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ORIGINAL ARTICLE

Effect of articaine on mental nerve anterior portion: Histological analysis in rats

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Abstract

Objectives. The aim of this study was to evaluate the possible toxic effects of articaine and lidocaine on mental nerve, due to the increasing number of paresthesia cases after nerve blocks. ***Materials and methods.*** The drugs were injected in the anterior portion of mental nerve of 24 rats, divided into three groups: G1—4% articaine with 1:100,000 epinephrine; G2—2% lidocaine with 1:100,000 epinephrine and G3—plain 1:100,000 epinephrine solution. These solutions were injected in the right side of the rat's mandible and the left side was used as control (0.9% saline solution). Previously to the injections, the animals were anesthetized with thiopental and, 24 h after the injections, their jaws were removed and submitted to routine histological techniques. A histopathological analysis was performed by optical microscopy. ***Results.*** An inflammatory infiltration was found around mental nerve, classified as intense for G3, moderate for G1 and light for both G2 and control groups. No injuries were found in nervous structure, despite the inflammatory reaction observed around it. ***Conclusion.*** The results suggest that articaine is not toxic to the nervous structure and further studies are necessary to explain the possible relation between articaine injection and paresthesia.

Key Words: *anesthesia, articaine, local anesthetics, mental nerve, paresthesia*

Introduction

Local anesthetics are among the most effective and safest drugs used in medicine and dentistry for the control and management of pain [1]. It is impracticable to provide effective dental care without the use of local anesthetics. Even though these drugs have an impressive history of efficacy and safety, all local anesthetics have the potential to produce significant toxicity.

Paresthesia is a possible adverse event, presented as a prolonged complete anesthesia or an altered sensation, ranging from complete numbness to burning, tingling or continual pain, that persists beyond the expected duration of action of a local anesthetic injection [2,3].

Articaine is a widely used local anesthetic in several European countries [4]. A number of advantages making it an attractive choice for specific cases has been related. In addition, it was recently considered as the best anesthetic choice in dentistry [5].

Usually, the local anesthetics contain benzene as the aromatic ring, but articaine has a thiophene ring providing great liposolubility. Cases of successful anesthesia after infiltration of the mandible are attributed to its high liposolubility and higher concentration when compared to lidocaine [6]. Previous studies showed greater success rates in the first molar after mandibular buccal infiltration with 4% articaine with 1:100,000 epinephrine than after 2% lidocaine with 1:100,000 epinephrine injection [7,8].

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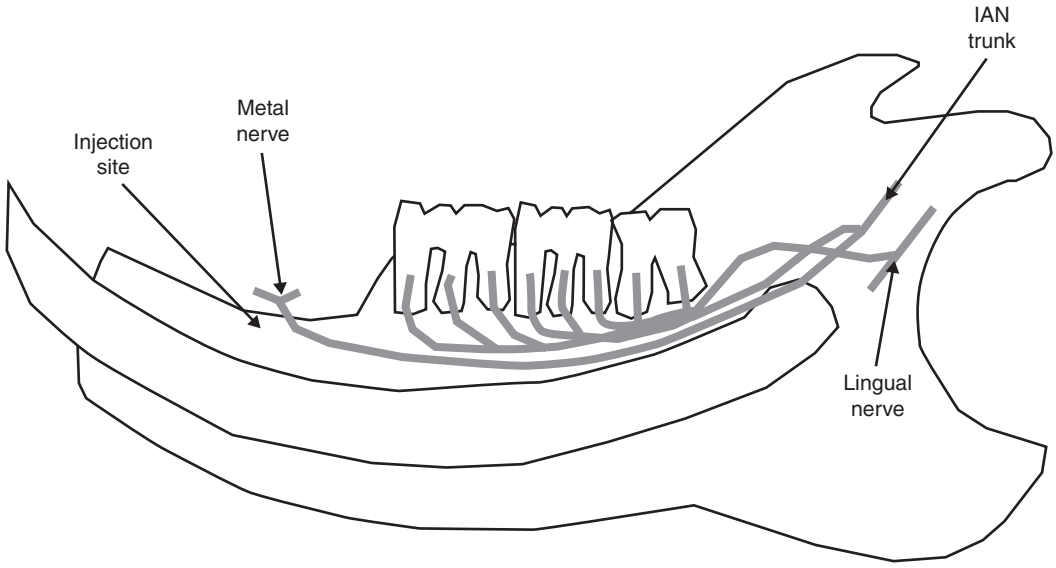

Figure 1. Injection site and diagram of inferior alveolar nerve branch distribution in the adult rat (adapted from Naftel et al. [18]).

Recently, Batista da Silva et al. [9] demonstrated that articaine promoted higher anesthesia success and longer duration of anesthesia than lidocaine after incisive/mental nerve block.

The ester group in the articaine molecule allows it to be hydrolyzed by plasma esterases as well as by hepatic microssomal enzymes [4], resulting in a shorter half-life when compared to other amides. For this reason, articaine presents less risk for systemic toxicity [6].

In spite of those advantages, an increase of paresthesia cases has been reported after inferior alveolar blocks with 4% articaine and prilocaine solutions when compared with other local anesthetics [10–13]. Despite the overall low incidence of paresthesia, the nerve damage could be permanent [13].

The possible mechanisms of paresthesia caused by articaine injection are still not clear. The aim of this study was to compare the toxicities of 4% articaine with 1:100,000 epinephrine and 2% lidocaine with 1:100,000 epinephrine after mental block infiltration in rats.

Methods

Materials

This study was approved by the Institutional Committee for Ethics in Animal Research of the University of Campinas (CEEA-Unicamp/Protocol Number #830-1).

Twenty-four male Wistar rats (*Rattus norvegicus* - albinus), 60 days of age and weighing 175 g ± 25 g, obtained from the Multidisciplinary Center for Biological Investigation of the University of Campinas (CEMIB/Unicamp, Campinas, SP, Brazil) were maintained at a controlled room temperature (21° ± 0.5° C) and a 12-h light-dark cycle. The animals were kept in plastic cages with access to food and water *ad libitum*. Prior to the surgical

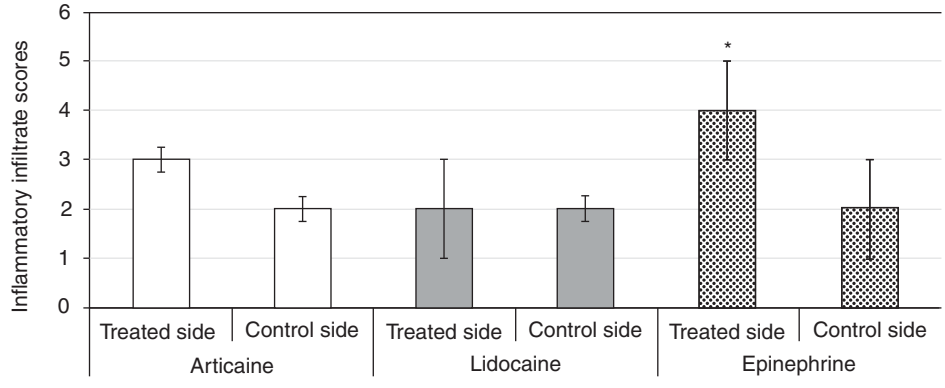

Figure 2. Average of the inflammatory scores for all groups.

Table I. Inflammatory infiltrate scores (median – interquartilic deviation) according to the groups.

	Inflammatory infiltrate scores (median – interquartilic deviation)	
	Treated side	Control side
Articaine	3 (0.25)	2 (0.25)
Lidocaine	2 (1)	2 (0.25)
Epinephrine	4 (1)	2 (1)

procedures, all animals were handled for 5 days to acclimate to the laboratory environment and experimental procedure.

The rats were divided into three groups (n = 8). This number was established according to studies that used local anesthetics in rats [14–17]. The groups were represented by: Group 1, articaine 4% with epinephrine 1:100,000; Group 2, lidocaine 2% with epinephrine 1:100,000; and Group 3, solution of epinephrine 1:100,000.

Anesthetic procedure

Slightly general anesthesia was induced by a subcutaneous injection of a thiopental sodium solution (Thiopentax® - Cristália, São Paulo, Brazil) in the concentration of 40 mg/kg before the administration of local anesthetic solution.

The previous described solutions (0.1 mL) were injected in the alveolar mucosa close to the lower-right first molar of rats (Figure 1); 0.1 mL of a saline solution (0.9% sodium chloride solution) was injected as negative control in the contralateral mucosa of all animals. After 24 h, the animals were killed and the samples (mandible and the soft tissue adhered) were removed. The mandibles were divided into the left (negative control) and the right half-mandibles.

Histological analysis

The specimens were placed in labeled plastic recipients containing 10% buffered formalin for 12 h for fixation of the organic tissue remnants. Afterwards, the specimens were washed for 1 h, decalcified in 4% EDTA (ethylene diamino tetraacetic acid), according to Warshawsky and Moore's [19] method, and embedded in paraffin. Transversal serial 6 m-thick cross-sections were obtained by using a microtome (E. Leitz®, Wetzlar, Hessen, Germany) and stained with hematoxylin and eosin. From each block, eight sections were chosen, after each 20 m, in the point that mental nerve started to cross the cortical bone.

The samples were examined with an optical microscope (Eclipse E 600; Nikon, Shinagawa-ku, Tokio, Japan) at 100 × and 400 × magnifications. The images were captured using Adobe Premiere 5.1 software (Adobe Systems Inc., San Jose, CA) and were analyzed using Corel Photo Paint 10 software (Corel Corporation Inc., Mountain View, CA).

The images were submitted to qualitative analysis in order to evaluate the intensity of leucocytes infiltration and/or any possible necrosis area. A preliminary pilot study conducted according to other previous studies [20,21] allowed the use of a qualitative score of the local tissue inflammation. The soft tissue and nervous structure near the injection site were analyzed. The score was defined based on the following descriptions: (1) no inflammatory infiltration, (2) very light inflammatory infiltration, (3) light inflammatory infiltration, (4) moderate inflammatory infiltration and (5) intense inflammatory infiltration.

Statistical analysis

Data were submitted to the Kruskal-Wallis and Dunn tests (alpha = 5%).

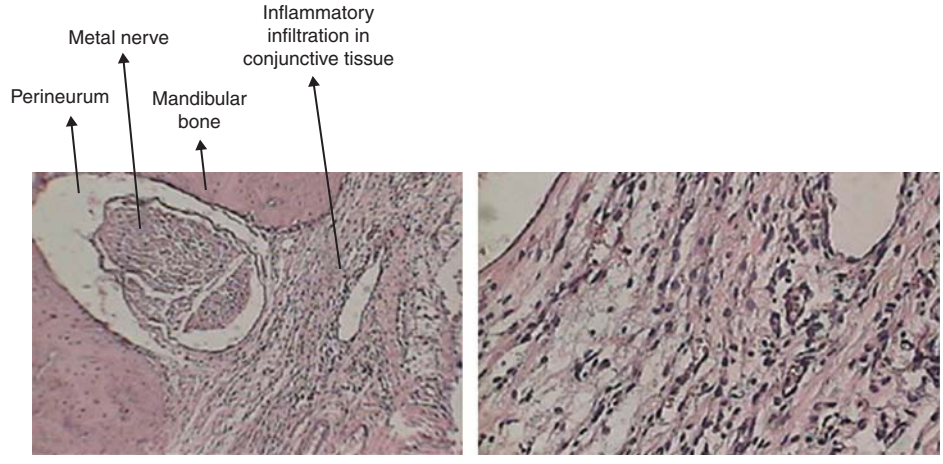

Figure 3. Histological slices from Group 1 (4% articaine with 1:100,000 epinephrine)–treated side (100 and 400 ×, respectively). Presence of moderate inflammatory infiltration around mental nerve.

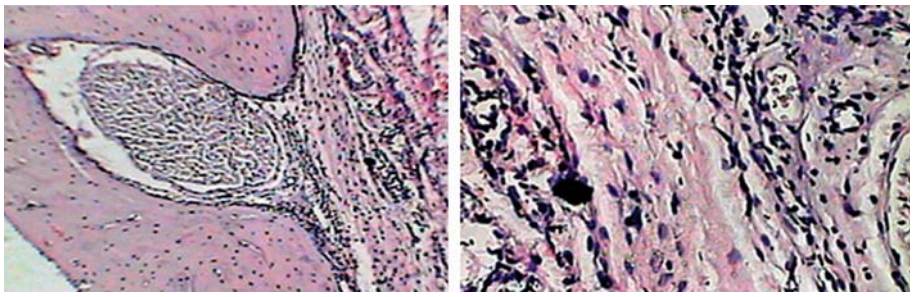

Figure 4. Histological slices from Group 1–control side (100 and 400 ×, respectively). Less inflammatory infiltration around mental nerve than treated side.

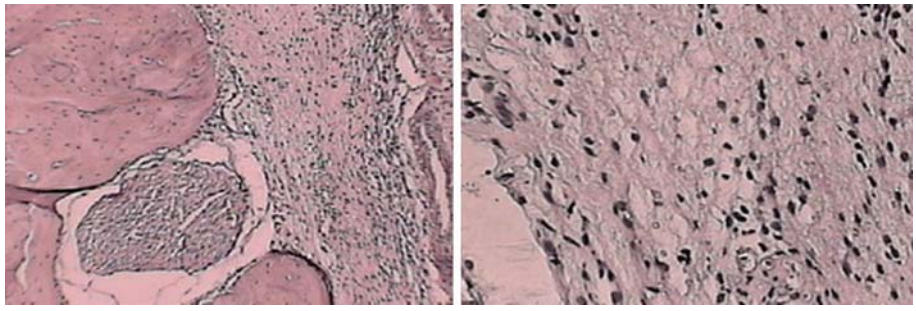

Figure 5. Histological slices from Group 2 (2% lidocaine with 1:100,000 epinephrine)–treated side (100 and 400 ×, respectively). Presence of light inflammatory infiltration around mental nerve.

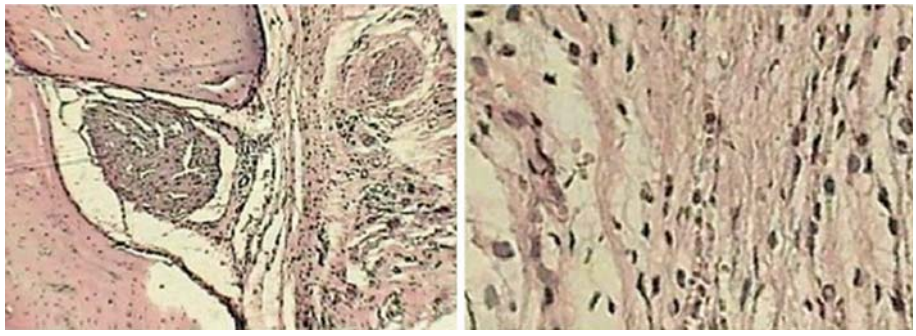

Figure 6. Histological slices from Group 2–control side (100 and 400 ×, respectively). Presence of light inflammatory infiltration around mental nerve.

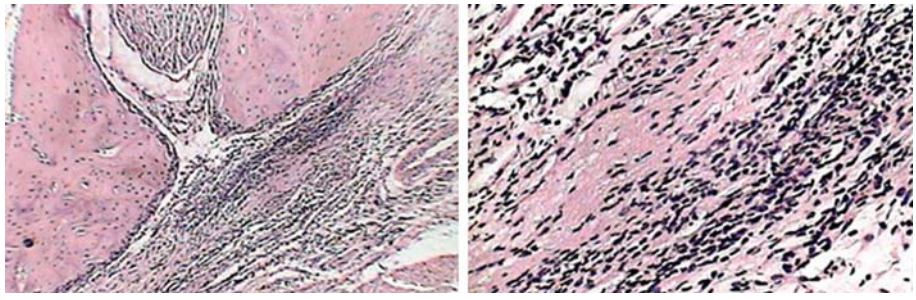

Figure 7. Histological slices from Group 3 (solution of 1:100,000 epinephrine)–treated side (100 and 400 ×, respectively). Presence of intense inflammatory infiltration around mental nerve.

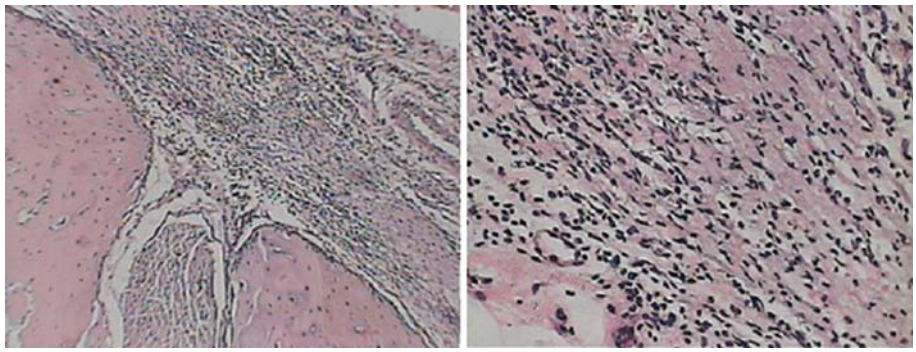

Figure 8. Histological slices from Group 3–control side (100 and 400 ×, respectively). Less inflammatory infiltration around mental nerve than treated side.

Results

Figure 2 and Table I show the scores obtained for the inflammatory cells 24 h after local injections (Groups 1–3). The mental nerve was not histologically affected by the treatments. However, higher scores ($p < 0.05$) of inflammation in connective tissue were observed when epinephrine was administered alone (Group 3) than associated with the local anesthetics (Groups 1 and 2).

Figures 3,4,5,6,7,8 show histological slices of mandibles from groups 1, 2 and 3; treated and control sides, respectively.

Discussion

Permanent paresthesia following a local anesthetic injection is a possible adverse event. Articaine, which is a widely used anesthetic in Europe, has been investigated with regard to its potential in causing paresthesia. Several authors [10–13] have reported that 4% solutions used in dentistry, articaine and prilocaine, are associated with paresthesia, mainly for inferior alveolar blocks. In addition, very few or no reports were attributed to lidocaine. Other follow-up studies have observed similar results [22–26]. So, it was suggested that an important cause of paresthesia could be related to the local anesthetic dose-dependent neurotoxicity. Some *in vitro* studies support this hypothesis [27,28].

Studies focusing on the toxicity of lidocaine showed that high concentrations of this local anesthetic resulted in irreversible conduction block while no effect was found in low concentrations [29,30]. In addition, prilocaine and lidocaine at the same concentration presented equivalent neurotoxicity in rats [31].

There are some reports of undesirable effects related to articaine use in dentistry [32–34]; however, most of them involve isolated cases. Unlike studies that support the hypothesis that articaine has neurotoxic potential, Leuschner and Leblanc [23] documented no pathomorphological systemic changes after the administration of 4% articaine in rats and dogs, even at toxic doses. Doses up to more than 10-times the maximum recommended dosage for humans revealed no evidence of harm to the fetus or to other reproduction phases. Local tolerance of this drug was considered by those authors to be 'good' to 'very good'.

Similarly, in the present study no significant increase in score inflammation was observed 24 h after articaine injection. Microscopic analysis of nervous structure showed no difference between the treated and the control sides. Ribeiro et al. [35] also observed no significant inflammatory reaction after subcutaneous articaine injection in rats when compared to lidocaine, bupivacaine and mepivacaine. In addition, the intensity of inflammatory reaction was not dose-dependent.

The same authors showed that lidocaine solution produced the least intense inflammation level, when compared to the other local anesthetic solutions. Similarly, the present study demonstrated that lidocaine was similar to saline solution and less toxic then articaine, although no statistical difference was observed.

The most intense inflammatory infiltration was observed when epinephrine was administered without local anesthetic (Group 3). This could be explained by the most intense vasoconstriction in the connective tissue, causing a more expressive hypoxia. This hypoxia is usually compensated by a vasodilation promoted in clinical doses by all local anesthetics used in dentistry [36]. Despite the intense inflammation observed in this group, there was no evidence of injury to the nervous structure.

Although the higher incidence of paresthesia associated to the use of 4% articaine formulation in comparison to other local anesthetics [10–13], the hypothesis that this local anesthetic may be neurotoxic was not confirmed in the present study.

The results of the present study suggest that articaine is not toxic to the nervous structure and that further studies are necessary to explain the possible relation between articaine injection and paresthesia.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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