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The influence of local anesthetic solutions storage on tissue inflammatory reaction

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Abstract

Objective: This study aimed to analyze the influence of storage conditions of local anesthetic solutions in the inflammatory reaction after injection in rats.

Study design: Twenty-four rats received in their oral mucosa the injection of 2% lidocaine with epinephrine 1:100.000 solutions (LA) submitted to the following storage conditions during a twelve-month period: G1 - inside the original packaging, in refrigerator ($5\pm 1^{\circ}\text{C}$); G2 - inside the original box, under light shelter, at room temperature; G3 - outside the original box at room temperature (exposed to artificial light for 12 hours/day) and G4 - brand new solution. For the controls tests, 0.9% sodium chloride solution was injected in the opposite side. After 6 and 24 hours, three animals of each group were sacrificed and their maxilla along with the soft tissue were removed and submitted to histological analysis (HE).

Results: The pH of LA was measured before and after the storage period and no statistically differences were observed between G1 and G4, but both were different from G2 and G3. All the scores of the testing solutions were higher than their respective negative controls, except for G1 at 6 hours. The order of the scores of inflammation after 6 hours was $G3 > G4 > G2 = G1$. After 24 hours the order was $G3 > G2 > G1 > G4$.

Conclusion: The study showed that the method of storage can influence the pH and the level of inflammatory reaction after the injection of 2% lidocaine with epinephrine 1:100.000.

Key words: Local anesthetics, storage conditions, lidocaine, rats.

Introduction

Local anesthetics (LA) are the safest and the most effective drugs for the control and management of pain in medicine and dentistry (1). The currently available solutions can be safely administered showing few and rare adverse effects such as tissue irritation or allergic reactions. In fact, complications associated with LA are rarely reported. In addition, almost all available solutions have shown efficacy, providing rapid onset of local anesthesia with adequate duration (2).

However, in some situations, LA can cause local tissue irritation resulting in inflammatory reaction, and consequently pain during and/or after anesthesia (3). The acidity of LA is one of the main causes of those adverse effects. The pH varies among the different anesthetic solutions due to many factors, being the LA with vasoconstrictors generally the most acid solutions (1).

Solutions without vasoconstrictors usually show pH (ranging between 5.5 and 7) close to the physiologic pH. LA with vasoconstrictor has lower pH (between 3.5 and 5.5) than the plain LA solutions being the low pH an important factor to avoid the oxidation of the vasoconstrictor (4,5). However, very low pH could result in tissue irritation and the very acid solutions are likely the primary source of pain (6).

The time and the storage conditions could contribute to the deterioration of LA solutions and significantly decrease the pH (7,8) affecting the vasoconstrictor concentration, which interferes in the LA performance (9,10).

The present study evaluated the tissue reaction induced in the oral mucosa of rats by injecting solutions of LA submitted to three different storage methods.

Materials and Methods

- Animals and drugs

Twenty-four male Wistar rats (*Rattus norvegicus*, albino), 60 days of age and weighing $175 \text{ g} \pm 25 \text{ g}$, were obtained from CEMIB-UNICAMP (Centro de Bioterismo – ICLAS Monitoring/Reference Center, Campinas, Brazil) where they were maintained under aseptic conditions. The protocol was approved by the Animal Ethical Committee - Institute of Biology - University of Campinas, Sao Paulo, Brazil (#504-1).

- Storage conditions and surgical procedure

The 2% lidocaine with epinephrine 1:100.000 (Lidocaina 100 – Alphacaine® - DFL, Brazil) anesthetic solutions were in the last month of their expiration date and they were previously submitted to three different storage methods during a twelve-month period.

The local anesthetic solutions (LAS) were divided into 4 groups: Group 1, inside the original box, in a refrigerator, at average temperature of 5°C; Group 2, inside the original box at room temperature, under light shelter; Group 3, outside the original box, at room temperature

(average temperature = 21.9°C), exposed to the artificial light during 12 hours/day. The Group 4 was composed by recently manufactured solution, not submitted to storage.

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

The LAS tested (0.1mL) were injected in the mucosa of the first upper-right molar of rats (six rats/group). 0.1mL of a saline solution (NaCl 0.9%) was injected in the mucosa of the opposite side of all animals (negative control). After six and 24 hours, three animals of each group were killed and the samples (maxilla and the soft tissue adhered) were removed.

Before the injections, the pH of the solutions was measured by using an ORION® model 290A pHmeter, with a LAZAR BNC microelectrode.

- Histological Analysis

The samples were fixed with 10% formalin solution and five cross-sections (6 µm thick, one each 40 µm of depth) were obtained from each animal. The samples were stained with hematoxylin and eosin.

The samples were blinded submitted to qualitative analysis in order to evaluate the intensity of leucocitary infiltration and/or any possible necrosis area. A preliminary pilot study conducted according other previous studies allowed the use of a qualitative score of the local tissue inflammation (11-13). The soft tissue and bone near the injection site were analyzed. The score was defined based on the following descriptions: (1) no inflammatory infiltration, (2) mild inflammatory infiltration, (3) moderate inflammatory infiltration, (4) intense inflammatory infiltration and (5) intense inflammatory infiltration with necrosis area.

- Statistical analysis

The pH obtained after one year in each storage method (groups 1 to 3) was compared to the pH obtained in group 4 by ANOVA and Dunnet tests. The data regarding inflammatory infiltration were submitted to the Kruskal-Wallis test for the comparison among different groups, and Wilcoxon test to compare the inflammatory infiltration observed in the right (test solutions) and left (negative control) sides. Differences of a P value of less than 0.05 were considered statistically significant.

Results

Figure 1 show the pH values obtained from the anesthetics of Groups 1 to 4.

The comparison (ANOVA, Tukey test) of the pH values showed no statistically significant differences between groups 1 and 4 ($p > 0.05$). However, the pH of both groups 1 and 4 were significantly higher ($p < 0.01$) than groups 2 and 3, being the pH of group 2 significantly higher than group 3 ($p < 0.05$).

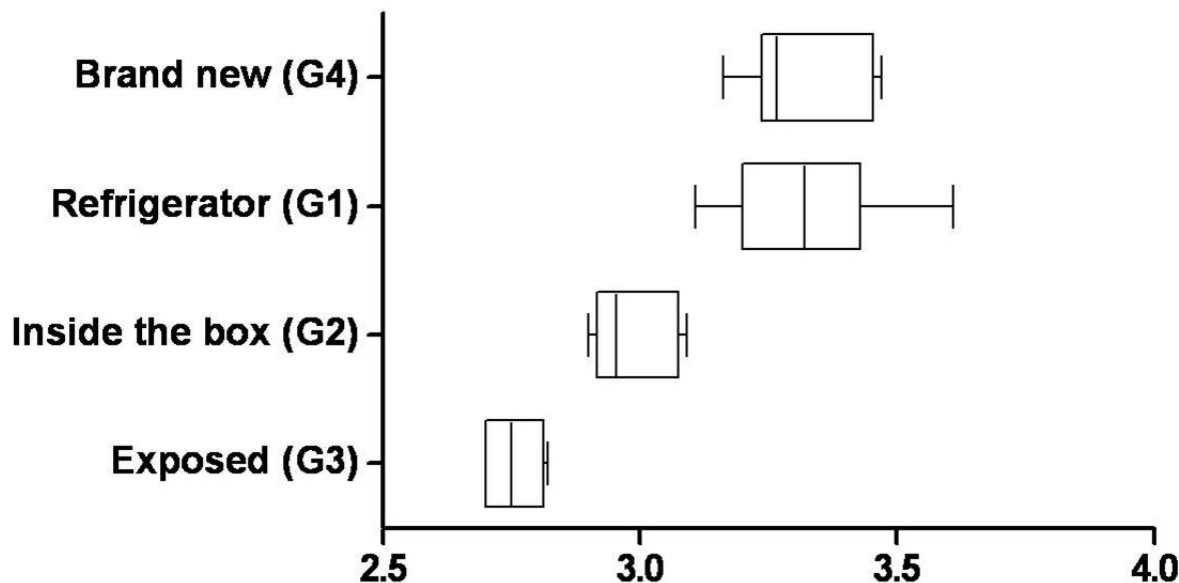


Fig. 1. PH of the tested anesthetic solutions. (Central line: median; Box: 1st and 3rd quartiles; Whisker: max and min values).

Table 1. Median (lower and upper quartiles) of the inflammatory scores for all groups in both periods of time.

Groups	6 hours		24 hours	
	Test	Control	Test	Control
Group 1 (Refrigerator)	2 (2 - 3)	2 (2 - 2)	3 (2 - 3)	2 (2 - 2)
Group 2 (Box)	3 (2 - 3)	2 (2 - 2.5)	3 (3 - 3.5)	2 (2 - 2)
Group 3 (Exposed)	4 (3 - 4)	2 (2 - 2)	4 (3 - 4)	2 (2 - 2.5)
Group 4 (Brand new)	3 (2.5 - 3)	2 (2 - 2)	3 (2 - 3)	2 (2 - 2)

The table 1 shows the scores obtained for the inflammatory cells after 6 and 24 hours of the administration of the anesthetics (Groups 1 to 4). No statistically significant differences were observed among the scores of the negative controls (Kruskal-Wallis, $p > 0.05$) at 6 or 24 hours. In addition, all the scores of the testing solutions were higher than their respective negative controls (Wilcoxon, $p < 0.05$), except for Group 1 at 6 hours (Wilcoxon, $p = 0.0679$).

Considering the six hours time, Group 3 showed the highest score among the other solutions and controls (Kruskal-Wallis, $p < 0.05$). There were no statistically significant differences (Kruskal-Wallis, $p > 0.05$) among the other groups.

No statistically significant differences (Kruskal-Wallis, $p > 0.05$) were found among the tested groups at the 24 hours period, but all tested solution showed higher inflammatory scores in comparison with their respective controls.

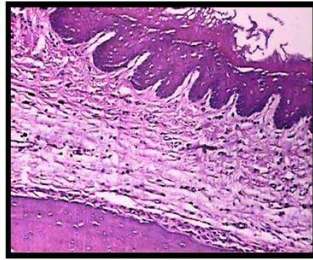
The level one of the inflammatory score was not found in the present study, because even the saline solution caused at least a mild inflammatory reaction. The figures 2 and 3 show the inflammatory reaction induced by each anesthetic solution in both periods of time.

Discussion

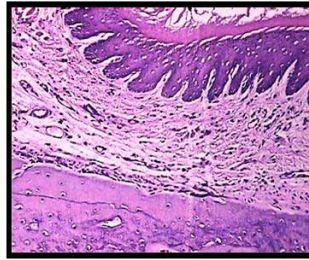
The inflammatory reaction induced by LAS was previously observed by De Carvalho et al. (13) and Ribeiro et al. (3). These studies observed polyethylene tubes containing absorbent-paper cones that were soaked in different anesthetic solutions, being subcutaneously implanted in the back of rats. This method could promote a more prolonged contact between LAS and tissues, easily allowing the localization of the implanted area. However, the oral mucosa close to the upper-first molar, as used in the present study, could better simulate the inflammatory reaction in the oral sites. The analysis of the results obtained in the control sides alone shows

6 HOURS

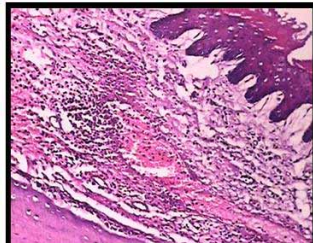
Group 1



Group 2



Group 3



Group 4

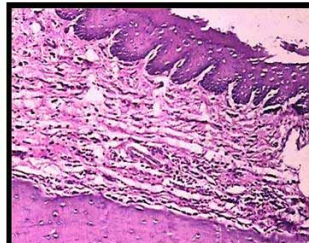
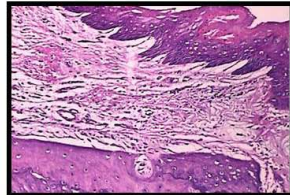


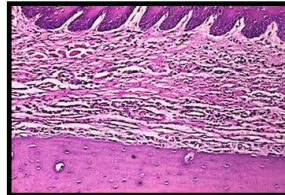
Fig. 2. Histological slides of soft tissue of rats after 6 hours of the injection of the anesthetic solutions (Groups 1, 2, 3 and 4). HE, magnification x10.

24 HOURS

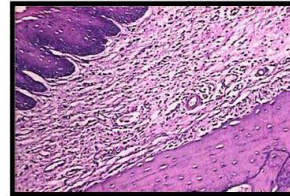
Group 1



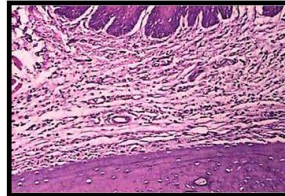
Group 2



Group 3



Group 4



CONTROL 24 HOURS

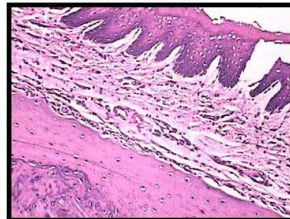


Fig. 3. Histological slides of soft tissue of rats after 24 hours of the injection of the anesthetic solutions (Groups 1, 2, 3 and 4). HE, magnification x10.

the homogeneity and the standardization of the method used in the present study, allowing a precise comparison of the effect of the LAS.

Previous studies showed lidocaine as the least irritating local anesthetic when compared to the other LAS commonly used in dentistry (3,13). Thus, we decide to observe this local anesthetic in our study.

Wakamatsu (14) observed that 2% lidocaine with or without epinephrine did not change the postextraction wound healing process. However, in the present study we observed that the lidocaine solutions caused higher levels of inflammation when comparing to their respective controls. The vasoconstrictor (epinephrine 1:100,000), which causes slow absorption and elimination of the LAS, could be the responsible for this difference.

Another explanation for this finding is that lidocaine (and other anesthetic agents), in clinical doses, has anti-inflammatory actions, thus inhibiting leukocyte adhesion, migration and phagocytosis, as well as inflammatory mediators synthesis, vascular hyperpermeability and edema formation (15). Our results, nevertheless, showed that the lidocaine solutions caused higher levels of inflammation when comparing to their respective controls except for Group 1 at 6 hours. This increased inflammatory reaction could be attributed to the pH of the LAS studied.

The pH of commercial LAS added with sympathomimetic amines (epinephrine, norepinephrine, levonordefrin and phenylephrine) usually varies from 3.5 to 5.5 (4), being these low pH values necessary to reduce the oxidation of these sympathomimetic amines (5). In our study, the measurement of the pH of LAS before their administration revealed that all of them were acid. The mean pH of all solutions was below 3.5, being the LAS conserved at room temperature inside the box and those exposed to the light the most acid (pH below 3.0) solutions. Although these values could be considered acceptable, they could also be a strong suggestion of the chemical deterioration of the solution, particularly of the vasoconstrictor.

It is well established that certain storage conditions can alter the pH of the LAS (7,9,10,16,17). The pH change is usually induced by the oxidation of the sulfites, which are used as antioxidants, into sulphates, causing protons liberation and resulting in pH decrease (18,19). A fast reduction of the vasoconstrictor concentration and pH decrease (7,8) by ultraviolet and infrared rays were observed when LAS were exposed to the sun light. The pH decrease was also observed in the present study, while the refrigerator kept the pH stable for one year at least. The low pH reflected in the inflammatory levels observed. Previous studies related adverse effects due to the use of acid and/or deteriorated solutions ranging from a slight burn sensation to a severe tissue inflam-

mation (20). These effects can cause discomfort and pain during the injection, and retard the beginning of the anesthesia (1,4,5). These finds are compatible with the results of the present study since the LAS expose to light and/or room temperature (Groups 2 and 3) caused more intense inflammatory reaction than the solutions stored in the refrigerator.

To the date any study was found in the literature showing the quality of the local anesthetic solutions utilized by the Brazilian dentists. Although the LAS are the drugs most used in dentistry, there is little information about the best way to storage them. Gerke et al. (8) showed a decreased epinephrine content when local anesthetic cartridges were exposed to sunlight, and recommended the professionals to avoid the sunlight exposition of the local anesthetics cartridges. This observation is corroborated by the results of the present study since the LAS stored in the refrigerator ($\pm 5^{\circ}\text{C}$) induced the less intense tissue reaction along with a more stable pH.

Based on the results presented in this study, it can be concluded that the method of storage influenced the pH and the level of inflammatory reaction after the injection of 2% lidocaine with epinephrine 1:100.000.

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