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Title Page

Concentration Dependent Reduction of Epidural Ropivacaine EC50 by Adjuvant Chlorprocaine for Labor Analgesia

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Abstract

Background: Chlorprocaine was popular in obstetric anesthesia and analgesia with its favorite characteristics as rapid onset and elimination, broad diffusion, and low toxicity. This study was to determine the effects of low-concentration of chlorprocaine on the median effective concentration (EC₅₀) and 90% effective concentration (EC₉₀) of epidural ropivacaine for maintenance of labor analgesia.

Methods: One hundred and twenty-three healthy, term primiparous women requesting epidural labor analgesia were randomly assigned into the RP group (Ropivacaine with 0.0% chlorprocaine), LP0.2 (Ropivacaine with 0.2% chlorprocaine) or LP0.3 (Ropivacaine with 0.3% chlorprocaine) group. Effective analgesia was defined as the VAS scores ≤ 3 within 60 minutes after loading dose. The concentration of ropivacaine for the first patient was 0.09%. Subsequent patients in each randomization group received a new concentration of ropivacaine as determined by the up-down allocation methodology with a 0.01% gradient. Probit regression was used to calculate the EC₅₀,

EC90 and 95% confidence interval (CI) of ropivacaine.

Results: Ninety-four patients completed this study. The EC50 values for epidural ropivacaine in combination with 0.0%, 0.2% or 0.3% chloroprocaine were 0.080%, 0.064% and 0.039%; the EC90 of which were 0.094%, 0.079% and 0.060%, respectively. Differences in EC50 values for ropivacaine were statistically significant as compared to the RP group, rather than EC90 values. This study preliminarily found that patients in the LP0.2 and LP0.3 groups achieved a lower onset time of analgesia, as well as a lower incidence of breakthrough pain, perineal distension and pain. The number of clinician-rescued bolus was significantly lower and maternal satisfaction with overall analgesia were significantly higher in the LP0.2 group than those in the LP0.3 group.

Conclusion: 0.2% and 0.3% chloroprocaine reduced the EC50 of ropivacaine by 21% and 50% during maintenance for labor analgesia.

Trial registration: This study was registered at the Chinese Clinical Trial Registry (<http://www.chictr.org.cn>, ChiCTR2300074933; Registration Date: August 21, 2023).

Keywords: epidural labor analgesia; chloroprocaine; ropivacaine; EC50; EC90

1 Introduction

Epidural labor analgesia is widely recognized as the gold standard for labor pain relief and is recommended by the World Health Organization (WHO) due to its excellent effectiveness and low potential for adverse effects^[1]. Current guidelines recommend

low concentrations of local anesthetics with sensory and motor separation (ropivacaine, bupivacaine) combined with minimal dosages of opioids (sufentanil, etc.) administered into the epidural space by an epidural catheter in a programmed interval infusion mode^[2]. This combination achieves effective and safe labor pain relief but has drawbacks such as a slow onset (15-20 minutes) and insufficient caudal spread^[3]. Clinically, parturients often complain about the slow onset and unbearable distension pain in the perineum and anus, with a significant number (30-80%) experiencing breakthrough pain^[4].

During the initial stages of labor, the stimulation of nociceptive afferent fibers by uterine contractions and cervical dilatation results in the generation of poorly localized visceral pain signals traveling through spinal nerves T10-L1. Additionally, as the fetal head descends and exerts pressure on the perineum and vagina, pain fibers are activated via the pudendal nerve and spinal roots S2-S4, leading to distension pain in the perineum and anus^[2]. Ropivacaine administered at the L2-3 epidural space can effectively block transmission from T10 to L1, but its diffusion to S2-S4 is less favorable^[5]. Chloroprocaine hydrochloride, a benzoyl ester local anesthetic, is considered safe for anesthesia and analgesia in pregnant women^[6-7] and infants^[8-9] due to its rapid onset, broad diffusion, and low toxicity. Previous studies have demonstrated that chloroprocaine effectively shortens onset time and manages breakthrough pain during labor^[4,10]. However, there is a lack of sufficient data on the use of chloroprocaine for maintaining labor analgesia.

Sultan et al. demonstrated that low concentrations of local anesthetics are preferable to higher concentrations, as they result in reduced motor blockade, a shorter second stage of labor, and a lower incidence of assisted vaginal delivery^[11]. Previous research reported the efficacy of 0.26% chloroprocaine in epidural labor analgesia with adjuncts of 3 µg/mL fentanyl^[12]. Coffman et al. also successfully used 0.3% chloroprocaine to initiate and maintain labor analgesia, using one-tenth the concentration typically required for surgical anesthesia^[13]. However, data on the combination of low-concentration chloroprocaine with ropivacaine is limited.

The objective of this study was to identify the optimal compatibility of epidural ropivacaine and chloroprocaine. Following the principle of avoiding motor block during the maintenance of labor analgesia, low concentrations of chloroprocaine (0.2% and 0.3%) were selected. The median effective concentration (EC₅₀) and 90% effective concentration (EC₉₀) of epidural ropivacaine in conjunction with various chloroprocaine concentrations for maintaining labor analgesia were determined using the up-down sequential allocation approach.

2 Methods

2.1 Ethical Approval

A double-blinded, sequential allocation study was conducted to investigate the EC₅₀ and EC₉₀ values of a combination of ropivacaine and varying concentrations of chloroprocaine for epidural labor analgesia. The study was approved by the Ethics

Committee of the Maternal and Child Medicine Center at Anhui Medical University (YYLL2023-07-18 for initial review and YYLL202401108-YJGZ-KSLL-01-1.0 for follow-up review), in accordance with the Declaration of Helsinki. The study was registered prior to subject enrollment at <http://www.chictr.org.cn> (ChiCTR2300074933) with a minor revision on grouping scheme and observation indicators. All participants were voluntary and signed informed consent.

2.2 Participants

Between August to December 2023, the study recruited healthy primiparous women in spontaneous labor seeking epidural analgesia at the Maternal and Child Medicine Center of Anhui Medical University. Inclusion criteria encompassed women aged 20 to 35, with American Society of Anesthesiologists (ASA) Physical Status I to II, BMI between 21 and 30 kg/m², gestational age of 37 to 41 weeks, all enter the latent period of the first stage of labor (cervical dilation <6 cm), and a visual analogue scale (VAS) pain score exceeding 5 during contractions (VAS scores: 0 = no pain, 10 = worst pain imaginable). Exclusion criteria comprised contraindications for intraspinal puncture, such as coagulation dysfunction, infection or injury at the puncture site, spinal lesions or severe spinal deformities, neurological diseases or neuropathies; allergy to local anaesthetics or opioids; multipara, abnormal fetal position, IVF; severe heart, lung, liver, kidney and other important system diseases; pathological obstetrics, such as pregnancy complications (severe preeclampsia, placental abruption, or placenta previa); those who cannot undergo vaginal delivery after obstetric evaluation; the inability of

pregnant women to cooperate due to combined mental illness or other conditions; used opioids, tranquilizers, or other analgesics 6 hours or recently before anesthesia.

Participants were discontinued from the study if they underwent cesarean section, labor induction, delivery within 2 hours of initial treatment, encountered issues with epidural catheter placement, or declined further participation.

2.3 Randomization and Blinding

A randomization scheme was devised by Dr. Jun-Ma YU, who was not engaged in data collection or patient care management. Allocation assignments were sealed in opaque envelopes, which the anesthesiologist opened upon the parturients' arrival in the analgesia room. The study solution was then prepared based on the numbers in the envelope. The assessment and documentation of outcomes were carried out by the anesthesia nurse. The participants (parturients) and outcome assessors (anesthesia nurses) were blinded to the details of group allocation.

The enrolled parturients were randomly allocated to one of three groups with different concentrations of chloroprocaine: Group RP (epidural administration 10 mL of ropivacaine with 0.0% chloroprocaine), Group LP0.2 (epidural administration 10 mL of ropivacaine with 0.2% chloroprocaine) or Group LP0.3 (epidural administration 10 mL of ropivacaine with 0.3% chloroprocaine).

The anesthetic agents employed included ropivacaine hydrochloride injection (10 mL: 100 mg; AstraZeneca plc, UK), chloroprocaine hydrochloride injection (10 mL: 300 mg, Jincheng Haisi Pharmaceutical, China), and sufentanil citrate injection (1 mL:

50 µg, Ren Fu Shu Fen, China).

2.4 Analgesia Procedure

Each participant signed informed consent before analgesia. Upon arrival in the analgesia room, the parturients had a peripheral venous catheter inserted and underwent standard physiological monitoring, which included heart rate, blood pressure, pulse oxygen saturation, uterine activity, and fetal heart rate assessments. The epidural puncture was performed at the L2-3 interspace using a 19-gauge Tuohy needle with the patient positioned in the left lateral decubitus. Subsequently, an epidural catheter was inserted cephalad into the epidural space, approximately 4 to 5 cm. Following verification of the negative aspiration test for blood and cerebrospinal fluid, a test dose of 1% lidocaine (3 mL) was administered in accordance with findings from our previous study. Observation for 3 minutes confirmed no vascular or subarachnoid response. A patient-controlled analgesia (PCEA) pump (ZZB-100, Jiangsu Aipeng Medical Devices Corp., China) in programmed intermittent epidural bolus (PIEB) mode was connected. The 10 mL study solution containing ropivacaine with 0.0% (Group RP), 0.2% (Group LP0.2), or 0.3% (Group LP0.3) chloroprocaine was administered as a bolus first.

2.5 Up-and-down sequential procedure

The EC50 and EC90 of ropivacaine were identified utilizing Dixon's up-and-down sequential allocation approach in each group. The initial concentration of ropivacaine for the first patient was 0.09%. Effective analgesia was defined as a VAS

score of ≤ 3 within 60 minutes after the loading dose. After receiving positive feedback on analgesia from one parturient, the concentration of ropivacaine was reduced by 0.01% for the next parturient. In contrast, if ineffective analgesia was reported, the ropivacaine concentration was increased by 0.01% for the next patient. The study necessitated the identification of eight crossover points involving either "positive versus negative response" or "negative versus positive response" to determine an adequate number of patients for the study.

2.6 Maintenance and rescue for analgesia

Forty-five minutes following the loading dose, the PCEA pump was initiated in PIEB mode to maintain labor analgesia. The set parameters comprised an 8 mL bolus infusion with a 45-minute interval. Parturients had access to PCEA infusion as needed, featuring an 8 mL dose per administration, a 30-minute lock time, and a maximum dose of 30 mL per hour. All analgesic solutions were routinely supplemented with 0.4 $\mu\text{g/mL}$ of sufentanil as an opioid adjuvant.

If a parturient reported effective analgesia, the same mixture as the study solution was used to maintain labor analgesia. If a parturient reported ineffective analgesia, a 6 mL bolus of 1.5% chloroprocaine was administered, and the solution for maintaining analgesia was changed to a standard formula (0.09% ropivacaine plus 0.4 $\mu\text{g/mL}$ sufentanil). If ineffective analgesia persisted, the anesthesiologist evaluated the position of the epidural catheter for issues such as a low puncture point or unilateral block. If a catheter misplacement was identified in a patient, their data were excluded

from the study analysis. Following this, the subsequent parturient was administered the same concentration of ropivacaine.

2.7 Outcome measures

The primary outcomes assessed in the study were the EC50 and EC90 values of ropivacaine across the three groups. Pain intensity was measured using VAS scores within 60 minutes after the administration of the loading dose, with scores ranging from 0 to 10 [14].

The secondary outcomes assessed the quality of epidural analgesia, which included: (1) the effect of analgesia: the onset of labor analgesia, defined as the first time to a VAS score ≤ 3 ; the rate of breakthrough pain, defined as a VAS score > 5 for which the parturient requested additional analgesia; the rate of perineal distension and pain, defined as a VAS score > 3 due to perineal distension and pain; maximum VAS score after analgesia; the mean VAS scores at the time points of before analgesia (baseline), 5, 15, 30, 45, 60, 90 and 120 minutes after epidural injection; times of rescue analgesia (anesthesiologists manually administer analgesics or PCEA); maternal satisfaction scores at 2 hours following delivery (assessed using a VRS from 0-10 scores: 0 very unsatisfied, 10 very satisfied); (2) the safety of analgesia: intensity of uterine contractions (IUCs) at baseline and at 5, 15, 30, 45, 60, 90, and 120 minutes after epidural injection; the thoracic sensory level to cold (tested with an ice bag) and motor blockade (tested using the Bromage scale) which were recorded 30 minutes after loading dosage; the adverse events related to epidural analgesia (shivering, nausea and

vomiting, pruritus, leg numbness, weakness in the legs, fever, fetal bradycardia within 60 min after analgesia); outcomes of delivery, mother and baby (mode of delivery, the duration of the first and second stages of labor, 1 and 5 minutes Apgar scores).

2.8 Statistical analysis

Based on Dixon's up-and-down sequential method protocol, it is recommended to accrue a sample size ranging from 20 to 40 cases to ensure a stable estimation of EC50, with a minimum of six reversals^[15-16]. We assumed that 34 study subjects would be needed in each group to get eight reversals of sequence. Accounting for a 20% dropout rate, 123 study subjects (41 patients in each group) were estimated to be needed to obtain eight pairs of reversals in the sequence.

The EC50, EC90, and 95% confidence intervals (CIs) of epidural ropivacaine were assessed utilizing probit regression based on the response categorizations of 'effective' and 'ineffective' utilizing Dixon's up-and-down allocation methodology. Differences in EC50 values were evaluated utilizing one-way ANOVA followed by the Bonferroni post hoc test. The differences in CIs for the EC50 and EC90 values among the three groups were tested using overlapping confidence interval methodology, with statistical significance determined by non-overlapping confidence intervals in at least 83% of cases^[17]. Concentration-response curves were plotted, and the area under the curve (AUC) calculation served as a sensitivity analysis.

The normality of quantitative data for the secondary outcomes was assessed utilizing the Kolmogorov-Smirnov test. Normally distributed data were reported as

means $\pm$ SD and analyzed utilizing one-way ANOVA followed by the Bonferroni post hoc test. Non-normally distributed data were shown as medians (Q1, Q3) and analyzed utilizing the nonparametric Kruskal-Wallis test. Categorical variables were expressed as numbers (percentages) and analyzed utilizing Pearson's chi-squared or Fisher's exact test. Multiple between-group comparisons were conducted using the α segmentation method, with significance at $P < 0.0125$.

A longitudinal study was conducted to analyze repeated measures data, including VAS pain scores and uterine contraction intensity, across different groups. A linear mixed model with restricted maximum likelihood method was utilized to account for patient-level clustering, incorporating a random intercept under an unstructured model^[18]. Adjusted Bonferroni post-tests were applied to multiple comparisons to assess group differences at each time point.

Statistical analysis was performed utilizing SPSS software (version 22.0, IBM Corp, USA) and GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was set at $P < 0.05$, and all analyses adhered to the intention-to-treat principle.

3 Results

3.1 Patient characteristics

A total of 123 parturients were initially screened for eligibility, of whom 111 were enrolled and randomized into three groups. Parturients were sequentially selected in each group until eight crossovers from effective to ineffective analgesia were achieved.

Of the 111 parturients enrolled, 17 patients were excluded for the following reasons: delivery within 2 hours after analgesia (12 patients), accidental dural puncture (1 patient), unsatisfactory block plane (1 patient), unilateral block (1 patient), and folding or blockage of the epidural catheter (2 patients). Ultimately, 94 patients (29 in the RP group, 31 in the LP0.2 group, and 34 in the LP0.3 group) were included in the final analysis. The CONSORT diagram outlining the study flow is depicted in Figure 1. Baseline parturient and obstetric characteristics were comparable among the three groups and are summarized in Table 1.

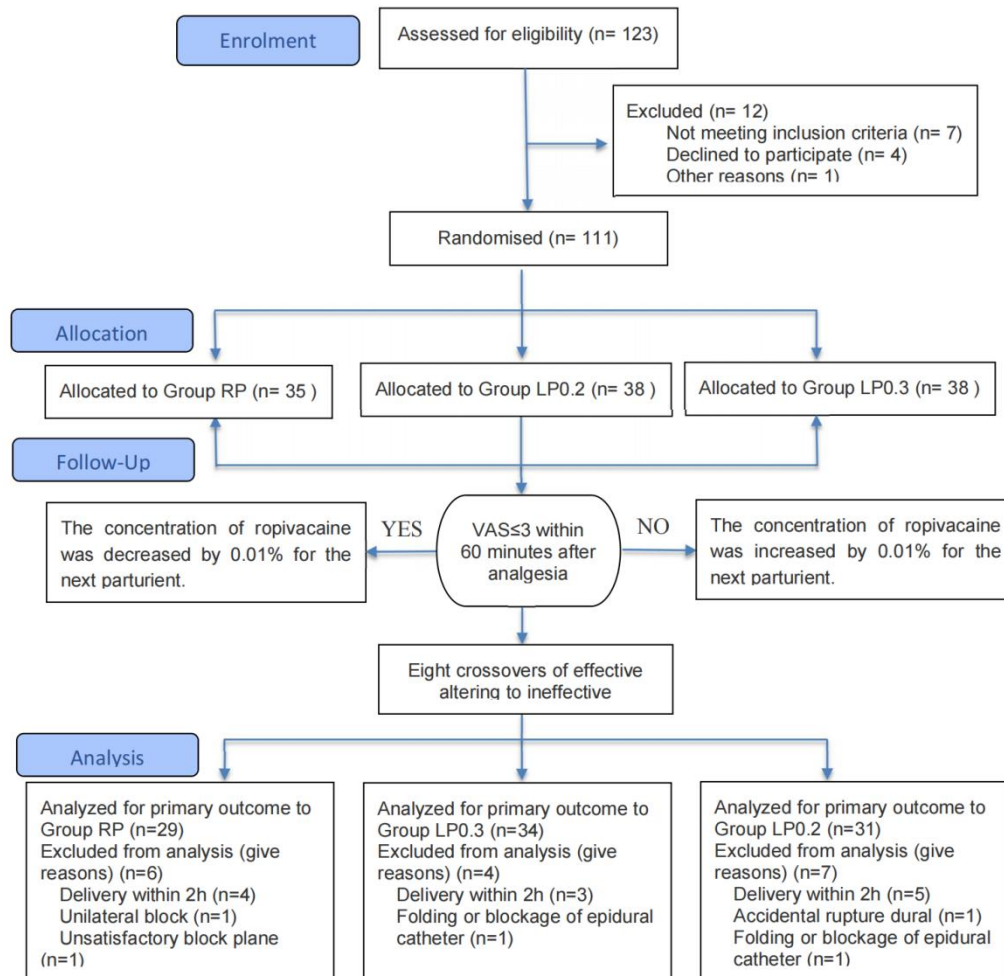


Figure 1 Flow diagram for the Dixon up-and-down method.

263 Abbreviations: RP, the group of ropivacaine with 0.0% chlorprocaine; LP0.2, the group of

Item	Group RP (n=29)	Group LP0.2 (n=31)	Group LP0.3 (n=34)	Statistical Value	<i>P</i> Value
Age, y	28.8±2.9	29.2±2.6	28.2±3.6	0.786	0.459
Height, cm	162.2±4.5	162.1±4.3	162.9±4.7	0.264	0.769
Weight, kg	70.1±9.1	70.4±6.0	68.3±6.1	0.845	0.433

264 ropivacaine with 0.2% chlorprocaine; LP0.3, the group of ropivacaine with 0.3% chlorprocaine.

265 **Table. 1 Demographic data of enrolled parturients in the three groups**

266 VAS, visual analogue scale score.

BMI , kg/m ²	26.6±2.7	26.8±2.3	25.8±2.3	1.703	0.188
ASA status, n (%)				1.620	0.474
I	16 (55.2)	20 (64.5)	24 (70.6)		
II	13 (44.8)	11 (35.5)	10 (29.4)		
Gestational week, W	40.1 (39.0-40.5)	39.7 (39.1-40.1)	39.7 (39.0-40.0)	1.099	0.577
Cervical dilatation, cm	2.0 (2.0-3.0)	3.0 (2.0-3.0)	2.0 (2.0-3.0)	1.812	0.404
Using oxytocin, n (%)	9 (31.0)	11 (35.5)	10 (29.4)	0.290	0.865
Baseline VAS score	10.0 (8.5-10.0)	10.0 (9.0-10.0)	10.0 (9.0-10.0)	0.035	0.982

Values are expressed as n (%), median (interquartile range), or mean (SD) as appropriate.

Abbreviations: ASA: American society of Anesthesiologists; VAS: visual analog scale; BMI: Body mass index.

3.2 Primary Outcome: the EC50 and EC90 values of ropivacaine

The results of up-down sequential allocation are presented in Figure 2. The EC50s (95% CIs) of ropivacaine, calculated utilizing probit regression, were 0.080% (95% CI, 0.072% ~ 0.088%), 0.064% (95% CI, 0.056% ~ 0.071%), and 0.039% (95% CI, 0.027% ~ 0.050%) in the RP, LP0.2, and LP0.3 groups, respectively. The EC90s (95% CIs) of ropivacaine were 0.094% (95% CI, 0.087% ~ 0.131%), 0.079% (95% CI, 0.072% ~ 0.110%), and 0.060% (95% CI, 0.050% ~ 0.115%) in the RP, LP0.2, and LP0.3 groups, respectively. Using the Bonferroni post-test and overlapping CIs methodology, the difference in EC50 values was statistically significant among the three groups ($P < 0.05$, Table 2 and Figure 3A). However, no significant variance was noted in EC90

280 values across the three groups ($P > 0.05$, Table 2).

281 The concentration-response curves, presented as a sensitivity test, are shown in Figure

282 3B. AUC of RP, LP0.2 and LP0.3 was 0.795 ($P = 0.007$, 95% CI: 0.632~0.959), 0.817

283 ($P = 0.003$, 95% CI: 0.664~0.971) and 0.791 ($P = 0.004$, 95% CI: 0.643~0.940),

284 respectively.

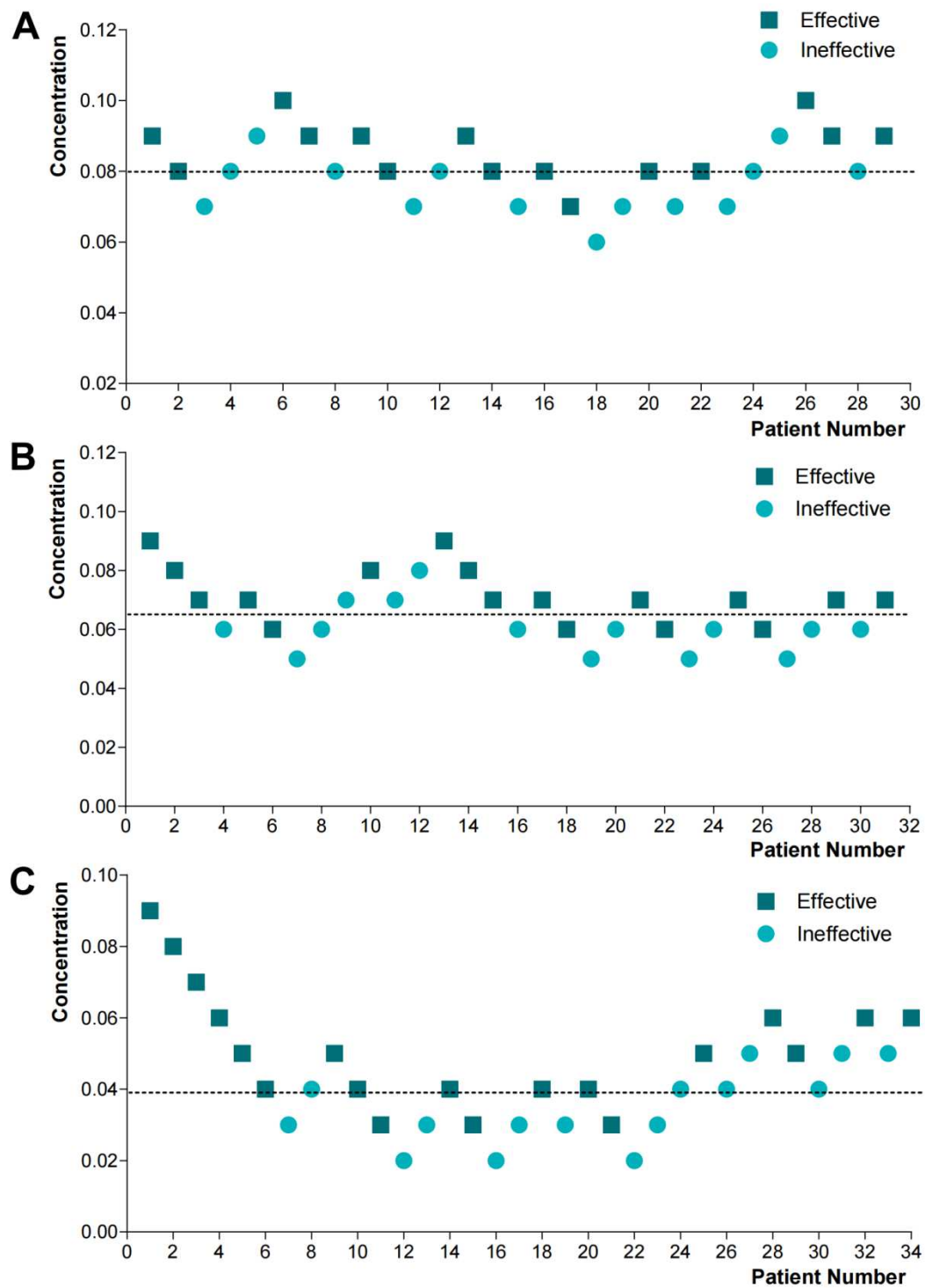
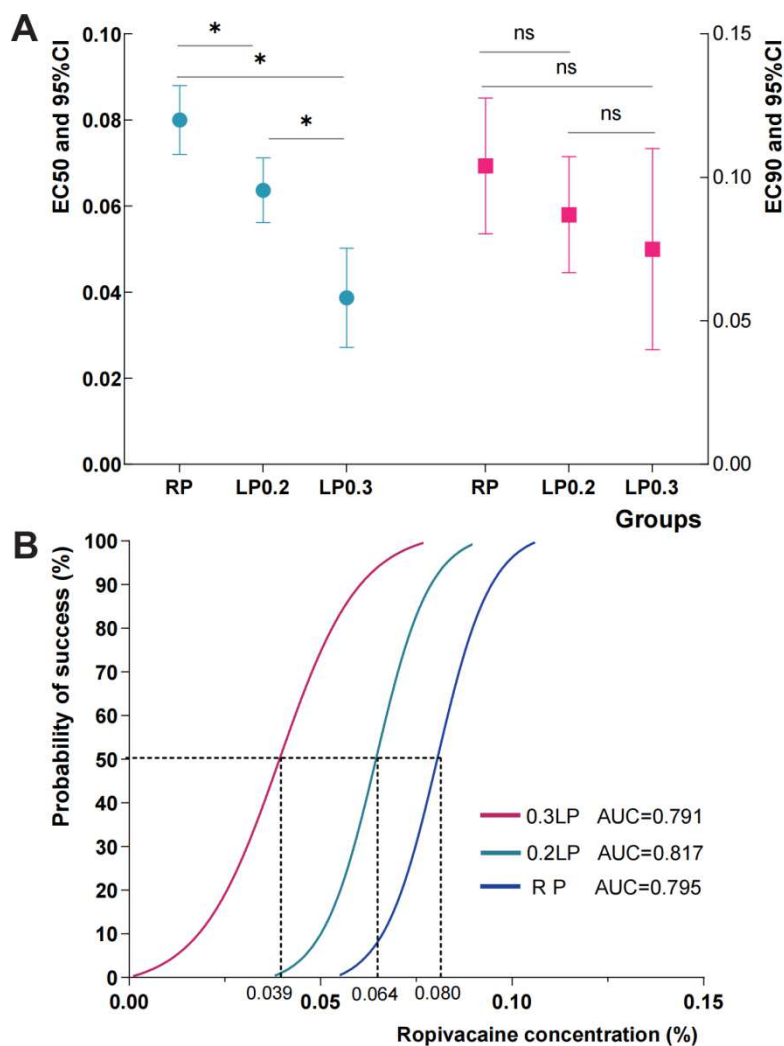


Figure 2 Individual response to the allocated epidural ropivacaine effect-site concentration in conjunction with various chloroprocaine concentrations using an up-and-down method.

289 (A) the RP group; (B) the LP0.2 group; (C) the LP0.3 group. “■” indicated a
 290 ropivacaine concentration of effective response; “●” indicated a ropivacaine
 291 concentration of ineffective response. To get eight crossovers, 29, 31 and 34 patients
 292 were included in the RP, LP0.2 and LP0.3 group, respectively.
 293



294
 295 **Figure 3 (A) The values of EC50 and 95% CI, EC90% and 95% CI in the RP,**
 296 **LP0.2 and LP0.3 group.** The statistical differences were calculated using overlapping
 297 confidence interval (CI) methodology and were considered statistically significant

when 83% of CIs did not overlap. **(B) Concentration-response curve for the EC50s of ropivacaine in three different concentrations of chloroprocaine.**

Table 2 EC50 and EC90 of ropivacaine in the three groups as determined by the up-down allocation method.

Item	Group RP (n=29)	Group LP0.2 (n=31)	Group LP0.3 (n=34)	Overlapping CI			P Value		
				RP	RP	LP0.2	RP	RP	LP0.2
				vs.	vs.	vs.	vs.	vs.	vs.
				LP0.2	LP0.3	LP0.3	LP0.2	LP0.3	LP0.3
EC50 ^a (%)	0.080	0.064	0.039						
	(95% CI, 0.072~0.088)	(95% CI, 0.056~0.071)	(95% CI, 0.027~0.050)	0%	0%	0%	< 0.05	< 0.05	< 0.05
EC90 ^a (%)	0.094	0.079	0.060						
	(95% CI, 0.087~0.131)	(95% CI, 0.072~0.110)	(95% CI, 0.050~0.115)	39.0%	34.6%	100%	> 0.05	> 0.05	> 0.05

^a Probit regression. $P < 0.05$ indicated statistically significant differences.

3.3 Secondary Outcomes: Quality of Epidural Labor Analgesia

The effect of labor analgesia

Table 3 summarizes the secondary outcomes related to the effect of labor analgesia. The onset time of analgesia ($P=0.020$, RP vs. LP0.2; $P=0.003$, RP vs. LP0.3), the rate of breakthrough pain ($P=0.034$, RP vs. LP0.2; $P=0.045$, RP vs. LP0.3), and perineal distension and pain ($P=0.031$, RP vs LP0.2; $P=0.016$, RP vs LP0.3) were lower in the LP0.2 and LP0.3 group than those in the RP group. The maximum VAS scores after analgesia ($P=0.001$, RP vs LP0.2; $P=0.022$, RP vs LP0.3) were also lower in the LP0.2 and LP0.3 groups than in the RP group. The time to first requiring rescue

administration ($P<0.001$, RP vs. LP0.2; $P=0.012$, RP vs. LP0.3) was longer in the LP0.2 and LP0.3 groups than in the RP group. The total epidural medication consumption ($P=0.015$, RP vs LP0.2) was lower in the LP0.2 group compared to the RP group. However, the number of rescue administrations was higher in the LP0.3 group than in the LP0.2 group ($P=0.017$, LP0.2 vs LP0.3). Maternal satisfaction with overall analgesia was greater in the LP0.2 group compared to the RP and LP0.3 groups ($P=0.041$, RP vs LP0.2; $P=0.009$, LP0.3 vs LP0.2). The three groups had no significant differences in Bromage score >0 and the highest sensory block ($P>0.05$).

Table 3 Effect of labor analgesia among the three groups

	Group R (n=29)	Group LP 0.2 (n=31)	Group LP 0.3 (n=34)	χ^2/H Value			Adjusted P Value		
				RP	RP	LP0.2	RP	RP	LP0.2
				vs. LP0.2	vs. LP0.3	vs. LP0.3	vs. LP0.2	vs. LP0.3	vs. LP0.3
Onset time, minutes	8 (7-9)	6 (5-8)	6 (5-7)	2.720	3.282	0.511	0.020	0.003	1.000
Breakthrough pain, n (%)	16 (55.2)	8 (25.8)	10 (29.4)	5.384	4.285	0.105	0.034	0.045	0.788
Perineal distension and pain, n (%)	15 (51.7)	6 (22.6)	7 (20.6)	5.480	6.676	0.038	0.031	0.016	1.000
Maximum VAS score after analgesia	6(5-7)	4(3-5)	4(3.8-6)	3.495	2.674	0.914	0.001	0.022	1.000
Time to first requiring rescue administration, minutes	37.5(23.8-59.3)	70.0(65.0-160.0)	60.0(48.8-70.0)	4.194	2.888	1.791	<0.001	0.012	0.220
Number of clinician boluses, times	1(0-1)	0 (0-1)	1(0.8-2)	1.355	1.337	2.770	0.527	0.544	0.017
Highest sensory block, T	10 (10-10)	10 (9-10)	10(9-10)	1.572	2.207	0.611	0.348	0.082	1.000
Bromage score >0 , n (%)	1 (3.4)	2 (6.5)	4 (11.8)	-	-	-	1.000	0.363	0.674
Total epidural medication	60.0 (42.0-78.5)	42.0 (30.0-60.0)	52.5 (39.8-76.5)	2.814	0.998	1.912	0.015	0.955	0.168

consumption, mL Maternal satisfaction with overall analgesia	9 (8-10)	10 (9-10)	9 (8-10)	-2.466	0.408	2.980	0.041	1.000	0.009
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323 Values are expressed as medians (Q1- Q3) , or number (%) as appropriate.

324 The statistic values for nonparametric Kruskal-Wallis test were expressed as H value. $P < 0.05$
325 indicated statistically significant differences. The statistic values pearson χ^2 was expressed as χ^2
326 value. Pairwise comparison between groups using α partitioning method, $P < 0.0125$ indicated
327 statistically significant differences.

328 The VAS pain scores during 120 minutes after analgesia are presented in Figure
329 4A. The linear mixed-effect model indicated that the time effect, the inter-group effect
330 over time and the group-by-time interaction effect among the three groups had
331 significant differences ($F_{\text{time}}=21.758$, $P_{\text{time}} < 0.001$; $F_{\text{group}}=460.442$, $P_{\text{group}} < 0.001$;
332 $F_{\text{group*time}}=7.027$, $P_{\text{group*time}} < 0.001$, respectively). Utilizing the adjusted Bonferroni
333 post-test for multiple comparisons among the three groups at each time point, the mean
334 VAS scores in the LP0.2 and LP0.3 groups were lower than those in the RP group at 5,
335 15, 30, 90, and 120 minutes post-analgesia (RP vs LP0.2: $P_{5\text{min}} < 0.001$, $P_{15\text{min}}=0.021$,
336 $P_{30\text{min}}=0.008$, $P_{90\text{min}}=0.004$, $P_{120\text{min}} < 0.001$; RP vs LP0.3: $P_{5\text{min}} < 0.001$, $P_{15\text{min}} < 0.001$,
337 $P_{30\text{min}} < 0.001$, $P_{90\text{min}}=0.001$, $P_{120\text{min}}=0.023$, respectively, Figure 4A). Differences
338 between the LP0.2 and LP0.3 groups were insignificant, except for the time point at 15
339 minutes following analgesia administration (LP0.2 vs LP0.3: $P_{15\text{min}}=0.005$).

340 **The safety of analgesia**

341 Table 4 summarizes the secondary outcomes related to the safety of analgesia,

including maternal and neonatal outcomes and adverse effects associated with analgesic administration. Our analysis revealed no statistically significant differences among the groups with regards to the duration of the first and second stages of labor, delivery method, blood loss, Apgar scores at the 1 and 5-minute marks, incidence of fetal bradycardia within the initial 30 minutes, and maternal adverse events related to labor analgesia ($P>0.0125$).

The IUCs during 120 minutes after analgesia are presented in Figure 4B. The linear mixed-effect model revealed no significant differences in the inter-group effect over time and the group-by-time interaction effect among the three groups ($F_{\text{time}}=39.020$, $P_{\text{time}}<0.001$; $F_{\text{group}}=1.966$, $P_{\text{group}}=0.146$; $F_{\text{group*time}}=1.663$, $P_{\text{group*time}}=0.061$). When the data of the three groups were analyzed separately, the time impact of IUCs in the three groups differed significantly, with a temporary decrease from 5 to 45 minutes after analgesia and then gradual recovery in the RP group ($F=7.976$, $P<0.001$), and a temporary decrease from 5 to 60 minutes after analgesia in the LP 0.2 ($F=17.048$, $P<0.001$) and LP0.3 ($F=19.956$, $P<0.001$) group, followed by gradual recovery.

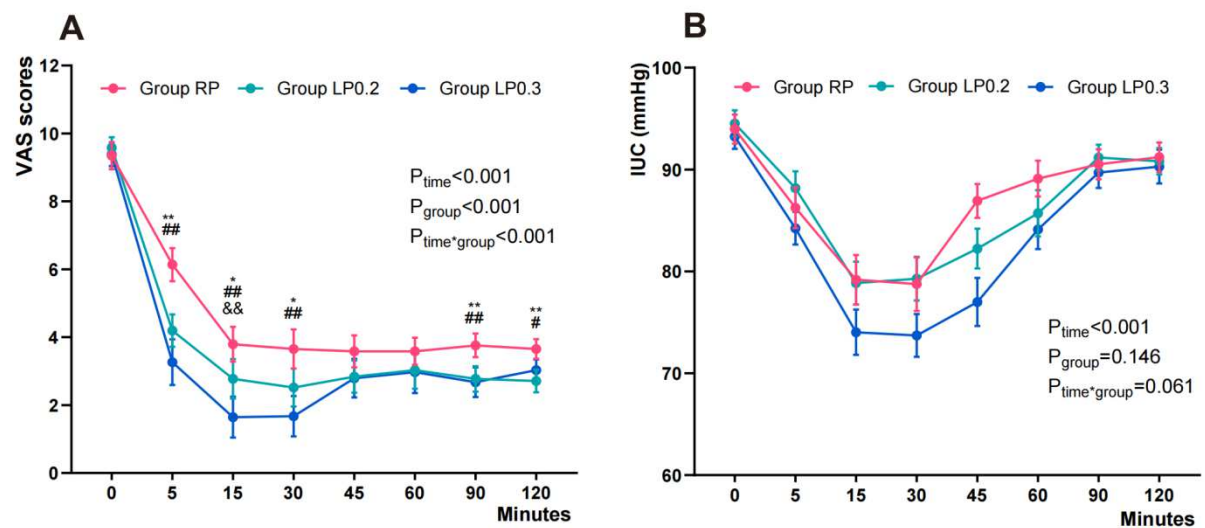


Figure4 (A) The mean VAS pain scores at the time points of before analgesia (baseline), 5, 15, 30, 45, 60, 90 and 120 minutes after epidural injection. (B) The mean IUC at the time points of before analgesia (baseline), 5, 15, 30, 45, 60, 90 and 120 minutes after epidural injection. VAS, Visual analog scale; IUC, intensity of uterine contraction. Data was presented as mean and 95% CIs, analyzed via the linear mixed mode. An adjusted Bonferroni post-test was used for multiple comparisons between group differences at each time point. $P < 0.05$ indicated a statistical difference.

4 Discussion

The prospective, randomized, double-blinded up-down sequential allocation investigation discovered that the EC50 values for epidural ropivacaine in combination with 0.0%, 0.2% or 0.3% chloroprocaine were 0.080%, 0.064% and 0.039%, respectively. The EC90 values were 0.094%, 0.079% and 0.060%, respectively. The EC50 values of ropivacaine were decreased by 20% and 51% in the LP0.2 and LP0.3

groups compared to the RP group, with statistically significant differences.

The emergence of preservative-free chloroprocaine has led to its reapplication as a spinal and epidural anesthetic^[19]. Rapid hydrolysis by plasma cholinesterase in maternal plasma prevents substantial drug transfer into the fetus^[20-21], making chloroprocaine attractive for obstetric anesthesia and analgesia^[4,6]. Previous studies have reported several case series in which epidural chloroprocaine infusion relieved labor pain in patients with a history of allergic reactions to amide local anesthetics, with concentrations ranging from 0.26%^[12], 0.3%^[13] to 1.5%^[19] with a low dosage of fentanyl adjuvant. It is known that lower concentrations of local anesthetics are preferable due to their lower likelihood of causing motor block and assisted vaginal delivery^[11]. Thus, we selected concentrations of chloroprocaine at 0.2% and 0.3% with a sufentanil adjuvant to explore the compatibility of low concentrations of chloroprocaine co-administered with ropivacaine. This study aimed to examine the EC50 and EC90 values of ropivacaine with varying concentrations of chloroprocaine using the up-down sequential allocation methodology.

A sequential method is a classical approach for determining the median effective dose of drugs, offering the advantage of conserving sample size. Using this method, the EC50 of epidural ropivacaine has been shown to decrease with the addition of adjuvants such as sufentanil or dexmedetomidine^[22-23]. Previous studies have determined the minimum local analgesic concentration (MLAC) of chloroprocaine in a 14 mL volume to be 0.42%^[24]. Another study reported that the MLAC of

chloroprocaine in a 20 mL volume during the first stage of labor was reduced from 0.43% to 0.26% by adding fentanyl^[12]. However, studies on the EC50 of epidural ropivacaine co-administered with chloroprocaine during labor are limited. Our results found that 0.2% and 0.3% chloroprocaine decreased the EC50 of epidural ropivacaine by 20% and 51%, respectively, and the EC90 of epidural ropivacaine by 16% and 36%, respectively. The EC50 was employed to evaluate potency, whereas the EC90 or EC95 were found to be more applicable in clinical practice^[15]. Although no statistically significant differences in EC90 were observed among the three groups using the overlapping CI methodology, these results have clinical guidance value. Fewer local anesthetics are preferable, especially during the latent phase of the first stage of labor, provided the same analgesic effect is achieved^[11,25].

Utilizing the rapid onset and excellent diffusion performance of chloroprocaine, our previous study revealed that administering 6 mL of 1.5% chloroprocaine resulted in a quicker onset of labor analgesia compared to 1% lidocaine^[10]. Ji et al. reported that 6 mL of 1.5% chloroprocaine achieved better pain relief for breakthrough pain than 0.125% epidural ropivacaine^[4]. In the present study, the onset time of analgesia and the incidence of breakthrough pain were significantly reduced in the LP0.2 and LP0.3 groups compared to the RP group, aligning with previous studies.

During the late first stage and early second stage of labor, pain commonly arises from the distention and traction on pelvic organs, often referred to as perineal distension and pain. This pain is primarily transmitted by the pudendal nerves through

the sacral plexus to the posterior nerve root ganglia at spinal levels S2 to S4. To alleviate this pain, a complete sacral block is necessary. However, achieving this with the combination of ropivacaine and sufentanil for epidural labor analgesia is challenging. The dural puncture epidural (DPE) technique is reported to improve sacral spread for labor analgesia^[3]. Nevertheless, the benefits of the DPE technique for labor analgesia are controversial, and it is not commonly used in clinical practice. A prospective randomized clinical trial demonstrated that DPE did not show a significant advantage over the standard epidural technique when maintained for labor analgesia using the PIEB method^[26]. The initial findings of this study indicated reduced VAS scores for perineal pain at 60 minutes post-analgesia and a decreased incidence of perineal distension and pain in the LP0.2 and LP0.3 groups compared to the RP group. These results imply that the inclusion of 0.2% or 0.3% chlorprocaine augmented the sacrococcygeal blockade efficacy of ropivacaine.

Unexpectedly, the LP0.3 group exhibited a higher incidence of clinician-rescued boluses compared to the LP0.2 group. Maternal satisfaction with overall analgesia was significantly greater in the LP0.2 group than in the RP and LP0.3 groups. Although the incidence of leg numbness and shivering seemed elevated in the LP0.3 group compared to the LP0.2 and RP groups, statistical significance was not observed, possibly due to the limited sample size. These results suggested that 0.3% chlorprocaine may not lead to better clinical analgesic effects than 0.2% chlorprocaine. No significant increase in adverse reactions or poor maternal and

infant outcomes was found in the LP0.2 or LP0.3 groups. The intensity of uterine contractions (IUC) showed a brief decrease within 60 minutes after analgesia in all groups but gradually recovered. Additional studies with an expanded sample size are necessary to validate these findings and evaluate the clinical effectiveness of analgesia.

This study has several limitations. Firstly, while the sample size in the up-down sequential allocation design was adequate for determining the EC50 and EC90, caution is advised when interpreting the secondary outcomes. The sample size may not have been large enough to detect statistically significant differences in these outcomes. Secondly, when comparison of quality of labor analgesia, the effective and ineffective cases obtained from sequential experiments were not differentiated in subsequent observations, which may lead to bias in the results. Therefore, since the EC50 and EC90 of ropivacaine with chloroprocaine have been determined, prospective case-control studies should focus on evaluating side effects, neonatal and obstetric results.

In conclusion, chloroprocaine at concentrations of 0.2% and 0.3% reduced the EC50 of ropivacaine by 21% and 50% during maintenance for labor analgesia. This study preliminarily showed that the combination of 0.2% chloroprocaine achieved better analgesic effects, particularly in reducing perineal distension and pain, without increasing adverse reactions. Future research should be conducted to assess the impact of labor analgesia on the quality of delivery, including side effects, neonatal outcomes, and obstetric results.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Maternal and Child Medicine Center at Anhui Medical University (YYLL2023-07-18 for initial review and YYLL202401108-YJGZ-KSLL-01-1.0 for follow-up review) and was registered prior to subject enrollment at <http://www.chictr.org.cn> (ChiCTR2300074933) with a minor revision on grouping scheme and observation indicators. All participants provided voluntary written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The data supporting the study findings are available from the corresponding author upon request.

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Figure legends

Figure 1 Flow diagram for the Dixon up-and-down method.

Abbreviations: RP, the group of ropivacaine with 0.0% chlorprocaine; LP0.2, the group of ropivacaine with 0.2% chlorprocaine; LP0.3, the group of ropivacaine with 0.3% chlorprocaine. VAS, visual analogue scale score.

Figure 2 Individual response to the allocated epidural ropivacaine effect-site concentration in conjunction with various chlorprocaine concentrations using an up-and-down method. (A) the RP group; (B) the LP0.2 group; (C) the LP0.3 group. “■” indicated a ropivacaine concentration of effective response; “●” indicated a ropivacaine concentration of ineffective response. To get eight crossovers, 29, 31 and 34 patients were included in the RP, LP0.2 and LP0.3 group, respectively.

Figure 3 (A) The values of EC50 and 95% CI, EC90% and 95% CI in the RP, LP0.2 and LP0.3 group. The statistical differences were calculated using overlapping

confidence interval (CI) methodology and were considered statistically significant when 83% of CIs did not overlap. (B) Concentration-response curve for the EC50s of ropivacaine in three different concentrations of chloroprocaine.

Figure4 (A) The mean VAS pain scores at the time points of before analgesia (baseline), 5, 15, 30, 45, 60, 90 and 120 minutes after epidural injection. (B) The mean IUC at the time points of before analgesia (baseline), 5, 15, 30, 45, 60, 90 and 120 minutes after epidural injection. VAS, Visual analog scale; IUC, intensity of uterine contraction. Data was presented as mean and 95% CIs, analyzed via the linear mixed mode. An adjusted Bonferroni post-test was used for multiple comparisons between group differences at each time point. $P < 0.05$ indicated a statistical difference.