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Pharmacokinetic study of liposome-encapsulated and plain mepivacaine formulations injected intra-orally in volunteers

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Abstract

Objectives The pharmacokinetics of commercial and liposome-encapsulated mepivacaine (MVC) injected intra-orally in healthy volunteers was studied.

Methods In this double blind, randomized cross-over study, 15 volunteers received, at four different sessions, 1.8 ml of the following formulations: 2% MVC with 1 : 100 000 epinephrine ($MVC_{2\%EPI}$), 3% MVC ($MVC_{3\%}$), 2% and 3% liposome-encapsulated MVC ($MVC_{2\%LUV}$ and $MVC_{3\%LUV}$). Blood samples were collected pre dose (0 min) and at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 min after injections. Liquid chromatography-tandem mass spectrometry was used to quantify plasma MVC concentrations.

Results Pharmacokinetic analysis showed that the maximum plasma concentration (Cmax) and the areas under the curves (AUC_{0-360} and $AUC_{0-\infty}$) after $MVC_{2\%LUV}$ and $MVC_{2\%EPI}$ injections were smaller ($P < 0.05$) than the equivalent figures for $MVC_{3\%}$ and $MVC_{3\%LUV}$. The time to maximum plasma concentration (Tmax) and the half-life of elimination ($t^1/_2$beta) obtained after the treatment with $MVC_{2\%LUV}$, $MVC_{2\%EPI}$, $MVC_{3\%}$ and $MVC_{3\%LUV}$ presented no statistically significant differences ($P > 0.05$). Cmax, AUC_{0-360} and $AUC_{0-\infty}$ after injection of the 2% formulations ($MVC_{2\%LUV}$ and $MVC_{2\%EPI}$) did not exhibit statistically significant differences ($P > 0.05$). The pharmacokinetics of $MVC_{2\%LUV}$ were comparable to the pharmacokinetics of $MVC_{2\%EPI}$.

Conclusion The liposomal formulation of 2% MVC exhibits similar systemic absorption to the local anesthetic with vasoconstrictor.

Introduction

Local anesthetics (LA) are among the classes of pharmacological compounds used to attenuate or eliminate pain. LA efficacy is dependent on drug concentration at the site of administration; on the other hand, side effects are dependent on high plasma concentrations. Commercially available formulations are used in a variety of doses and routes of administration, but the relatively short duration of analgesia due to their transfer and redistribution from the site of injection still restricts their clinical use.[1]

The development of LA drug delivery systems, such as liposomal formulations, has improved the therapeutic effects of these agents.[1] Liposomes have been extensively described in the literature for their use as drug delivery systems. The distinct advantage of liposomes is their structural versatility combined with their ability to encapsulate different compounds, including LAs.[1–3]

The advantages of liposome-encapsulated LAs are slow drug release, prolonged anesthetic effect and reduced toxicity for both the cardiovascular and central nervous system.[4–6] The pharmacological effectiveness of many liposomal–anesthetic preparations has been demonstrated by studies using animals[4,7–12] and human beings.[13–17]

Mepivacaine (MVC) was the second amide to be introduced as a local anesthetic. It has a fast onset, similar to that of lidocaine, and is a popular choice for a wide range of regional anesthetic procedures due to its safety.[18] Liposome-encapsulated MVC increases the analgesic effect in relation to amplitude and anesthetic duration.[11] Moreover,

encapsulation of MVC in liposomes greatly enhances the infraorbital nerve block in rats when compared with the standard pharmaceutical solution of this local anesthetic.[19] Liposome-encapsulated MVC also increases the duration of intra-oral anesthesia and reduces the injection discomfort caused by the vasoconstrictor-associated formulations in healthy volunteers.[17]

Determining drug concentratiosn in biological fluids provides fundamental information needed for the development of new sustained-release pharmaceutical formulations, such as liposome-encapsulated LAs. No previous studies have reported the pharmacokinetics of liposomal MVC formulations after clinical application in dentistry. Thus, the aim of this study was to determine the plasma levels of commercial and liposomal preparations of MVC after intra-oral injections in healthy volunteers.

Methods

The trial registration number of this study was NCT01032798.

Chemicals and reagents

The commercial anesthetic solutions used in this study were 2% MVC with 1 : 100 000 epinephrine ($MVC_{2\%EPI}$) (Mepiadre®-DFL; batch n°0408011) and plain 3% MVC ($MVC_{3\%}$) (MepiSV®-DFL; batch n°0407A09). The 2% and 3% liposomal MVC formulations ($MVC_{2\%LUV}$and $MVC_{3\%LUV}$) were prepared with MVC hydrochloride salt obtained from DFL Ind. Com. S.A. (RJ, Brazil). Egg phosphatidylcholine, cholesterol and α-tocopherol were purchased from Sigma Chemical Co. (MO, USA). All 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.[4,20] 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 for 5 min. Unilamellar-liposome vesicles (0.4 μm) 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.[21] MVC was added directly to the liposomes after extrusion, up to concentrations of 2% and 3%. 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). Afterwards, the sterility was evaluated by microbiological testing with brain–heart infusion and apyrogenicity was assessed by the Endosafe® Limulus Amoebocyte Lysate test.

The liposomal formulations used in this study were identical to those described previously and exhibited the same *in vitro* characteristics.[11,17,19,21]

Volunteer selection

Fifteen healthy volunteers (seven men and eight women) aged from 20 to 45 years (mean = 24.2 ± 4.1 years) were randomized in a double-blind manner, using a scheme described by a previous numbered table. The person who executed the allocation sequence was different from the person who recruited the participants. The volunteers were free from cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and haematological disease, psychiatric disorders and allergy to local anesthetic. They did not use any medication, except oral contraceptives, one week prior to or during the experiment.

The study was approved by the Human Research Ethics Committee of Piracicaba Dental School (Protocol #058/2007), which follows the requirements of the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. All volunteers signed a written consent form.

Clinical protocol

In this double-blind study, the formulations were codified by an individual who was not involved in the administration of the anesthetic formulations or in blood collection procedures. The formulations ($MVC_{2\%EPI}$, $MVC_{3\%}$, $MVC_{2\%LUV}$ and $MVC_{3\%LUV}$) were randomly applied to the subjects at four different sessions with a washout interval of seven days. In each session, the volunteers received a buccal maxillary infiltration performed in the upper right canine region by the same operator with a hypodermic syringe and a 30G 1-inch needle. The volume and the rate of the injections were standardized at 1.8 ml and 1 ml/min, respectively.

Blood samples (5 ml) from an antecubital vein were collected via a heparinised cannula pre-dose (0 min) and at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min following drug administration and placed in EDTA-coated tubes. A heparin saline solution (0.9% NaCl and heparin, 9.8 : 0.2) injection (0.4 ml) was flushed into the cannula to prevent blood clotting after each blood sampling. Just before each blood sample was collected, heparin in the heparin-locked catheter was discarded with 0.5 to 0.7 ml blood. Immediately after each blood collection, the samples were centrifuged at 3000 × *g* for 15 min and plasma was separated and stored at −70°C until analysis.

Blood collection was carried out at the ambulatory clinic of Piracicaba Dental School, UNICAMP, São Paulo, Brazil. The

volunteers were monitored during the period of blood sampling and told to report any adverse effects after this period.

LC–MS/MS assay: apparatus and chromatographic conditions

MVC plasma concentrations were determined using a Waters® HPLC system (2795) coupled to a Micromass Quattro Premier XE triple stage quadrupole mass spectrometer (LC-MS/MS), equipped with an API electrospray source. All separations were carried out on a Polaris C_{18} column (50 mm × 2 mm id, 5 μm particle size). 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 MVC and ropivacaine (internal standard) were (*m/z*) 247.50 > 150.50 and 275.00 > 126.13, respectively. The data were integrated using the MassLynx 4.1 (Waters®) software. Quality control samples, prepared by mixing drug-free plasma with appropriate volumes of working solutions, were used to validate the method.

Plasma sample preparation

The frozen plasma samples (−70°C) were thawed at room temperature. The extraction procedure was adapted from Koehler *et al*. (2005)[22] and was performed by transferring 200 μl of plasma to test-tubes, followed by the addition of 25 μl of internal standard work solution (ropivacaine 1 ng/ml). The samples were vortexed for 1 min and 200 μl of acetonitrile was added. The samples were vortexed for 2 min and centrifuged at 1200*g* for 5 min at −4°C. A sample of 0.20 ml of the organic liquid layer was transferred to LC-MS/MS system vials for further injection (5 μl).

Preparation standards and quality control

Stock solutions of MVC were prepared in acetonitrile. Aliquots of the stock solutions were used to spike blank human plasma in order to obtain calibration standards of 1.00, 5.00, 10.00, 50.00, 100.00, 500.00 and 1000.00 ng/ml. Three levels of quality control fixed at 3.00, 400.00 and 800.00 ng/ml (low, medium and high) were prepared using the same blank plasma.

Method validation

Precision and accuracy for this method were controlled by calculating the intra-batch and inter-batch variation at three concentrations (3.00, 400.00 and 800.00 ng/ml) of quality control samples in five replicates. The method's accuracy was shown as error (RE) and calculated based on the difference between the mean calculated and nominal concentrations. Precision was evaluated as the relative standard deviation. Three calibration curves were plotted as the peak area ratio versus MVC concentration in the range of 1.00–1000.00 ng/ml. The limit of quantification (LOQ) was defined as the lowest concentration at which precision and accuracy were within 20% of the true value.

Pharmacokinetic assessment

The concentration–time data obtained in the study were analysed by the non-compartmental approach. The pharmacokinetic parameters were calculated using WinNonlin software (WinNonlin version 5.3, Pharsight Corporation, CA, USA).

Statistical analysis

The plasma MVC concentrations were analysed by one-way ANOVA and the Tukey–Kramer test (post-hoc) considering each period of time separately ($\alpha = 0.05$). The pharmacokinetic parameters were analysed using the Kruskal–Wallis test and Student–Newman–Keuls as the *post hoc* test ($\alpha = 0.05$).

Results

The analysis of MVC using the MRM function was highly selective, with neither interfering compounds nor significant ion suppression from endogenous substances observed at the retention times for MVC and the internal standard. Figure 1 (a and b) shows the mass spectrum of MVC and the chromatogram of MVC (MRM mode) in blank human plasma. The calibration curve for MVC showed a good response over the range of 1.00–1000.00 ng/ml. The assay was linear; RSDs were less than 15%. The relative error of the mean of measured concentrations ranged from 0.86 to 8.90%. The determination coefficients (r^2) were greater than 0.99 for all curves. The intra-batch accuracy and precision were calculated to be from 92.45 to 105.5% and from 0.4 to 9.8%, respectively. Inter-batch accuracy and precision were calculated to be from 95.72 to 98.00% and from 0.69 to 3.36% respectively. These results indicate that the method is reliable and reproducible within its analytical range.

In addition, the LOQ was set as the lowest measurable concentration with acceptable accuracy and precision. The acceptance criterion was a deviation of less than ±20%. The LOQ for MVC was set to 1.00 ng/ml.

No statistically significant difference ($P > 0.05$) was observed between the plasma levels of $MVC_{2\%LUV}$ and $MVC_{2\%EPI}$ at all periods of time, except at 120 min. $MVC_{3\%}$ and $MVC_{3\%LUV}$ induced higher ($P < 0.05$) plasma concentrations than the 2% formulations at all periods of time. The highest measured plasma MVC concentration for an individual subject was 1448.71 ng/ml, recorded 15 min after the administration of 3% plain MVC. Figure 2 shows the graph of

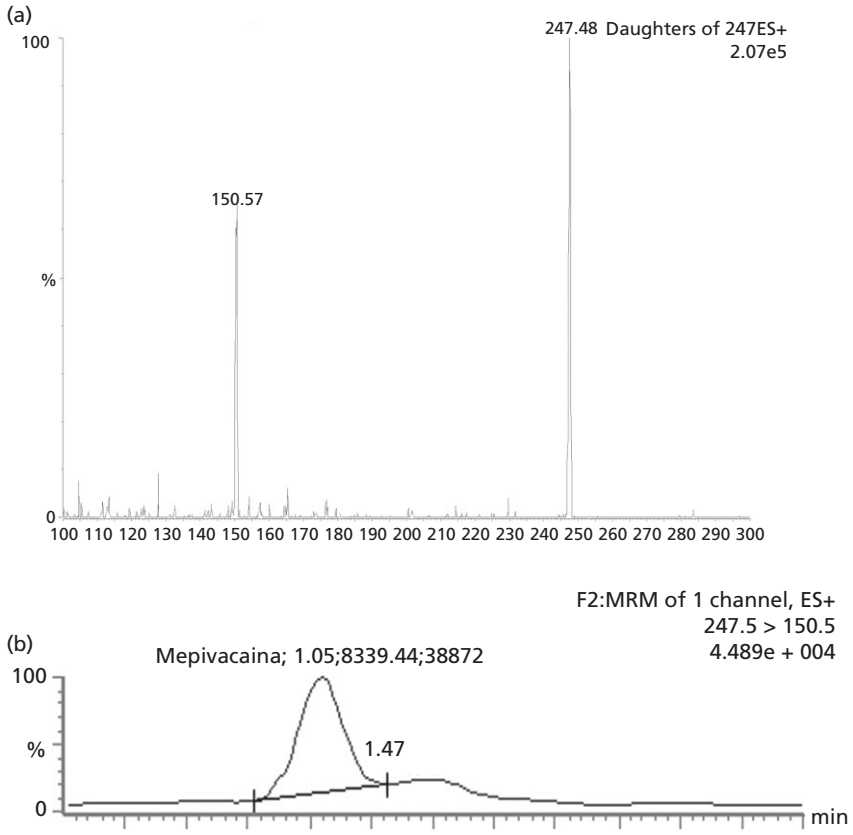

Figure 1 (a) Mass spectrum of mepivacaine in the sequential mode *m/z* 247.48, generating ion fragments at *m/z* 150.57 at collision energy of 20 eV. (b) Chromatogram of mepivacaine (MRM mode).

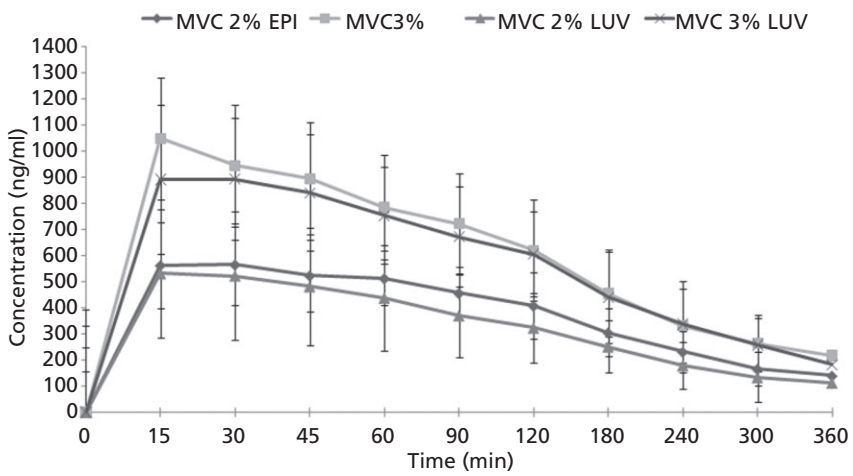

Figure 2 Graph of mean plasma concentration versus time after injecting $MVC_{2\%EPI}$, $MVC_{3\%}$, $MVC_{2\%LUV}$ and $MVC_{3\%LUV}$.

mean plasma concentrations versus time after injecting $MVC_{2\%EPI}$, $MVC_{2\%LUV}$, $MVC_{3\%}$ and $MVC_{3\%LUV}$.

Table 1 shows the mean values of the pharmacokinetic parameters obtained after intraoral injections of the tested formulations. Pharmacokinetic analysis showed that the maximum plasma concentration (Cmax) after $MVC_{2\%LUV}$ and $MVC_{2\%EPI}$ injections was about half of the value obtained after the administration of $MVC_{3\%}$ and $MVC_{3\%LUV}$ ($P < 0.05$). The

Table 1 Mean (±SD) of pharmacokinetic parameters

	$MVC_{2\%EPI}$	$MVC_{3\%}$	$MVC_{2\%LUV}$	$MVC_{3\%LUV}$
Cmax (ng/mL)	620.341 ± 126.230 **a**	1073.284 ± 225.510 **b***, d ***, f**	606.920 ± 289.160 **a**	1037.937 ± 262.760 **c***, e***, f**
AUC_{0-360} (ng-min/ml)	32.306 ± 9.047 **a**	50.019 ± 16.470 **b***, d ***, f**	26.603 ± 13.777 **a**	47.652 ± 14.110 **c***, e***, f**
$AUC_{0-\infty}$ (ng-min/ml)	41.382 ± 13.775 **a**	63.756 ± 25.190 **b***, d ***, f**	34.258 ± 21.745 **a**	58.550 ± 22.873 **c***, e***, f**
Tmax (min)	41.000 ± 42.221 **a, b, c**	26.000 ± 16.497 **b, d, f**	32.000 ± 41.610 **a, d, e**	37.000 ± 41.610 **c, e, f**
$t^1/_2$beta (min)	149.328 ± 37.155 **a, b, c**	143.437 ± 36.924 **b, d, f**	129.798 ± 57.759 **a, d, e**	128.720 ± 46.545 **c, e, f**
Vd (ml/kg)	0.050 ± 0.010 **a, b, c**	0.050 ± 0.010 **b, d*, f**	0.060 ± 0.020 **a, e***	0.050 ± 0.010 **c, f**
CL (ml/min/kg)	0.016 ± 0.007 **a**, b, c**	0.016 ± 0.007 **b, d**, f**	0.027 ± 0.020 **e****	0.017 ± 0.006 **c, f**

Data expressed as mean (±SD). Statistical analysis: $P < 0.001$ (***), $P < 0.01$(**), $P < 0.05$(*), $P > 0.05$ (not significant). ANOVA/Tukey Kramer results: a, $MVC_{2\%EPI} \times MVC_{2\%LUV}$; b, $MVC_{2\%EPI} \times MVC_{3\%}$; c, $MVC_{2\%EPI} \times MVC_{3\%LUV}$; d, $MVC_{2\%LUV} \times MVC_{3\%}$; e, $MVC_{3\%LUV} \times MVC_{2\%LUV}$; f, $MVC_{3\%} \times MVC_{3\%LUV}$.

areas under the curves (AUC_{0-360} and $AUC_{0-\infty}$) obtained after the injection of $MVC_{2\%LUV}$ and $MVC_{2\%EPI}$ were smaller ($P < 0.05$) than those obtained with $MVC_{3\%}$ and $MVC_{3\%LUV}$. The volume of distribution (Vd) after the injection of $MVC_{2\%LUV}$ was larger than those obtained with $MVC_{3\%}$ and $MVC_{3\%LUV}$. Cmax, AUC_{0-360}, $AUC_{0-\infty}$ and Vd after the injection of 2% formulations ($MVC_{2\%LUV}$ and $MVC_{2\%EPI}$) did not exhibit statistically significant differences ($P > 0.05$) between them. The same pattern was observed with the 3% formulations ($MVC_{3\%}$ and $MVC_{3\%LUV}$) ($P > 0.05$). The time to maximum plasma concentration (Tmax) and the half-life of elimination ($t^1/_2$beta) obtained after the treatment with $MVC_{2\%LUV}$, $MVC_{2\%EPI}$, $MVC_{3\%}$ and $MVC_{3\%LUV}$ exhibited no statistically significant differences ($P > 0.05$). Clearance for $MVC_{2\%LUV}$ presented small but statistically significant differences ($P < 0.05$) to the other tested formulations.

Discussion

Liposomal formulations are designed to slowly release the local anesthetic to prolong the analgesic effect and to reduce plasma peak concentrations and systemic toxic effects.[23] Local anesthetic systemic toxicity can be caused by accidental rapid intravascular injection, exceeding the maximum recommended dose, or by rapid absorption after injection into a highly vascularised site. The severity of central nervous system toxicity (CNS) correlates with plasma local anesthetic concentrations. CNS toxicity is characterised by an initial excitement followed by depression as the plasma LA concentration increases. Cardiovascular system toxicity also shows this biphasic behavior, although it usually follows CNS toxicity. The encapsulation of LAs into liposomes reduces the plasma concentration of the drug due to slow release, allowing the safe administration of larger doses.[24]

The crossover randomized study reported here assessed the pharmacokinetic profile of liposomal MVC formulations compared with commercial formulations of the same anesthetic salt. Determining drug concentration and pharmacokinetics profiles provides information regarding the effectiveness of the carrier system and whether the formulation exhibits typical properties of slow-release formulations, since the encapsulated form should promote lower systemic concentrations of the drug when compared to the free form. Several studies have assessed the pharmacokinetics of liposome-encapsulated LAs in animals.[4,9,10,25,26] These studies demonstrated that liposomes are able to alter the pharmacokinetic behaviour of LAs. Usually, liposomal formulations have constant or lower plasma concentrations when compared to a non-encapsulated anesthetic, suggesting that encapsulation into liposomes delays the transfer of the anesthetic to the bloodstream.

Davidson *et al.* (2010)[23] and Franz-Montan *et al.* (2010)[27] studied the pharmacokinetics of liposomal formulations of bupivacaine and ropivacaine in volunteers and the same pattern of slow release with liposomal formulations was observed. Franz-Montan *et al.* (2010)[27] determined the pharmacokinetic parameters of liposomal ropivacaine after dental anesthesia in 14 healthy volunteers in a randomized, double-blind crossover study, the volunteers received maxillary infiltration of liposomal 0.5% ropivacaine and 0.5% ropivacaine with 1 : 200 000 epinephrine in two different sessions. No differences were observed between the formulations regarding Cmax, Tmax, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$ and plasma ropivacaine concentrations. Similarly to Franz-Montan *et al.*,[27] our study showed that the pharmacokinetics of 2% liposome-encapsulated MVC are comparable to those of MVC plus epinephrine. These similar results could be explained by the use of the same route of administration and the same kind of liposomes.

The pharmacokinetics of high-dose bupivacaine loaded into multivesicular liposome was evaluated by Davidson *et al*. (2010).[23] Eight volunteers received subcutaneous injections of plain 0.5% bupivacaine and 2% liposomal bupivacaine. The authors observed similar Cmax and AUC values for both formulations, despite a fourfold increase in total bupivacaine dose in the liposomal preparation. Our study found similar values of Cmax and AUC for the 2% formulations of MVC (liposomal and non-liposomal), but these values were not similar to those for the 3% formulations. Unlike the observation of Davidson *et al*.,[23] the increased dose in the MVC 3% formulation produced higher Cmax and AUC values. These authors also observed higher Tmax and $t_{1/2}$ values for the liposomal bupivacaine preparation. In our study there was no difference in the Tmax or $t_{1/2}$ values for any tested formulations. These differences may be explained by the use of different routes of administration and different kinds of liposome.

The 3% liposomal MVC did not reduce the plasma concentration when compared with the plain commercial 3% formulation. These results could be explained by the discrete fraction of MVC encapsulated into the liposomes of 18.4% ($\pm$3.9%),[11] which means that a significant amount of MVC remains free in the 3% formulation. Unfortunately the separation of free and liposome-encapsulated MVC is not feasible since egg phosphatidylcholine-cholesterol liposomes are at the liquid crystalline disordered phase at ambient temperature and the leakage of LAs can easily occur from such vesicles.[1] Fast exchange is also the reason why we did not try to remove non-encapsulated LA molecules from our liposome formulations. Instead, optimisation of the anesthetic transport was obtained with MVC loaded also in the external aqueous phase: the 3% liposomal formulation presented 19.43 mM of encapsulated MVC and 86.62 mM was non-encapsulated. Thus, it seems that the drug in the plasma must be the fraction of non-encapsulated local anesthetic of the 3% liposome–MVC system.

Since equal liposome concentrations were used, the rate of encapsulated MVC (18.4%) was the same in the 2% and 3% liposomal formulations, which means that 13.00 mM of MVC was encapsulated and 57.70 mM remains free in the first case. The 2% liposomal MVC exhibited properties of a slow-release formulation, since the encapsulation of 2% MVC reduced the plasma concentrations in a similar way to the addition of a vasoconstrictor. Epinephrine is a vasoconstrictor often associated with LAs; it is used to improve their efficacy by modifying their residence time in the injection site.[28] In both cases the clearance of local anesthetic from the site of action was affected: by vasoconstriction or by the reservoir effect of the lipid bilayers. Nevertheless, in a previous paper we observed that the nerve block induced by 2% MVC with epinephrine was more effective than 2% liposomal MVC[17], showing that the vasoconstriction action of epinephrine prevailed upon the enhanced LA concentration provided by the liposome reservoir. This resulted in a high anesthetic concentration near the nerve tissue for a longer period of time.

Conclusion

The efficacy after intra-oral injection of 2% liposomal MVC was previously described.[17] $MVC_{2\%LUV}$ was able to produce a similar duration of anesthesia as the 3% commercial formulation of MVC, despite a 50% decrease in the anesthetic concentration. Also, the encapsulation of 2% MVC reduced the injection discomfort caused by vasoconstrictor-associated formulations. These features, in addition to lower plasma concentrations, indicate a promising future for the 2% liposomal formulation, which may be used as an alternative for patients who cannot receive simpathomimetic amines like epinephrine.[29]

Declarations

Declaration of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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