Encapsulation of mepivacaine prolongs the analgesia provided by sciatic nerve blockade in mice

[La micro-encapsulation de la mépivacaïne prolonge l'analgésie fournie par le bloc du nerf sciatique chez la souris]

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Purpose: Liposomal formulations of local anesthetics (LA) are able to control drug-delivery in biological systems, prolonging their anesthetic effect. This study aimed to prepare, characterize and evaluate *in vivo* drug-delivery systems, composed of large unilamellar liposomes (LUV), for bupivacaine (BVC) and mepivacaine (MVC).

Methods: BVC and MVC hydrochloride were encapsulated into LUV (0.4 μm) composed of egg phosphatidylcholine, cholesterol and α-tocopherol (4:3:0.07 molar ratio) to final concentrations of 0.125, 0.25, 0.5% for BVC and 0.5, 1, 2% for MVC. Motor function and antinociceptive effects were evaluated by sciatic nerve blockade induced by liposomal and plain formulations in mice.

Results: Liposomal formulations modified neither the intensity nor the duration of motor blockade compared to plain solutions. Concerning sensory blockade, liposomal BVC (BVC_{LUV}) showed no advantage relatively to the plain BVC injection while liposomal MVC (MVC_{LUV}) improved both the intensity (1.4–1.6 times) and the duration of sensory blockade (1.3–1.7 times) in comparison to its plain solution ($P < 0.001$) suggesting an increased lipid solubility, availability and controlled-release of the drug at the site of injection.

Conclusion: MVC_{LUV} provided a LA effect comparable to that of BVC. We propose MVC_{LUV} drug delivery as a potentially new therapeutic option for the treatment of acute pain since the formulation enhances the duration of sensory blockade at lower concentrations than those of plain MVC.

Objectif : *Des préparations liposomales d'anesthésiques locaux (AL) peuvent contrôler l'administration de médicaments dans les systèmes biologiques, prolongeant leur effet anesthésique. Notre objectif était de préparer, caractériser et évaluer des systèmes d'administration de médicaments in vivo, composés de gros liposomes unilamellaires (GLU), pour la bupivacaïne (BVC) et la mépivacaïne (MVC).*

Méthode : *Le chlorhydrate de BVC et de MVC a été mis en capsules dans des GLU (0,4 μm) composés de lécithine d'œuf, de cholestérol et de α-tocophérol (concentration molaire 4:3:0,07) pour obtenir des concentrations finales de 0,125, 0,25, 0,5 % pour la BVC et 0,5, 1, 2 % pour la MVC. La fonction motrice et les effets antinociceptifs ont été évalués par le blocage du nerf sciatique induit par des préparations liposomales et des préparations simples chez des souris.*

Résultats : *Les préparations liposomales, comparées aux préparations simples, n'ont pas modifié l'intensité ni la duré du bloc moteur. Quant au bloc sensitif, la BVC liposomale (BVC_{LUV}) n'a pas présenté d'avantage par rapport à l'injection de BVC simple tandis que la MVC liposomale (MVC_{LUV}) a amélioré l'intensité (1,4–1,6 fois) et la duré du bloc sensitif (1,3–1,7 fois) comparée à la solution simple ($P < 0,001$). Ce qui laisse croire à une meilleure solubilité lipidique, à une disponibilité accrue et à une meilleure administration du médicament à libération contrôlée au site de l'injection.*

Conclusion : *La MVC_{LUV} fournit un effet AL comparable à celui de la BVC. Nous proposons l'administration de MVC_{LUV} comme un nouveau choix possible de traitement de la douleur aiguë, puisque la préparation augmente la durée du bloc sensitif à des concentrations plus faibles que celles de la MVC simple.*

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BUPIVACAINE (BVC) and mepivacaine (MVC) are local anesthetics (LA) that are used worldwide for pain relief.[1] BVC is the drug of choice in surgery due to its moderate onset and long duration of action, as well as its significant differentiation between motor and sensory blockade. Although chemically related to BVC, MVC has lower systemic and cardiotoxicity, induces rapid onset of anesthesia but has a restrained duration of action.[2]

The development of LA formulations in carriers - such as liposomes - offers the possibility to control drug delivery in biological systems, prolonging their anesthetic effect.[3] Because of their amphiphilic nature, when bound to model membrane systems, LA reside mainly in the bilayer region[4–6] while another fraction remains in the aqueous core.[7] We can take advantage of this strong interaction between LA and liposomes to control the release of the drug, in order to obtain a prolonged duration of action and reduce central nervous and/or cardiovascular system toxicity.[8,9]

We aimed to prepare, characterize and evaluate two drug-delivery systems for the amino-amide anesthetics BVC and MVC. Motor and sensory blockade in mice were used to compare liposomal formulations and the LA preparations available commercially.

Methods

BVC and MVC hydrochlorides were donated by Cristália Prod. Quím Farm Ltda (Itapira, SP, Brazil). Egg phosphatidylcholine, cholesterol, α-tocopherol and HEPES buffer were purchased from Sigma Chemical Company (St. Louis, MO, USA). BVC and MVC solutions were prepared in 0.9% saline (154 mM NaCl), whereas LA liposomal formulations were prepared in 20 mM HEPES buffer plus 154 mM NaCl at pH 7.4.

Preparation and *in vitro* tests - including liposome size distribution, partition coefficient determination and release kinetics - are presented in the Appendix available as Additional Material at www.cja-jca.org.

In vivo efficacy studies were performed using groups of eight to ten male Swiss adult albino mice, weighing 30 to 35 g and obtained from CEMIB-UNICAMP (Centro de Bioterismo - State University of Campinas - UNICAMP, Campinas, SP, Brazil). The protocol was approved by the UNICAMP Institutional Animal Care and Use Committee (Protocol 322-1) which follows the recommendations of the Guide for the Care and Use of Laboratory Animals.

Animals selected for the motor blockade test were treated by infiltration (0.1 mL) with plain and liposomal formulations of BVC and MVC. The needle was inserted into the popliteal space on the posterior surface of the knee, in the area of the sciatic nerve. Motor and sensory blockade were assessed simultaneously. Drugs were given in a randomized order (animals were assigned to the groups by using a previous numbering list) and the same investigator performed all the experiments. The animals were observed for 24 hr after treatment in order to detect possible toxic (systemic, such as seizures or obits) effects or nerve damage (lack of recovery of normal movement on the injected limb) caused by the procedure. The experimental groups included: control (treated with drug-free liposomes - large unilamellar liposomes (LUV) - or plain LA solutions - BVC_{plain} or MVC_{plain}) and liposomal (BVC_{LUV} or MVC_{LUV}) treated mice. The sciatic nerve model was used to assess motor blockade. Before starting the experiment, the ability of each mouse to walk normally with four limbs on both the top and inverted side of a wire mesh screen (1 mm diameter wire, 5 mm mesh) was evaluated. Only animals that showed this behaviour were selected for the experiment. LA activity was assessed by the loss of motor control in the injected limb with the scores: 0 (normal movement); 1 (unable to flex the limb completely); and 2 (total paralysis).[10] Any animal that could not use the injected limb to walk normally on the top and on the inverted wire mesh screen was considered to have a positive response to local anesthesia. The efficacy of motor blockade was evaluated every minute, from one to five minutes, and thereafter every ten minutes up to one hour following the injection. Latency (time between injection and the loss of motor function), time to reach the maximum score (T_{max}), time for motor function recovery and the total LA effect (estimated by the area under the effect *vs* time curve and expressed by score/hour; AUC)[11] were evaluated using Origin 6.0 (Microcal™ Software, Inc., Northampton, MA, USA).

Evaluation of sensory blockade was done by the paw pressure test or mechanical nociception.[12] Pain was measured with an analgesimeter (Ugo Basile, Varese, Italy), which exerts an increasing - at a constant rate - force (in grams) on the paw (Figures 1A and B). In order to avoid analgesia induced by excessive stress, each animal was gently wrapped in a small towel so that only the limbs and head were free. The withdrawal reflex was considered representative of the pain threshold or paw withdrawal threshold to pressure (PWTP). The baseline of the PWTP test was measured before injecting vehicle or drugs, in order to determine the pain threshold of the animal. Baseline values of 30 to 50 g were selected as the pain threshold and animals that presented lower or higher values

TABLE Latency, T_{max}, time for recovery and total effect of motor blockade (AUC) for plain and liposomal bupivacaine and mepivacaine formulations

Groups	*Concentration (%)*	*Latency (min)*	*T_{max} (min)*	*Time for recovery (min)*	*AUC (score/hr)*
BVC_{plain}	0.125	1 (1–2)	3 (2–3)	20.0 (-)	22.5 (19.0–28.0)
	0.25	1 (1–2)	2 (2–3)	40.0 (30.0–40.0)	41.0 (37.0–48.0)
	0.5	1 (-)	1 (-)[a***]	50.0 (30.0–60.0)[a***]	58.0 (50.0–88.0)[a**]
BVC_{LUV}	0.125	1 (1–2)	4 (2–5)	20.0 (20.0–30.0)	21.5 (17.0–38.0)
	0.25	1 (-)	2.5 (1–3)	40.0 (30.0–50.0)	57.0 (19.0–78.0)
	0.5	1 (-)	1 (1–2)[b**]	40.0 (40.0–50.0)[b*]	68.0 (58.0–98.0)[b***]
MVC_{plain}	0.5	1 (1–2)	2 (2–3)	25.0 (20.0–40.0)	28.5 (20.0–58.0)
	1	1 (-)	2 (1–4)	30.0 (20.0–40.0)	37.5 (27.0–48.0)
	2	1 (-)	1.5 (1–3)	40.0 (40.0–50.0)[c*]	68.0 (39.0–78.0)[c*]
MVC_{LUV}	0.5	1 (1–2)	2.5 (1–3)	20.0 (20.0–40.0)	27.0 (19.0–38.0)
	1	1 (-)	2.5 (1–3)	40.0 (30.0–40.0)	47.5 (37.0–58.0)
	2	1 (-)	1 (1–3)	40.0 (40.0–50.0)[d**]	58.0 (47.0–78.0)[d**]

Data are expressed as median (minimum–maximum; n = eight per group). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Tmax = time to reach the maximum score; BVC = bupivacaine; MVC = mepivacaine; LUV = large unilamellar liposomes. Statistical differences between: a, 0.5% BVC_{plain} and 0.125% BVC_{plain}; b, 0.5% BVC_{LUV} and 0.125% BVC_{LUV}; c, 2% MVC_{plain} and 0.5% MVC_{plain}; d, 2% MVC_{LUV} and 0.5% MVC_{LUV}. AUC = areas under the curve.

than that of baseline were excluded. The established antinociception cut-off value was 150 g, considered to be representative of the anesthetic state. After drug or vehicle administration, measurements were carried out at intervals of 15 min during the first hour, 30 min in the second and third hour and finally 60 min up to five hours after treatment.

Size distribution of liposomes was compared by the one-way analysis of variance (one-way ANOVA) and *in vitro* release tests were analyzed by unpaired t test. Motor block data (latency, T_{max}, time for recovery and AUC) were analyzed by the Kruskall-Wallis test and expressed as medians (minimum and maximum limits). Sensory blockade data were analyzed by one-way ANOVA with Tukey-Kramer as a post hoc test. Statistical significance was defined as $P < 0.05$.

Results

Motor function was affected by the injection of BVC and MVC (plain or liposomal) so that the mice lost motor control in the injected limb. The LUV group did not show any motor blockade. Latency, T_{max}, time for recovery and total LA effects were compared between all the experimental groups (Table). Dose-dependent effects were observed on motor blockade for liposomal and plain formulations of LA. No significant differences were observed between liposomal and plain formulations for any of the LA.

T_{max}, time for recovery and AUC were greater with 0.5 compared to 0.125% BVC_{plain} ($P < 0.001$). Similar effects were observed between 0.5% and 0.125% BVC_{LUV} for the same variables ($P < 0.01$, $P < 0.05$ and $P < 0.001$, respectively).

Time for recovery and AUC were greater with 2% compared to 0.5% MVC_{plain} ($P < 0.05$) and with 2% compared to 0.5% MVC_{LUV} ($P < 0.01$).

Equivalent effects were found between 0.25% BVC_{LUV} and 0.5% BVC_{plain} and between 0.5% MVC_{LUV} and 1% MVC_{plain}, both in time for recovery and AUC.

Dose-response relationships were established for BVC and MVC (plain and liposomal) in mice by the PWTP test (Figures 2 and 3). Time-course of analgesia was recorded for the groups: drug-free LUV, BVC or MVC plain and BVC_{LUV} or MVC_{LUV}, at three different concentrations of the anesthetics. Figures 2 and 3 show that vehicle infiltration in the region of the sciatic nerve did not modify baseline values (30–50 g) of the pain threshold, while all LA formulations tested were different from their control-vehicle groups ($P < 0.001$) regarding sensory blockade.

BVC_{LUV} showed similar intensity (evaluated from the average response on the PWTP test) and duration of sensory blockade when compared to BVC_{plain}. A higher intensity of sensory blockade were found only at 15 min for 0.125% BVC_{LUV} ($P < 0.01$; Figure 2A);

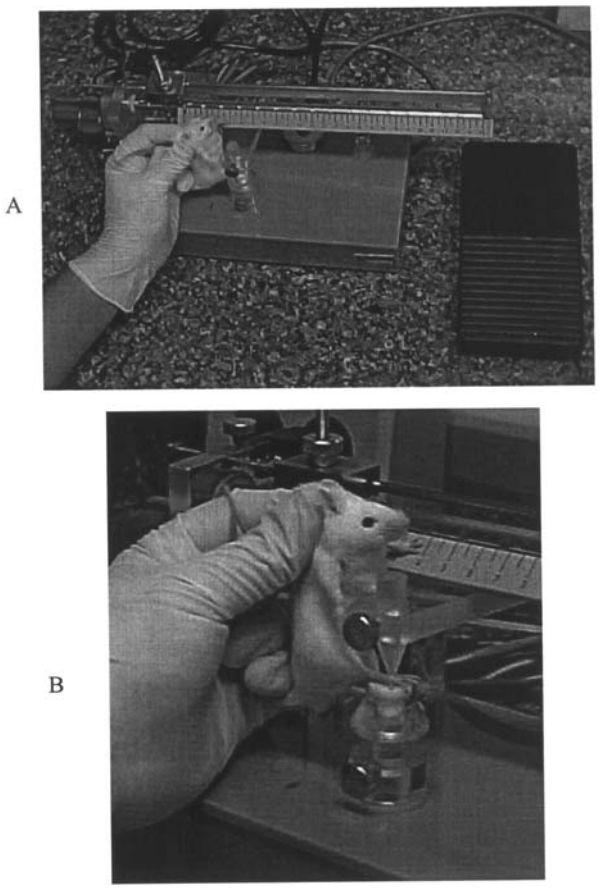

FIGURE 1 Paw withdrawal threshold to pressure (PWTP) test (A). The mouse's hindpaw is placed under a pressure-pad (B) and the force (in grams) applied to obtain limb withdraw is recorded.

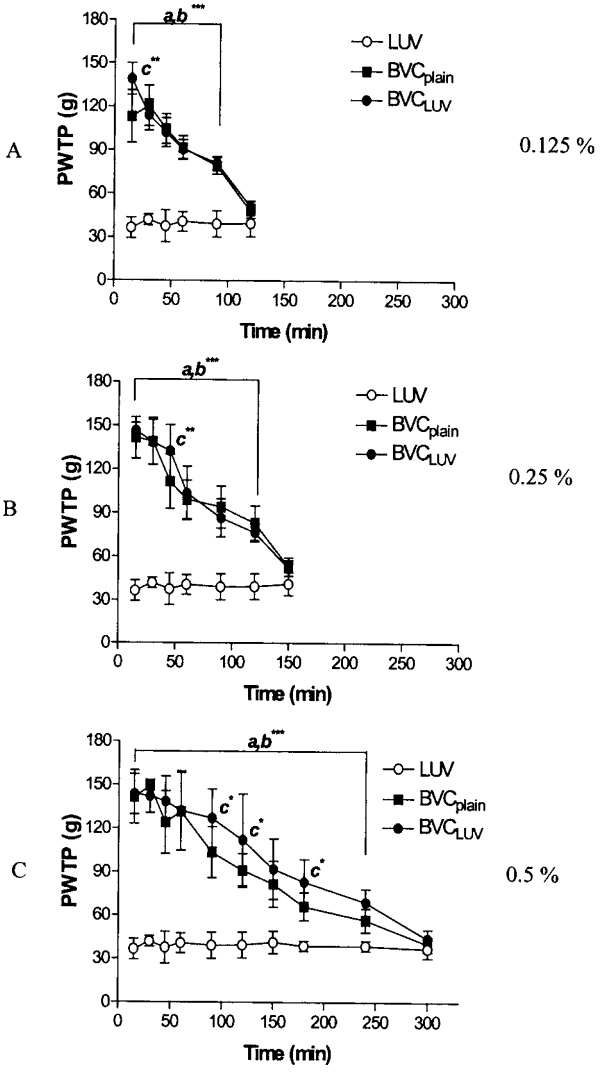

FIGURE 2 Time-course (min) on the paw withdrawal threshold to pressure (PWTP) test showing control group large unilamellar liposomes (LUV), bupivacaine $(BVC)_{plain}$ and BVC_{LUV} formulations at 0.125% (A), 0.25% (B) and 0.5% (C) BVC concentration. Values are expressed as mean ± SD (n = ten per group). Differences between: a, BVC_{LUV} and LUV; b, BVC_{plain} and LUV; c, BVC_{LUV} and BVC_{plain}. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

at 45 min for 0.25% BVC_{LUV} ($P < 0.01$; Figure 2B) and at 90, 120 and 180 min ($P < 0.05$) for 0.5% BVC_{LUV} (Figure 2C), relatively to their plain solution.

Treatment with 0.5% MVC_{LUV} and 0.5% MVC_{plain} (Figure 3A) showed similar analgesic profiles in the first 120 min after infiltration, but subsequently MVC_{LUV} prolonged analgesia up to 180 min with differences in the PWTP observed after 150 min ($P < 0.001$). PWTP were higher from 90 to 180 min after injection of 1% MVC_{LUV}, compared to MVC_{plain} ($P < 0.05$ and $P < 0.001$, respectively). At 180 min a 1.6 times increase in the PWTP was measured; analgesia was prolonged up to 300 min after MVC_{LUV} (Figure 3B). PWTP were higher, from 45 to 240 min after treatment with 2% MVC_{LUV} in comparison to 2% MVC_{plain} ($P < 0.01$ and $P < 0.001$, respectively). At 240 min after injection, PWTP was increased 1.4 times compared to the plain formulation; analgesia was prolonged up to 300 min (Figure 3C). These data show that infiltration of MVC_{LUV} increased the intensity and duration of analgesia up to 240 min (0.5%, $P < 0.001$) and 300 min (1% and 2%, $P < 0.01$ and $P < 0.001$, respectively) compared to their plain solution.

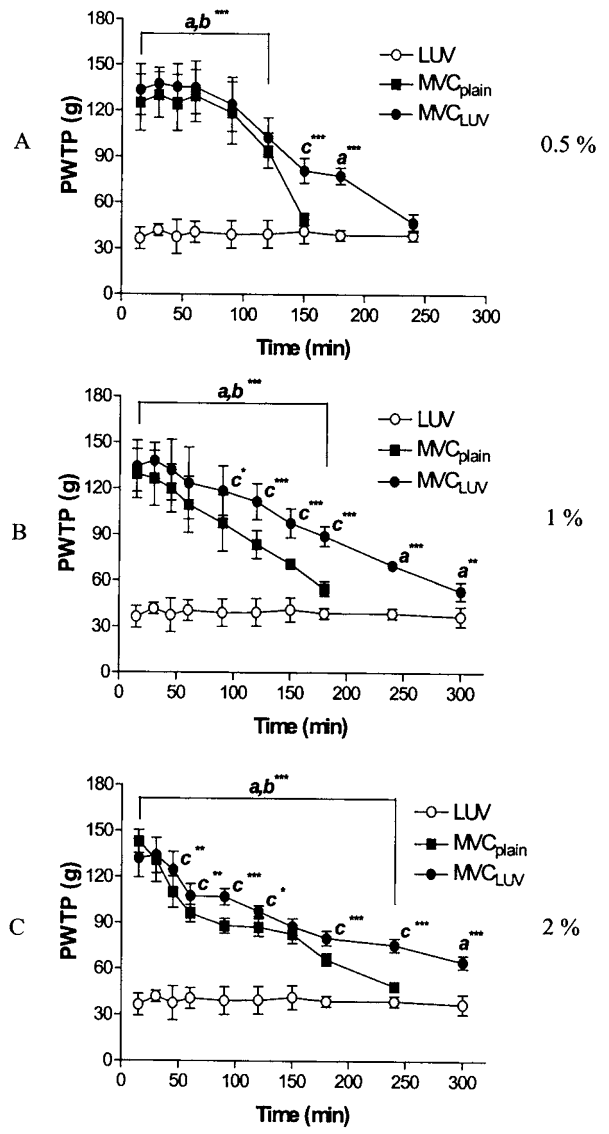

FIGURE 3 Time-course (min) on the paw withdrawal threshold to pressure (PWTP) test showing control large unilamellar liposomes (LUV), mepivacaine (MVC)$_{plain}$ and MVC$_{LUV}$ formulations at 0.5% (A), 1% (B) and 2% (C) MVC concentration. Values are expressed as mean ± SD (n = ten per group). Differences between: a, MVC$_{LUV}$ and LUV; b, MVC$_{plain}$ and LUV; c, MVC$_{LUV}$ and MVC$_{plain}$. *P < 0.05, **P < 0.01, ***P < 0.001.

Discussion

The ideal LA must have a long duration of action, low systemic toxicity and selectivity for sensory rather than for motor blockade. In order to achieve this goal, numerous approaches have been used so far, such as the organic synthesis of new LA molecules, the management of LA pH formulations and pharmaceutical associations. In the last decade, the development of LA formulations in carriers - such as liposomes - has provided the possibility to control drug-delivery in biological systems, prolonging the anesthetic effect and/or reducing its toxicity.[3] Here we studied the encapsulation of BVC and MVC into liposomes. These compounds belong to the cyclic amino-amide series of LA. Despite being chemically related, they exhibit differences in physicochemical properties such as partition coefficients,[13] ionization and solubility,[4–7] features that are determinant in their anesthetic potency.[1]

Sciatic nerve blockade was used as an experimental model, providing information about the intensity and duration of blockade induced by LA agents.[10] Many studies have utilized motor or sensory blockade to assess LA activity in rats[14–17] and mice.[18–20] Our experimental model was shown to be appropriate to monitor the duration of motor and sensory blockade induced by LA in mice. Furthermore, the choice of a baseline PWTP was important to limit injury and avoid excessive stimulation of the nociceptors and stress-induced analgesia in mice.

BVC$_{LUV}$ and MVC$_{LUV}$ were not able to modify the intensity or the duration of LA motor blockade, but MVC$_{LUV}$ improved the antinociceptive effects on the PWTP test relatively to its plain solution. Our results suggest that the concentration of the anesthetic available at the site of action was improved due to the partition of MVC into LUV. Increasing the local availability of MVC to the nerve fibre, the liposomal formulation enhanced the duration of analgesia and the differential nerve blockade induced by MVC. Controlled release formulations of LA formulations have been used to maintain a high therapeutic index in the treatment of acute pain[19] or in the management of postsurgical pain,[21] reducing systemic toxicity and blocking sensory rather than motor fibres. This last feature, analgesia without excessive motor blockade, has enormous clinical relevance.[9,21,22]

Encapsulation of BVC in MLV[21,23,24] has been reported to prolong its effects and decrease local/systemic toxicity.[25,26] Nevertheless our study did not show any improvement in BVC action after encapsulation into LUV, despite the greater osmotic stability of LUV compared with MLV.[8,27,28] Since the diffusion of drugs in liposomes that have a single lipid bilayer is faster[9] than that in MLV and also P (partition coefficient) values are smaller,[5] these should have contributed to the low efficiency of our BVC system. Another consideration is that BVC has a non-ideal partitioning, determined by its non-favourable water

solubility[4–6] that can limit its entrance into the membrane. Moreover, our liposomal system was prepared in a different manner than that described previously, in order to carry higher amounts of uncharged BVC. This may explain the differences in analgesic effects observed between BVC liposomal formulations.

Our study suggests that the administration of 0.5%, 1% and 2% MVC_{LUV} can induce sensory blockade of similar duration to 0.5% BVC_{plain}. We suggest that MVC_{LUV} is comparable to BVC_{plain}, regarding analgesic effects, at clinically relevant concentrations. Duration and intensity of analgesia with MVC was enhanced by encapsulation while this effect was not observed with BVC_{LUV}. Despite differences between this *in vivo* model and clinical conditions, encapsulated MVC could be an interesting therapeutic option for the treatment of acute pain, since the duration and intensity of analgesia are comparable to those of BVC.

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