Liposomal system designed for the sustained release of ropivacone

Silva, C.M.G.; Casadei, B.R.; Damasio, V.A.G.; Cereda, C.M.S. and de Paula, E.

1Biochemistry Department, Institute of Biology, State University of Campinas, SP, Brazil

cmorais.gs@gmail.com

ABSTRACT SUMMARY

Ropivacone (RVC) is a local anesthetic largely used, worldwide, in surgical procedures [1]. This work aimed to evaluate the in vivo effect of RVC when encapsulated into a combined liposomal drug-delivery system. The liposome combination used in this study included: multivesicular donor vesicles (LMVV) containing a kosmotropic (sulfate) anion [2] at pH 7.4 in their inner compartment and unilamellar acceptor vesicles (LUV) with internal pH (5.5) gradient, both at external pH = 7.4.

INTRODUCTION

Ropivacone (RVC) has been increasingly used in clinical practice since 1985, due to its lower toxicity regarding bupivacone, the drug of choice for anesthesia in surgical procedures [3]. Local anesthetics have a relative short duration of action, and the use of drug delivery carriers such as liposomes can prolong their residence time at the site of injection [4]. In order to prolong the anesthetic effect of RVC we have developed modified liposomes (with internal ionic gradient), to be used as a donor-acceptor pair. With this approach the protonated form of RVC was trapped in the inner aqueous core of the vesicles, allowing for the sustained release of the anesthetic. Multivesicular donor liposomes (LMVV) and unilamellar acceptor vesicles (LUV) were prepared with soy phosphatidylcholine (HSPC): cholesterol containing either excess amounts of kosmotropic anions from the Hofmeister series [2] (250 mM sulfate) or protons (pH 5.5), respectively, in their inner aqueous compartments. RVC was incorporated to a final 0.75% concentration.

EXPERIMENTAL METHODS

Multilamellar liposomes were formed by hydration of the lipid film and vortex agitation. Their extrusion trough polycarbonate membranes at 400 nm gave rise to LUVs, while LMVV [5] where prepared by extrusion through 100 nm pores followed by 10 freeze-thawing cycles.

Characterization studies were performed by light scattering analysis to determine the diameter, polydispersity, and Zeta potential of the vesicles; membrane organization was measured by electron paramagnetic resonance (EPR) using stearic-acid (SASL) spin probes. The time for recovery after RVC (free and liposome-encapsulated) injection was evaluated with the tail flick test, in mice.

RESULTS AND DISCUSSION

Although LMVV (7.4 + sulfate) vesicles have been extruded through filters of 100 nm, the subsequent freeze-thawing process increased the vesicles size (~900 nm) and polydispersity (~0.7) while LUV (5.5) were found to be more homogenous (polydispersity index ~0.2) with vesicles diameter in the range of 500 nm. The liposomes combination system presented large vesicles (> 1000 nm diameter) and high polydispersity (~0.7). Zeta potentials were always negative for all liposomes and no significant changes were registered after RVC encapsulation.

Diameter, polydispersity and Zeta potential of the combined formulation were followed during 6 months of storage at 2-8°C, and the results indicating stability over that time.

Figure 1 shows the spectra of the 5-SASL incorporated (1 mole %) into multivesicular and unilamellar liposomes at pH 7.4. The segmental order parameter (S) [6] was measured from such spectra (S = 0.82±0.01 and 0.84±0.01 for LMVV and LUV, respectively) showing that HSPC/cholesterol liposomes are highly ordered, when compared to egg phosphatidylcholine liposomes (S = 0.65) [7], or either to biological (erythrocyte, S = 0.76) membranes, that are considered well organized due to the presence of proteins [8]. Such organization is a result of the major lipid component (HSPC) and contribute to the sustained release of RVC.
Figure 1 - Electron paramagnetic resonance (EPR) spectra of 5-SASL inserted in multivesicular (LMVV) and unilamellar (LUV) HSPC/cholesterol liposomes, at pH 7.4 and room temperature.

Figure 2 shows the results of tail flick tests. RVC in solution promoted around 3 h of anesthesia, while the liposomes combination promoted ca. 7 h. Moreover, the antinociceptive effect obtained with the combined liposomes was longer than that evoked by each liposome - LMVV (7.4+sulfate) or LUV (5.5) – alone: ca. 4 h of anesthesia. In the combined formulation RVC molecules released by donor liposomes were encapsulated by the acceptor vesicles before becoming available at the site of injection, as described before by Barenholz & Garbuzenco [9] for bupivacaine.

Figure 2 – Tail-flick pain response test in mice after 0.75% ropivacaine injection, at 7.4. RVC was tested free or encapsulated into HSPC/cholesterol liposomes: containing an internal sulfate (■) or protons (▲) gradient, or a combination of those (▲■). Statistics: two-way ANOVA with Bonferroni: *p<0.05; **p<0.01 and ***p<0.001, n=7. MPE = percentage of maximum possible effect.

CONCLUSION

The combined delivery system of liposomes with transmembrane ionic gradient provided improved anesthetic effect (ca. 7 hours) with 0.75% RVC, in comparison to simple liposomes (LMVV 7.4+sulfate and LUV 5.5 = 4 h) or free RVC (3 h) of equivalent concentration. The results show that this formulation has a good potential for future use in long surgical procedures or postoperative anesthesia.

REFERENCES


ACKNOWLEDGMENTS

Financial support: FAPESP (# 11/21735-3) and FAEPEX-UNICAMP (#1316/12).