Effect of Lidocaine- and Prilocaine-Based Topical Anesthetics on the Inflammatory Exudates in Subcutaneous Tissue of Rats

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The aim of this present study was to evaluate the irritative potential of 2 topical anesthetics used in intrapocket anesthesia for periodontal scaling/root planing when applied in subcutaneous tissue of rats. Sixty animals were divided into 4 groups: group 1, saline solution (control); group 2, poloxamer gel (thermosetting vehicle); group 3, lidocaine and prilocaine poloxamer thermosetting gel; group 4: EMLA, a lidocaine and prilocaine dermatological cream. Injections of 2% Evans blue were administered intravenously into the lateral caudal vein. In order to analyze vascular permeability, the tested substances were injected intradermally. The rats were sacrificed 3, 6, and 9 hours after injection of the substances. The dorsal skin was dissected and removed. The vascular permeability was evaluated by the measurement of area of dye extravasation and the dye was subsequently extracted after immersion in formamide. Statistical analyses were made by ANOVA with Bonferroni's post hoc test and Pearson correlation. The 2 methods to analyze the exudative phase of the inflammatory process showed statistically significant difference among the groups and periods of evaluation ($P < .05$). Both methods had a significant correlation ($P < .0001$). Under the tested conditions, the anesthetic agents showed mild initial inflammatory response when implanted in subcutaneous connective tissue.

Key Words: Biocompatibility testing; Anesthetics local; Lidocaine; Prilocaine.

Control of pain constitutes an important aspect of dental treatment. Several means exist, including pharmacologic and psychologic strategies, to reduce pain and unpleasantness. In spite of the documented efficacy of local injections with anesthetics, fear of pain and the needle are still common reasons for avoidance of dental treatment in odontophobics.¹

Periodontal scaling/root planing is an unpleasant and painful procedure for which local anesthesia is frequently used. The main anesthetic techniques used

in conjunction with periodontal scaling/root planing are nerve block/infiltration anesthesia alone or in combination with topical anesthesia. The main drawbacks of existing topical products are a lack of efficacy due to inadequate depth of penetration, too short duration of action, and difficulties of administration. Because many patients fear injection needles and there is a general desire to avoid numbness of the lip and tongue, there is a need for a fast-acting and effective topical anesthetic.^{2,3}

In recent years new topical anesthetic agents have been developed.⁴ Topical agents based on a combination of 2.5% lidocaine and 2.5% prilocaine in a eutectic mixture have been used to provide pain control in conjunction with periodontal scaling/root planing, following local application into the periodontal pockets.^{1,5}

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EMLA cream 5% is a 1 : 1 oil/water emulsion of a eutectic mixture of lidocaine (2.5%) and prilocaine (2.5%) bases. The eutectic mixture has a lower melting point (17°C) compared with the respective individual melting points of the lidocaine base (66–69°C) and the prilocaine base (36–38°C). This physical property allows the lidocaine/prilocaine eutectic mixture to be liquid at mouth temperature and thus facilitates rapid absorption of the bases.^{4,6} Because EMLA is not registered for intraoral use, a new thermosetting topical anesthetic gel (Oraqix) has been introduced in the field for intraoral application. At room temperature this poloxamer-based thermosetting formula is a low-viscosity fluid, whereas at body temperature it becomes an elastic gel. The new noninjection local anesthetic agent, which is made up of 5% lidocaine/prilocaine gel, provides pain relief during periodontal probing and scaling and root planing.^{3,6,7}

Tissue reactions induced by the anesthetic agents could be one factor in provoking pain after anesthesia. Mepivacaine and articaine induced a less inflammatory reaction than bupivacaine. Lidocaine solution produced the least inflammatory response in subcutaneous tissue of rats.⁸ Vascular permeability analysis is a method often employed in order to evaluate the irritant potential of several substances.⁹ This method physicochemically assesses the plasmatic exudate because of the irritating potential of the test agent.¹⁰ The methodology is useful not only to quantify the inflammatory exudate elicited by phlogistic agents, but also to evaluate the efficacy of anti-inflammatory drugs.¹¹ This model has been successfully used in previous studies to assess the irritating potential of a variety of endodontic drugs and dental adhesives.^{10,12–14}

Because of the limited availability of information in the literature about the effect of topical anesthetic on the initial phases of the inflammatory process (exudative phase) after periodontal scaling/root planing, this study aimed to evaluate the irritative potential of 2 topical anesthetic agents when applied in the subcutaneous tissue of rats.

METHODS

Study Design

This study was performed in accordance with the Ethical Principles of Animal Experimentation (COBEA–Brazilian College of Animal Experimentation) and was approved by the local Research Ethics Committee.

Sixty 2–3-month-old male rats (*Rattus norvegicus*, Albinus Wistar) weighing between 200 and 250 g were used. The animals were kept in an acclimatized room and received a balanced diet and water ad libitum. The tested substances were divided into 4

groups: group 1, saline solution 0.9% (control); group 2, poloxamer gel (thermosetting vehicle); group 3, lidocaine (25 mg/g) and prilocaine (25 mg/g) poloxamer thermosetting gel; and group 4, EMLA, lidocaine (25 mg/g), and prilocaine (25 mg/g) dermatological cream (AstraZeneca do Brasil Ltda, Coita, SP, Brazil).

The rats were anesthetized with an intraperitoneal injection (ketamine 75 mg/kg and xylazine 10 mg/kg). The dorsal skin was shaved and disinfected with 5% iodine in alcohol. Then, rat tails were washed and dried to facilitate the injections of 2% Evans blue (20 mg/kg) administered intravenously into the lateral caudal vein. In order to analyze vascular permeability, 0.1 mL of the tested substances was injected intradermally into 2 experimental sites (higher and lower). The animals were sacrificed 3, 6, and 9 hours after injection of the substances. The dorsal skin was dissected and removed.

The samples were photographed (digital camera, CiberShot DSC707, Sony do Brasil, São Paulo, SP, Brazil) and images of 5.1 megapixels were obtained. These stored digitalized images were not enhanced or transformed. The image analysis software used was Image Pro Plus Version 4.5.0.29 (Media Cybernetics, Silver Spring, Bethesda, Md). Each image was calibrated individually with a standard scale (mm). The vascular permeability was evaluated by the measurement of the dye extravasation area (mm²). Two measurements (higher and lower) were taken for each animal and the mean was calculated. The same examiner performed all the measurements, after testing the data reproducibility.

After that, this area was cut into small fragments and the dye was extracted after immersion in 7 mL of 99.9% formamide for 72 hours at 37°C. After centrifugation at 2500 rpm for 10 minutes and filtration with glass wool, 200 µL of each sample was placed in a 96-well plate. The absorbance was measured at 630 nm in a spectrophotometer (BioTek Instruments, Inc, Winooski, Vt). Each measurement was repeated 5 times. The total amount of dye extracted from samples was calculated by means of a standard calibration curve.

Statistical Analysis

Intraexaminer reproducibility (dye extravasation area) was tested with intraclass correlation coefficient. Comparisons among groups and also the periods of analysis were tested by 2-way ANOVA with Bonferroni's post hoc test. The normality of the distribution of data was confirmed using the Kolmogorov-Smirnov test. The relationship between the extravasation area of

Table 1. Means and SDs of the Extravasation Area of Blue Dye in Dorsal Skin Tissue of Rats (mm²) in Experimental Groups According to Periods*

Period, h	Substances			
	Control (Saline Solution 0.9%)	Poloxamer Gel (Thermosetting Vehicle)	Anesthetic Gel (5% Lidocaine/Prilocaine Poloxamer Gel)	EMLA™ (5% lidocaine/Prilocaine Cream)
3	1.55 ± 0.59 ^A	3.95 ± 1.04 ^A	409.73 ± 88.43 ^B	339.96 ± 91.69 ^B
6	0.06 ± 0.06 ^A	31.64 ± 18.28 ^A	452.59 ± 346.02 ^B	330.47 ± 165.68 ^B
9	0.02 ± 0.01 ^A	10.56 ± 9.34 ^A	382.60 ± 190.63 ^B	336.83 ± 50.85 ^B

*Horizontally, different letters indicate statistically significant differences among substances ($P < .05$). There were no statistically significant differences within each substance in different periods ($P < .05$). Two-way ANOVA with Bonferroni's post hoc test.

blue dye (mm²) and the amount of dye extracted (µg/site) was obtained using the Pearson correlation. The significance level was set at $\alpha = 5\%$ ($P \leq .05$). The analyses were performed using a statistical program (GraphPad Prism 5.00, GraphPad Software, San Diego, Calif).

RESULTS

The intraexaminer intraclass correlation coefficient was 0.98 for the extravasation area of the blue dye (mm²), considered very good.

The 2 methods to analyze the exudative phase of the inflammatory process showed statistically significant difference among the groups ($P < .05$). There was statistically significant difference considering the periods of evaluation ($P < .05$) only within the experimental groups (thermosetting anesthetic gel and EMLA). Interaction (agent and period) did not show statistically significant difference with $P > .05$ (Tables 1 and 2).

Both methods (extravasation area of blue dye and amount of dye extracted) showed similar results with a significant correlation ($P < .0001$; Pearson correlation; Figure).

DISCUSSION

This study evaluated the effect of topical anesthetic on the initial phase of an inflammatory response when applied in subcutaneous tissue of rats. The initial

phase of an inflammatory response is characterized by vasodilatation, an increase in vascular permeability, and cellular migration. During the initial inflammatory process, fluids, electrolytes, and proteins escape in high amounts through the vascular endothelium and are collected in the intercellular space to induce the inflammatory edema.^{10,11} The most widely used method to measure the rate of edema is based on the leakage of protein-bound dye into the inflammatory area. Some vital dyes, such as Evans blue, when administered intravenously, interact with the basic nitrogen of plasmatic albumin and are suitable as plasmatic markers for detecting leakage of proteins into inflammation sites.^{9,12,15}

The model used in the present study allows a rapid primary screen to evaluate the inflammatory potential of a variety of chemical agents. Nevertheless, this method may not provide an accurate representation of the irritating potential of the test agents when they cause extensive damage. This tissue destruction occurs because of the thin tissue around the test site, which deteriorates along with its diffusion. Sometimes, the size of the external blue zone does not correspond to the size of the lesion in the underlying connective tissue when freely dissected.^{12,16}

Some studies have used the method of vascular permeability to standardize the biopsy area (1–3 cm).^{10,12–14} In this present research, the biopsy size was not previously determined; the subcutaneous tissue was removed with a safety margin. On the other hand, if the margin had been previously standardized,

Table 2. Means and SDs of the Amount of Dye Extracted (µg/Site) in Experimental Groups According to Periods*

Period, h	Substances			
	Control (Saline Solution 0.9%)	Poloxamer Gel (Thermosetting Vehicle)	Anesthetic Gel (5% Lidocaine/Prilocaine Poloxamer Gel)	EMLA (5% Lidocaine/Prilocaine Cream)
3	0.41 ± 0.39 ^{AC}	1.37 ± 1.24 ^{AC}	6.24 ± 3.21 ^{AC}	5.52 ± 2.90 ^{AC}
6	0.26 ± 0.24 ^{AC}	1.91 ± 1.83 ^{AC}	7.24 ± 5.68 ^{ABC}	9.13 ± 7.80 ^{BCD}
9	0.42 ± 0.36 ^{AC}	0.65 ± 0.64 ^{AC}	16.88 ± 14.00 ^{BD}	11.32 ± 6.29 ^{BCD}

*Horizontally, different letters (A and B) indicate statistically significant differences among substances ($P < .05$). Vertically, different letters (C and D) indicate statistically significant differences for each substance in different periods ($P < .05$). Two-way ANOVA with Bonferroni's post hoc test.

Pearson correlation test among methods (extravasation area of blue dye amount of dye extracted) to evaluate the exudative phase of the inflammatory process (vascular permeability). Significant correlation ($P < .0001$).

an underestimation of the dye quantification is possible if this surpasses the limits of the predetermined biopsy area.

It is possible to verify in the literature different periods of observation ranging from 30 minutes to 14 days. This time variation could be related to different formulations (liquid and solid) of tested materials (endodontic irrigants, dental adhesives, intracanal medications, and root canal sealers).^{10–14} Thermosetting anesthetic gel and EMLA are considered semisolid formulations; therefore, a long period of observation would not be necessary to evaluate the initial phases of the inflammatory process (exudative phase). Thus, in this present study only 3 evaluation periods (3, 6, and 9 hours) were determined. Late phases of inflammatory process could be better assessed using histopathological techniques.

In this present research, vascular permeability was analyzed by 2 different methods: analysis of dye extravasation area (mm^2) and dye quantification ($\mu\text{g}/\text{site}$). Both methods had similar results regarding control and poloxamer gel (vehicle) groups. The analysis of dye quantification was more sensitive than the method of dye extravasation area, after 6 and 9 hours, because the former showed statistically significant difference for thermosetting anesthetic gel and EMLA according to the time points. On the other hand, the dye extravasation area had no significant results at initial time.

The findings of this study revealed a higher vascular permeability induced by thermosetting anesthetic gel and EMLA groups in comparison with control (saline solution) and poloxamer gel (vehicle). Both anesthetic agents demonstrated an increase in the range of dye values from 3 to 9 hours, but these results were not observed with the dye extravasation method. The agent properties (EMLA cream 5% is a 1 : 1 oil/water emulsion of a eutectic mixture and thermosetting poloxamer gel 5% lidocaine/prilocaine) have a low

viscosity (semisolid formulation); consequently, the extracellular local defense processes were unable to completely neutralize the anesthetic substances tested during the evaluation periods. For this reason, the blood system reacted with an increase in vascular permeability and inflammatory exudates with the aim of diluting those injurious agents. Both tested agents had a higher initial inflammatory response than poloxamer gel (vehicle based on triblock copolymers of polyethylene oxide and polypropylene oxide) and saline solution (control), probably because of the irritating properties of anesthetic agents (lidocaine and prilocaine). On the other hand, thermosetting anesthetic gel and EMLA did not seem to impair wound healing when placed in the experimental oral laceration.⁷

Comparisons among the results obtained in this present study are difficult because of the different methods that were used to calculate the amount of dye extracted from the tissue. Therefore, wide variations in results can be observed.^{10,12–14} There have been no other studies found that evaluate the initial inflammatory response of topical agents used for intrapocket anesthesia in periodontal scaling/root planing.

EMLA and thermosetting anesthetic gel were used directly in the periodontal pocket. During the scaling/root planing, both intrapocket anesthetic agents are rapidly absorbed into the oral tissue.^{2,3} Topical anesthetic substances in direct contact with the connective tissue of the periodontal pocket could induce tissue irritation and would be sufficient to contribute to discrete post anesthetic pain after nonsurgical periodontal treatment.

CONCLUSION

Under the tested conditions, both anesthetic agents showed mild initial inflammatory response when implanted in subcutaneous connective tissue. Nevertheless, further investigations must be carried out to assess the effects on inflammatory cells for longer periods.

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