Regional Anesthesia and Pain

Liposomal formulations of prilocaine, lidocaine and mepivacaine prolong analgesic duration

[Des préparations liposomiques de prilocaine, de lidocaïne et de mépivacaïne prolongent la durée de l’analgésie]

Cíntia Maria Saia Cereda PhD, Giovana Bruschini Brunetto MSC, Daniele Ribeiro de Araújo PhD, Eneida de Paula PhD

**Purpose:** A laboratory investigation was undertaken to compare the in vivo antinociceptive effects of 2% liposomal formulations of prilocaine (PLC), lidocaine (LDC) and mepivacaine (MVC) compared to plain solutions of each of these three local anesthetics.

**Methods:** Large unilamellar vesicles were prepared by extrusion (400 nm), at pH 7.4. The membrane/water partition coefficients were obtained from encapsulation efficiency values, after incorporation of each local anesthetic to the vesicles. The anesthetic effect of each liposomal formulation was compared to the respective local anesthetic solution in water, using the infraorbital nerve-blockade test, in rats.

**Results:** The partition coefficients were: 57 for PLC, 114 for LDC and 93 for MVC. In vivo results showed that local anesthetic-free liposomes, used as control, had no analgesic effect. In contrast, the encapsulated formulations induced increased intensities of total anesthetic effect (35.3%, 26.1% and 57.1%) and time for recovery (percentage increases of 30%, 23.1% and 56%), respectively, for PLC, LDC and MVC when compared to the plain solutions ($P < 0.01$).

**Conclusions:** These results indicate that liposomes provide effective drug-delivery systems for intermediate-duration local anesthetics. Mepivacaine was affected to the greatest extent, while LDC benefited least from liposome encapsulation, possibly due to greater vasodilatory properties of LDC.

From the Department of Biochemistry, Institute of Biology, State University of Campinas – UNICAMP, Campinas, São Paulo, Brazil.

**Address correspondence to:** Dr. Eneida de Paula, Departamento de Bioquímica, Instituto de Biologia, UNICAMP, C. P. 6109, CEP 13083-970, Campinas, SP, Brazil. Phone: +55 19 3788 6143; Fax: +55 19 3788 6129; E-mail: depaula@unicamp.br

Financial support: Cíntia M.S. Cereda was recipient of a fellowship from CAPES (Comissão de Aperfeiçoamento de Pessoal do Ensino Superior) and Eneida de Paula of a research fellowship from CNPq (Conselho Nacional de Pesquisa). This work was also supported by FAPESP (Fundaçao de Amparo a Pesquisa do Estado de São Paulo - 01/12476-2).

Accepted for publication March 27, 2006.
Revision accepted April 12, 2006.

This article is accompanied by an editorial. Please see Can J Anesth 2006; 53: 1074–7.
PRilocaine (PLC), lidocaine (LDC), and mepipvacaine (MVC) are structurally related local anesthetics (LA), commonly used for regional anesthesia, with fast onset and intermediate durations of action (90–240 min in clinical studies). The structure of these aminoamide LA comprises two major components: a lipophilic fraction (an aromatic group) and a polar region, connected by an intermediate carboxyl group in an amide bond (Figure 1).

The structure and physicochemical features of each LA molecule determines drug potency, onset of action, duration of sensory block, and toxicity. Water solubility is an important property influencing transportation of the anesthetic molecule to the nerve fibres, as well as the ionization equilibrium that guarantees the presence of charged and uncharged LA species in the axoplasm, at physiologic pH. In contrast, hydrophobicity is also crucial for drug partitioning into the axon so that a sufficient amount of LA molecules remain within that membrane in order to maintain the voltage-gated sodium channel protein in the inactive, non-conducting state.

Amongst the desirable properties of an ideal LA molecule are long duration of action, low toxicity and adequate solubility in water and lipids. While the search for ideal molecules continues, we speculated that it may be possible to enhance the effects of currently-available LA by their encapsulation into liposome delivery systems. Liposomes are lipid vesicles that have been extensively described in the literature as effective drug-carriers, since they are able to enhance drug bioavailability, reduce systemic toxicity, and increase the half-lives of LA in vivo.

The present study was undertaken to compare the in vivo antinociceptive effects of intermediate-duration LA, when encapsulated in large unilamellar liposomes (LUV). Prilocaine, LDC and MVC (Figure 1) were used at the same (2%) concentration, and each drug was administered to rats either in both plain solutions and encapsulated liposomal formulation. To better understand the interactions of LA molecules with liposomes, the results are related to the physicochemical properties of these molecules.

Methods

Materials and animal model
Prilocaine, LDC and MVC hydrochloride formulations, and thiopental, were obtained from Cristália – Produtos Químicos e Farmacêuticos Ltda (SP, Brazil). Egg phosphatidylcholine (EPC), cholesterol (Ch) and α-tocopherol (α-TC) were purchased from Sigma Chemical Co. (MO, USA). All other reagents were of analytical grade.

Male Wistar rats, 250–350 g, were obtained from CEMIB – UNICAMP (Centro de Bioterismo - State University of Campinas – UNICAMP, SP, Brazil) and were given free access to water and food throughout the study. The experiment was approved by the Institutional Committee for Ethics in Animal Research of UNICAMP (Protocols 824-1 and 559-1), which follows the recommendations of the Guide for the Care and Use of Laboratory Animals.

Liposomal LA and plain solution preparations
A dry lipid film, containing EPC, Ch and α-TC at a 4:3:0.07 molar ratio was prepared by solvent evaporation under nitrogen flow. 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. Unilamellar liposomes 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. Since LA exhibit a fast equilibrium between EPC membranes and the aqueous phase, LA molecules were added directly to the liposomes after extrusion, up to a concentration of 2% (corresponding to 77.9 mM of PLC, 73.8 mM of LDC and 70.7 mM of MVC). Plain LA solutions with the same therapeutic LA concentrations were prepared in 0.9% saline (154 mM NaCl). Liposome LA formulations were incubated for 12 hr and stored at 4°C until further use.

The selection of LA concentration, 2%, was determined by the clinical efficacy of LDC and MVC. Comparisons among the drugs were directed by determination of their partition coefficient and the relationship between these values and enhanced analgesic effect provided by encapsulation into the liposomes is discussed.

Partition coefficient determination
The partition coefficient (P) between liposome/water was obtained from the encapsulation efficiency values, according to equation 1:

\[
P = \frac{n_m/V_m}{n_w/V_w}
\]

where: n corresponds to the number of moles of the anesthetic and V, to membrane volume, and m and w refer to the liposome and aqueous phase, respectively.

The encapsulation efficiency was determined by centrifugation (120,000 × g, two hours, 10°C) of liposome suspensions (4 mM lipid concentration), in the presence of an appropriate LA concentration.