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| ORIGINAL

Does hypersensitive teeth show pulp inflammation?

Dentes hipersensíveis apresentam inflamação pulpar?

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ABSTRACT

Objective: This study investigated the presence of inflammatory response in the dental pulp of rats showing hypersensitive dentin, induced by erosive episodes. **Methods**: Sixteen Wistar rats were fed with commercial sucrose-free pellet diet for 12 hours; whereas the food was removed during the remainder of the day, and the animals received mineral water or a lemon-based sucrose-free soft drink, according to the group to which they belonged. Eight animals consumed the soft drink to induce hypersensitivity, while the other 8 animals received mineral water (control). After six weeks, the animals were euthanized, the mandible was removed and subjected to a median incision in the sagittal plane, to obtain right and left hemimandibles. The slides stained with hematoxylin-eosin were analyzed using light microscopy. **Results**: Histological evaluation of the control and experimental groups revealed no inflammatory process in the pulp tissue, and the presence of inflammatory cells, such as lymphocytes, plasma cells, eosinophils and macrophages, was not observed. In addition, there was no edema or dilated and congested blood vessels. The Mann-Whitney test showed no significant difference (p = 1.000) between the experimental and the control groups. **Conclusion**: In the animal model used, dentin hypersensitivity does not trigger dental pulp inflammatory response.

Indexing terms: Dentin; Hypersensitivity; Pulp inflammation; Animal model; Dental erosion.

RESUMO

***Objetivo**: Este estudo investigou a presença de resposta inflamatória na polpa dentária de ratos apresentando hipersensibilidade dentinária induzida por episódios erosivos. **Métodos**: Dezesseis ratos Wistar foram alimentados com ração peletizada isenta de sacarose por 12 horas; enquanto a dieta estava ausente no restante do período diário, os animais receberam água mineral ou um refrigerante dietético à base de limão, de acordo com o grupo ao qual pertenciam. Oito animais consumiram o refrigerante para a indução do quadro de hipersensibilidade, enquanto os demais receberam água mineral (controle). Após seis semanas, realizou-se a eutanásia dos animais, os quais tiveram suas mandíbulas removidas e seccionadas medialmente no plano sagital, a fim de serem obtidas hemimandíbulas direita e esquerda. Lâminas coradas com hematoxilina-eosina foram analisadas sob microscopia óptica. **Resultados**: A avaliação histológica dos grupos controle e experimental revelou ausência de processo inflamatório no tecido pulpar e também*

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não foi observada a presença de células inflamatórias como linfócitos, plasmócitos, eosinófilos ou macrófagos. Em adição, não houve edema ou vasos sanguíneos dilatados e congestos. O teste de Mann-Whitney demonstrou inexistência de diferença significativa (p = 1,000) entre os grupos experimental e controle. ***Conclusão****: No modelo animal adotado, a hipersensibilidade dentinária não desencadeou resposta inflamatória na polpa dentária.*

Termos de indexação: *Dentina; Hypersensibilidade; Inflamação pulpar; Modelo animal; Erosão dentária.*

INTRODUCTION

Dentin hypersensitivity is characterized by chronic, yet short-lasting pain, caused by thermal, osmotic, chemical or evaporative stimuli due to the exposure of the dentinal tubules to the oral environment [1,2] producing symptoms that cannot be explained by any other dental pathology [3].

Among the sources responsible for the exposure of the dentin tubules is dental erosion, caused mainly by dietary or gastric acids [4]. Dental erosion has been associated not only with the localization but also the initiation of dentin hypersensitivity [2,5]. In fact, acidic substances can cause dental loss and tubular opening [6], which has been substantiated by clinical data showing that the frequent contact between dentin and acids is directly related to the occurrence of dentin hypersensitivity [7].

From a pathophysiological point of view, dentin hypersensitivity has been attributed to the movement of fluid inside the dentin tubules, which would trigger the nerve endings in both the vicinity and inside the pulp tissue [1]. It has been suggested, however, the possibility that an inflammatory response within the dental pulp may coexist [6]. There have been, however, only very few initiatives in the literature that intended to investigate the pulp reactions in hypersensitive dentin [8-10].

The first difficulty in verifying such a hypothesis would be the experimental model. In humans, there are ethical impediments to the induction of erosive lesions to create hypersensitivite dentin. Nonetheless, animal models appear promising. In this respect, in two previous studies using rat models [11,12], acidic beverages were capable of creating lesions on dental hard tissues. However, it is worth noting that in the quoted papers the animals had been previously inoculated with cariogenic microorganisms and the acidic beverages had sucrose. Thus, it is likely that in these experimental rat models there were acid from bacterial origin as well, making it difficult to achieve an actual erosion lesion. More recently, erosion lesions [13], dentinal tubules exposure and dentin hypersensitivity [14,15] were more representatively induced in animal studies in which rats consumed acidic drinks but were not inoculated with cariogenic bacteria.

Based on the potential relationship between dentin hypersensitivity and pulp inflammation and considering the feasibility of employing animal models to provide such conditions, this study aimed to investigate the existence of inflammatory response in the pulp of rats upon creating dental erosion to induce dentin hypersensitivity.

METHODS

Ethical aspects

All procedures performed in this study were in accordance with the ethical standards in compliance with national and international guidelines for care and welfare of animals. This study was reviewed and approved by the Ethics Committee on Animal Experimentation - CEUA, São Leopoldo Mandic Institute and Research Center (protocol # 0361).

Experimental design

The sample consisted of 16 male Wistar rats weighing approximately 250 g placed into quarantine for 10 days to ascertain absence of disease. The animals were divided randomly into two groups (n = 8), according to the beverage ingested during the experimental period: sucrose-free soda lemonade (Sprite Zero, Coca Cola Co., Porto Real, RJ, Brazil) and mineral water, as control (Crystal, Coca Cola Co., Mogi das Cruzes, SP, Brazil, as a negative control group). The response variable was the presence of pulp inflammation, evaluated histologically.

Accommodation of the animals

The animals were housed in groups of four, in polypropylene cages containing pine shavings in an open system, ventilated shelves with temperature ranging

between 20 and 24 ° C, in the vivarium of the São Leopoldo Mandic Dental School, set to 12 hours of light and 12 hours of dark.

Erosive diet regime

During the 12 hours of light, the animals were offered commercial pelleted feed ad libitum (Premix Mineral AIN-93 M zero sucrose, Rhoster, Sorocaba, SP, Brazil), consisting of corn starch, milk casein, dextrin starch (maltodextrin), soybean oil, cellulose, sucrose-free microcrystalline, mineral mix AIN-93M (maintenance) AIN-93 vitamin mix, L-cystine, choline bitartrate and tert-butylhydroquinone. The equivalent proportions in the composition of the feed are described in table 1.

During the dark period, the feed was removed and, according to the group they belonged to, the animals were given mineral water or the lemon-based soda drink (Sprite Zero) ad libitum in bottles.

After six weeks, symptoms of dentin hypersensitivity was checked by applying a jet of cold water (4°C, 0.5 ml) with a syringe, for 5 s, on the labial surface of molars. The animal's response were scored as follows: 0 = no response; 0.5 = slight contraction of the body; 1 = body contraction; 2 = strong body contraction and a short vocalization; 3 = strong body contraction and a prolonged vocalization) [16].

The animals were euthanized with an intramuscular injection of ketamine solution (200 mg/Kg body weight) and intraperiotoneal injection of thiopental at 150 mg/Kg and lidocaine at 1 mg/mL. The mandible of the rats was removed and incised in the median sagittal plane to obtain the right and left hemimandibles.

Table 1. Basic composition of the pelleted feed used in this study.

Components	% per 100 g
Corn starch	56.57
Casein	14.00
Dextrin starch (maltodextrin)	15.50
Soybean oil	4.00
Sucrose	zero
Cellulose	5.00
Mix Mineral AIN-93M (maintenance)	3.50
Vitamin Mix AIN-93	1.00
choline bitartrate	0.25
L-Cystine	0.18
Tert-Butylyidroquinone	0.0014

Processing for histological analysis

After identification, the mandibles were placed in cassettes, fixed in 10% formaldehyde solution (pH 7) for 24 hours followed by decalcification in formic acid solution at 20%. Then, the paraffin blocks were prepared. The slides sections were semi-serial thickness of 5 µm being chosen for analysis of each central block sections.

The slides were immersed in xylene twice for 5 min and dehydrated in alcohol baths (absolute, 90% and 80%) at room temperature for 5 min, respectively. After further washing in water for 5 min, the tissues were immersed in hematoxylin (1 min), rinsed in water for 5 min and ethanol 80% (2 min). The slides were immersed in eosin (10 min), rinsed in water, dehydrated in baths of increasing concentration of alcohol (80%, 90% and absolute) and immersed in xylene-I and then placed in xylene-II until mounting.

Histological analysis

The histological analyses were performed under an optical microscope (Infinity 1, Osasco, São Paulo, Brazil), coupled to a camera Nikon Eclipse CI H550S through an image scanning software (Infinity Analyse, Lumenera Co., Ottawa, ON, Canada). The criteria used for histologic evaluation were disorganization of the odontoblast layer, absence or presence of inflammation cells (lymphocytes, plasma cells, eosinophils or macrophages), interstitial edema and dilation of blood vessels. Inflammation was evaluated according to a 4-point scoring system: 0 (no inflammation), 1 (mild - up to 10% of the tissue), 2 (moderate - from 11% to 50% of the tissue), 3 (intense - greater than 50% of the tissue).

Statistical analysis

The data were submitted to the Mann-Whitney test, adopting a significance level of 5%. Statistical calculations were conducted on SPSS 20 (SPSS Inc., Chicago, IL, USA).

RESULTS

The control group showed histological findings similar to those found for the experimental group (whose rats ingested lemon-based sucrose-free soft drink), as shown in figures 1 and 2.

Histological evaluation of the experimental group demonstrated that the odontoblasts remained organized with no evidence of an inflammatory process within the pulp. As well as the inflammatory cells such as lymphocytes, plasma cells, eosinophils and macrophages, interstitial edema or dilated and congested blood vessels were not present either.

The histological analysis of the control group had a score of 0 on the assessment scale of pulp inflammation, since the structures and tissue conditions observed in the sections featured no alterations (figure 1).

As for the analyses of the experimental group using the same criteria as the control group (disorganization of the odontoblast layer, presence or absence white blood cells, interstitial edema and dilation of blood vessels), no alterations were observed in the experimental group in terms of inflammation (figure 2).

When comparing both groups (experimental and control), the Mann-Whitney test showed no significant difference between them ($p = 1.000$).

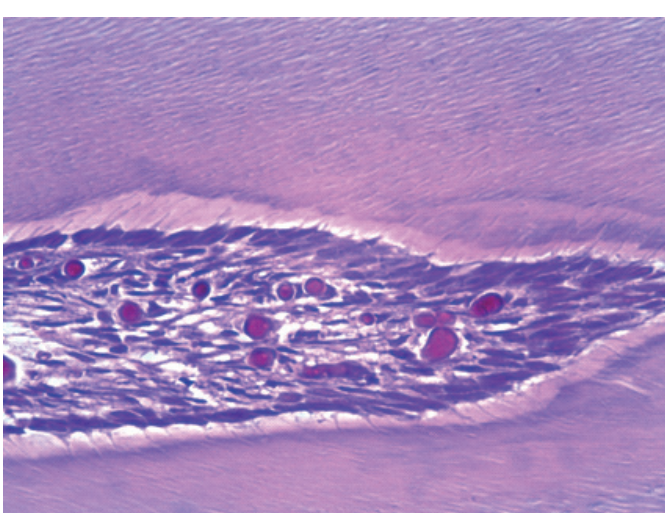

Figure 1. Histological section of the pulp tissue (x40) from the control group showing the layer of odontoblasts in palisade and the absence of an inflammatory cell infiltrate.

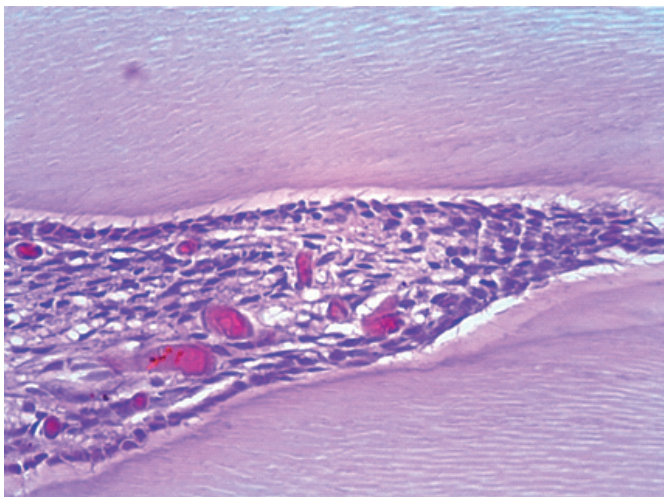

Figure 2. Histological section of the dental pulp (x40) from the test group showing odontoblasts in palisade and no inflammatory cell infiltrate.

DISCUSSION

In the context of dentin hypersensitivity, in addition to the well-accepted hydrodynamic theory, based on the facts that nerve activation may release neuropeptides and consequently neurogenic inflammatory reactions [10], and that non-steroidal anti-inflammatories can alleviate dentin hypersensitivity [6], it has been hypothesized that this condition could be related to the occurrence of pulp inflammation [6]. This study, therefore, aimed to investigate this hypothesis using an animal model to simulate dental erosion in order to expose the dentinal tubules so that pulp response could be evaluated.

Unlike Bowen and Lawrence's work [11], which used a cola-based soda drink, and the model adopted by Bergamini et al. [14,15] which used a sport drink, this study opted up for inducing erosive lesions using a sucrose-free soda lemonade due to its low pH and high erosive potential, as per revealed in a previous study [16], as apposed to the sugary version, which has been proven milder [16]. Similar to the present study, Aldosari et al. [15] also used a sucrose-free soda lemonade in their rat model and found moderate and advanced erosion lesions at the end of four weeks. In our model, however, in order to increase de percentage of advanced erosion lesions and therefore, allow dentin hypersensitivity, the rats consumed the acid drink for 6 weeks rather than 4. Even though under both duration times acid beverage can induce dentin hypersensitivity, it has been shown that after 6-week period

animals respond with higher scores of pain after cold water stimuli [14]. We assumed that increased pain would more likely be associated with pulp inflammation.

Unlike Bergamini et al. [14] in the present study we opted to alternate the intake of drinks and food so that the effect of the drink could be enhanced, as combining food and drink at the same time could reduce the erosive potential of the acidic beverage. Still aiming at potentiating the erosive effect and therefore the possibility of dentin hypersensitivity, soda was provided at nighttime, when the animals are most active [17]. In effect, while the control animals showed no response (score 0) or slight contraction of the body (score 1) when the jet of cold water was applied, among the rats that ingested the soda lemonade 5 of 8 (62.5%) presented strong body contraction and short or prolonged vocalization.

It is worth mentioning that the present study used commercial sucrose-free pelleted feed to ensure the nutritional needs of the animals while controlling the production of acids by eventual cariogenic bacteria that could have been present in the oral cavity of the animals.

In this study we used the presence or lack of inflammatory cells and vascular changes to evaluate pulp inflammation. No inflammation evidence was found in dental pulps either from experimental or control groups. No lymphocytes, plasma cells, eosinophils and macrophages were observed. In addition, there was no edema or dilated and congested blood vessels.

Therefore, our findings do not support the hypothesis that tubular exposure would cause pulp inflammation. One reasonable explanation may be the fact that cellular debris and plasma proteins may have occluded dentin tubules precluding pulpal inflammatory reactions [18]. In conjunction with this possible phenomenon, one can speculate that the time course of the dentin exposure may have not been long enough to activate inflammatory cells and induce vascular changes.

One should, however, bear in mind that dentin hypersensitivity can generate neurogenic inflammation due to the release of neuropeptides [10], which does not necessarily cause noticeable cells and vascular changes in the histological analyses. Therefore, in addition to assessing histological inflammatory changes, it seems reasonable to proceed one step further and investigate the release of such neuropeptides.

CONCLUSION

The animal model used in this study revealed that dental pulp of hypersentive teeth did not show evidence of histological inflammatory response.

Collaborators

DP FAGUNDES-DE-SOUZA, carried out the experiment and wrote the first draft of the manuscript. MH NAPIMOGA, supervised the methodological steps and participated of the data/results analysis and discussion. AB SOARES, performed the histological analysis and color-edited the histological images. VC ARAÚJO, performed the histological analysis. CP TURSSI, designed the study, carried ou the statistical analysis and wrote the final version of the manuscript

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