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Anaesthetic efficacy of unilamellar and multilamellar liposomal formulations of articaine in inflamed and uninflamed tissue

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Abstract

We compared the efficacy of articaine encapsulated in multilamellar and unilamellar liposomes with that of articaine with epinephrine, after infiltration into inflamed and uninflamed tissue in rats. We encapsulated 4% articaine in multilamellar (articaine:multi) and unilamellar (articaine:uni) liposomes and compared them with 4% articaine with 1:100 000 epinephrine (articaine:epinephrine), in inflamed (plantar incision into the hind paw) and uninflamed (infraorbital nerve block) tissue in rats. Anaesthetic formulations (0.1 ml) were injected near the right infraorbital foramen in uninflamed tissue, where success and duration of anaesthesia were assessed by pinching the upper lip every 5 minutes. For inflamed tissue the anaesthetic formulations (0.1 ml) were injected laterally into a surgical wound made 24 hours earlier in the plantar region of the rat's right hind paw. The degree of anaesthesia was assessed by application of forces laterally to the wound with electronic von Frey filaments. Articaine:uni resulted in less successful anaesthesia than both articaine:multi ($p=1.1x10^{-5}$) and articaine:epinephrine ($p=4.3x10^{-8}$) in uninflamed tissue, but there were no differences in duration or success of anaesthesia between articaine:epinephrine and articaine:multi. In inflamed tissue articaine:epinephrine gave significantly more effective anaesthesia for longer than articaine:uni ($p=2.3x10^{-6}$), and articaine:epinephrine ($p=1.8x10^{-6}$) formulations, which did not differ from each other. Multilamellar liposomal articaine

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could be an option for local anaesthesia in uninflamed tissues. However, articaine with epinephrine gave better results than liposomal formulations in inflamed tissue.

Keywords: Local anaesthesia; Dental anaesthesia; Articaine; Epinephrine; Liposome

Introduction

Since the introduction of local anaesthesia many additives have been studied to try and improve the efficacy and safety of the anaesthetics used, and since 1905 vasoconstrictors such as epinephrine have been added to help the anaesthetic last longer.[1] Drug delivery systems, including cyclodextrins, nanocapsules, and liposomes, have also been suggested.[2]

In dentistry unilamellar liposomes have been unable to increase the duration of mepivacaine, prilocaine, or ropivacaine anaesthesia compared with vasoconstrictors.[3–5] However, case reports[6,7] and clinical studies[8–10] have shown that the encapsulation of bupivacaine in liposomes improved its efficacy compared with plain bupivacaine and the epinephrine-containing bupivacaine solutions.[9]

Articaine has singular chemical characteristics, particularly the tiophene ring with an ester side radical. Compared with lignocaine, it provides longer anaesthesia after buccal mandibular infiltration[11] and has a shorter half-life.[12] However, some adverse effects have been reported.[13] Lowering the concentration of articaine or combining it with drug release systems could therefore help to reduce its toxicity.

As multilamellar formulations of liposomes seem to provide longer anaesthesia than that obtained with epinephrine,[8,9] we have compared the efficacy of articaine in multilamellar and unilamellar liposomes with that of articaine with epinephrine, after infiltration into inflamed and uninflamed tissue in rats.

Methods

All experiments were approved by the Ethics Committee on Animal Experimentation of the University of Campinas (CEEA-Unicamp # 1938-1) and conducted in accordance with the *Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals*, as issued by the International Association for the Study of Pain.[14] Sixty male Wistar rats (250-300 g) were obtained from the Multidisciplinary Center for Biological Investigation of the University of Campinas (CEMIB/Unicamp, Campinas, SP, Brazil), and were housed in plastic cages with soft bedding, free access to water and food, and a 12-hour day/night cycle at 22 (±1) °C room temperature.

The effects of multilamellar liposomal 4% articaine (articaine:multi) and unilamellar liposomal 4% articaine (articaine:uni), were compared with that of the commercial 4% articaine with 1:100,000 epinephrine (Articaine® 100, DFL Ind Com Ltda, Rio de Janeiro, RJ, Brazil) formulation (articaine:epinephrine). Multilamellar and unilamellar liposome suspensions, and 0.9% saline (Ind. Equiplex Farm., Brazil), were used as controls. The pH of the formulations were: 6.7 (±0.2), 6.6 (±0.3), and 3.7 (±0.3), respectively.

Liposomal formulations were prepared as previously described.[15] Briefly, a dry lipid film that contained egg phosphatidylcholine, cholesterol, and α-tocopherol 4:3:0.07 M was prepared by evaporation of the solvent under nitrogen flow. Multilamellar liposomes were obtained by adding HEPES buffer 20 mmol, pH 7.4, to the dry lipid film and spinning the mixture for 5 minutes. Unilamellar liposomes were prepared by extrusion of the multilamellar vesicles (12 cycles, using a 400 nm polycarbonate membrane, at 25 °C). The total lipid concentration of the two liposomes was 8 mmol. Articaine (kindly donated by DFL Ind. Com. SA, Rio de Janeiro, Brazil) was added directly to the two preparations to a concentration of 4%. The formulations were sterilised by autoclaving (121 °C, 1 atm, for 15 minutes), stored at 4° C, and used within 15 days.[16]

Uninflamed tissue: infraorbital nerve block

A power calculation indicated that for uninflamed tissue a sample size of 8 animals/group would provide 90% power to detect a difference of 10 minutes of anaesthesia among groups, accepting a probability of less than 0.05 as significant.

Twenty-four rats were given an infraorbital nerve block, as described by Fink et al.[17] After light sedation with sodium pentothal (25 mg/kg), a 26G 13 × 4.5 needle (BD PrecisonGlide®, Becton Dickinson Ind. Cirúrgicas Ltda, Curitiba, PR, Brasil) was inserted in the maxillary mucobuccal fold halfway between the incisor and the first molar. One of the anaesthetic formulations (0.1 ml) was injected into the right side, and the same volume of the respective control into the left side. Five minutes after the injection, both sides of the upper lip were pinched with forceps to test the anaesthetic effect. The absence of a response indicated that the upper lip was anaesthetised.

Inflamed tissue: incision into the plantar surface of the right hind paw

A power calculation indicated that a sample size of 6 animals/group for inflamed tissue would provide 95% power to detect a difference of 5 minutes of anaesthesia among groups, assuming a probability of less than 0.05 as significant.