Effect of nitric oxide inhibitor and donor substances on the inflammatory process caused by endodontic irrigants

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ABSTRACT

Nitric oxide (NO) has been considered a key molecule in inflammation. Objective: The aim of this study was to evaluate the effect of treatment with L-NAME and sodium nitroprussiate, substances that inhibit and release NO, respectively, on tissue tolerance to endodontic irrigants. Material and Methods: The vital dye exudation method was used in a rat subcutaneous tissue model. Injections of 2% Evans blue were administered intravenously into the dorsal penial vein of 14 male rats (200-300 g). The NO inhibitor and donor substances were injected into the subcutaneous tissue in the dorsal region, forming two groups of animals: G1 was inoculated with L-NAME and G2 with sodium nitroprussiate. Both groups received injections of the test endodontic irrigants: acetic acid, 15% citric acid, 17% EDTA-T and saline (control). After 30 min, analysis of the extravasated dye was performed by light absorption spectrophotometry (620 nm). Results: There was statistically significant difference (p<0.05) between groups 1 and 2 for all irrigants. L-NAME produced a less intense inflammatory reaction and nitroprussiate intensified this process. Conclusions: Independently of the administration of NO inhibitors and donors, EDTA-T produced the highest irritating potential in vital tissue among the tested irrigating solutions.

Key words: Nitric oxide. Inflammation. Root canal irrigants.

INTRODUCTION

Chemical substances should act without being aggressive to the pulp and periapical tissues. Considering that the chemical substances used during chemomechanical preparation of root canals can extrude to the periapical region, and that the chemical agents used are tissue irritatings, it is important to know the consequences of their contact with vital tissues, in order to minimize postoperative complications. All substances that come into contact with vital tissues require previous biocompatibility tests.

When a tissue is damaged, a normal protective response is set off: inflammation. The inflammatory response starts by the release of chemical mediators, produced by the cells of the affected tissue, which promote vasodilation and increase the
blood flow, resulting in an accumulation of liquid and blood cells. The vasodilation phenomenon occurs as a result of the action of a substance produced in the endothelium, denominated endothelium derived relaxation factor (EDRF). Palmer, Ferrige and Moncada (1987) and Ignarro, et al. (1987) suggested that this factor was nitric oxide (NO) because of the similarities in their physicochemical characteristics.

In 1992, the scientific journal Science recognized the importance of this substance in various areas of Medicine, and named it the “Molecule of the Year”. Since then, a increased number of studies on NO and its metabolites have progressively allowed an understanding of some of its main biological functions: participation in the immunological system, neurotransmission and vasodilation. Furthermore, a variety of cardiovascular and cerebral problems and inflammatory and infectious diseases may be related to a high or low NO level in the organism.

NO is synthesized by the enzyme nitric oxide synthase (NOS), which is present in a variety of different cell types, or is induced by an external mechanism, such as immunological and inflammatory stimuli. Dental pulp presents the potential to produce NO, since NO is present in endothelial cells, odontoblasts, nerve tissues, white blood cells and vascular smooth muscles.

Analyzing NOS production, NO has been found to be synthesized in root cysts and inflamed periapical tissues, playing a decisive role in the regulation of chronic, periapical infection.

After obtaining good results with inhibitory substances on cells removed from periapical cysts, Takeichi, et al. (1999) suggested the use of NO inhibitors in the root canal as a pharmacological treatment for periapical lesions.

There are several studies correlating NO with endodontic sealers or periapical lesions, but only one relating it to chemical irritant substances. Laboratory determination of NO is complex, and characterization of its specific activators and inhibitors constitutes a new challenge to the understanding and treatment of various diseases.

The solutions commonly used in the final irrigation of endodontic therapy are citric acid and EDTA, and more recent research has also suggested acetic acid. The use of these irrigants has been extensively studied. It would be interesting to accelerate the healing process, in order to obtain a better control of the inflammatory process, and to provide more comfort to the patient. Thus, the aim of this study was to evaluate the effects of the treatment with L-NAME and sodium nitroprussiate, substances that block and release NO, respectively, in connective tissue inflammation caused by acetic acid, citric acid, EDTA-T irrigants.

**MATERIAL AND METHODS**

This study was approved by the Ethics Committee of the Dental School of the University of Taubaté, Brazil (protocol 07/2005).

The following endodontic irrigants, prepared at Fórmula & Ação pharmacy (São Paulo, SP, Brazil), were evaluated:

- 17% EDTA-T (ethylenediaminetetraacetic acid disodium– tergentol) – The salt EDTA was weighed (17 g), diluted in 100 mL of tergentol and the pH was adjusted to 7.3 with sodium hydroxide solution.
- 15% citric acid - The salt (Merck S.A., São Paulo, SP, Brazil) was weighed, diluted in deionized, and the pH was adjusted to 1.0 using pH meter (B371; Micronal, São Paulo, SP, Brazil).
- 10% acetic acid - 10 g of 2-hydroxypropane tricarboxylic acid was diluted in (qsp) 100 mL of bidistilled water.
- Saline solution (used as control).
- L-NAME (N²-nitro-L-arginine methyl ester) and sodium nitroprussiate were purchased from Tocris Cookson Inc. (St. Louis, MO, USA).

**Vital dye exudation technique**

The experiment was performed according to the technique proposed by Udaka, et al. (1970). Fourteen male Wistar rats (Rattus novergicus albinus), weighing between 200-300 g, were obtained from the Animal Care Facility of the Araquarara Dental School, São Paulo State University, Brazil. They were kept in cages, in a suitable environment, at the Pharmacological Laboratory of the Medical School of the University of Taubaté, Brazil.

The animals were anesthetized with intraperitoneal administration of Thiopentax (0.3 mL for every 100 g/rat) in the lateral caudal region. After, 2% Evans vital blue dye (Sigma Chemical Co., St. Louis, MO, USA) was injected intravenously in the dorsal penial vein, at the dose of 20 mg/kg of body weight. A sufficient period was waited to ensure that the dye injection had been successfully administered (around 3 h) observing the blue stain in the irises of the rats. After that, the dorsal region was shaved and the animals were randomly divided into 2 groups (n=7):

Group 1: at four predetermined sites in the dorsal region of the rats, 0.1 mL of L-NAME, a NO inhibitor (30 mg/kg dissolved in 5 mL of saline solution), was injected into the subcutaneous tissue. Afterwards, 0.1 mL of each tested solution (EDTA, citric acid, acetic acid and saline) was injected in each point.

Group 2: at four predetermined sites in the subcutaneous tissue, 0.1 mL of sodium nitroprussiate, a NO donor (4 mg/kg dissolved in 40 mL of saline), was injected into the subcutaneous tissue. Afterwards, 0.1 mL of each tested solution
(EDTA, citric acid, acetic acid and saline) was injected in each point.

The substances were used in a system of rounds, in such a way that the same animal would receive the three irrigants, in addition to the control solution. Concomitantly with NO inhibitor or donor substances, the irrigants were applied, according to their respective groups.

After 30 min the animals were sacrificed by anesthetic overdose (Thiopentax®-0.6 mL for every 100 g body weight) and their dorsal skins were excised using an iron ring with an active tip (3 cm in diameter), with a safety margin of 20 mm beyond the bluish colored edema haloes formed by the medication.

The tissue obtained was fragmented, immersed in 10% formalin and kept in water at 37°C for 72 h for complete extraction of the dye. After 72 h, the resulting solutions were subjected to spectrophotometric analysis (Cary 50 Bio UV Visible Spectrophotometer; Varian Inc., San Francisco, CA, USA) at 620 nm wavelength (maximum absorbance capacity of the Evans blue dye), and calibrated for optical density reading, according to the evaluation criteria of the irritating potential of substances proposed by Nagem-Filho and Pereira14 (1976) (Figure 1).

Statistical Analysis
The obtained data were examined for the adherence to the normality curve and submitted to statistical analysis by the Kruskal-Wallis test for individual comparison at a level of significance of 5% (p<0.05).

<table>
<thead>
<tr>
<th>Absorbance values</th>
<th>Degree of irritation potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000 - 0.1249</td>
<td>Not significant</td>
</tr>
<tr>
<td>0.1249 - 0.3010</td>
<td>Discrete</td>
</tr>
<tr>
<td>0.3010 - 0.6201</td>
<td>Moderate</td>
</tr>
<tr>
<td>0.6201 - 2.0000</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Figura 1 - Criteria for evaluating the irritating potential of substances according to the absorbance values (A620) - Nagem-Filho and Pereira14 (1976)

Table 1 - Mean and standard deviation of optic density reading obtained by the spectrophotometer, corresponding to the degree of irritation - Nagem-Filho and Pereira14 (1976).

<table>
<thead>
<tr>
<th>Tested substances</th>
<th>Sodium Nitroprussiate (Mean±Standard deviation)</th>
<th>Degree of irritation</th>
<th>L-NAME (Mean±Standard deviation)</th>
<th>Degree of irritation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>0.279±0.344a</td>
<td>discrete</td>
<td>0.021±0.011a</td>
<td>not significant</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.072±0.045b</td>
<td>not significant</td>
<td>0.008±0.008b</td>
<td>not significant</td>
</tr>
<tr>
<td>EDTA-T</td>
<td>1.300±0.418b</td>
<td>severe</td>
<td>0.155±0.111b</td>
<td>discrete</td>
</tr>
<tr>
<td>Saline solution</td>
<td>0.077±0.057b</td>
<td>not significant</td>
<td>0.011±0.006b</td>
<td>not significant</td>
</tr>
</tbody>
</table>

Statistical differences are indicated by different letters in rows (Kruskal Wallis test, P<0.05).
Effect of nitric oxide inhibitor and donor substances on the inflammatory process caused by endodontic irrigants

Based on a previous study that evaluated the ideal concentration of EDTA ranging from 10% and 15%, it is important to emphasize that this potential in vital tissues than the other irrigants. However, it is observed that, for both NO inhibitor and donor substances, EDTA-T had the highest irritating potential in vital tissues than the other irrigants. Moreover, it can be concluded that: 

1. Sodium nitroprussiate and L-NAME changed the inflammatory reaction produced by tested irrigants.
2. The inflammatory reaction was more intense in the group submitted to treatment with sodium nitroprussiate.
3. In the group in which the NO inhibitor (L-NAME) was administered, there was a reduction in the inflammatory process.
4. Independently of the administration of NO inhibitor or donor substances, EDTA-T had the highest irritating potential in vital tissue among the tested irrigating solutions.

REFERENCES


CONCLUSION

Based on the conditions established by the experiment, it can be concluded that:

1. Sodium nitroprussiate and L-NAME changed the inflammatory reaction produced by tested irrigants.
2. The inflammatory reaction was more intense in the group submitted to treatment with sodium nitroprussiate.
3. In the group in which the NO inhibitor (L-NAME) was administered, there was a reduction in the inflammatory process.
4. Independently of the administration of NO inhibitor or donor substances, EDTA-T had the highest irritating potential in vital tissue among the tested irrigating solutions.