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Liposomal delivery system for topical anaesthesia of the palatal mucosa☆

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Abstract

An effective topical agent to reduce pain during local anaesthesia of the palate is not yet available. The aim of the present study was to evaluate the efficiency of liposome-encapsulated ropivacaine in different concentrations for topical anaesthesia of the palatal mucosa. In this single-blinded, placebo-controlled, crossover study 40 (20 male) healthy volunteers were randomised to be given: liposome-encapsulated 2% ropivacaine, liposome-encapsulated 1% ropivacaine, a eutectic mixture of 2.5% lidocaine and 2.5% prilocaine (EMLA), and liposomal placebo gel, topically on to the palatal mucosa of the right canine region for 5 min each, at four different sessions. Pain associated with insertion of a 30G needle, and with injection of a local anaesthetic, was rated on a visual analogue scale (VAS). The effect of liposomal ropivacaine 1% and 2% did not differ from that of placebo ($p = 0.3$ and $p = 0.1$, respectively) in reducing pain during insertion of the needle. Lower VAS were obtained with EMLA. In this group VAS were lower in women than men ($p = 0.007$). There was no difference in VAS among groups ($p = 0.3$) as far as injection of the local anaesthetic was concerned. In conclusion, liposomal-encapsulated ropivacaine formulations did not reduce the pain of insertion of a needle into the palatal mucosa. None of the anaesthetic formulations tested, including the positive control (EMLA), were effective in reducing the pain of an injection of local anaesthetic compared with placebo.

Keywords: Local anaesthetics; Ropivacaine; Oral mucosa; *In vivo*; Anaesthetic efficacy

Introduction

Local anaesthesia of the palatal mucosa is important to enable manipulation of palatal soft tissue without pain during dental procedures.[1] However, this region has a thick, keratinised layer that is more resistant to the effects of topical anaesthetics than other intraoral sites, mainly the anterior region.[2,3] Infiltration of anaesthetic into the palatal mucosa can be extremely painful because the mucosa is firmly attached to the underlying periosteum and has numerous accessory nerves.[4] According to Harker, the pain during palatal injections is more associated with the dislocation of the mucoperiosteum than with the puncture.[5] Because palatal mucosa is one of the most painful sites to anaesthetise locally, it is the strictest test to which a topical anaesthetic can be submitted to assess its efficacy.[3,6]

An effective topical agent to reduce pain during local anaesthesia in the palate has been sought since 1979.[7] Several studies have shown that the most used topical agent, 20% benzocaine, failed to reduce pain from insertion of a needle and from an injection of local anaesthetic in this region.[7–10]

The first studies of EMLA, the eutectic mixture of 2.5% lidocaine and 2.5% prilocaine for dermal use, that were made on the oral mucosa showed promising results. In most the cream was effective on the palatal mucosa in alleviating

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pain from insertion of a needle,[6,11–13] injection of a local anaesthetic,[9,14] and removal of a leaf fibroma.[15] According to Meechan this was the unique effective topical anaesthetic with which to reduce pain during palatal injection.[2]

The liposomal encapsulation of local anaesthetics has been widely studied for dermal topical application. Liposomes are phospholipid vesicles used to carry drugs that have been shown to increase cutaneous and percutaneous penetration, provide slow release of the local anaesthetic, and better superficial anaesthesia.[16–20] In the oral mucosa the liposomal encapsulated ropivacaine was of similar efficacy to EMLA in reducing pain during insertion of a needle into the maxillary buccal fold after a 2-min application.[21]

The aim of the current study was to evaluate the efficacy of liposomal encapsulated ropivacaine at different concentrations in reducing pain during insertion of a needle and injection of a local anaesthetic into the palatal mucosa.

Subjects and methods

The study was approved by the Ethics Committee of Piracicaba Dental School, University of Campinas, SP, Brazil (#059/2008), and the trial registration number was NCT01054547.

A power calculation indicated that a sample size of 40 subjects would provide 95% power to detect a difference of 10 mm in visual analogue scale (VAS) between the two groups, assuming a level of significance of 5% (two-tailed).

All the subjects were undergraduate or graduate students at Piracicaba Dental School and were in good health. Exclusion criteria were a history of allergy to any of the local anaesthetics used, intake of drugs that would alter perception of pain, pregnancy, and presence of a lesion at the site of application. After being informed verbally about the study, the volunteers who agreed to participate were asked to read and sign the consent form.

Topical preparations

Liposomal and gel formulations were prepared at the Department of Biochemistry, Institute of Biology, University of Campinas, as described by de Araujo et al.[22] The liposomes consisted of large unilamellar vesicles of homogeneous sizes calculated with quasielastic light scattering (400 nm) at the final concentration of 1% or 2%. These formulations were sterilised by autoclaving (121 °C, 101 kPa for 15 min).

Liposome-encapsulated 1% and 2% ropivacaine gel and the placebo gel were prepared based on a patented method (Silva et al. Pharmaceutical composition, manufacturing process and the use of an effective quantity of anaesthetic and jelly agent. Product and treatment method. 2009 Patent number (Brazil) PIO704542-5). These formulations were prepared by the same operator (not involved in the use or evaluation of anaesthetic efficacy).

The commercial topical formulation used was a eutectic mixture of 2.5% lidocaine and 2.5% prilocaine (EMLA®, Astra-Zeneca, Cotia, Brazil – batch n° 10102AB).

Anaesthetic

In this single-blind, random, crossover, and four-period study the volunteers were given 100 mg (previously weighed) of the following topical formulations applied by the same operator: liposome-encapsulated 2% ropivacaine gel; liposome-encapsulated 1% ropivacaine gel; liposome-placebo gel; and EMLA® (2.5% lidocaine and 2.5% prilocaine) on four separate occasions spaced at least one week apart. EMLA® was used as a positive control because of its efficacy in reducing pain in the palatal mucosa after insertion of a needle, [6,11–13] and injection of local anaesthetic.[9,14]

Before topical anaesthesia, the palatal mucosa in the right canine region was dried with sterile gauze. The topical anaesthetic was applied with a cotton swab for 5 min, and then removed with sterile gauze.

Immediately after removal, a second operator inserted a 30G needle attached to an aspirating syringe at the place of application (about 1.0 cm away from the gingival margin) until contact was made with the bone, and 2% lidocaine 0.3 ml with 1:100 000 adrenaline (Alphacaine® – DFL Ind. Com. Ltda) was injected at a rate of 1 ml/min. The volunteers were informed about the insertion of the needle and injection of the anaesthetic. Because EMLA has physical characteristics that are different from those of the other formulations and to ensure that the study remained blind, the volunteers and the operator who evaluated the efficacy of the anaesthetic were unaware of the formulations applied.

After this procedure, the volunteers were asked to rate pain during insertion of the needle, and during injection of the anaesthetic on two VAS, each of which consisted of a 100 mm line the left end (0) of which indicated “no pain” and the right end (100) of which indicated “unbearable pain”.

Statistical analyses

Data were analysed with the help of BioEstat, version 5.0 (Mamiraua Institute, Belem, PS, Brazil). We used the Kruskal–Wallis and Student–Newman–Keuls tests to assess the significance of differences between sexes and treatments. Probabilities of less than 0.05 were accepted as significant.

Results

We studied 20 men and 20 women, mean (SD) age 22 (3), range 19–29 years.

There was no “session effect” on VAS for pain as far as insertion of a needle or injection of an anaesthetic was concerned ($p = 0.2$).

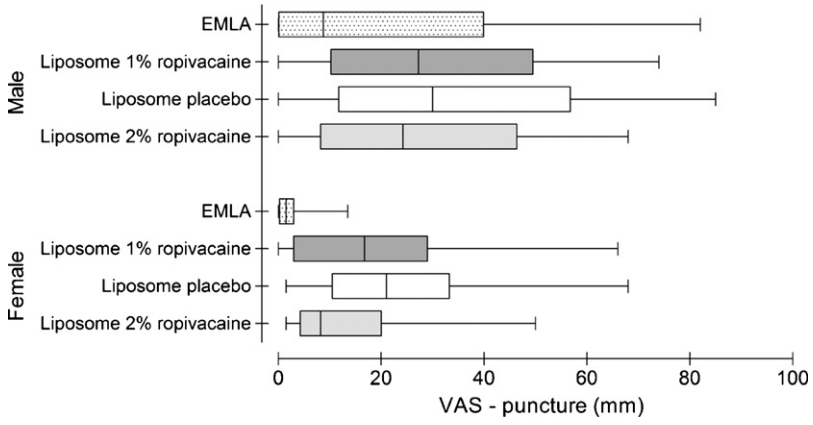

Fig. 1. Visual analogue scores rated by volunteers during needle puncture (central line = median; box = lower and upper quartiles; whisker = range of values). EMLA reduced pain more effectively during insertion of a needle than liposome-encapsulated ropivacaine at 1% ($p = 0.0004$; $p = 0.04$ for women and men, respectively); at 2% ($p = 0.004$; $p = 0.048$ for women and men, respectively); and also more than liposomal placebo ($p < 0.0001$; $p = 0.014$ for women and men, respectively). Liposome ropivacaine 1% and 2% did not differ from placebo ($p = 0.32$; $p = 0.10$, respectively).

Fig. 1 shows medians of VAS for pain during insertion of a needle for all groups. There was a sex-related effect in VAS in the EMLA group, the use of which resulted in significant lower VAS for pain in women than in men ($p = 0.007$). EMLA was also more effective at reducing pain than liposome-encapsulated ropivacaine at 1% and 2% and also than liposomal placebo. Liposomal ropivacaine at 1% and 2% did not differ from placebo (Fig. 1).

There was no significant difference among topical anaesthetics used ($p = 0.29$), and Fig. 2 shows median VAS for all groups concerning pain during injection of local anaesthetic.

Discussion

Topical anaesthetics are commonly used to reduce pain during dental anaesthesia, but published results about their efficacy are contradictory and their effects depend on the topical anaesthetic agent used, and the site and duration of application.[2]

According to Meechan and co-workers pain during insertion of a needle is more intense in the anterior region of the palate than in the posterior region.[3] Harker attributed the pain associated with giving local anaesthetics to the dislocation of the mucoperiosteum.[5] Hutchins et al. agreed, and stated that the efficacy of a topical anaesthetic is better evaluated if it is injected rather than only simulated.[9]

In the present study we decided to test liposomal ropivacaine in a strict model for oral topical anaesthetic: injection of local anaesthetic into the anterior palate.

It has been shown that liposome-encapsulated 1% ropivacaine was equivalent to EMLA in reducing pain during insertion of a needle (no anaesthetic solution was injected) in the maxillary buccal fold after a 2-min application.[21] In the present study, however, even in double concentration (2%) and with a longer application time (5 min) liposome-encapsulated ropivacaine was not effective in reducing pain on insertion of a needle as recorded by VAS.

EMLA, however, was effective in reducing pain during insertion of a needle in both sexes, which confirmed the results of other authors who found EMLA superior to other

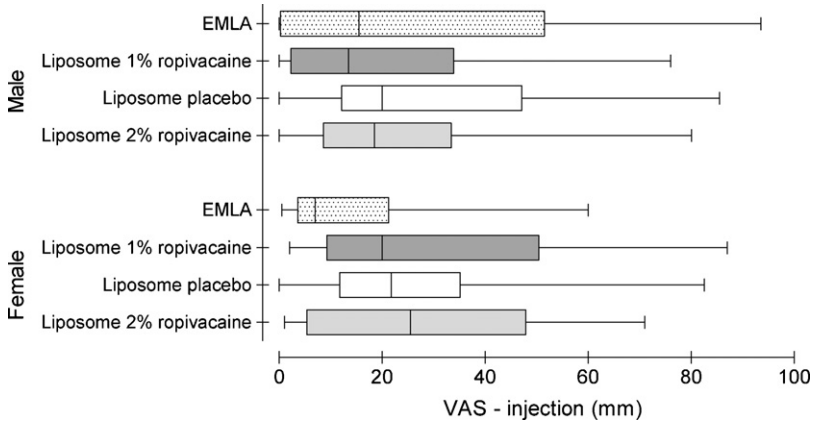

Fig. 2. Visual analogue scores rated by volunteers after injection of local anaesthetic (central line = median; box = lower and upper quartiles; whisker = range of values).

topical anaesthetics or placebo in reducing the pain of a needle being inserted into the palate.[6,11–13]

There was no difference between men and women during insertion of a needle, except when EMLA was used. These results are similar to those of Meechan et al. who found no difference between men and women concerning VAS after insertion of needles in the anterior and posterior regions of the palate.[3]

Liposome-encapsulated local anaesthetics have been thought to have equal or superior effects than EMLA[19,20] and non-encapsulated tetracaine[16,23] in reducing the pain of insertion of a needle into the skin after 30 and 60 min of application. Differences in the methods such as the patients' ages, number of volunteers, and the inclusion of a placebo group could explain the difference in results between these and the present study. Other possible causes for the difference in the results are the size of liposome used and the amount of local anaesthetic encapsulated, which are not mentioned in most studies, except for that by Gesztes and Mezei in which multilamellar liposomes were used. In the present study ropivacaine was encapsulated in unilamellar liposomes with 24% encapsulation.[16]

Two studies have evaluated liposomal local anaesthetics in oral mucosa (Zed CM, et al. Topical liposome encapsulated tetracaine versus benzocaine: a clinical investigation Abstract 1840 J Dent Res 1996;75:247).[21] In the latter study[21] liposomal amethocaine was effective in reducing pain during insertion of needles and injection of anaesthetic (no mention was made of the exact site and duration of application). In the later study pain was reduced after insertion of a needle after application of liposomal ropivacaine in the buccal fold mucosa, a region known to be less painful than the palate.[2]

However, as shown in the present study, the results after application to the palate were disappointing, and the hypothesis that penetration by liposomal-encapsulated ropivacaine through the keratinised palatal mucosa is increased was not confirmed.

None of the preparations reduced the pain related to injection of local anaesthetic, and these results confirm those of Hutchins et al. who found no difference between 20% benzocaine and application of placebo before injection of anaesthetic into the palate.[9]

Reduction in pain scores after injection when EMLA[14] had been applied, and the removal of a soft tissue lesion with only a topical anaesthetic,[15] have been reported. However, these studies used larger amounts of local anaesthetic and for a longer time than those used in the present study. Meechan used EMLA 0.5 g applied for 15 min,[15] which is considered too long for use in clinical dentistry. In addition, longer times may cause mucosal necrosis, as reported by Franz-Montan et al. when they applied EMLA to the buccal mucosa for 30 min.[24]

In the study by Meechan and Winter, EMLA was more effective than placebo and transcutaneous electronic nerve stimulation in reducing the pain of injection into the palate, with no difference in perception of pain between the anterior and posterior regions of the palate.[14] Although there was a large number of patients ($n = 100$) used to compare the treatments, it was not designed as a crossover study. A more recent crossover study reported more discomfort during insertion of a needle into the anterior than into the posterior palatal region.[3] Based on these results, Meechan et al. concluded that the penetration of the needle into the anterior palatal mucosa was the most stringent test for assessing the effectiveness of topical anaesthetics.[2] The ideal intraoral topical anaesthetic is not yet available.

In conclusion, liposome-encapsulated formulation of ropivacaine, although effective in the buccal mucosa, did not reduce the pain of insertion of a needle into the palatal mucosa. EMLA was the only topical anaesthetic to be effective in reducing pain during insertion of a needle, but none of the anaesthetic formulations tested were effective in reducing pain related to injection of a local anaesthetic when compared with placebo. There is still a need to develop better topical anaesthetics for use on the palatal mucosa.

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