Safety of blood reinfusion after local infiltration analgesia with ropivacaine in total knee arthroplasty

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Abstract. Objective: The authors hypothesized that it is safe to combine local infiltration analgesia (LIA) in total knee arthroplasty (TKA) with a retransfusion drain since ropivacaine concentrations would not exceed the arterial toxicity threshold concentrations of 4.3 mg/L for total and 0.56 mg/L for unbound ropivacaine. Materials and methods: 22 patients scheduled for primary TKA were included. During surgery three peri-articular injections with ropivacaine (300 mg) were given. Plasma and shed blood samples were taken at 0, 1, 3, 6, 7, and 24 hours postoperatively. Results: At 6 hours postoperatively, the total ropivacaine plasma concentration ranged from 0.26 to 1.53 mg/L and unbound ropivacaine from 0.03 to 0.12 mg/L. At 7 hours, the total ropivacaine plasma concentration ranged from 0.19 to 1.71 mg/L and unbound ropivacaine from 0.02 to 0.09 mg/L. In the collected shed blood, a total of 0.27 to 12.8 mg (median 3.73 mg) unbound ropivacaine was present. Reinfusion would lead to an addition of 3.73 mg (median) unbound ropivacaine that would be reinfused into the patient. The calculated (modeled) estimation regarding the maximum unbound ropivacaine plasma concentration showed a median value of 0.114 mg/L (IQR: 0.09, 0.12 mg/L). All concentrations were well below reported toxicity thresholds. Conclusions: The combination of LIA and reinfusion presented here-in are considered safe. However, differences in pain protocol lead to changes in the safety evaluation. Compared with previous studies, the technique of administration is of greater importance for the effect on unbound ropivacaine because of unknown mechanisms.

Introduction

As part of a multimodal approach, local infiltration anesthesia (LIA) has been intro-
duced. Large case series have been described with satisfactory results for pain relief and despite the high concentration, side effects of local infiltration techniques occur infre-
quently [1, 2].

The combination of LIA with autologous blood transfusion, that is postoperatively giv-
ing back the patient’s own blood collected in a transfusion bag during the first 6 hours after surgery, might lead to infusion of consider-
able amounts of local anesthetics. In this respect, continuous infusion issues, plasma concentrations and toxicity are clinical phar-
macological issues that have to be considered in the safety evaluation of intra-operative local infiltration techniques with ropivacaine in hip and knee arthroplasty combined with autologous blood transfusion.

In plasma, ropivacaine is mainly bound to α1-acid glycoprotein (AAG) which is an acute-phase protein whose concentration has been shown to increase in response to surgical stress [3]. As ropivacaine is eliminated by hepatic metabolism, with an intermediate to low extraction ratio [4, 5], its rate of elimination should, theoretically, depend on the unbound ropivacaine concentration in plasma [6]. The total plasma clearance is expected to vary with changes in the unbound fraction, i.e., a postoperative increase in the AAG concentration will decrease the free fraction (due to increased protein binding), which will decrease the total clearance and result in a relative increase in total plasma concentrations [6, 7]. This is important, as it is the unbound plasma concentration that is related to systemic pharmacodynamic effects and toxicity. Since the rate of diffusion across a membrane is propor-
Thomassen, Touw, van der Woude, et al. 2

The unbound drug concentration, the rapid appearance of systemic central nervous system and cardiovascular toxicity of local anesthetics is determined by the unbound concentration as opposed to the total plasma drug concentration.

Only six small studies have been published about (possible) reinfusion following total knee arthroplasty (TKA) using ropivacaine [8, 9, 10, 11, 12, 13]. Hitherto, reinfusion was studied in two studies with different doses (200 mg vs. 150 mg), additional bolus injections (twice vs. none) and even other comparators such as tourniquet usage and hip and knee arthroplasty [9, 10].

The aim of this study was to determine the safety of LIA in combination with a retransfusion drain. Total ropivacaine plasma concentrations should not exceed 4.3 mg/L (arterial) as these are associated with toxicity; as for the unbound ropivacaine plasma concentrations – supposing a protein binding of 87% – concentrations > 0.56 mg/L (arterial) are associated with toxicity [14]. Since protein binding may vary considerably between and within subjects, we measured total and unbound ropivacaine plasma concentra-

tions as well as ropivacaine concentrations in the retransfusion device.

Materials and methods

Our regional ethics committee approved the study (CCMO no. NL33364.098.10) and the trial was registered in the Dutch trial registry (NTR2677). Patients planned for a primary TKA with preoperative hemoglobin (Hb) concentrations > 7.5 mmol/L were eligible for participation in the study. Other inclusion criteria were age between 18 and 90 years, American Society of Anesthesiologists (ASA) Classification I or II, lumbar spinal anesthesia (L2-3 or L3-4 level with 3 mL of bupivacaine 0.5%), an estimated GFR with the modification of diet in renal disease (MDRD) formula > 48 mL/min. Patients gave their informed consent after having received oral and written information.

An intravenous cannula was inserted into a vein of each arm, one for routine monitoring of the patient during surgery and one (ante-cubital) in order to obtain blood samples. One orthopedic surgeon performed all operations according to standard procedure. Cemented cruciate retaining components (PFC Sigma, DePuy, Johnson&Johnson, Warsaw, IN, USA) were placed and patella resurfacing was done when necessary. A tourniquet was used during surgery which was inflated to 300 mmHg. The tourniquet was released after skin closure and compressive bandaging. Perioperative and postoperative surveillance of the patient was performed by routine cardiac monitoring which took place for at least 3 hours after surgery.

Three syringes each containing 50 mL ropivacaine 0.2% (Fresenius Kabi, Hamburg, Germany) and 0.33 mg epinephrine were injected with an 18 gauge spinal needle. Before placement of the final implants the posterior capsule and deep peri-articular tissues were infiltrated with the first syringe. The second syringe was used to infiltrate the capsule and the borders of the incised quadriceps and patellar tendons, infra-patellar ligament and possible remnants of the fat pad. The third syringe was used to infiltrate the subcutaneous layer around skin incision.

At the end of surgery an autologous blood transfusion (ABT) drain (Bellovac
ABT, AstraTech, Mölndal, Sweden) was placed intra-articular in the knee joint. The drain was opened at the recovery unit after the first blood sample was taken (T = 0). At 1, 3, 6, 7, and 24 hours after the first sample another blood sample was taken from the patient. At 1, 3, and 6 hours the drainage bag was disconnected, volume noted and a sample was taken. All samples were drawn with EDTA tubes and samples were centrifuged at 3,000 rpm for 10 minutes, plasma samples were then stored at –70 °C until LC-MSMS analysis. The Bellovac ABT drain was removed after 24 hours.

A standardized pain therapy protocol was used (Table 1). The Visual Analog Scale (VAS) for pain was asked before blood samples were taken.

**Analyses**

The main analyses of this study consisted of total and unbound ropivacaine plasma concentrations in the different patients' and shed blood samples. Ropivacaine concentrations were measured with liquid chromatography-tandem mass spectrometry (LC-MSMS, Agilent Technologies 6410 Triple Quad, Agilent, Amstelveen, The Netherlands) [8]. The accuracy of the ropivacaine analyses was 2.2 – 4.4% and intermediate precision was 2.0 – 2.9% [12].

The free ratio of ropivacaine for each patient was calculated by dividing the unbound ropivacaine plasma concentration by the total ropivacaine plasma concentration of the different samples (Psff = patient-specific free fraction).

Subsequently, the theoretical maximum unbound ropivacaine plasma concentration was modeled if the blood from the retransfusion device would have been instantly returned to the patient. Circulating plasma volume per patient was estimated according to the blood volume formulae of Lemmens et al. [15], patient specific (preoperative) hematocrit value and body weight. Next, the theoretical maximum unbound ropivacaine plasma concentration was estimated as follows:

$$((R_{bel} \times V_{bel} \times Psff_{6}) + (R_{pl6} \times patient-specific circulating plasma volume))/(patient specific circulating plasma volume + V_{bel}).$$

(Rbel = unbound ropivacaine plasma concentration in mg/L in the retransfusion device after blood passage through the filter cascade, Vbel = volume of shed blood in L, Psff_{6} = patient-specific free fraction of ropivacaine at 6 hours postoperatively, Rpl6 = unbound ropivacaine plasma concentration in mg/L at 6 hours postoperatively).

In this calculation the assumption that most of the unbound ropivacaine present in the blood collected in the retransfusion device would instantly bind to plasma protein when reinfusion was made.

**Pharmacokinetic analysis**

Individual pharmacokinetic parameters for ropivacaine were calculated with the MW/Pharm pharmacokinetic software package (version 3.70, Medi/Ware, The Netherlands). MW/Pharm consists of Bayesian modeling software and uses an a priory population pharmacokinetic model [16]. Using Bayesian techniques the model was fitted to the measured ropivacaine data. “A priori” pharmacokinetic parameters were taken from our previous study [12] and consisted of a one-compartment open model with a metabolic clearance (CLm) of 11 ± 5.98 L/h, fraction excreted unchanged in the urine (fr) of 1%, volume of distribution 0.5099 ± 0.3674 L/kg LBMc, (LBMc is lean body mass corrected), absorption constant from the site of injection 0.0313 ± 0.0114 h⁻¹ and a lag time until absorption of 0.1267 ± 0.1364 hours. When modeling the data, the fraction excreted unchanged was fixed to the literature value of 0.01 because of lack of data on renal elimination [17].

**Statistics**

Continuous data are presented as the number of subjects, mean, standard deviation, minimum and maximum concentrations. Categorical data expressed as frequencies and percentages. Data were tested for normality. Normally distributed data are presented as mean ± standard deviation; in case of a non-normal distribution, median and inter-quartile range (IQR) was used.

Data were analyzed using SPSS 17.0 (SPSS for Windows, SPSS Inc. Chicago, IL, USA).
Results

A total of 24 consecutive patients were enrolled in the study, 22 patients were included in the final analysis. Two patients were excluded because no blood samples could be drawn.

The average age was 67.7 ± 9.8 years, body mass index (BMI) was 27.8 ± 3.4 kg/m^2. The majority of patients was male (59%) and were operated upon the right knee (64%) with patella resurfacing in 9 cases (41%). 86% of the patients had American Society of Anesthesiologist (ASA) Classification II, eGFR ranged between 63 and 109 mL/min (average 81.5 mL/min). The average length of hospital stay was 3.69 ± 0.98 days. The three LIA blocks were given on average respectively 47, 66 and 76 minutes after start of surgery. The first sample was taken 50 minutes (SD: 9 min) after the first intra-operative injection.

Mean total and unbound ropivacaine plasma concentrations are shown in Table 2 and Table 3. The plasma concentrations 6 and 7 hours postoperatively are important for the potential reinfusion of shed blood. Plasma concentrations 6 hours postoperatively ranged from 0.26 to 1.53 mg/L for total ropivacaine and from 0.03 to 0.12 mg/L for unbound ropivacaine. At 7 hours the concentrations were 0.19 – 1.71 mg/L for total ropivacaine and 0.02 – 0.09 mg/L for unbound ropivacaine. Lowest total and unbound ropivacaine plasma concentrations were found in patient 20 at T = 0 and were 0.023 and 0.003 mg/L, respectively. In the same person the highest concentrations were found in shed blood at T = 1, 141.98 mg/L for total ropivacaine and 111.49 mg/L for unbound ropivacaine. The highest unbound ropivacaine plasma concentration was found at T = 6 (0.12 mg/L) and the highest total ropivacaine plasma concentration was 1.80 mg/L (at T = 24), both in the same person.

The time points where patients reached peak concentrations varied widely. For total ropivacaine peak plasma concentrations were reached at 3 hours (8 patients), 6 hours (5 patients), 7 hours (5 patients), and 24 hours (4 patients). The unbound ropivacaine plasma concentrations reached their peak at 3 hours (7 patients), at 6 hours (7 patients), at 7 hours (7 patients), and 2 patients at 24 hours.

The median of postoperative shed blood volume was the sum of the 4 time points (1, 3, 6, and 24 hours postoperative) and was 707 mL (IQR: 403, 909 mL). The median of postoperative shed blood (collected blood until 6 hours postoperative) that would be returned to the patient was 463 mL (IQR: 306, 669 mL).

Table 2. Total and unbound ropivacaine concentrations in plasma.

<table>
<thead>
<tr>
<th>T</th>
<th>Unbound median (IQR)</th>
<th>Total median (IQR)</th>
<th>Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.009 (0.006 – 0.013)</td>
<td>0.106 (0.081 – 0.192)</td>
<td>6.498 (5.596 – 8.286)</td>
</tr>
<tr>
<td>1</td>
<td>0.016 (0.014 – 0.022)</td>
<td>0.269 (0.209 – 0.375)</td>
<td>6.581 (5.762 – 7.544)</td>
</tr>
<tr>
<td>3</td>
<td>0.032 (0.027 – 0.043)</td>
<td>0.466 (0.428 – 0.609)</td>
<td>6.791 (5.870 – 7.432)</td>
</tr>
<tr>
<td>6</td>
<td>0.043 (0.036 – 0.050)</td>
<td>0.585 (0.455 – 0.663)</td>
<td>7.538 (6.208 – 9.272)</td>
</tr>
<tr>
<td>7</td>
<td>0.045 (0.003 – 0.062)</td>
<td>0.593 (0.189 – 1.708)</td>
<td>7.560 (6.706 – 9.622)</td>
</tr>
<tr>
<td>24</td>
<td>0.024 (0.017 – 0.039)</td>
<td>0.433 (0.278 – 0.578)</td>
<td>6.195 (4.777 – 7.094)</td>
</tr>
</tbody>
</table>

Unbound and total values are shown in mg/L as median (IQR), fraction is unbound concentration in % of the total concentration.

Table 3. Total and unbound ropivacaine concentrations in shed blood.

<table>
<thead>
<tr>
<th>T</th>
<th>Volume (mL)</th>
<th>Unbound median (IQR)</th>
<th>Total median (IQR)</th>
<th>Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ABT</td>
<td>100 (60 – 225)</td>
<td>15.793 (9.634 – 24.666)</td>
<td>27.462 (18.996 – 42.725)</td>
<td>57.731 (54.214 – 59.762)</td>
</tr>
<tr>
<td>6 ABT</td>
<td>200 (100 – 234)</td>
<td>2.774 (1.269 – 3.380)</td>
<td>8.049 (5.373 – 9.551)</td>
<td>31.841 (22.445 – 36.939)</td>
</tr>
</tbody>
</table>

Volume presented in mL. Unbound and total values are shown in mg/L as median (IQR), fraction is unbound concentration as % of the total concentration.
When the shed blood would have been returned to the patient, the volume and concentrations collected during the first 6 hours are important (Table 3). In the cumulative collected shed blood from the first 6 hours a total of 0.27 to 12.8 mg (median 3.73 mg) unbound ropivacaine was present. The median unbound amount per time point (1, 3, and 6 hours postoperatively) was, respectively, 2.18 mg (IQR: 1.21, 3.51 mg), 0.73 mg (IQR: 0.36, 1.4 mg), and 0.44 mg (IQR: 0.15, 0.69 mg). The amount of blood collected was quite equal during these time points but unbound ropivacaine concentrations decreased significantly from T = 1 to T = 3 and T = 6. Reinfusion would lead to an addition of 3.73 mg (median) unbound ropivacaine to the patient’s blood.

The calculated (modeled) estimation, assuming reinfusion, regarding the maximum unbound ropivacaine plasma concentration showed a median value of 0.11 mg/L (IQR: 0.09, 0.12 mg/L).

Pharmacokinetic analyses are presented in Table 4. Mean metabolic clearance in the pharmacokinetic analyses was 12.41 ± 6.32 L/h. The Ka and Tlag were 0.05 ± 0.11 hours and 0.16 ± 0.11 hours, respectively. The volume of distribution for ropivacaine averaged 0.61 ± 0.36 L/kg.

Routine cardiac monitoring perioperative and 3 hours postoperatively did not show any remarkable changes that could be addressed to ropivacaine toxicity. None of the patients experienced any adverse events attributable to the LIA procedure during hospitalization and until 6 weeks postoperative. Furthermore, no non-specific adverse events, such as headache, were experienced by the patients during and after the procedure.

Additionally, the VAS scores were good for all patients. The first two patients with too much pain (VAS > 4) were reported at 3 hours postoperative. The patients that reported too much pain at the following time points were successfully treated with the escape medication mentioned in the pain protocol.

### Discussion

There is sufficient evidence that LIA is safe and can reduce postoperative pain following TKA [18]. We studied the safety of LIA in combination with a retransfusion drain. Total and unbound concentrations of ropivacaine were used as surrogate parameters for safety. The individual unbound ropivacaine plasma concentrations at 6 hours postoperative are below the published arterial toxicity threshold of 0.56 mg/L, and no cardiac symptoms related to ropivacaine or other adverse effects were observed in all patients studied [14]. Cardiac symptoms of ropivacaine toxicity were measured by perioperative and postoperative surveillance of the patient, monitoring took place for at least 3 hours after surgery.

If we should add 3.73 mg unbound ropivacaine (via shed blood) and assume instant binding, unbound ropivacaine plasma concentrations would rise by a median of 0.11 mg/L. Also the maximum unbound ropivacaine plasma concentration (0.44 mg/L) does not exceed the toxic threshold especially if we take into account the elimination of ropivacaine between 6 and 7 hours postoperative.
We previously performed a similar study with a slightly different design. In the former study patients received LIA with two syringes of 50 mL ropivacaine 0.375% and continuous infiltration after surgery during the next 24 hours. Plasma samples were taken at the same time points, excluding T = 1 and T = 7 and shed blood was only collected at 6 hours postoperative [12]. The sample handling, laboratory and ropivacaine analysis were performed by the same persons and the same validated analytical technique, only some samples were stored longer than others which is inherent to the inclusion of eligible patients. Also the pharmacokinetic data analysis was performed by the same person with the same software. However, both studies revealed some contradictions which could not be explained by difference in study design alone.

The mean free fraction in the current study (7.3%) was higher than the population average of 5% and even significantly higher than the 4.8% in the former study [12]. This difference could not be explained by the included patients, because both patient groups were equal in co-morbidity, ASA classification, and medication usage. In the former study, continuous infusion was given in the first 24 hours postoperative which could lead to saturation of AAG. It is known that AAG, the protein responsible for ropivacaine binding, has high inter- and intra-individual variability and therefore concentrations and binding capacity can largely differ over time and between patients. Furthermore, AAG concentrations are influenced by surgery, myocardial infarction and inflammatory processes [3]. In the study by Essving et al. [19] it is noted that even though the total plasma concentration showed increasing concentrations, the free fraction decrease with time. This is in line with the fact that ropivacaine is mainly bound to AAG, and AAG availability has been associated with an increase in the protein binding of ropivacaine during long-term infusion after surgery [7, 20]. This is also seen in both studies performed, where the mean unbound ropivacaine fraction decreased after 24 hours.

Secondly, the unbound and total ropivacaine concentrations at T = 0 were lower in the current study despite the higher concentration of ropivacaine given at that time point (300 mg vs. 150 mg). The fluid management during surgery showed no differences between both studies. In the former study an average of 1,300 mL (median 1,500 mL) was given, in the current study an average of 1,227 mL (median 1,250 mL) was infiltrated perioperatively. All patients received intravenous sodium chloride and Ringer’s solution. Also the intraoperative sedation (propofol) beside the spinal anesthesia during surgery could potentially explain the differences between the two groups. The intravenously given fat emulsion (vehicle for propofol) is prone to absorb ropivacaine [21]. However, in both groups the same number of patients, 14 vs. 13, respectively, in the current and former study group received propofol infusion (same dose) during surgery.

Thirdly, we had expected that the ropivacaine concentrations in the shed blood would be much lower because no continuous infusion with ropivacaine was given in the current study. But the median unbound concentrations differed only 1.8 mg per liter in shed blood between the two studies.

The pharmacokinetic data analyses had some remarkable findings. The metabolic clearance was much lower than expected, 12.4 L/hour (13.6 L/hour previous study) compared to the 26 L/hour as expected from literature. The volume of distribution of ropivacaine (0.61 L/kg current and 0.63 L/kg previous study) was comparable with literature value (0.67 L/kg) [17]. The lower absorption rates were to be expected because the injection site was intra-articular and intramuscular and furthermore ropivacaine was combined with epinephrine which causes a contraction of the blood vessels which leads to slower release from the injection site.

There is not much data to compare our results with. The studies that focus on ropivacaine infusion and injection site do not focus on specific pharmacokinetics and pharmacodynamics.

Two studies focus on the pain intensity after intra- vs. extra-articular continuous infusion [22, 23]. One study has shown that after synovial procedures, where an extensive raw surface is created, there is an increased absorption of bupivacaine. Furthermore tourniquet inflation seems to reduce absorption, however longer tourniquet ischemia may lead to enhanced post-ischemic reperfusion with enhanced systemic absorption. In
our studies no differences in tourniquet inflation and duration was seen. Cederholm et al. [24] investigated the different concentrations of ropivacaine in combination with or without epinephrine on the skin blood flow. They found a reduction of skin blood flow compared to saline. This reduction was more pronounced with lower concentrations of ropivacaine (< 0.5%: tested 1%, 0.5%, 0.375%, 0.125% and 0.063%). All the above factors except for the concentrations in the LIA injections were equal, 0.375% in 100 mL (375 mg) and 0.2% in 150 mL (300 mg) in both studies.

Six other small studies have so far been published where blood has been collected postoperatively in wound drains and analyzed for ropivacaine [8, 9, 10, 11, 12, 13]. The ropivacaine doses for LIA ranged between 150 and 400 mg. Only in three studies shed blood was returned to the patients after LIA with ropivacaine. In comparison with the other studies the total ropivacaine amount in shed blood found in the current study was higher (median 8.2 mg, range 1.3 – 19.5 mg) than in the other studies (range 0.28 – 7 mg). The only other study that looked at unbound ropivacaine concentrations had a lower unbound ropivacaine plasma concentration (< 0.038 mg/L) than the 0.043 mg/L unbound ropivacaine in the current study without reinfusion. The mean calculated unbound plasma concentration after potential reinfusion was 0.11 mg/L. This is remarkable because our ropivacaine concentrations in shed blood (range 0.27 – 12.8 mg) are comparable with the ranges found by Breindahl et al. [9] (0.49 – 7.2 mg), but our plasma concentrations are much higher. Also, no explanation could be found when comparing study data in which the same retransfusion systems are being compared.

Furthermore, it is quite extraordinary that the unbound ropivacaine plasma concentration that is related to systemic pharmacodynamic effects and toxicity is scarcely measured in studies, because total and unbound concentrations are related to each other.

Limitations of our study include the assumption of reinfusion that has not actually been performed out of safety considerations. Also this study has no large groups of patients studied and furthermore pain protocols were not completely comparable with the first study performed. Additionally to the performed laboratory analysis actual AAG concentrations would have given us more insight in the apparent anomalies in free-fraction.

**Conclusion**

Based on unbound ropivacaine concentrations and the absence of signs of ropivacaine-related cardiotoxicity, the combination of LIA and reinfusion presented herein can be considered as safe. However, as seen in the comparison of this study with the former study performed by this same group, differences in pain protocol lead to unexplainable changes in the pharmacokinetic evaluation. The mode of administration is of greater importance than expected which is difficult to explain because of an unknown gradient/hydrostatic effect in the human body. The pharmacokinetic analysis showed that the results are unpredictable because they depend upon unknown variables.

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**Conflicts of interest**

None to declare.

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