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RESEARCH ARTICLE

Liposomal-based lidocaine formulation for the improvement of infiltrative buccal anaesthesia

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ABSTRACT

This study describes the encapsulation of the local anaesthetic lidocaine (LDC) in large unilamellar liposomes (LUV) prepared in a scalable procedure, with hydrogenated soybean phosphatidylcholine, cholesterol and mannitol. Structural properties of the liposomes were assessed by dynamic light scattering, nanoparticle tracking analysis and transmission electron microscopy. A modified, two-compartment Franz-cell system was used to evaluate the release kinetics of LDC from the liposomes. The *in vivo* anaesthetic effect of liposomal LDC 2% (LUV_{LDC}) was compared to LDC 2% solution without (LDC_{PLAIN}) or with the vasoconstrictor epinephrine (1:100 000) (LDC_{VASO}), in rat infraorbital nerve blockade model. The structural characterization revealed liposomes with spherical shape, average size distribution of 250 nm and low polydispersity even after LDC incorporation. Zeta potential laid around −30 mV and the number of suspended liposomal particles was in the range of 10^{12} vesicles/mL. Also the addition of cryoprotectant (mannitol) did not provoke structural changes in liposomes properties. *In vitro* release profile of LDC from LUV fits well with a biexponential model, in which the LDC encapsulated (EE% = 24%) was responsible for an increase of 67% in the release time in relation to LDC_{PLAIN} ($p < 0.05$). Also, the liposomal formulation prolonged the sensorial nervous blockade duration (~70 min), in comparison with LDC_{PLAIN} (45 min), but less than LDC_{VASO} (130 min). In this context, this study showed that the liposomal formulations prepared by scalable procedure were suitable to promote longer and safer buccal anaesthesia, avoiding side effects of the use of vasoconstrictors.

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Introduction

Local anaesthetics (LA) are small molecules that are quickly removed from the site of injection, limiting the duration of the antinociceptive effect. Drug–delivery systems provide an interesting approach to prolong anaesthesia. By encapsulating LA agents in carriers such as liposomes, there is a sustained release at the site of injection, prolonging the anaesthesia time and reducing the systemic toxicity (Grant *et al.* 2004, de Paula *et al.* 2012, Rogobete *et al.* 2016).

Liposomes are ideally suited to act as carrier system, being biocompatible, biodegradable and non-immunogenic (Torchilin 2012, Bozzuto and Molinari 2015, Bulbake *et al.* 2017). They are lipid vesicles that enclose an aqueous compartment into which guest molecules can be loaded, as well as in-between the lipids of the bilayer (Allen and Cullis 2013). Liposomes have been shown to prolong the duration of LA effect in animals (Malinovsky *et al.* 1997, Grant *et al.* 2000, Grant and Bansinath 2001, de Araújo *et al.* 2008, Silva *et al.* 2016, 2017) and in humans (Grant *et al.* 2004, Taddio *et al.* 2005, Franz-Montan *et al.* 2010, 2012, 2013, 2015). Also, liposomes provide safer formulations than plain anaesthetic solutions due to the decrease in the rate of absorption, reducing the systemic side effects of LA (Tófoli *et al.* 2012, Cereda *et al.* 2016).

Lidocaine (LDC) is a gold standard LA with moderate action that is used in a wide range of dentistry procedures, mainly in association with vasoconstrictors, in order to increase the duration of nerve blockade. Nevertheless, the use of vasoconstrictors is either not recommended or contraindicated in many clinical conditions (Perusse *et al.* 1992a, 1992b, Eidelman *et al.* 2005). In this context, we have previously reported that liposomal formulation with prilocaine is able to prolong the duration of anaesthesia when compared to plain prilocaine (without vasoconstrictor). Also this formulation showed similar effects of felypressin-containing prilocaine suggesting that liposomal encapsulation is able to replace the vasoconstrictor in LA formulations to dentistry uses (Cereda *et al.* 2004), especially when the vasoactive compound is contraindicated.

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So, in this study we evaluated the effectiveness of a liposomal formulation with LDC, in comparison to commercially available solutions of LDC for infiltrative use (with or without vasoconstrictor). The effectiveness was evaluated by the use of rat infraorbital nerve blockade model. Moreover, the liposomes were prepared using scalable procedure (spray-drying with addition of a cryoprotectant) and composition with hydrogenated lecithin:cholesterol that favour the increase in shelf-life of products.

Materials

LDC hydrochloride and thiopental were donated by Cristália Ind. Quim. Farm. Ltda (Itapira, Brazil). 2% LDC solution containing 1:100 000 epinephrine was purchased from DFL-Ind. Com. S.A. (Rio de Janeiro, Brazil); Epikuron 200SH (hydrogenated soybean phosphatidylcholine, HSPC) was supplied from Lukas Meyer Inc. (Hamburg, Germany); mannitol was obtained from Labsynth Prod. Lab. Ltda (Diadema, SP) and cholesterol (Chol) was purchased from Sigma Chem. Co. (St Louis, USA). All other reagents were of analytical grade.

Liposomal LDC preparation

A solid film was prepared by spray-drying (Goldbach *et al.* 1993) of a mixture of HSPC:Chol and mannitol (3:1:1.25 mol%), at 110 °C. Multilamellar liposomes were obtained by adding 20 mM Hepes buffer pH 7.4 to the freeze-dried material, stirred for 1 h at 65 °C. Unilamellar liposomes (LUV) were prepared by extrusion (Mowat *et al.* 1996) of the multilamellar vesicles (15×) through 400 nm polycarbonate membrane, using a (Lipex Biomembranes Inc., Vancouver, Canada) extrusion unit under N_2 flux at 65 °C, i.e. above the main transition temperature of HSPC ($T_m = 52$ °C) (Darwis and Kellaway 2001). The phospholipid content of the liposomes was determined by phosphate quantification in the samples, according to Rouser *et al.* (1970). The total lipid concentration in the LUV was set to 5 mM (Cereda *et al.* 2004, de Araújo *et al.* 2004).

After extrusion, LDC was added to the LUV, to reach a final 2% concentration (equivalent to the commercially available epinephrine-containing and epinephrine-free LDC, used in the anaesthesia procedures), and the system left for equilibrium for 2 h at 65 °C. As in previous studies, we did not remove the unencapsulated LDC from the aqueous phase (Cereda *et al.* 2004, 2006, de Araújo *et al.* 2004, 2008), since the LA equilibrium between the membrane and water phase is rather fast (Paula and Schreier 1995, de Paula and Schreier 1996), and such procedure would decrease the amount of LDC available for nerve blockade, below its clinical dose.

Determination of particle size, polydispersity, zeta potential and liposomal concentration

The mean particle size and distribution (polidispersity, PDI values), and Zeta potential of the liposomes – with and without LDC – were analysed by dynamic light scattering (DLS), using a Nano ZS90 (Malvern Instruments, UK) equipment. The average diameter and size distribution (Span) assessed using a NS300 NTA instrument equipped with a 532 nm laser (Nanosight, UK) that also allowed determination of the concentration of liposomes (number of vesicles/mL). All measurements were performed in freshly diluted samples, at room temperature ($n = 3$).

Transmission electron microscopy

The morphology of the vesicles – liposomal formulations containing or not LDC – was analysed by transmission electron microscopy (TEM). In order to provide contrast, 2% of uranyl acetate was added to the diluted samples that were deposited into copper grids coated with a carbon film and dried up to remove the solvent. The micrographs of the formulations were elucidated using a JEOL 1200 EXII microscope, operated at 80 kV.

Encapsulation efficiency

The encapsulation efficiency of LDC into liposomes was determined by phase separation (centrifugation of the liposomal suspensions at 120 000 × g for 2 h, 20 °C). LDC concentration in the supernatant was detected by UV absorption ($\varepsilon^{M}_{260\,nm} = 380$) (de Paula and Schreier 1995). The amount of anaesthetic in the supernatant was subtracted from the initial LDC concentration, in order to determine the fraction of anaesthetic bounded to the liposomes (expressed as percent of encapsulation efficiency – EE%).

In vitro *release study*

The *in vitro* LDC release from liposomes was investigated using a modified two-compartment method Franz-cell system, as described by Paavola *et al.* (1995). In brief, the liposome formulation was added into a donor compartment (1 mL) separated by a cellulose membrane (Spectra/Pore 12 000–140 00 Da) from an acceptor compartment (100 mL), containing 20 mM Hepes buffer, pH 7.4. Aliquots of 1 mL were withdrawn from the acceptor compartment periodically, replacing the withdrawn volumes. The amount of LDC released to the acceptor compartment was determined at 260 nm, and expressed as percent values. Mathematical modelling was used to analyse the obtained LDC release profiles. The best fits were found with monoexponential (Equation (1)) and biexponential (Equation (2)) models, as revealed by the correlation coefficients obtained with Sigma Plot 8.0 software (Systat Inc, San Jose, USA).

$$C = 1 - \left(C_0 e^{-kt}\right) \quad (1)$$

$$C = 1 - \left(a.e^{-k_1 t} + b.e^{-k_2 t}\right) \quad (2)$$

where C is the concentration of LDC released at time t, C_0 the drug loading, k, k_1 and k_2 are the observed kinetic rate constants, a and b parameters reflect the portion of the initial concentrations of LDC that contributed to the burst and sustained phases, respectively.

The release efficiency (RE%) was used to compare the drug-release profiles (Costa and Lobo 2001).

$$RE = \frac{\int ydt}{y_{100}.t} \times 100 \quad (3)$$

where $\int ydt$ is the area under the release curve up to a certain time, t; $y_{100}.t$ is the area of the rectangle described by complete (100%) drug-release in the same time. Each replicate ($n = 4$) was used to calculate the RE% values of the formulation, which are expressed as mean ± SD.

Infraorbital nerve blockade tests

The antinociceptive test protocol was approved by the Institutional Committee for Ethics in Animal Research of the University of Campinas – UNICAMP (Protocol no. 1004-1), which follows the recommendations of the guide for the care and use of laboratory animals. Male Wistar rats, 250–350 g, were obtained from CEMIB (Centro de Bioterismo – UNICAMP) and were given free access to water and food throughout the study.

To evaluate the anaesthetic effect, the rat infraorbital nerve blockade test was used, as adapted from Fink *et al.* (1975). The infraorbital nerve of the rat, with a diameter of 2–3 mm innervates the upper lip and the whisker area; it emerges from the skull in the infraorbital notch, situated above a gap between the posterior molars and the anterior incisor, in each side of the rat jaw. The anaesthetic preparations were injected into this site, after the animals were lightly anesthetized with intraperitoneal thiopental (25 mg/kg). The degree of sedation did not interfere with the aversive response to upper lip pinching, induced with an artery forceps.

The anaesthetic effect was assessed by observation of aversive response to rat upper lip pinching, according to the scores: 0 (aversive response) or 1 (no aversive response). These values were expressed as percent LA activity. Each group ($n = 7$–10 animals) received 0.1 mL of the following preparations: group I – control liposomes, without LDC (LUV); group II – plain LDC (LDC_{PLAIN}); group III – epinephrine (1:100 000)-containing LDC (LDC_{VASO}) and group IV – liposomal LDC (LUV_{LDC}). Equivalent (2%) LDC concentrations were used for LDC_{PLAIN}, LDC_{VASO} and LUV_{LDC}. All preparations were randomly evaluated and performed by the same operator. The samples were injected unilaterally into the right side of the rat upper lip, and the intact left side served as control. The animals were tested at every 5 min, up to detection of the first aversive sign, in the injected side.

The efficacy of the infraorbital nerve blockade was taken from the time needed for the sensory function recovery or analgesia duration (time for recovery), from maximum possible effect (MPE), and from the total LA effect. This last parameter was estimated by the area under the (effect *vs.* time) curve (AUC) calculated using the trapezoidal rule (Gantenbein *et al.* 1997) expressed by score/h. Both parameters were calculated using the Origin 6.0 program (Microcal TM Software, Inc., Northampton, USA), and expressed as means ± standard error of mean (SEM). Statistical analysis among the groups was analysed by one-way ANOVA, followed by Tukey's *post hoc* test, with $p = 0.05$ significance level.

Results and discussion

LA are frequently used in combination with a vasoconstrictor agent, typically epinephrine, in order to enhance the intensity and duration of their action (Covino and Vassallo 1976, Tucker and Mather 1980). In the last decades, a large number of approaches have attempted to increase the duration of LA action without increasing its systemic toxicity, such as the development of liposomal drug-delivery systems that achieves slow anaesthetic release over an extended period of time (Boogaerts *et al.* 1994, Lafont *et al.* 1996, Malinovsky *et al.* 1997, Grant *et al.* 2000, Dyhre *et al.* 2001, Cereda *et al.* 2004, de Araújo *et al.* 2004). Nevertheless, the production of liposomes in large scale, maintaining the chemical and physical stability of the particles, is a known limiting step in the development of these drug-delivery systems (Li and Deng 2004).

Indeed, literature reports encouraging results obtained with LDC -containing liposomal formulations, by different research groups (Mashimo *et al.* 1992, Bucalo *et al.* 1998, Taddio *et al.* 2005) and ours (Cereda *et al.* 2006, Franz-Montan *et al.* 2012, 2015). However, the development of scalable formulations, which is essential to reach the market, remains a challenge (Li and Deng 2004, Allen and Cullis 2013, Bozzuto and Molinari 2015, Sercombe *et al.*, 2015). To face that, the preparations of novel liposome formulations that are suitable for scale-up process are needed. Unlike the previously published liposomal–LDC formulations with unsaturated lipids (Zucker *et al.* 2009, Yeagle 2012), here we prepared vesicles with HSPC:cholesterol that were not prone to peroxidation. Moreover, the liposomes were prepared by freeze-drying (Goldbach *et al.* 1993), and in the presence of a cryoprotectant (mannitol), in order to guarantee proper reconstitution of the formulation as large unilamellar vesicles (LUV) (Cabral *et al.* 2004).

Structural characterization of the liposomal–LDC formulation

The physicochemical characterization of liposomal formulation and its control (without LDC) was performed by DLS, nanoparticle tracking analysis (NTA) and TEM methods. Table 1 shows characterization data of LUV and LUV_{LDC}, regarding particle size, polydispersity (PDI and Span indexes), Zeta potential and liposome concentration (vesicles/mL).

DLS data revealed a monodisperse population of liposomes with particle size around 240 nm and low PDI (0.18); after LDC incorporation, the average size and polydispersity increased to 260 nm and 0.26, respectively. Zeta potentials were negative, far from 0 (higher than – 30 mV) and did not change significantly upon LDC addition, contributing to the stability of the formulation in solution (Attama *et al.* 2012).

Table 1. *In vitro* characterization of liposomal LDC formulation, regarding particle size, polydispersity, Zeta potential and liposomes concentration (vesicles/mL), assessed by DLS and NTA.

	DLS			NTA		
Formulation	Size (nm)	Dispersity (PDI)	Zeta (mV)	Size (nm)	Dispersity (Span)	[LUV] (vesicles/mL)
LUV	240.0 ± 1.8	0.181 ± 0.03	−31.7 ± 0.6	102.6 ± 52.4	1.1	$2 \times 10^{12} \pm 0.2 \times 10^{12}$
LUV_{LDC}	260.1 ± 5.5	0.260 ± 0.09	−32.3 ± 1.4	135.9 ± 4.4	1.5	$3 \times 10^{12} \pm 0.4 \times 10^{12}$

Values are displayed as mean ± S.D ($n = 3$).
LUV: control liposomes; LUV_{LDC}: liposomes containing 2% LDC.

Similar results were obtained with video-tracking analysis of individual particles (NTA): the mean size distribution for LUV and LUV_{LDC} were 102 and 135 nm, with polydispersity Span values of 1.1 and 1.5, respectively. The Span index, calculated from the cumulative size distribution of liposomes, should have values around 1 for monodisperse samples, as observed here (Bender *et al.* 2012).

Together, the two analytical (DLS and NTA) methods provided comparable results. Both techniques showed the same effect in the liposomes after LDC entrapment, i.e. a slight increase in particle size and polydispersity. NTA also allowed determination of the liposomes concentration ($2–3 \times 10^{12}$ vesicles/mL), a mandatory parameter to describe colloidal formulations designed for drug delivery (Ribeiro *et al.* 2018).

The slightly increase in particle size and the more heterogeneous distribution of LUV_{LDC} sizes was justified by the anaesthetic incorporation (EE% = 24%, see below). NTA size distributions are always smaller than those determined by DLS from the scientific principles of the technique, which is supported by the particle-by-particle approach. Differently from DLS, the existence of a small amount of large particles in the sample does not affect the particle size determination by NTA. As for DLS, its particle size (average) calculation is affected by the presence of dispersed large particles, interfering with quantification of the small ones, and leading to overestimated average sizes (Filipe *et al.* 2010).

It is important to note that mannitol, used as excipient in the freeze-drying process (to favour liposomes' large-scale production), caused no drastic effects in the liposomal structural properties. Indeed, TEM images revealed the spherical morphology of liposomes with well-delimited contours, confirming the efficient preparation of LUV and LUV_{LDC} (Figure 1).

The size distribution and the average size of the individual liposomes were calculated from the micrographs (at 100 000 magnification, through the ImageJ software). The obtained results (237 and 250 nm for LUV and LUV_{LDC} samples, respectively) showed good agreement with those determined by DLS.

In general, the results obtained by DLS, NTA and TEM demonstrated that the presence of LDC did not disturb the structural properties of the liposomes, which maintained suitable structural properties (size, polydispersity, Zeta potential, vesicles concentration and morphology) for drug delivery. Therefore, these results exemplify the importance of using a set of biophysical methods to understand the structural organization of the colloidal systems (Ribeiro *et al.* 2018).

Encapsulation efficiency of LDC in the liposomes

LUV_{LDC} was able to incorporate 24% of the LDC, in agreement to previous studies from our group, in which EE% = 22% was achieved for LDC in phosphatidylcholine:cholesterol:α-tocopherol (4:3:0.07 mol%) % (Cereda *et al.* 2006, Franz-Montan *et al.* 2015). The low level of LDC encapsulation into the liposomes reveals its less hydrophobic nature, when compared to other aminoamide anaesthetic agents such as etidocaine and bupivacaine (de Paula and Schreier 1995). Regarding the lipid composition, liposomes composed of lipids of high T_m such as hydrogenated soybean phosphatidylcholine are known to produce less fluid liposomes, decreasing the incorporation of guest molecules, as also reported for beclomethasone (Darwis and Kellaway 2001). Then, the bilayer formed by hydrogenated soy-bean lecithin, being more ordered than those of (unsaturated) egg lecithin, should curb the accommodation of LDC molecules in-between the lipids (Zucker *et al.* 2009). But the presence of cholesterol counteracts the influence of the high T_m of HSPC, facilitating LDC incorporation in the bilayer. Moreover, HSPC is less prone to peroxidation than egg-PC (Torchilin 2012, Yeagle 2012), favouring the shelf stability of the liposomal formulation.

In vitro *drug release*

The kinetics of LDC release from the liposomes was evaluated, as shown in Figure 2. LDC in solution, hereon identified as PLC_{PLAIN} and liposomal LDC presented quite different deliveries: 92 and 71%, respectively, after 300 min.

The release profiles for LDC_{PLAIN} and LUV_{LDC} were modelled using monoexponential and biexponential models. LDC_{PLAIN} presented the best experimental data according to the monoexponential model ($r = 0.9997$), with a constant rate of $0.0396 \pm 0.001\ min^{-1}$. For LUV_{LDC} the best data fitting was obtained with the biexponential model ($r = 0.9998$); the observed rate constants for the burst (k_1) and sustained (k_2) phases of 0.052 ± 0.015 and $0.0253 \pm 0.005\ min^{-1}$, respectively. From the rate constants, the release equilibrium time was assigned at 142.3 and 225.9 min, for LDC_{PLAIN} and LUV_{LDC}, respectively.

LUV_{LDC} showed slower release rate than plain LDC. The burst and sustained profiles of LUV_{LDC} (Figure 2) reflect the contribution of free and encapsulated LDC (24% of the total LDC in the formulation). The presence of liposomes prolonged the time for LDC release up to 60% in comparison to plain LDC, as revealed from the decreased release efficiency

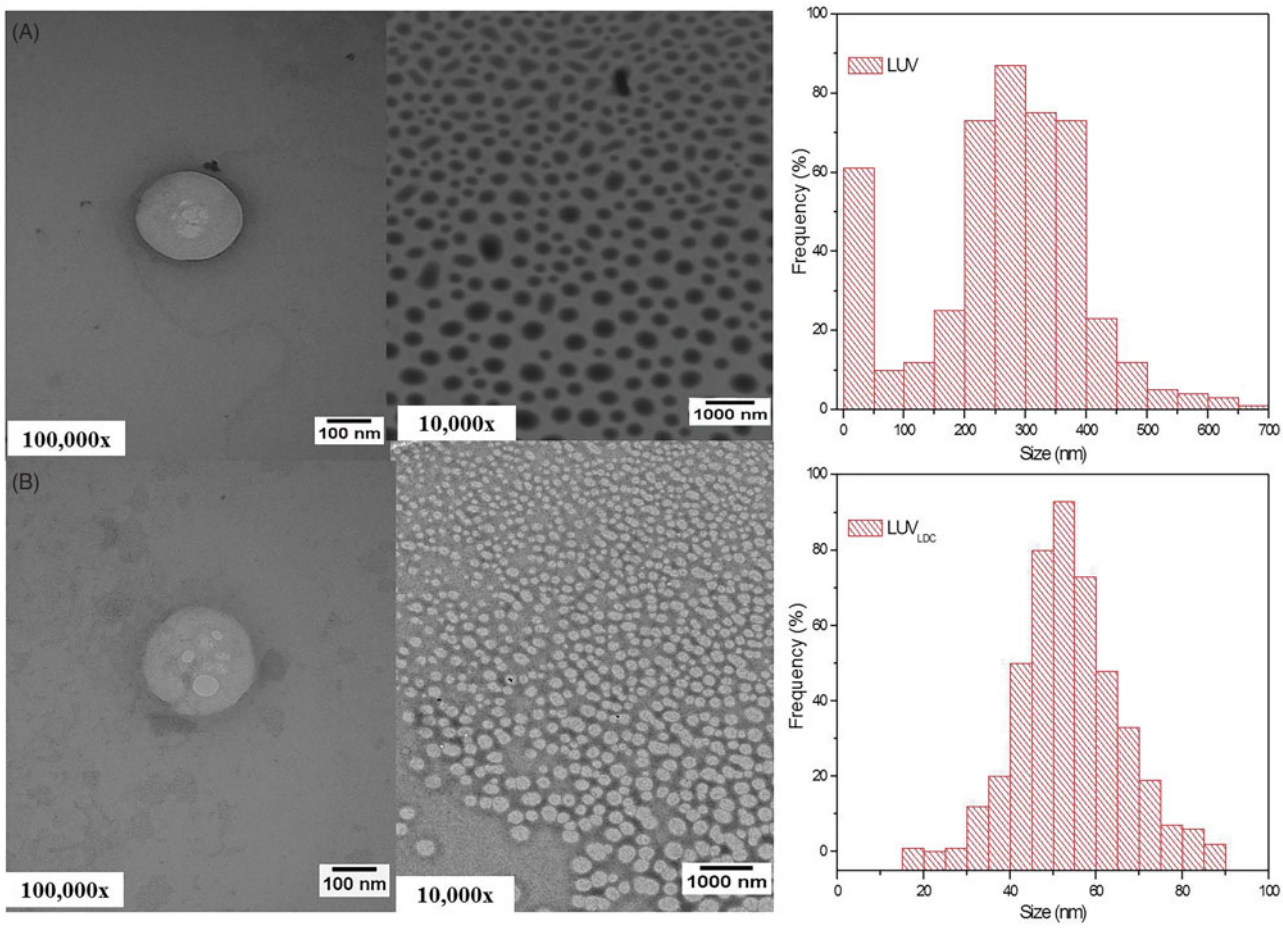

Figure 1. TEM images of LUV (A) and LUV$_{LDC}$ (B) and their respective size distribution, as calculated using the ImageJ software. Scale bar and magnifications are given in the micrographs.

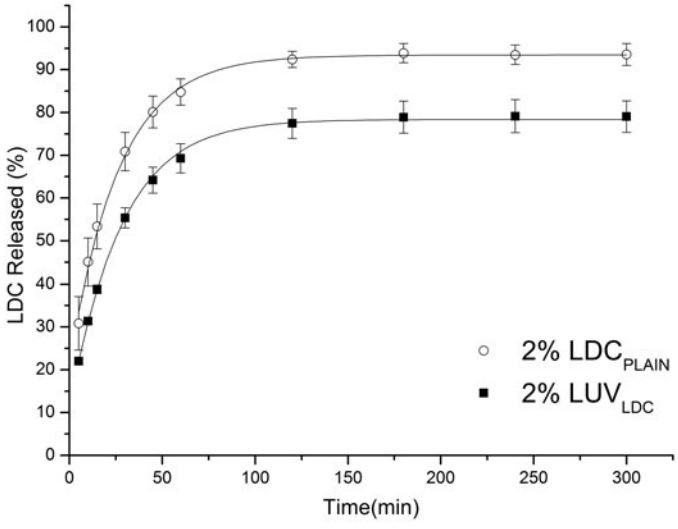

Figure 2. *In vitro* release kinetic profiles (mean ± SD) of LDC in solution (LDC$_{PLAIN}$) and liposomal LDC formulation (LUV$_{LDC}$) at pH 7.4, $n = 4$.

values. Using that approach we succeed to produce a novel liposome formulation, of equivalent LDC encapsulation to previous liposomal formulation (Cereda *et al.* 2006) but suitable for scale up production (by the lipid composition and incorporation of cryoprotectant), which also produced prolonged anaesthesia, in rats.

In vivo *antinociceptive evaluation*

The antinociceptive effect induced by LUV$_{PLAIN}$ and LUV$_{LDC}$ in the infraorbital nerve of rats is shown in Figure 3. The results are expressed as the percentage of MPE *vs.* time (min). Injection LDC-free liposomes, control group I (LUV) failed to induce any appreciable blockade (data not shown).

The results in Figure 3 show that duration of nerve blockade after treatment with LUV$_{LDC}$ was significantly longer than that produced by LDC$_{PLAIN}$. Nevertheless, the longest blockage was obtained with LDC$_{VASO}$. The total effect of nerve blockade (AUC and recovery time) produced after LDC$_{PLAIN}$, LDC$_{VASO}$ and LUV$_{LDC}$ injection are given in Table 2.

Treatment with LUV$_{LDC}$ significantly prolonged ($p < 0.05$) the analgesia time to 70 min after injection, when compared to LDC$_{PLAIN}$ (45 min). Analysing the AUC values, one can see that LUV$_{LDC}$ produced a 67% increase in the infraorbital nerve blockade, in comparison to LDC$_{PLAIN}$ ($p < 0.05$).

This *in vivo* blockade data agrees with those of the *in vitro* release kinetics, since both the blockade and the release time were prolonged with the liposome LDC formulation. The antinociceptive effects are also in accordance to previous reports in the literature that showed a prolonged analgesia in animals injected with egg phosphatidylcholine-based liposomes containing LDC (Mashimo *et al.* 1992), bupivacaine (Malinovsky *et al.* 1999), prilocaine (Cereda *et al.* 2004),

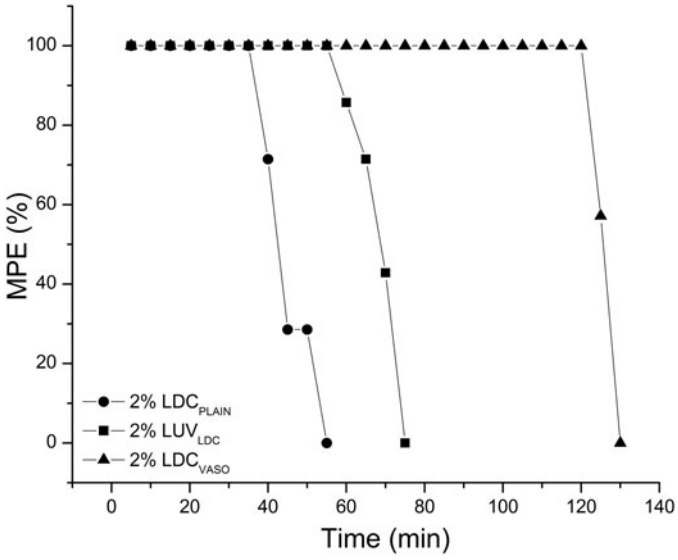

Figure 3. MPE (%) (mean ± SEM) of LDC (LDC$_{PLAIN}$), LDC plus epinephrine (LDC$_{VASO}$) and liposomal LDC (LUV$_{LDC}$) formulations, as evaluated by the rat infraorbital nerve blockade test.

Table 2 Total effect of sensory blockade (AUC) and recovery time for LDC$_{PLAIN}$ (in presence and absence of vasoconstrictor) and LUV$_{LDC}$ formulations.

Formulations	Time for recovery (min)	AUC (score/h)
LDC$_{PLAIN}$	45 (40–55)	37.5 (32.5–47.5)
LUV$_{LDC}$	70 (60–75)[a]*	62.5 (52.5–62.5)[a,]*
LDC$_{VASO}$	130 (125–130)[b]***,[c]*	122.5 (117.5–122.5)[b,]***,[c]*

Data are expressed as means (minimum – maximum) ($n = 7$/group). LDC content = 2%.
Statistical differences from the variance analysis (one-way ANOVA), with significance of:
[a]LDC$_{PLAIN}$ vs. LUV$_{LDC}$ – $p < 0.05$ (*)
[b]LDC$_{PLAIN}$ vs. LDC$_{VASO}$ – $p < 0.001$ (***)
[c]LUV$_{LDC}$ vs. LDC$_{VASO}$ – $p < 0.05$ (*).

mepivacaine (de Araújo *et al.* 2004) or either for LDC in soybean phosphatidylcholine–diacylglycerol liposomes (Dyhre *et al.* 2001).

Nevertheless, in our study (Figure 3) the duration of the nerve blockade after treatment with 2% LDC containing epinephrine (1:100 000) was significantly longer than the nerve blockade produced by LUV$_{LDC}$ or LDC$_{PLAIN}$. We have observed before (Cereda *et al.* 2006) that the intrinsic vasodilator activity of LDC probably counterbalance the prolonged release of LDC from liposomes and favours its clearance, leaving less LDC molecules available for neural blockade, explaining why the antinociceptive effect of LUV$_{LDC}$ was not comparable to that of LDC$_{VASO}$. Besides, the effect of epinephrine association with LA has been reported by Fink *et al.* (1975) using the infraorbital test in rats, who reported an increase of 80% in the blockade induced by 1% LDC associated to 1:200 000 epinephrine. But vasoconstrictors such as epinephrine can potentially cause serious systemic effects when included in LA preparations (Perusse *et al.* 1992a, 1992b), and its use may be avoided whenever possible.

In summary, the data presented here are very encouraging, since this novel soya-lecithin-based liposomal formulation significantly prolonged the duration of analgesia in more than 50%, when compared to plain LDC in the blockade of the infraorbital nerve in rats. Besides, the composition of the LDC liposomal formulation makes it suitable for large-scale preparation.

Conclusions

The composition of the liposomes presented in this study was desirable for the encapsulation of the LA LDC. Liposomal formulations exhibited compatibility among the excipients (including with the cryoprotectant mannitol) and desirable structural properties. The incorporation of LDC by LUV prolonged the release profile and increased the analgesic activity of LDC evaluated in rats in comparison with plain LDC. This system has been shown to be promising for scale-up process.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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