

Pharmacokinetic Profile of Liposome-Encapsulated Ropivacaine after Maxillary Infiltration Anaesthesia

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O objetivo do presente estudo foi determinar os parâmetros farmacocinéticos da ropivacaína encapsulada em lipossomas após anestesia local em 14 voluntários sadios. Neste estudo randomizado, cruzado e duplo-cego, os voluntários receberam anestesia infiltrativa na maxila de ropivacaína a 0,5% encapsulada em lipossomas e ropivacaína 0,5% com epinefrina a 1:200.000 em duas sessões distintas. Amostras de sangue foram coletadas antes e após (de 15 a 1440 min) a administração das formulações de ropivacaína. A quantificação da concentração plasmática de ropivacaína foi feita por meio de HPLC com detecção por UV. Os parâmetros farmacocinéticos AUC_{0-24} (área sob a curva de concentração \times tempo do tempo 0 até 24 horas), $AUC_{0-\infty}$ (área sob a curva de concentração \times tempo do tempo 0 até o infinito), C_{max} (concentração máxima da droga), CL (clearance renal), T_{max} (tempo em que ocorre a concentração máxima); $t_{1/2}$ (meia vida de eliminação) e V_d (volume de distribuição) foram analisados pelo teste de Wilcoxon. Nenhuma diferença ($p > 0,05$) foi observada entre as duas formulações em cada parâmetro farmacocinético avaliado e as concentrações plasmáticas de ropivacaína, considerando cada período de tempo. Ambas as formulações apresentaram perfil farmacocinético semelhante, indicando que a formulação lipossomal poderia ser uma opção mais segura para o uso deste anestésico local, devido à ausência de vasoconstritor.

The aim of this study was to determine the pharmacokinetic parameters of liposomal ropivacaine after dental anesthesia in 14 healthy volunteers. In this randomized, double-blind and crossover study, the volunteers received maxillary infiltration of liposome-encapsulated 0.5% ropivacaine and, 0.5% ropivacaine with 1:200,000 epinephrine in two different sessions. Blood samples were collected before and after (from 15 to 1440 min) the administration of either ropivacaine formulation. HPLC with UV detection was used to quantify plasma ropivacaine concentrations. The pharmacokinetic parameters AUC_{0-24} (area under the plasma concentration \times time curve from baseline to 24 h), $AUC_{0-\infty}$ (area under the plasma concentration-time curve from baseline to infinity), C_{max} (maximum drug concentration), CL (renal clearance), T_{max} (maximum drug concentration time), $t_{1/2}$ (elimination half-life) and V_d (volume of distribution) were analyzed using the Wilcoxon signed-rank test. No differences ($p > 0.05$) were observed between both formulations for any of the pharmacokinetic parameters evaluated and plasma ropivacaine concentrations, considering each period of time. Both formulations showed similar pharmacokinetic profiles, indicating that the liposomal formulation could be a safer option for use of this local anesthetic, due to the absence of a vasoconstrictor.

Keywords: ropivacaine, local anesthesia, dentistry, liposome, pharmacokinetic

Introduction

Prolonged-action local anesthetic is required when postoperative pain and discomfort are expected, especially after major surgical procedures.¹ In many countries, bupivacaine, the racemic mixture of *S*- and *D*-bupivacaine, is the only long-acting local anesthetic available in dental practice.

Ropivacaine (RVC), another long-acting local anesthetic of the cyclic aminoamide family, is synthesized in the *S*-enantiomer form and presents lower toxicity to the cardiovascular and central nervous systems, compared to bupivacaine.²

Traditionally, most of local anesthetic formulations are administered together with a vasoconstrictor in order to increase anesthesia duration and to reduce systemic absorption rate.³ It was recently demonstrated that the use of these formulations increased, especially those containing epinephrine 1:100.000.⁴ In spite of the known safety of these drugs, there is evidence concerning adverse reactions involving the autonomic system, been the most common those associated to the medical status of the patient.⁵

Alternative drug delivery systems, such as liposomes, have been used to prolong the duration of action of many drugs, including local anesthetics.⁶ Liposomes are phospholipid vesicles that have been demonstrated to be effective drug carriers, improving the effectiveness and reducing the toxicity of anesthetics in both cardiovascular and central nervous systems.⁶⁻¹⁰ These vesicles are nontoxic and nonimmunogenic because their components (phosphatidyl choline and cholesterol) are also found in biological membranes.¹¹ These characteristics have been demonstrated *in vivo* (animal models) for liposome-encapsulated bupivacaine using multilamellar vesicles,¹²⁻¹⁶ and large unilamellar vesicles.¹⁷

Previous authors have shown that liposomal encapsulation of bupivacaine altered its pharmacokinetic profile after extradural injection in rabbits, resulting in lower concentrations of the drug in plasma, liver and myocardium.¹⁸ Grant and co-workers¹⁶ observed that when encapsulated in liposomes, bupivacaine remained at the injection site for a significantly longer period of time after subcutaneous injection in mice.

Attempting to simulate an accidental intravascular injection of a local anesthetic, Boogaerts and co-workers⁸ assessed the acute CNS (central nervous system) and cardiac toxicities induced by intravenous infusion in rabbits of 0.25% bupivacaine, with and without epinephrine (1:200,000), compared to liposome-encapsulated bupivacaine. They demonstrated a reduction of CNS and cardiac toxicities using liposome-encapsulated

bupivacaine. The addition of epinephrine to the plain solution did not decrease the CNS and cardiac toxicities induced by bupivacaine.

It was recently demonstrated in animal studies, which used sciatic and infraorbital nerve blockades, that encapsulation of ropivacaine into unilamellar vesicles increased the duration and the intensity of analgesic effects.⁶

Although long-acting local anesthetics are normally used in low doses in dentistry, high doses may be required for removal of four impacted third molars in a single session.¹⁹ According to Zink and Graf,²⁰ ropivacaine seems to have the greatest margin of safety of all the long-acting local anesthetics, and could be useful in lengthy dental procedures.

The present study is the first attempt to measure the pharmacokinetic parameters of ropivacaine after maxillary infiltration anesthesia using a liposome-encapsulated formulation in healthy volunteers. The pharmacokinetic parameters of an RVC formulation containing epinephrine (vasoconstrictor) were also assessed for comparison.

Experimental

Materials

RVC hydrochloride was donated by Cristalia Prod. Quim. Farm. Ltda (Itapira, SP, Brazil). Egg phosphatidylcholine (EPC), cholesterol (Ch) and α -tocopherol (α -T) were purchased from Sigma Chemical Company (St Louis, MO). All other reagents used were of analytical grade.

RVC-liposome formulation

The liposomal RVC formulations were prepared as described by Araújo and co-workers.⁶ Briefly, EPC-Ch- α -T (4:3:0.07, molar ratio) films were obtained by evaporating stock chloroform solutions under a stream of wet nitrogen, followed by vacuum for 2 h. Films were suspended in HEPES buffer (20 mmol L⁻¹, 154 mmol L⁻¹ NaCl, pH 7.4), and multilamellar vesicles were obtained after vortexing at ambient temperature (5 min, 25 °C). Large unilamellar vesicles were prepared by extrusion (15 cycles) of the multilamellar vesicles within 400 nm membrane filters (25 °C), using a Lipex Biomembranes Inc. (Vancouver, Canada) extruder. RVC was added directly to the liposomes after extrusion, to reach a final concentration of 0.5%. This formulation was sterilized by autoclaving (121 °C, 1 atm during 15 min), as described previously by Cereda and co-workers.¹⁰

The following liposomal characterization was previously determined by Araújo and co-workers.⁶ The mean diameter and size distribution analysis, performed

by photon correlation spectroscopy (Malvern Mastersizer-Malvern Instruments, France), showed an average size of 371 ± 7 nm (85% of the population), which did not change after RVC encapsulation. All the measurements presented a homogeneous distribution of the liposomal population obtained (polydispersity index of 0.12-0.17). The encapsulation efficiency of RVC into the liposomes, of samples containing 2 mmol L⁻¹ RVC and 4 mmol L⁻¹ liposomal suspensions determined by ultracentrifugation, was around 24%.

Subjects

This research was approved by the Ethical Committee of Piracicaba Dental School, University of Campinas (approval #164/2006). Fourteen healthy volunteers (seven males and seven females) aged 24 (± 3.1) years old were selected, and signed a written informed consent. The volunteers presented no systemic or oral disorders, had no history of allergy to any of the local anesthetics used, and were not taking any medication, as determined by oral questioning and by their documented health histories.

Prior to the beginning and right after the end of the study, all the subjects were submitted to laboratory tests to confirm that they were in good health and that the females were not pregnant. The same tests were performed at the end of the study to ensure that all results previously obtained were not altered by the anesthetic formulations tested. These tests included cross-reactive protein, blood-hemoglobin, lymphocyte count, platelet count, erythrocyte sedimentation rate, serum (S)-sodium, S-potassium, S-chloride, S-albumin, S-alkaline phosphate, S-gamma-glutamyl-transferase, S-aspartate transaminase, S-alanine transaminase, S-creatine, plasma glucose, urea, cholinesterase, total protein, bilirubin, uric acid, urine glucose, urine leukocyte count, urine protein, and urine hemoglobin. Serology tests for human immunodeficiency virus and hepatitis B and C were also performed. Female subjects underwent a urine β HCG pregnancy test.

Ambulatory procedures

Anesthetic procedures

In this double-blind and crossover study, the volunteers randomly received 1.8 mL of 0.5% ropivacaine with 1:200,000 epinephrine, and liposome-encapsulated 0.5% ropivacaine, for infiltration anesthesia at the apex of the right maxillary canine, in two different sessions spaced one week apart.

Ropivacaine with 1:200,000 epinephrine was obtained by simple dilution of the commercially available solution

of ropivacaine (Naropin® 10 mg mL⁻¹, AstraZeneca, São Paulo, Brazil) immediately before application. Under sterile conditions, 5 mL of 1% ropivacaine was diluted with 5 mL of 1:100,000 (v/v) epinephrine (Drenalin®, Ariston Ind. Quim. Farm. Ltda, São Paulo, SP, Brazil).

The local anesthetics (1.8 mL) were placed into coded sterile 3 mL Luer-Lok syringes (Becton Dickinson, Curitiba, Brazil) fitted with disposable needles (30G, one-inch, Becton-Dickinson Company, Franklin Lakes, NJ, USA). After topical anesthesia on the injection site with 20% benzocaine, the formulations were injected at the maxillary buccal fold of the right-canine region at an injection rate of 1 mL min⁻¹. The same operator performed the maxillary infiltration anesthesia in all the subjects.

Blood sampling and drug analysis

Blood samples (4.5 mL) from a forearm vein were collected with a heparinized cannula before and 15, 30, 45, 60, 75, 90, 120, 240, 420, 600 and 1440 min after administration of either of the ropivacaine formulations. 0.4 mL of a heparinized saline solution (0.9% NaCl and heparin, 9.8:0.2 v/v) was injected into the cannula after each blood sampling, to prevent clotting. The last sample was obtained using a sterile syringe and needle. Immediately after each blood collection, the samples were centrifuged at $3000 \times g$ for 15 min, and plasma was removed and stored at -70 °C.

Detection of ropivacaine concentrations in the plasma samples was performed by high-performance liquid chromatography (HPLC), following a method adapted from Kawata and co-workers.²¹ Briefly, chromatographic separations were carried out using an ODS column (TSK-GEL, 150 mm, 4.6 mm i.d., TOSOH) at room temperature. The detection wavelength was set at 215 nm. The analytical calibration curve was obtained by diluting ropivacaine in drug-free human plasma (concentration range: 0.03-10 μ g mL⁻¹; concentration used 10.0, 5.0, 2.5, 1.25, 0.62, 0.31, 0.15, 0.075, 0.030 μ g mL⁻¹, peak area = $20.21[\text{RVC}] + 0.50$, $r = 0.9998$, $n = 3$). The specificity was tested in the presence of plasma components, and it was demonstrated that these factors did not affect RVC quantification. The limit of detection was determined according to the ICH guidelines (2005).²²

The mobile phase consisted of acetonitrile, methanol and 0.05 mol L⁻¹ phosphate buffer (10:30:60, v/v), adjusted to pH 4.0 and pumped at a flow rate of 1.0 mL min⁻¹. The HPLC system consisted of a Varian 9012 pump, a Varian diode-array detector (ProStar 335 DAD) coupled with Galaxie software integrator, and a Varian autosampler (ProStar 410).

Plasma samples (250 μL) were extracted by adding 125 μL of 0.1 mol L^{-1} sodium hydroxide in a 2.0 mL tube. The mixture was submitted to agitation and addition of 1 mL ethylacetate in order to extract ropivacaine. The 2.0 mL tube was vortexed for 1.5 min and centrifuged at $1500\times g$ for 6 min. The upper organic phase was transferred to another 2.0 mL tube, and 1 mL of ethylacetate was added. The upper organic phase was removed to a new 2.0 mL tube. After evaporation to dryness at room temperature, the residue was dissolved in 30 μL of the mobile phase, injected into the HPLC system and quantified using the analytical curve.

Pharmacokinetic and statistical analyses

The following pharmacokinetic parameters were evaluated by computer software (PK Solutions, non-compartmental pharmacokinetics data analysis, 2001; Summit Research Services, Montrose, CO, USA): C_{max} (maximum drug concentration); T_{max} (maximum drug concentration time); AUC_{0-24} (area under the plasma concentration- time curve from baseline to 24 h); $\text{AUC}_{0-\infty}$ (area under the plasma concentration-time curve from baseline to infinity); CL (renal clearance); $t_{1/2}$ (elimination half-life) and Vd (volume of distribution).

Statistical analysis was performed using the Student's *t*-test in order to compare the ropivacaine concentrations between the groups at each time interval. Pharmacokinetic parameters were compared using the Wilcoxon signed-rank test. The significance level was set at 5%, and the tests were performed with BioEstat 5.0 (Fundação Mamirauá, Belém, PA, Brazil)

Results and Discussion

In an earlier study, Araújo and co-workers⁶ demonstrated that the size distribution of liposomal formulations containing RVC presented two modes, one with a maximum at 371 nm (85%), and another with a peak at 128 nm (15%). The efficiency of encapsulation was around 24%, which was sufficient to modify the release profile of the pharmaceutical, with a reduction of the release rate over a one-hour period from 76 to 58%. In the same study it was also shown that, compared to RVC alone, the liposomal RVC formulation increased the duration and intensity of analgesic effects in sciatic and infraorbital nerve blocking experiments.

Extending the earlier work of Araújo and co-workers⁶ here we report on the first attempt to assess the pharmacokinetic parameters of ropivacaine after maxillary infiltration anesthesia using a liposome-encapsulated ropivacaine formulation in healthy volunteers, comparing

the results with a commercial RVC formulation containing epinephrine vasoconstrictor.

The calibration curve for determination of plasma ropivacaine (Figure 1a) was linear in the concentration range 0.030-10 $\mu\text{g mL}^{-1}$ ($R^2 = 0.9998$), showing that the HPLC procedure was sufficiently sensitive to quantify ropivacaine in plasma. The concentration of RVC was determined using the equation: peak area = 20.21[RVC] + 0.50 ($n = 3$). The limit of detection of ropivacaine in plasma, determined as described by ICH guidelines (2005),²² was 0.030 $\mu\text{g mL}^{-1}$. Its retention time was 7 min, and no interference from other plasma components was observed (Figure 1b). Selectivity and sensitivity were similar to those previously reported by Kawata and co-workers.²² The detection limit for ropivacaine observed in our study (30 ng mL^{-1}) was close to the limit observed by those authors (25 ng mL^{-1}).

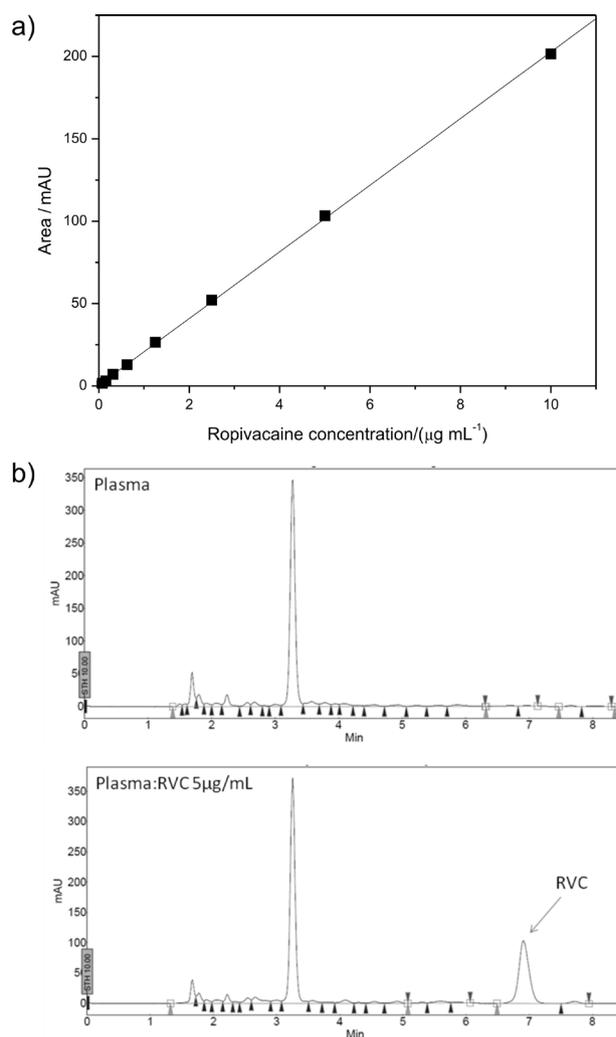


Figure 1. a) Calibration curve of plasma concentration of ropivacaine and peak area as measured by HPLC (see Methods section); b) HPLC chromatogram of plasma and plasma with 5 $\mu\text{g mL}^{-1}$ of RVC (HPLC conditions as described in methods section).

Mean plasma concentrations of RVC are plotted as a function of time in Figure 2 for both the liposomal RVC formulation, and for RVC with epinephrine. The pharmacokinetic parameters (C_{\max} , T_{\max} , AUC_{0-24} , $AUC_{0-\infty}$, CL, $t_{1/2}$ and Vd) were subsequently calculated (Table 1). No statistically significant differences ($p > 0.05$) were observed between the formulations for all of the pharmacokinetic parameters evaluated.

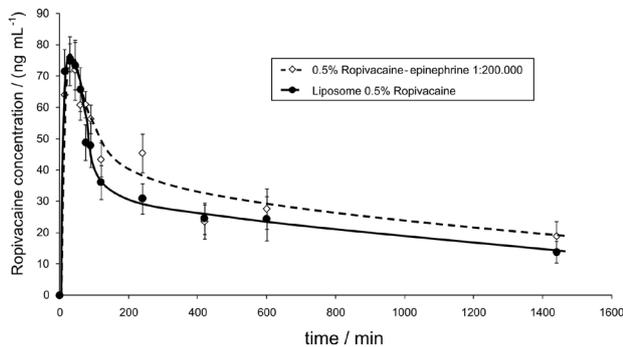


Figure 2. Mean (\pm SEM) values and regression curve for plasma concentration of ropivacaine after maxillary infiltration of liposome-encapsulated 0.5% ropivacaine and 0.5% ropivacaine with 1:200,000 epinephrine.

Kawata and co-workers²² studied the topical application of 5 mL of 0.5% viscous ropivacaine, held in the mouths of only two volunteers for 10 min. They observed a C_{\max} of $107 (\pm 25.5)$ ng mL⁻¹ and a T_{\max} of $50 (\pm 14.1)$ min. Despite methodological differences, these results were similar to those observed in the present study (Table 1).

Many substances are added to local anesthetics to improve their efficacy by modifying their pharmacodynamic

and pharmacokinetic properties, with epinephrine being the most commonly used. Lee and co-workers²³ demonstrated that the addition of epinephrine significantly reduced the concentration of ropivacaine after epidural anesthesia in humans during the first hour, in both arterial and venous blood. Here, we have found no difference between the pharmacokinetic profiles of both formulations, showing that liposome encapsulation of ropivacaine was as effective as epinephrine in reducing ropivacaine absorption.

Several animal studies have also demonstrated that liposomal encapsulation of long-acting local anesthetics is able to alter their pharmacokinetic behavior, with lower plasma concentrations and toxicity compared to the plain solution.^{13,15,16,24}

The use of a liposomal formulation instead of epinephrine containing local anesthetic could be an advantageous since, it was demonstrated that local anesthetic solutions containing sympathomimetic vasoconstrictors could promote changes in heart rate and blood pressure,^{25,26} and dysrhythmias,^{27,28} increasing the risk of morbidity, especially in cardiovascular patients when higher doses or more stressful procedures are being carried out.²⁹

Despite differences in liposolubility, partition coefficient, and some other physico-chemical/pharmacokinetic parameters, ropivacaine and bupivacaine have some similarities, such as pka , protein binding and molecular weight. In addition, they have similar onset times and blocking durations when used in epidural blockade.² No differences in anesthetic efficacy parameters were found between these two local anesthetics after maxillary infiltration.³⁰

Table 1. Median pharmacokinetic parameters following maxillary infiltration of liposome-encapsulated 0.5% ropivacaine and 0.5% ropivacaine with 1:200,000 epinephrine.

Pharmacokinetic parameters	Groups	Median	Quartiles		<i>p</i> value
			First	Third	
C_{\max} (ng mL ⁻¹)	liposome-encapsulated 0.5% ropivacaine	92.9	82.7	97.7	0.6378
	0.5% ropivacaine with 1:200,000 epinephrine	93.4	63.2	114.7	
T_{\max} (min)	liposome-encapsulated 0.5% ropivacaine	30.0	15.0	56.3	0.9645
	0.5% ropivacaine with 1:200,000 epinephrine	37.5	30.0	45.0	
AUC_{0-4} (ng-min mL ⁻¹)	liposome-encapsulated 0.5% ropivacaine	40.4	26.3	55.2	0.6378
	0.5% ropivacaine with 1:200,000 epinephrine	32.4	20.1	44.0	
$AUC_{0-\infty}$ (ng-min mL ⁻¹)	liposome-encapsulated 0.5% ropivacaine	71.9	28.1	138.6	0.7794
	0.5% ropivacaine with 1:200,000 epinephrine	78.5	4.9	102.6	
Vd (mL kg ⁻¹)	liposome-encapsulated 0.5% ropivacaine	2.6	1.5	4.4	0.5754
	0.5% ropivacaine with 1:200,000 epinephrine	2.8	1.5	13.8	
$T_{1/2}$ (min)	liposome-encapsulated 0.5% ropivacaine	869	349	1512	0.9738
	0.5% ropivacaine with 1:200,000 epinephrine	868	142	1498	
CL (mL min ⁻¹ kg ⁻¹)	liposome-encapsulated 0.5% ropivacaine	0.0013	0.001	0.0029	0.8182
	0.5% ropivacaine with 1:200,000 epinephrine	0.0017	0.0009	0.0041	

Grant and co-workers¹³ compared 0.5% plain bupivacaine with 2% liposomal bupivacaine, and even with a 4-fold higher concentration of bupivacaine in the liposomal formulation the plasmatic levels of bupivacaine decreased when the liposomal formulation was used for wound analgesia in rats. In the present study, the pharmacokinetics of liposome-encapsulated ropivacaine was comparable to that of epinephrine-associated ropivacaine, suggesting the same profile observed by Grant and co-workers¹³ with encapsulation into liposome vesicles delaying transfer of the anesthetic into the blood.

According to Grant and Bansinath³¹ liposome composition affects the release kinetics of encapsulated drugs. Drugs tend to be released more rapidly from liposomes composed of a single lipid bilayer, while the release tends to be retarded from multilamellar vesicles.^{13,15} In our study, unilamellar vesicles were able to delay ropivacaine absorption, since both formulations presented similar pharmacokinetic profiles. Further studies are necessary to evaluate how the changes in liposome composition affect both the absorption of ropivacaine from the injection site and its plasmatic concentration after dental anesthesia.

Another factor that could maintain a constantly low plasma concentration for hours, resulting in a prolonged effect, is the percentage of encapsulated drug.³² According to a previous study⁶ that used the same liposome employed in the present study, the encapsulation efficiency of ropivacaine was 24%, while other reports in the literature have given higher encapsulation efficiency values.^{16,33,34} Ostergaard and co-workers³⁵ showed that ropivacaine had lower liposome affinity than bupivacaine. De Araújo and co-workers⁶ also suggested that enhancement of liposome encapsulation could prolong the analgesic effect and decrease cytotoxicity.

Conclusions

In conclusion, the present work demonstrates that the HPLC technique can be used to quantify RVC in plasma samples during pharmacokinetic experiments. Results showed that liposome-encapsulated ropivacaine had a pharmacokinetic profile that was similar to that of ropivacaine associated with epinephrine, suggesting that liposomal formulations could be a safer alternative during clinical use of this local anesthetic.

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