, respectively add ropivacaine working solution10 μL with concentration of 20 ng/ml and 1500 ng/ml, shake it evenly, achieve whole blood ropivacaine concentration of 2 and 150 ng/ml respectively, immediately treat the sample, the concentration is respectively 2.55±0.26 ng/ml and 184.1±6.3 ng/ml, sample introduction analysis is implemented after storage for 4h, the concentration is respectively 2.60±0.14 ng/ml and 174.9±2.6 ng/ml, and analysis shows that ropivacaine has excellent stability in whole blood.

4.9. Verification of Ropivacaine Concentration Determination Method in Pig Myocardium

Endogenous substances do not interfere determination of ropivacaine and corresponding bupivacaine internal standard, the ropivacaine and internal standard bupivacaine resolution is consistent with requirements, and respectively implement weighted ($1/X^2$) linear regression on corresponding concentration (C, X) according to ropivacaine peak area and bupivacaine peak area ratio (As/AIS, Y) within the scope of 1-500 ng/g, wherein standard curve is Y=1.87X+3.06, r=0.99, within-run precision of ropivacaine quality control samples with concentration of 1, 2, 150 and 375 ng/ml is respectively 11.03%, 4.37%, 2.57% and 3.52%, the accuracy is 91.33%-102.93%, 2 and 375 ng/g ropivacaine pig myocardium quality control sample is stored at room temperature for 4 h after homogenation, it is centrifuged for obtaining supernatant and introducing sample through membrane, the testing concentration is respectively 1.94±0.09and 369.9±6.9 ng/g, and the stability is in line with the requirements.

4.10. Verification of Ropivacaine Concentration Determination Method in Pig Skin

Endogenous substances in pig skin do not interfere determination of ropivacaine and corresponding internal standard, the retention time of different determination materials is respectively shown as follows: ropivacaine: 0.65 min, internal standard bupivacaine: 0.83 min, the specificity is excellent, sample blank pig skin, add ropivacaine working solution with different concentrations, shake it evenly for achieving ropivacaine concentration in pig skin equivalent to 1, 5, 20, 50, 100, 200, 300 and 500 ng/g, test sample after treatment, respectively implement weighted ($1/X^2$) linear regression to corresponding concentrations (C, X) according to ropivacaine peak area and bupivacaine peak area ratio (As/AIS, Y), and conclude that the standard curve of ropivacaine in pig skin is Y=1.8X+2.09, R=0.99, RSD=5.75%. The within-run precision of ropivacaine quality control samples with concentration of 1, 2, 150 and 375 ng/ml is respectively 5.90%, 2.48%, 3.65% and 2.55%, the accuracy is 94.70%-106.19%, 2 and 375 ng/g ropivacaine pig myocardium quality control sample is homogenized and stored at room temperature for 4 h, it is tested then, the concentration is respectively 1.90±0.04 ng/g and 397.8±1.66 ng/g, and the stability is in line with the requirements.

4.11. Verification of Benzyl Alcohol Determination Method in Pig Skin

Blank pig skin and pig skin quality control samples as well as pig skin of drug administration site were treated with above mentioned method and the benzyl alcohol concentration were determined by a HPLC. The results showed that endogenous substances in pig skin do not interfere phenylcarbinol determination, phenylcarbinol retention time is 8.01 min (**Figure 3**), and the specificity is excellent.

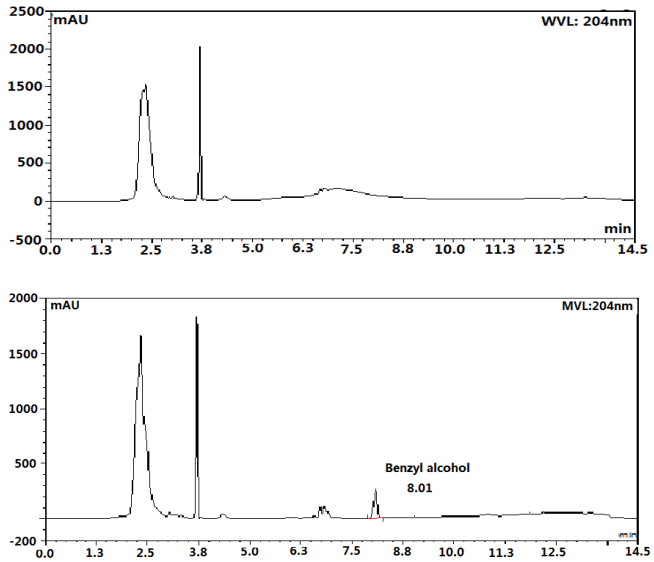

Figure 3: Chromatogram of benzyl alcohol in blank porcine skin and samples, blank sample atlas(up) and benzyl alcohol (down).

Prepare blank pig skin sample, add phenylcarbinol control solution with different concentrations, make phenylcarbinol concentration in pig skin equivalent to 2, 5, 10, 50, 100 and 200 μg/g, treat biological sample for analysis, and test quantitatively with peak area external standard method, implement weighted ($1/X^2$) linear regression on corresponding concentration (C, X) according to phenylcarbinol peak area, thereby obtaining the standard curve Y=0.5X+0.1, R=0.99 of phenylcarbinol in pig skin sample.

Prepare respectively five phenylcarbinol pig skin samples with concentration of 4, 80 and 160 μg/g, implement sample pretreatment and analyze it, and determine 3 batches continuously, wherein within-run precision is respectively 6.03%, 1.97% and 1.40%, the accuracy is 100.4-114.6%, batch-to-batch precision is respectively 7.30%, 6.10% and 6.20%, and the accuracy is 99.5-110.2%.

Prepare phenylcarbinol pig skin sample with concentration of 4 and 160 μg/g, store sample for 6 h after homogenization, determine the concentration as 4.379±0.33 and 165.2±3.24 μg/g, store the sample for 6 days at 80 after homogenization, determine the concentration as 4.06±0.16 and 149.3±1.46 μg/g, store the sample at - 80 for 9 days after homogenization, determine the concentration as 4.35±0.03 and 156.9±0.92 μg/g, obtain supernatant after sample treatment, place it at room temperature for 8h, and determine the concentration as 4.55±0.02 and 164.7±2.50 μg/g after treatment, wherein the stability is in line with the requirements.

4.12. Establishment of Incisional Pain Model in Mini Pig and Determination of Mechanical Hyperalgesia Domain

Seven mg/kg propofol was injected to the right hind of mini-pig for anesthesia induction, anesthesia quantity was kept at 3.5mg/kg. The experimental animal was in the anesthesia condition during the operation. Next, the scalp needle was used to fixed the mini-pig on the thermostatic (32°C) Table with supine position. Incisional pain surgery and external jugular vein intubation surgery were carried out. Skin was cut at the direction parallel to the trachea and 4cm away from the left trachea, the external jugular vein was exposed by blunt separation, and 1mm caliber silicone tube was inserted, fixed and ligated. After the hair in the back neck skin was removed and the skin was disinfected, one incision was made to lead the silicone tube to the back for blood sample collection. Next, normal saline was used for rinse the tube and heparin normal saline (5 mg/ml, 0.2 ml) was further used for washing and closing. After disinfection in pig left hind, 3cm incision was made at left hind shank front area (2 cm away from the bottom), skin and fascia were separated, fascia was cut for six times with scalpel, and the incision was sutured after stanching bleeding.

Mini-pigs were divided into four groups, for ropivacaine oil solution group, 3.5 mg/kg, 0.94 ml was administered; for ropivacaine hydrochloride injection group, 2.3mg/kg, 1.4ml was administered, for the model group, 1.4 ml normal saline was given; for the solvent control group, 0.94 ml solvent was given. For all groups, the drug was injected into the subcutaneous skin, which was 0.5cm away from the incision site.

Pig was fixed on a frame and the experiment was started after it became quiet. The von Frey probe was used for stimulating the skin 1 cm around the incision site, and mechanical hyperalgesia determination was carried out at four positions around the incision site (**Figure 4**), each point was measured twice, the average value was regarded as the threshold of the animal mechanical hyperalgesia. The maximum stimulation pressure was 200 g. If the animal did not shrink legs, the pain threshold was 200 g. Determination time was before drug administration, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h and 24 h after drug administration.

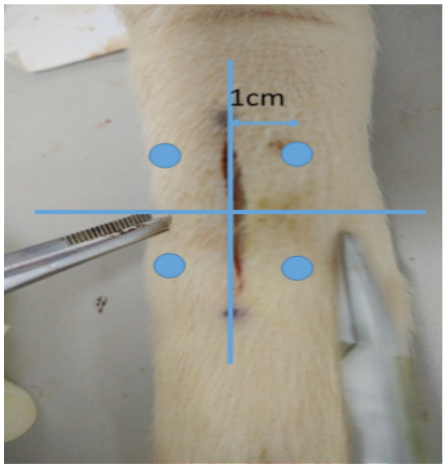

Figure 4: Four irritants for the determination of mechanical pain in mini-pigs.

4.13. Biological Sample Collection and Treatment

To collect the blood sample, external jugular vein cannulation was performed with heparin as the anti-coagulation agent. The blood was collected before the drug administration, 2 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h and 24 h after drug administration. Next, the blood samples were centrifuged at 4000 rpm for 5 min, and plasma was collected and stored at -80°C.

The pig heart and wound skin were collected 24 h after drug administration. The heart and skin were stored at -80°C after being cleaned and dehydrated with filter paper. Similarly, the drug injection site of the pig skin were collected 24 h after drug administration, and the skin were sheared immediately. Next, 100 mg sample was placed in a 2 mL centrifuge tube, in which 400 μL methanol and a small magnetic bead was added. The mixture were then homogenized for 3 times (60 Hz, 30 sec/time). The homogenization was stored at -80°C in sealed condition for phenylcarbinol residue determination.

5. Data Analysis

Graphpad Prism 5.0 software was used for statistical analysis. All data was expressed as mean ± SD, one-way analysis of variance was used in the statistical test, wherein $P<0.05$ is referred to statistical significant.

6. Results

6.1. Analgesic Effect of Ropivacaine Oil Solution to Mini-pig Model of Incisional Pain

As shown in Figure 5, there is no significant difference among pigs in four groups regarding the post-operative mechanical hyperalgesia thresholds at time 0 and the mechanical hyperalgesia thresholds is between 10 and 20 g. Compared with the model group, at t=5 min, the mechanical hyperalgesia thresholds in solvent control group is prominently higher ($P<0.05$). However, for other time points, there is no mechanical hyperalgesia thresholds difference between the two groups. For ropivacaine oil solution (3.5 mg/kg), the analgesia onset time is 5 min after administration, the mechanical hyperalgesia threshold is 200 g, analgesic duration is 2 h, its significance was higher than pain threshold maintenance time of 14 h; While for ropivacaine hydrochloride injection (2.3 mg/kg), the analgesia onset time is 5 min, and the analgesic duration is 4 h.

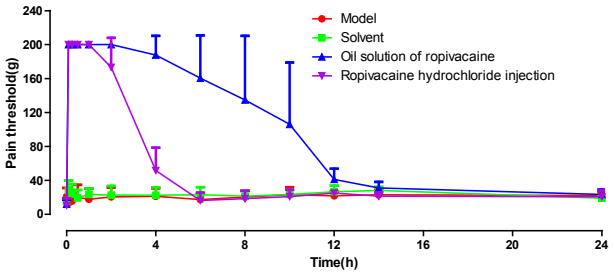

Figure 5: Oil solution of ropivacaine for local anesthetic analgesia in mini-pigs with incision pain ,Mini-pigs were subcutaneously injected with ropivacaine oil solution (3.5mg/kg) or with ropivacaine hydrochloride (2.3 mg/kg), n=6.

6.2. Pharmacokinetics of Ropivacaine Oil Solution

The plasma concentration of ropivacaine at different time after the administration of ropivacaine oil solution (3.5 mg/kg) is shown in **Figure 6**. The T_{max} is between 0.5-1 hour, The C_{max} is over 1400 ng/ml, and plasma concentration of ropivacaine could be kept over 40 ng/ml for as long as 14 h.

The plasma concentration of ropivacaine at different times after the administration of ropivacaine hydrochloride injection (2.3 mg/kg) is shown in **Figure 7**. The T_{max} is 0.083 h, and the C_{max} is between 4000-5000 ng/ml. Next, the ropivacaine of plasma is quickly eliminated and the blood concentration is around 50 ng/ml at 8 h after administration.

As shown in **Figure 8**, compared with ropivacaine hydrochloride injection, ropivacaine oil solution, has a longer and slower absorption phase, longer elimination phase and therefore, we can conclude that the ropivacaine in the oil solution is slowly released form the formulation.

The pharmacokinetics parameters in the ropivacaine oil solution group and ropivacaine hydrochloride injection group are shown in **Table 2 and 3** respectively. Compared with ropivacaine hydrochloride injection group, T_{max} and $MRT_{0-\infty}$ of ropivacaine oil solution group was significantly prolonged, and the C_{max} was significantly reduced.

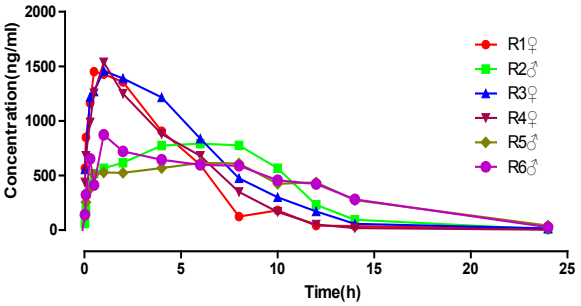

Figure 6: Plasma concentration-time curve of ropivacaine oil solution of 3.5mg/kgafter subcutaneous injection , n=6.

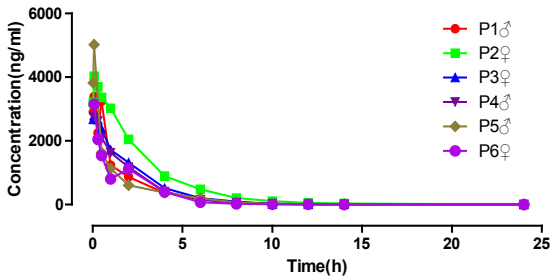

Figure 7: Plasma concentration-time curve of ropivacaine hydrochloride injection of 2.3 mg/kg after subcutaneous injection in mini-pigs, n=6.

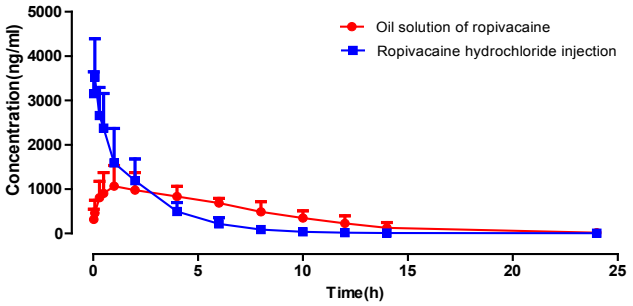

Figure 8: Plasma ropivacaine concentration-time curve in mini-pigs plotted as a function of time. Mini-pigs were subcutaneously injected with ropivacaine oil solution (3.5mg/kg)or with ropivacaine hydrochloride (2.3 mg/kg), n=6.

Table 2: Pharmacokinetic parameters of ropivacaine oil solution in mini-pigs

Sex	AUC_{0-t}	$AUC_{0-\infty}$	$MRT_{0-\infty}$	C_{max}	T_{max}	$t_{1/2}$	CL	V_z
Number	ng·h/mL	ng·h/mL	h	ng/ml	h	h	L/h/kg	L/kg
R1♀	7964	8016	4	1451	0.5	4.7	0.44	2.97
R2♂	8552	8554	6.6	793	6	1.6	0.41	0.92
R3♀	10434	10506	4.9	1459	1	3.3	0.33	1.6
R4♀	8177	8177	4	1537	1	1.3	0.43	0.78
R5♂	8648	8855	8.7	616	6	3.5	0.4	2.02
R6♂	9315	9429	7.9	874	1	3	0.37	1.59
Mean	8848	8923	6	1122	2.6	2.9	0.4	1.65
Standard deviation	905	925	2	405	2.7	1.3	0.04	0.8

Table 3: Pharmacokinetic parameters of ropivacaine hydrochloride injection in mini-pigs.

Sex	AUC_{0-t}	$AUC_{0-\infty}$	$MRT_{0-\infty}$	C_{max}	T_{max}	$t_{1/2}$	CL	V_z
Number	ng·h/mL	ng·h/mL	h	ng/ml	h	H	L/h/kg	L/kg
P1♂	5756	5756	2.1	3392	0.083	1.7	0.4	0.98
P2♀	11657	11674	2.8	4028	0.083	3.2	0.2	0.9
P3♀	7094	7095	2.5	2793	0.083	12	0.32	0.93
P4♂	6062	6062	2.2	2753	0.083	1.6	0.38	0.9
P5♂	5249	5251	2.2	5021	0.083	2.7	0.44	1.71
P6♀	4741	4749	2.1	3155	0.083	4.7	0.48	3.29
Mean	6760	6765	2.3	3524	0.083	4.3	0.37	1.45
Standard deviation	2528	2533	0.3	869	0	3.9	0.1	0.96

Table 4: Ropivacaine concentration in myocardial tissue of mini-pigs (ng/g).

oily solution of ropivacaine	right atrium	ventriculus dexter	ropivacaine hydrochloride injection	right atrium	ventriculus dexter
R1♀	1.186	1.189	P1♂	-	-
R2♂	1.119	0.823	P2♀	-	2.209
R3♀	3.393	18.26	P3♀	2.123	1.316
R4♀	-	-	P4♂	-	-
R5♂	9.844	9.596	P5♂	3.696	24.12
R6♂	5.742	5.534	P6♀	-	-

Note: "-" indicates that the concentration is below the lower limit of quantification.

6.3. Ropivacaine Concentration in Pig Myocardial Tissue After Local Administration

The drug concentrations in pig myocardial tissue at 24 h after administration is shown in **Table 4** and there is no significant difference between the ropivacaine oil solution and ropivacaine hydrochloride injection group.

6.4. Ropivacaine Concentration in Pig Wound Site after Drug Administration

The drug concentration at wound site are shown in **Table 5**. The drug concentration in pig wound site of ropivacaine oil solution group is between 292~2700 ng/g, and drug concentration ofropivacaine hydrochloride injection group is between 915~6718 ng/g.

6.5. Benzyl Alcohol Residue in Skin at the Injection Site after Drug Administration

The benzyl alcohol residue in skin at the injection point was sampled 24 h after drug administration and the results are shown in **Table 6**. For roivacaine oil solution group, the benzyl alcohol residue is 4.28~140.9 μg/g, and for solvent group, the residue is 6.77~117.0 μg/g.

Table 5: Ropivacaine concentration in wound skin of pigs (ng/g).

oil solution of ropivacaine	concentration	ropivacaine hydrochloride injection	concentration
R1♀	1836	P1♂	914.8
R2♂	1662	P2♀	3249
R3♀	2510	P3♀	5916
R4♀	731.9	P4♂	2018
R5♂	2700	P5♂	6718
R6♂	291.9	P6♀	3412

Table 6: Residual concentration of benzyl alcohol in pigs skin (μg/g).

oil solution of ropivacaine	concentration	Solvent	concentration
R1♀	10.48	V1♂	117
R2♂	50.16	V2♀	17.64
R3♀	140.9	V3♂	11.1
R4♀	4.28	V4♀	28.7
R5♂	5.616	V5♀	10.08
R6♂	5.37	V6♂	6.767

17. Discussion

The structure of ropivacaine has been optimized based on the structure of bupivacaine and mepivacaine, and its cardiovascular toxicity and neurotoxicity is significantly reduced. However, there were still some adverse effects in clinic, when high dose ropivacaine was administered. J. D. Griffiths [7], etc. reported that among 30 patients received 2.5 mg/kg ropivacaine solution in abdominal muscles after gynecological surgery, 3 patients showed toxic symptoms in nervous system, whose and their blood concentration was about 2.70 ug/ml. Achir A. Al Alami reported an adhd patient became confused with slower speaking rate, quivering muscle, quicker breathing rate and faster heart rate 10 minutes after receiving 400 ng ropivacaine subcutaneous injection to the patient [8]. Currently, the risk of adverse events is 8 per 100,000 patients. Therefore, the adverse effects of ropivacaine is also examined in our study.

The concentration of ropivacine in blood, myocardial tissue and administration site was examined by an LC-MS/MS Bupivacaine is the internal standard whose LLOD is 1 ng/ml. Besides, its recovery rate, precision, accuracy and stability under different storage conditions are consistent with the requirements. Meanwhile, an external standard method was adopted for benzyl alcohol content determination. The specificity, accuracy, precision, and stability under different storage and determination conditions are consistent with requirements.

Currently, the dosage of ropivacaine hydrochloride injection is 100 mg/time in clinic, the dosage of ropivacaine oil solution is proposed to be 150 mg/time. The corresponding dosage in pig is respectively 2.3 mg/kg and 3.5 mg/kg respectively. The pharmacokinetic study showed that the mean residence time of ropivacaine oil solution is 3 times longer that of ropivacaine hydrochloride injection (6.0±2.0 h for ropivacaine oil solution *VS* 2.3±0.3 h for ropivacaine hydrochloride injection); the maximum drug concentration of ropivacaine oil solution is 1122±405 ng/ml, which is significantly lower ($P<0.05$) than that of ropivacaine hydrochloride injection (3524±869 ng/ml); the time of maximum concentration of ropivacaine oil solution is 2.6±2.7 h, which is significantly slower ($P<0.05$) than that of ropivacaine hydrochloride injection (5min). The pharmacodynamics study shows that the analgesic duration of ropivacaine oil solution is three times longer than that of ropivacaine hydrochloride injection, besides no adverse effects was observed. Therefore, the pharmacokinetics and analgesic effect of ropivacine oil solution are positively correlated. After being administered subcutaneously, ropivacaine oil solution slowly released, absorbed and eliminated, the residence time is prolonged, thereby the analgesic effect is also prolonged. Besides, the maxicum drug concentration (C_{max}) is significantly decreased, thereby leading to a lower systemic toxic and side effect.

Tiny residue of ropivacaine is observed in the injection site. 24 h after drug administration, the drug concentration in ropivacaine hydrochloride injection group is higher than that of oil solution group. This may be due to the the polarity difference in two groups. In ropivacaine hydrochloride injection group, the high polarity of ropivacaine hydrochloride leads to a slow penetration of drug in skin. However, for ropivacaine oil solution, the oily preparation leads to a fast penetration of drugs across the skin. 24 h after the administration, the ropivacaine concentration in pig myocardial tissue is 0.82~18.22 ng/g for ropivacaine oil solution which is slightly lower than that of ropivacaine hy-

drochloride injection group. Individual sample concentration is lower than quantitative lower limit, and the safety factor is relatively high.

Benzyl alcohol is generally used in clinic as a solvent, subcutaneous or intramuscular injection of benzyl alcohol has analgesic effect in some extent, however, there are some associated adverse reactions. 24 h after administration, there are some benzyl alcohol residue in the skin for ropivacaine oil solution and the solvent group. There are no obvious adverse reactions such as swelling, induration, canker, etc. In the administration site and no abnormal reactions of animals after being touched. Therefore, the benzyl alcohol concentration used in the formulation is within the safe dose range.

In summary, after being administered, the drug absorption phase, elimination phase and mean residence time are prolonged. The maximum drug concentration in the oil solution group is 1500ng/ml, which is significantly lower than that of ropivacaine hydrochloride injection group, which leads to a smaller systemic side effects. This pharmacokinetic behavior of ropivacaine oil solution is closely correlated to its pharmacodynamics behaviors, in which the formulation has a slower analgesic effect with longer analgesic duration when compared with ropivacaine hydrochloride injection.

8. Conclusion

Ropivacaine oil solution shows a slow release profile, long duration anesthetic effect and no obvious toxicity.

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