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RESEARCH ARTICLE

Pharmacokinetic and local toxicity studies of liposome-encapsulated and plain mepivacaine solutions in rats

Giovana Radomille Tofoli^{1,4}, Cíntia Maria Saia Cereda¹, Daniele Ribeiro de Araujo^{1,2}, Eneida de Paula¹, Rui Barbosa Brito Júnior³, José Pedrazzoli Júnior⁴, Eduardo Meurer⁴, Fábio Alessandro Proença Barros⁵, Francisco Carlos Groppo⁶, Maria Cristina Volpato⁶, and José Ranali⁶

¹Department of Biochemistry, Institute of Biology, State University of Campinas, Campinas, SP, Brazil, ²Human and Natural Sciences Center, Federal University of ABC, Santo André, SP, Brazil, ³Department of Molecular Biology, São Leopoldo Mandic Dental Research Institute, Campinas, SP, Brazil, ⁴UNIFAG - Clinical Pharmacology and Gastroenterology Unit, São Francisco University, Bragança Paulista, SP, Brazil, ⁵Core Clinical Research, Bragança Paulista, SP, Brazil, and ⁶Department of Physiological Sciences, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, Brazil

Abstract

The pharmacokinetics and the local toxicity of commercial and liposome-encapsulated mepivacaine formulations injected intra-orally in rats were studied. Animals were divided in groups ($n=4-6$) and treated with 0.1 mL of the formulations: 2% mepivacaine with 1:100,000 epinephrine ($MVC_{2\%EPI}$), 3% mepivacaine ($MVC_{3\%}$), and 2% liposome-encapsulated mepivacaine (MVC_{LUV}). The results showed that the 2% liposome-encapsulated mepivacaine reduced C_{max} , prolonged $AUC_{0-\infty}$ and $t_{1/2}$, compared with 3% plain and 2% vasoconstrictor-associated mepivacaine, after intraoral injection. In addition, it was also observed that liposomal mepivacaine might protect the tissue against local inflammation evoked by plain or vasoconstrictor-associated mepivacaine, giving supporting evidence for its safety and possible clinical use in dentistry.

Keywords: Local anesthetics; liposomes; mepivacaine; pharmacokinetics; local toxicity

Introduction

Local anesthesia is the most widely used method for controlling pain during the intra-operative period (Whiteside & Wildsmith, 2001). Although it is an effective method, the conventional local anesthetic agents clinically used are characterized by short duration of analgesia and both systemic and local toxicity (Cox et al., 2003). For this reason, care should be taken regarding the right choice of local anesthetic with a preference for one that is most effective and has the lowest concentration of anesthetic salt and vasoconstrictor.

Liposomes have been extensively described in the literature for their use as drug carriers (Torchilin, 2005; Samad et al., 2007). The distinct advantage of liposomes

is their structural versatility combined with their ability to encapsulate different compounds, including local anesthetics (Felnerova et al., 2004).

The advantages of liposome encapsulated local anesthetics are slow drug release, prolonged anesthetic effect, and reduced toxicity for both the cardiovascular and central nervous system (Gesztos & Mezei, 1988; Langerman et al., 1992; Boogaerts et al., 1993a; b; Mowat et al., 1996; Bucalo et al., 1998; de Araujo et al., 2003). The vasoconstrictor is usually absent in liposome encapsulated anesthetics formulations. The pharmacological effectiveness of many liposomal-anesthetic preparations has been demonstrated by studies using animals (Mashimo et al., 1992; Boogaerts et al., 1993a; b; Malinovsky et al., 1997; Grant & Bansinath, 2001;

Address for Correspondence: Dr Eneida de Paula, Department of Biochemistry, Institute of Biology, State University of Campinas, PO Box 6109, CEP 13083-970, Campinas, SP, Brazil. Tel: +55 19 3521 6143. Fax: +55 19 3521 6129. Email: depaula@unicamp.br

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Yu et al., 2002) and human beings (Lafont et al., 1994; 1996; Boogaerts et al., 1994; Grant et al., 2004).

Mepivacaine was the second amide to be introduced as a local anesthetic. It has a fast onset; similar to that of lidocaine, and it is a popular choice for a wide range of regional anesthetic procedures due to its safety (McLure & Rubin, 2005). Liposome encapsulated mepivacaine increased the analgesic effect in relation to amplitude and anesthetic duration (de Araujo et al., 2004). Moreover, encapsulation of mepivacaine in liposomes greatly enhanced the infraorbital nerve block in rats when compared with the plain solution of this local anesthetic (Cereda et al., 2006).

In developing a new pharmaceutical form, such as liposomal mepivacaine, the International Conference on Harmonization requires pharmacokinetic and local toxicity assessments in animals. Thus, the aims of this study were to determine plasma concentrations and to evaluate the tissue reaction induced by liposomal mepivacaine compared with commercial formulations after intra-oral injections in rats, looking forward to its clinical use in dentistry.

Materials and methods

2% Mepivacaine with 1:100,000 epinephrine-MVC_{2%EPI} (Mepiadre®-DFL Indústria e Comércio S.A., Rio de Janeiro, RJ, Brazil) and plain 3% mepivacaine-MVC_{3%} (MepiSV®-DFL Ind. Com. S.A., Rio de Janeiro, RJ, Brazil) were purchased in local commerce. Liposomal suspension of 2% mepivacaine-MVC_{LUV} was prepared with mepivacaine hydrochloride salt supplied by Cristália Ind. Quim. Farm. Ltda (Itapira, SP, Brazil). EDTA titriplex was purchased from Merck KGaA and sodium thiopental solution (Thiopentax®) from Cristália Ind. Quim. Farm. Ltda. Alpha-chloralose, urethane, alpha-tocopherol acetate, egg phosphatidylcholine, and cholesterol were obtained from Sigma Chem. Co (St Louis, MO). Acetonitrile used was of HPLC grade. Other reagents were of analytical grade.

Liposomal formulations: Preparation and sterilization

A dry lipid film, containing egg phosphatidylcholine, cholesterol, and α -tocopherol at a 4:3:0.07 molar ratio was prepared by solvent evaporation under nitrogen flow (Boogaerts et al., 1993a; b; Fraceto et al., 2002). Multilamellar liposomes were obtained by adding 20 mM HEPES buffer, pH 7.4 (containing 154 mM NaCl) to the dry lipid film and vortexing the mixture during 5 min. Unilamellar-liposome vesicles (0.4 μ m) were prepared by extrusion (12 cycles through 400 nm polycarbonate membrane, at 25°C) of the multilamellar vesicles. The total lipid concentration in the LUV was 5 mM

(Cereda et al., 2008). Mepivacaine was added directly to the liposomes after extrusion, up to a concentration of 2% (corresponding to 70.7 mM of mepivacaine). Liposome formulations were incubated for 12 h and stored at 4°C until further use.

The preparations were sterilized by autoclaving (121°C, 1 atm, and 15 min). Afterward, the sterility was evaluated by microbiological test with Brain-Heart Infusion (BHI) and apyrogenicity was assessed by Endosafe® Limulus Amoebocyte Lysate Test.

Animals

Male Wistar rats, 250–350 g, were obtained from CEMIB-UNICAMP (Centro de Bioterismo, State University of Campinas, UNICAMP, SP, Brazil) and they received water and food *ad libitum*. Animals were randomly divided in groups ($n=4-6$ /group) and protocols were approved by the Institutional Committee for Ethics in Animal Research of the State University of Campinas (protocols #1067-1 and 871-1), which follows the recommendations of the Guide for the Care and Use of Laboratory Animals.

Pharmacokinetic study: Experimental design

The animals were divided into three groups and they received intra-orally 0.1 mL of one of the following treatments: Group 1, 3% plain mepivacaine (MVC_{3%}); Group 2, 2% mepivacaine with 1:100,000 epinephrine (MVC_{2%EPI}); and Group 3, liposomal 2% mepivacaine (MVC_{LUV}). The selection of local anesthetic concentration in the liposomal formulation was determined by the efficacy of 2% liposomal mepivacaine shown in previous studies (de Araujo et al., 2004; Cereda et al., 2006).

General anesthesia was performed with α -chloralose (50 mg/kg) and urethane (1 g/kg) before the injections. A prior pilot assay (data not shown) showed no influence of general anesthesia on the mepivacaine pharmacokinetics. An intravascular catheter was inserted in the femoral vein and 0.2 mL blood samples were collected right before the anesthetic injection (0 min) and at 15, 30, 45, 60, 120, 180, 240, 300, 360, and 420 min after the anesthetic injection. These intervals were defined to obtain 10 samples between the baseline (0 min) and ~ 4 -times the $t_{1/2}$ (half-life) of mepivacaine (1.5 h) (Covino & Vassallo, 1976). This geometric progression scheme is commonly used and supplies information about drug disposition (Bourne, 1995). Samples were transferred into heparinized tubes and the separated plasma was stored at -70°C .

LC-MS/MS assay: Apparatus and chromatographic conditions

A Waters® HPLC system (2795) coupled to a Micromass Quattro Premier XE triple stage quadrupole mass

spectrometer, equipped with API electrospray source was used. Separations were performed using a C18 polaris 5 μm , 50 mm \times 2 mm i.d. The mobile phase was 80% acetonitrile and 20% water (with 0.1% formic acid). The total run time was 3.0 min. The mass spectrometer was run in the positive mode (ES+) and set for multiple reaction monitoring (MRM). The full-scan single-mass spectrum and the daughter ion-mass spectrum for mepivacaine and Venlafaxine (internal standard; IS) were (m/z) 247.5 > 150.5 and 278.1 > 215.31, respectively. The data were integrated using the MassLynx 4.1 (Waters®) software. The WinNonlin 5.2 computer program (Pharsight, USA) was used to calculate the pharmacokinetic parameters. Quality control samples (QC) prepared by mixing drug-free plasma with appropriate volumes of working solutions were used to validate the method.

Plasma sample preparation

The extraction procedure was performed by transferring 80 μL of plasma to test tubes, followed by the addition of 25 μL of IS working solution. The samples were vortexed for 1 min and 50 μL of 1 M NaOH were added followed by 1 min vortexing; 1000 μL of hexane/ethyl acetate (1:1, v/v) was added to each tube. After centrifugation (5 min), 800 μL of the organic phase were removed to clean tubes and the solvent was evaporated under nitrogen flow (N_2) at 37°C. Each sample was then diluted in 100 μL of mobile phase, agitated for 2 min, and transferred to LC-MS/MS system vials, for further injection (5 μL).

Local toxicity study: Surgical procedure

Slightly general anesthesia was induced by an intraperitoneal injection of sodium thiopental solution (40 mg/kg), before the administration of the local anesthetic formulations. The animals were divided into four groups and received 0.1 mL of one of the following formulations: MVC_{2%EPI}-2% mepivacaine with 1:100,000 epinephrine; MVC_{3%}-3% mepivacaine; MVC_{LUV}-liposomal 2% mepivacaine; and LUV-mepivacaine-free liposome.

The animals received one of the formulations in the oral mucosa of the upper right first molar, as previously described by Cereda et al. (2008). The same amount of saline solution (NaCl 0.9%) was administered in the left side as control. Animals were sacrificed under anesthesia (urethane 1 g/kg and alpha-chloralose 50 mg/kg) 6 h, 24 h, and 4 days after treatment, and the maxilla bones along with soft tissues were removed. Before the administration of the formulations, their pH values were measured with a pHmeter ORION®, model 290A, with a microelectrode LAZAR BNC.

Histological analysis

The samples were fixed with Bowin solution during 24 h and with 10% formalin solution for 48 h, and decalcified with EDTA titriplex (Merck KGaA). Five cross-sections (6 μm thick, 40 μm deep) were obtained from each animal sample. The cross-sections were stained with hematoxylin and eosin. The cross-sections were submitted to qualitative analysis in order to evaluate the intensity of the leucocitary infiltration and/or any area of necrosis. The cross-sections were photographed by a photomicroscope (Zeiss-AXIOSKOP2-PLUS). The analyzed region was the site of the injection and it enclosed the connective tissue in the most internal portion of the anterior maxillary fornix. A preliminary pilot study conducted using methods developed in previous studies (Scheib & Garner, 2004; Shipper et al., 2005) allowed the use of a qualitative score of the local tissue inflammation. The score was defined based on the following descriptions: (1) no infiltrate; (2) minimal infiltrate; (3) mild infiltrate; (4) severe infiltrate; and (5) severe infiltrate with necrosis areas (Cereda et al., 2008).

The images were codified and two individuals blinded to treatment conditions evaluated the images according to the qualitative score previously described. The replication of the classification method between the two examiners was calculated by the intra-class correlation test for continuous data.

Statistical analysis

The plasmatic mepivacaine concentrations were analyzed by one-way ANOVA and the Tukey-Kramer test (post-hoc) considering each period of time separately ($\alpha = 0.05$). The pharmacokinetic parameters (C_{max} , T_{max} , AUC_{0-420} , $\text{AUC}_{0-\infty}$, $t_{1/2}$) were analyzed using the Kruskal-Wallis test and Student-Newman-Keuls as post hoc test ($\alpha = 0.05$). The results obtained in each time interval (6 h, 24 h, and 4 days) were compared considering each group and considering the control side. Data were analyzed with the Kruskal-Wallis test considering each group (inter-group analysis). The tissue reaction was also analyzed by Wilcoxon paired test considering treated and control sides (intra-group analysis). The level of significance was set at 5%.

Results and discussion

Pharmacokinetic study

The calibration graph for mepivacaine was generated with increasing amounts of the drug standards. A good response over the range of 1.0–1000.0 ng/mL was shown and presented linearity with high correlation coefficient ($r = 0.99972$). The precision and accuracy of the

method were assessed by determination of seven concentrations in three independent series of spiked serum samples. The accuracy and precision were calculated to be from 92.45–105.5% and from 0.4–9.8%, respectively. The 95% confidence intervals were 92.97–106.83% considering accuracy and 6.84–7.03% for precision. In addition, the limit of quantification (LOQ) was set as the lowest measurable concentration with acceptable accuracy and precision. The LOQ for mepivacaine was set to 1 ng/mL.

Figure 1 shows the graph of mean plasma concentrations vs time after injecting MVC_{2%EPI}, MVC_{3%}, and MVC_{LUV}. Table 1 reports the median (lower and upper quartiles) values of the pharmacokinetic parameters obtained after intra-oral injections of the tested formulations. MVC_{3%} induced higher plasma concentrations than MVC_{LUV} after intra-oral injection for periods of time up to 240 min ($p < 0.05$). After this period, no statistically significant difference was observed between the two formulations ($p > 0.05$). MVC_{3%} also induced higher plasma concentrations than MVC_{2%EPI} at 15, 120, 180, and 240 min ($p < 0.05$). Statistical differences were not observed for plasmatic concentrations between MVC_{2%EPI} and MVC_{LUV} for all periods of time ($p > 0.05$) (Figure 1).

Regarding the pharmacokinetic parameters, MVC_{LUV} induced higher AUC_{0-∞} and $t_{1/2}$ values when compared to MVC_{3%} (2.2- and 10-times, respectively) and MVC_{2%EPI} (12- and 15-times, respectively), as observed in Table 1 ($p < 0.01$). However, MVC_{3%} showed statistically different ($p < 0.001$) C_{max} values compared to MVC_{LUV}.

In the present study, mepivacaine analysis was discontinued at 420 min since the blood collection period could not be prolonged due to the volume of blood collected at each time point (0.4 mL). However, the analysis demonstrated that the plasma concentrations obtained with aqueous mepivacaine solution were higher than those obtained with the liposome formulation, similar to the results obtained by Boogaerts et al. (1993b).

Determining drug concentration in biological fluids provides the fundamental information needed for the development of new drug forms (Altun et al., 2004), such as liposome preparations of local anesthetics. Moreover, pharmacokinetic determinations are fundamental in clinical practice (Adams et al., 1989). Although there are several methods for quantifying drug components, liquid chromatography with mass spectrometry (LC-MS/MS) is considered the technique of choice for biological fluid analysis (Cobb & Andersson, 2005). The LC-MS/MS system used in this study is one of the most utilized analytical tools for determining pharmacokinetic parameters of a drug with high precision and reliability (Korfmacher, 2005).

There are no previous reports that compare the absorption of local anesthetics, free as well as liposome-encapsulated, after an intra-oral injection. Several studies assess the absorption of liposome-encapsulated local anesthetics such as: epidural in dogs (Mashimo et al., 1992), intra-articular in rabbits (Hou & Yu, 1997), subcutaneous in rats (Yu et al., 2002), and during axillary block in rabbits (Boogaerts et al., 1993b). Despite these methodological differences, the results obtained in the present study are compatible with those obtained by those authors.

Hou and Yu (1997) evaluated the pharmacokinetics of aqueous lidocaine and liposome lidocaine after an intra-articular injection in rabbit knees. These authors observed that the liposome drug was absorbed to a lesser extent than non-vasoconstrictor-containing lidocaine, which was similar to the findings of this study. This may have occurred due to the slow release of the anesthesia by the liposomes.

Boogaerts et al. (1993b) compared plasma concentrations of bupivacaine after administration of free bupivacaine and a complex with multilamellar liposomes during axillary block in rabbits. Those animals given liposome bupivacaine had lower plasma levels during the first 10 min and then higher levels after 24 h,

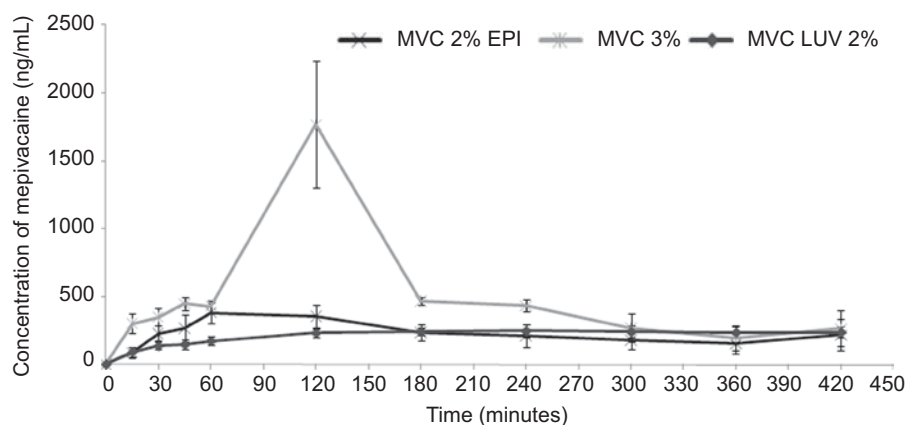


Figure 1. Graph of mean plasmatic concentration vs time after injecting MVC_{2%EPI} (1:100,000); MVC_{3%}, and MVC_{LUV}.

demonstrating that the slow release of the anesthetic was responsible for its prolonged action.

Mashimo et al. (1992) showed the pharmacokinetics of free and liposome encapsulated lidocaine in dogs after epidural administration. The areas under the curve (AUC) and T_{\max} were greater in animals given liposome encapsulated lidocaine, contrary to the observations in this study that revealed smaller parameters for liposome formulations compared with free formulations of the local anesthetic. These differences may be explained by the type of local anesthetic agent used in this study as well as the distinct concentrations and vasoconstrictors, since the commercial formulations with mepivacaine used here were similar to those used in clinical dentistry. Moreover, the different route of administration may have contributed towards these differences.

Yu et al. (2002) studied the pharmacokinetics of free and liposome bupivacaine after injecting these formulations in rat tails. In this study, the blood was collected from the animals hourly for 480 min. These authors

described that C_{\max} value was 5-times greater for the non-encapsulated anesthetic. In the present study, the C_{\max} obtained after injecting MVC_{3%} was 7-fold greater than that obtained after injecting MVC_{LUV}. Likewise, Yu et al. (2002) obtained smaller AUC values for liposomal formulations. Even if statistical differences were not observed, the area under the curve (AUC_{0-t}) after treatment with MVC_{LUV} was less than half that obtained after injecting MVC_{3%}. This difference is probably not only due to encapsulation of the anesthetic but also due to the different mepivacaine concentrations in these formulations. The results presented in the present study are similar to those observed by Yu et al. (2002), showing the liposomal anesthetic preparation maintained a constant plasmatic level when compared with a non-encapsulated common anesthetic without a vasoconstrictor. In this manner, the results obtained by the present study as well as the documented anesthetic efficacy (de Araujo et al., 2004; Cereda et al., 2006) indicate a promising future for liposomal formulations of mepivacaine.

Table 1. Pharmacokinetic parameters of mepivacaine from intra-orally administered formulations.

	C_{\max} ($\mu\text{g/mL}$)	T_{\max} (min)	AUC ₀₋₄₂₀ ($\mu\text{g/min/mL}$)	AUC _{0-\infty} ($\mu\text{g/min/mL}$)	$t_{1/2}$ (min)
MVC _{LUV}	0.27 (0.21-0.33)	150 (120-195)	92.7 (72.3-111.9)	776.1 (424.1-1221.5) ^{a,c,**}	1465.2 (911.6-2822.4) ^{a,c,**}
MVC _{3%}	1.90 (1.24-2.42) ^{a,**}	120 (120-120)	241.4 (221.0-248.9) ^{a,b,**}	349.3 (257.0-454.7)	145.2 (49.0-315.9)
MVC _{2%EPI}	0.40 (0.33-0.48)	90 (60-195)	66.7 (61.2-115.9)	60.1 (46.5-65.2)	94.9 (89.7-226.5)

Data expressed as median (lower and upper quartiles) ($n=4$).

^a MVC_{LUV 2%} vs MVC_{3%}; ^b MVC_{3%} vs MVC_{2%EPI}; ^c MVC_{LUV 2%} vs MVC_{2%EPI}. ** $p < 0.01$.

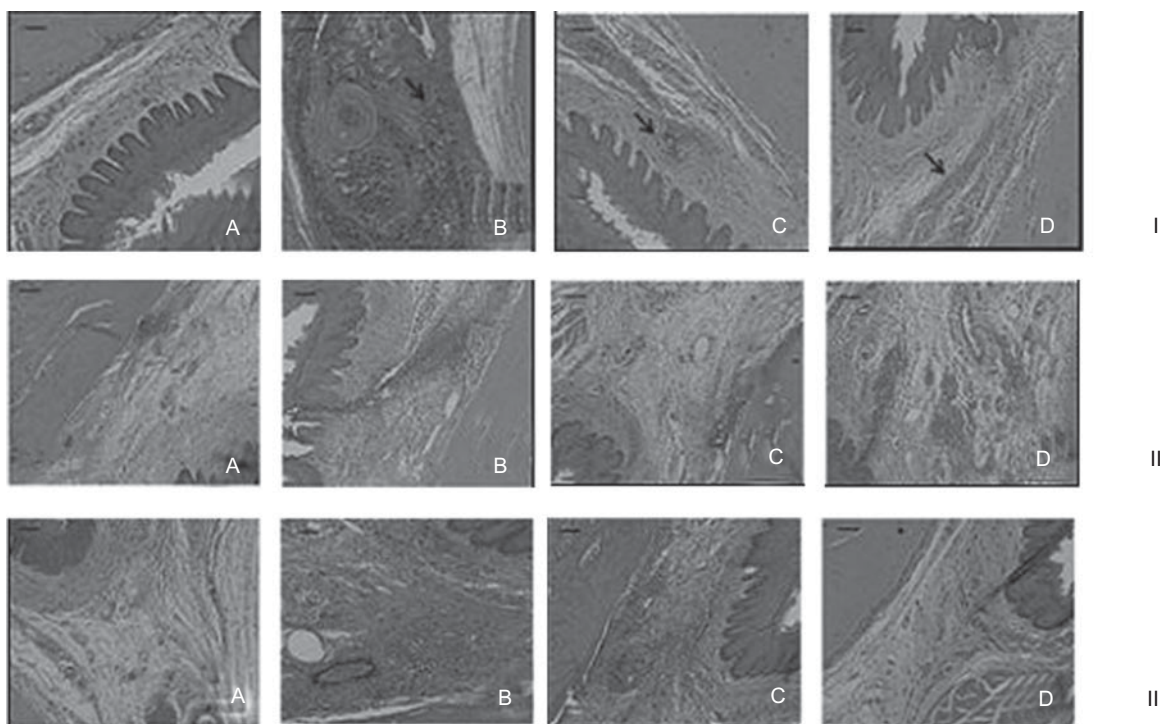


Figure 2. Images obtained after 6 h (I), 24 (II) h, and 4 days (III) of the treatment with: (A) MVC_{LUV}; (B) MVC_{2%EPI}; (C) MVC_{3%}, and (D) LUV. Scale bar: 20 μm .

Local toxicity evaluation

Figure 2 shows transverse sections of the maxilla bones and their surroundings soft tissues 6 h, 24 h, and 4 days after the injections of MVC_{LUV}, MVC_{3%}, MVC_{2%EPI} or empty liposomal vesicles (LUV). The pH values of each formulation were verified as follows: commercial mepivacaine formulation without vasoconstrictor, 0.9% NaCl, and mepivacaine with vasoconstrictor formulation showed similar pH values of 5.5, 5.8, and 3.8, respectively. The liposomal mepivacaine (7.1) and the mepivacaine free liposome (liposome vesicles) (7.3) had a pH value on the physiologic range. As quality control assay of the histological examination, the intra-class correlation test showed an index of 0.96 ($p < 0.0001$), indicating agreement between both examiners.

Figure 3 shows the median scores for intensity of the inflammatory reaction after the injection of different mepivacaine formulations (inter-group analysis). At 6 h after injection, MVC_{2%EPI} had higher inflammatory scores compared to MVC_{LUV} ($p = 0.0043$) and LUV ($p = 0.0012$). However, MVC_{3%} did not show statistically significant differences from MVC_{2%EPI} ($p > 0.05$). In addition, no

differences were observed between MVC_{LUV}, LUV, and MVC_{3%} ($p > 0.05$). After 24 h, the results followed the same profile observed at 6 h. However, after 4 days the inflammation scores were higher for MVC_{2%EPI} compared to MVC_{LUV} ($p = 0.0096$).

The right (treated) and the left (control) sides of the maxilla of each animal were compared (intra-group analysis). Considering the intra-group analysis after 6 and 24 h of treatment, the commercial solutions MVC_{2%EPI} and MVC_{3%} showed higher inflammatory reaction scores ($p < 0.05$) when compared to their controls. Non-significant differences were observed after the administration of MVC_{LUV} and LUV when compared to their controls ($p > 0.05$). Four days after the treatment, the group treated with MVC_{2%EPI} still had higher scores when compared with the control side ($p < 0.05$). Non-significant differences were observed after the administration of MVC_{3%}, MVC_{LUV}, and LUV when compared to their controls ($p > 0.05$). Table 2 demonstrates the median values of the scores obtained after the treatments and their controls.

The tissue reaction induced by local anesthetic solutions or by liposomal formulations of local anesthetics

Table 2. Median (minimum–maximum limits) of the inflammatory scores for all treatments and their controls 6 h, 24 h, and 4 days after the treatment.

	Time after treatment					
	Test side			Control side (saline solution)		
	6 h	24 h	4 days	6 h	24 h	4 days
MVC _{2%EPI}	4.8 (4.2–5.0) ^{a***,b***}	4.0 (3.8–4.0) ^{a***,b***}	2.5 (2.0–3.0) ^{c**}	2.5(2.0–3.0) ^{d***}	2.0 (2.0–2.4) ^{d***}	1.7 (1.0–2.0) ^{d***}
MVC _{3%}	3.3 (3.0–4.2)	3.0 (2.6–3.2)	2.0 (1.8–2.0)	2.1 (2.0–2.6) ^{c**}	2.0 (2.0–2.4) ^{c**}	1.6 (1.4–2.0)
MVC _{LUV}	2.5 (2.0–3.2)	2.0 (2.0–2.6)	1.3 (1.0–2.4)	2.5 (2.0–3.0)	2.1 (2.0–2.8)	1.6 (1.0–2.0)
LUV	2.3 (2.0–2.8)	2.1 (2.0–2.8)	1.9 (1.6–2.4)	2.4 (2.0–3.4)	2.0 (2.0–2.8)	1.9 (1.4–2.2)

Statistical analysis: $p < 0.001$ (***) , $p < 0.01$ (**), $p < 0.05$ (*), $p > 0.05$ (non-significant).

Inter-groups (Kruskal–Wallis): ^a MVC_{2%EPI} vs MVC_{LUV}; ^b MVC_{2%EPI} vs LUV; ^c MVC_{2%EPI} vs MVC_{LUV}. Intra-groups (Wilcoxon paired test): ^d MVC_{2%EPI} vs control (saline); ^e MVC_{3%} vs control (saline).

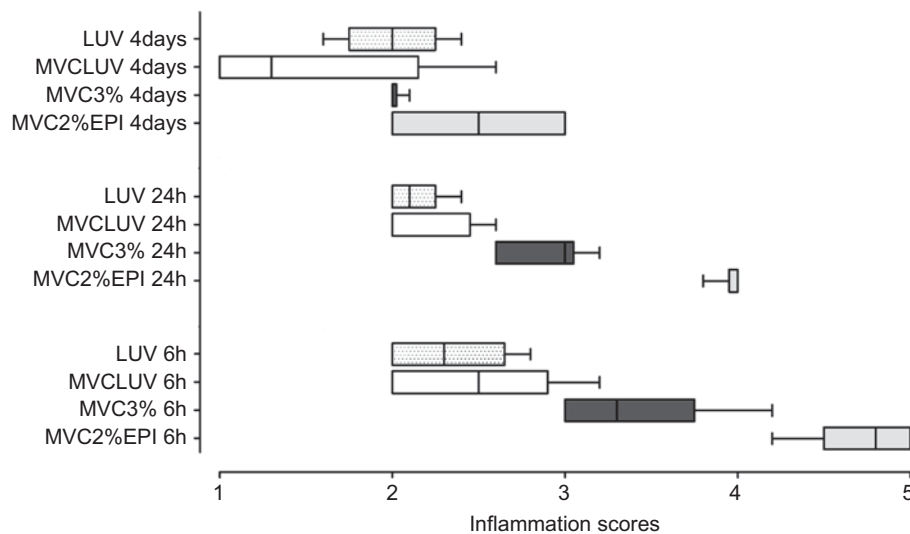


Figure 3. Scores of the intensity of the inflammatory reaction (Central line: median; Box: lower and upper quartiles; Whisker: maximum and minimum values) induced by MVC_{2%EPI}, MVC_{3%}, MVC_{LUV} and LUV.

was previously evaluated (Malinovsky et al., 1997; Ribeiro et al., 2003). However, the present study evaluated the oral tissue reaction to mepivacaine liposomal anesthetic formulation. This local toxicity evaluation is necessary for future use in human subjects. Those studies were performed using polyethylene tubes containing absorbent-paper cones soaked in different anesthetic solutions (lidocaine, mepivacaine, articaine, and bupivacaine), implanted subcutaneously in the backs of rats. According to this previous study, this method could promote a more prolonged contact between local anesthetics and tissues and easily would allow the localization of the implanted area (Ribeiro et al., 2003). In the present study, the site of the injection was the oral mucosa close to the upper first molar. This area was used to better simulate the inflammatory reaction in oral sites and it also allowed the use of the upper first molar to locate the injection area. Besides the differences in methodological approaches, both of the studies showed that local anesthetics induce inflammatory reactions.

A 0.9% NaCl solution induced a more intense inflammatory reaction when compared with plain 2% lidocaine solution, probably due to the vasodilator effect of lidocaine, which could cause a rapid absorption and elimination of the anesthetic from the site of the injection (Ribeiro et al., 2003). In our study the plain mepivacaine solution evoked more intense inflammatory reactions compared to the 0.9% NaCl solution at 6 and 24 h periods after treatment. The low vasodilator effect of mepivacaine allows longer residence in the site of injection and increases the possibility of tissue irritation, and could be responsible for the more intense inflammatory reaction.

Along with the local anesthetic properties, another important factor to consider regarding the inflammatory response was the presence of vasoconstrictors. In fact, the pH of local anesthetics with sympathomimetic amines (epinephrine, norepinephrine, levonordephrin, and phenylephrine) is usually in the pH range of 3.5–5.5 (USP XXII). This is necessary to avoid the oxidation of the sympathomimetic amines (Murakami et al., 1994). In this study, the pH of the 2% mepivacaine with 1:100,000 epinephrine solution was 3.8. This low pH could have contributed for the high levels of inflammatory reaction induced by this solution at 6 and 24 h after administration. Previous studies reported adverse effects associated with the use of acidic solutions. These solutions could cause discomfort and pain during or after the injection, and retard the onset of anesthesia (Oikarinen et al., 1975; Murakami et al., 1994; Meehan & Day, 2002; Wahl et al., 2002).

Previous studies showed that a single intrathecal injection of plain epinephrine (up to 0.5 mg) is not associated with histological injury in rabbits (Oka et al., 2001) or rats (Bahar et al., 1984; Hashimoto et al., 2001).

However, epinephrine worsens histological spinal cord injury when added to 5% lidocaine in rats (Hashimoto et al., 2001) or 1–2% tetracaine in rabbits (Oka et al., 2001). Thus, it seems that the addition of epinephrine to a potential irritating substance (local anesthetic) worsens the tissue reaction. In our study the injection of 1 µg of epinephrine with 2% mepivacaine produced the most intense inflammatory reaction when compared to the saline control and to the other formulations. The low pH and the prolonged tissue exposure to the local anesthetic caused by the vasoconstrictor action of epinephrine could explain the more intense inflammatory reaction of MVC_{2%EPI}. In addition, the use of vasoconstrictors evokes ischemia, hypoxia, and important cell injury (Kumar et al., 2007). This feature could explain the increased toxic effects observed after the injection of the MVC_{2%EPI} formulation. The liposomal formulations, with a pH in the physiologic range, with or without mepivacaine, did not promote an inflammatory reaction higher than the one evoked by the injection of 0.9 % NaCl solution. Histopathology characteristics of the spinal cord of rabbits were evaluated after an intra-cisternal injection of plain and liposomal bupivacaine, both at 0.5%, and bupivacaine free liposomes (Malinovsky et al., 1997). These authors observed that the injection of liposomal bupivacaine did not produce histopathological changes in the spinal cord different from those evoked by the plain solution. Despite methodological differences, our study showed similar results, since the injection of liposomal mepivacaine did not produce an inflammatory reaction more intense than the injection of plain mepivacaine (MVC_{3%}), mepivacaine free-liposomes, and 0.9 % NaCl solution.

Conclusion

Results from our study indicate that 2% liposome-encapsulated mepivacaine reduced the C_{max} and prolonged AUC_{0-∞} and $t_{1/2}$ compared with 3% plain and 2% vasoconstrictor-associated mepivacaine, after intra-oral injection. In addition, liposomal mepivacaine might protect the tissue against local inflammation evoked by plain or vasoconstrictors-associated mepivacaine, supporting its safety and possible clinical use in dentistry. This formulation could be considered an alternative to the use of vasoconstrictor-containing local anesthetic formulations when the vasoactive compound is contraindicated.

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Declaration of interest

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