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THE REACTION OF DENTAL PULP TO *ESCHERICHIA COLI* LIPOPOLYSACCHARIDE AND *ENTEROCOCCUS FAECALIS* LIPOTEICHOIC ACID

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ABSTRACT

This research evaluates the effects of the lipopolysaccharides (LPS) from *Escherichia coli* and lipoteichoic acid (LTA) from *Enterococcus faecalis* on dental pulp. These molecules are components of the Gram-negative and Gram-positive bacteria cell wall, respectively. Ten dogs were used in the experiment. Inoculation in surgically opened pulp and coronal restoration with glass ionomer was the method chosen. The evaluation times were 1, 7, 15, 30 and 60 days. The results showed that the LPS and LTA, at 150 µg/ml, produced a negative interference in the pulp leading to destruction. LTA caused less damage than LPS.

Key words: lipopolysaccharide, endotoxin, lipoteichoic acid, dental pulp, bacteria

Lipoteichoic acid (LTA) and lipopolysaccharides (LPS) are components of the bacterial cell wall that can induce pulp inflammation and periapical infections, including lethal cellular aggression (9). The lipid portion of the cellular membrane associated with teichoic acids from the cell wall form lipoteichoic acids which are encountered solely in Gram positive bacteria (8). Lipopolysaccharide can be identified as a lipid complex called lipid A, bound to a polysaccharide present in the outer membrane of Gram-negative bacteria. This endotoxin was associated with repeated sepsis and inhibition of DNA production, diminishing protein synthesis (3).

This experiment assessed the inflammatory response in the dental pulp of dogs to *Escherichia coli* lipopolysaccharide and *Enterococcus faecalis* lipoteichoic acid, at 150 µg/ml concentration.

Ten three-year-old healthy male dogs were selected. They received food and water *ad libitum*. The dogs were submitted to anesthesia with Rompum (Bayer do Brasil) and Thionembutal, e.v. (Abbott Laboratorios do Brasil) and the surgical field was isolated with a rubber dam leaving only the experimental teeth exposed. Prophylaxis of the teeth was performed with a water-refrigerated rubber point. Antisepsis was carried out using a 2% iodide alcohol.

A low-speed, water refrigerated #2 diamond bur was used on the bucal surface to obtain access to the pulp. Access was considered to be achieved upon bleeding. Once opened, the cavity was washed with saline solution and then dried with a cotton pellet. After inoculation with 0.05 ml of test material, cavities were sealed with glass ionomer cement (Vidrion R - SS White - Rio de Janeiro - RJ). After the intervention, animals were confined with food and water *ad libitum*.

LPS was obtained from *Escherichia coli* Serotype 055.B5, L-4524. Lot 11H4085, and LTA from *Enterococcus faecalis*, L4015 – Lot. 104H4039 and Lot 11344013 (Sigma Laboratory – USA) (150 mg/ml). Four teeth from each animal were used. Group I (control group) consisted of unprepared canines (i.e., only healthy pulp). The third right pre-molars, which were prepared with surgical access and ionomer restoration, formed Group II. The 4th right pre-molars, which were prepared with access, LPS and ionomer restoration, formed Group III. The 4th left pre-molars, which were prepared with access, LTA and ionomer restoration, formed Group IV. Evaluation times were 1, 7, 15, 30 and 60 days.

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In each period, two dogs were killed by Thionembutal, e.v. (Abbott Laboratorios do Brasil) overdosage. Samples were obtained by bone resection. Pieces were washed and decalcified with nitric acid at 5%. The histological preparation was undertaken concluding with hematoxylin and eosin staining. The staining technique described by Brown and Brenn (1) was used to detect the presence of any bacterial strain in pulp tissue during experimentation.

The results showed that LPS and LTA enhanced inflammation in the dental pulp of dogs. The inflammatory response was high with an increase in leukocyte population. The tissue structure was irregular presenting some necrosis areas.

Group I (healthy pulp) displayed connective tissue characterized by normal cells and regular organization. Group II (with surgical trauma) showed a limited damage identified by fibrosis and coagulation. The tissue structure presented tecidual reorganization.

In LPS samples (Group III) significantly high mononuclear infiltration was observed between hemorrhage points, more evident on the thirtieth and sixtieth days. The tissue disorganization with necrosis areas was due to inflammation (Fig. 1).

In the present study, the beginning of the reaction was similar to the trauma caused by mere access to the pulp (control group). After 7 days, there was a modification, signalled by an increase in polymorphonuclear cells and inflammatory edema. The edema associated with the particular location, entirely enclosed in a mineralized tissue, the dentin, caused metabolic collapses with vascular stasis. At 15 days, the breakdown of the pulp matrix was more evident. The effect after 30 days was the enhancement of inflammation with increased presence of mononuclear leukocytes and necrosis areas. At 60 days the tissue was irregular with necrosis and hemorrhage points.

The inflammatory effects of endotoxin are numerous. LPS induces prostaglandins, leukotrienes, PAF, complement 3a and 5a, and interleukin-1 (3). Cell growth inhibition has been related to endotoxin. Nakane *et al.* (7) have shown that the relation is time and dose dependent. Such inhibition was observed in the present experiment. The destructive capacity of these molecules was likewise observed.

In LTA samples (Group IV) less enhanced inflammatory tissue was observed than in the LPS group. It was initially similar to LPS with ingurgitated vessels, but at the thirtieth and sixtieth days LTA showed high mononuclear infiltration with few necrosis areas. In this study endotoxin proved to be more aggressive than LTA with great destruction of pulp tissue and several instances of necrosis. The increase of mononuclear leukocyte stimulation has also been observed by Keller *et al.* (5) in analysis of macrophage response to lipoteichoic acids. The tissue structure was irregular with hemorrhage points (Fig. 2).

Sugiyama *et al.* (8) have shown that LTA from various Gram-positive bacteria enhance the production of hepatocyte growth factor (HGF) by human gingival fibroblasts in culture. The control of these substances is an important step toward solving general infections.

Pharmacological substances for eliminating LPS and LTA have been studied. Buck *et al.* (2) have observed LPS deactivation by irrigation substances.

This study demonstrated that, 150 µg/ml, LPS and LTA affect pulp, leading to destruction of the pulp tissue. Less damage was caused by LTA than by LPS.

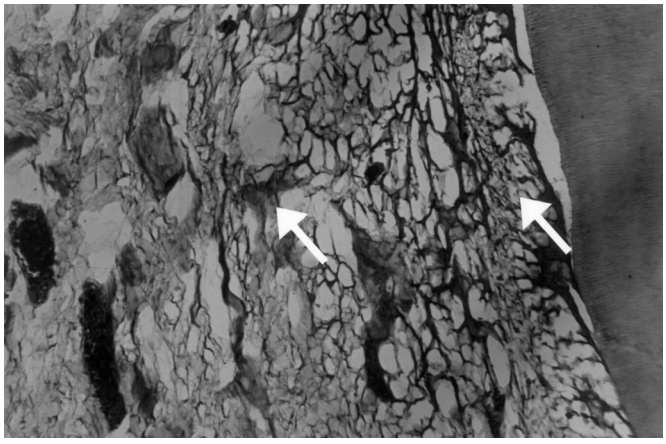

Figure 1. Photomicrograph of pulp inoculated by 150 µg/ml *E. coli* LPS at 60 days showing complete breakdown of pulp matrix with necrosis areas - arrows. (HE; 400 X).

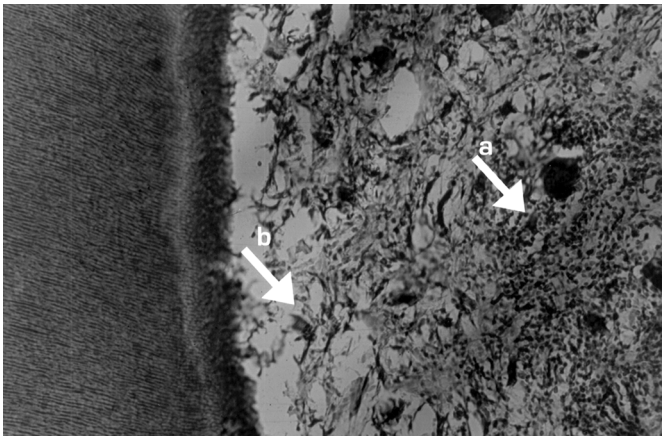

Figure 2. Photomicrograph of pulp tissue inoculated by 150 µg/ml *S. faecalis* LTA at 60 days showing high mononuclear infiltration (a) with necrosis areas (b) - arrows.(HE; 400 X).

RESUMO

Reação da polpa dental ao lipopolissacarídeo de *Escherichia coli* e ao ácido lipoteicóico de *Enterococcus faecalis*

O presente trabalho avaliou os efeitos do ácido lipoteicóico (LTA) e do lipopolissacarídeo (LPS) no tecido pulpar. Essas moléculas estão presentes nas paredes das bactérias Gram-positivas e Gram-negativas, respectivamente. Utilizaram-se dez cães para inoculação das substâncias em polpas expostas cirurgicamente. Os períodos de avaliação foram de 1, 7, 15, 30 e 60 dias. Os resultados demonstraram que 150 µg/ml de LPS e LTA interferem negativamente na polpa promovendo destruição tecidual. O LTA estabeleceu um padrão citotóxico menos agressivo que o LPS.

Palavras-chave: lipopolissacarídeo, endotoxina, ácido lipoteicóico, polpa dental, bactéria

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