Braz Dent J (2003) 14(2): 95-98 ISSN 0103-6440

Tissue Reactions to a Component of Root Canal System Bacteria: Lipoteichoic Acid

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Lipoteichoic acid (LTA), present in Gram-positive microorganisms, has physiochemical characteristics that allow it to act as an immunogen. Due to polymicrobial characteristics of root canal infections, LTA can participate in the development of periapical disease. The reaction of the rat subcutaneous tissue to Teflon tube implants, filled with Fibrinol soaked in lipoteichoic acid (concentration of 150 µg/ml), was observed. Lipoteichoic acid provoked an inflammatory tissue reaction.

Key Words: lipoteichoic acid, tissue reaction, Gram-positive microorganisms.

INTRODUCTION

Pulp and periapical tissues are commonly affected by microorganisms and their byproducts, resulting in inflammation. The concern with microorganisms is not recent (1). A wide variation of microbial flora including Gram-positive and Gram-negative microorganisms is routinely present in infected root canals (2,3). Therefore, components of Gram-negative microorganisms (e.g. the endotoxins lipopolysaccharides - LPS) and fragments of Gram-positive microorganisms (e.g. lipoteichoic acid - LTA) are also present.

After microbial death, components of microorganism cellular walls, such as LTA, persist inside macrophages for prolonged periods and can cause chronic inflammation (4). LTA is classified as an amphypatic molecule possessing hydrophilic and hydrophobic groups, with the potential to initiate biological reactions (5).

In the last 20 years, it has been speculated that microorganisms of the endodontic flora may be related to host tissue molecular reactions; thus, bacterial cell wall products, cellular membranes and bacterial LPS have been evaluated (6). Studies on lipoteichoic acid (7-11) have demonstrated several tissue alterations, such as bone resorption (9), kidney, liver and heart cell destruction (6), activation of the classic (11) and alternative pathways (7) of the complement system, and macrophage activation with production of tumor necrosis factor alpha (TNF-α) (10). Many of them are similar to bacterial LPS.

The possible action of LTA in the beginning and during the development of pulp and periapical alterations seems to exist. The purpose of this research was to evaluate rat subcutaneous tissue reaction to LTA from Gram-positive microorganisms.

MATERIAL AND METHODS

Sixteen Albinus-Wistar adult rats (weight, 250-300 g) were used in the study. During the observation period, the animals received ration and water *ad libitum*.

The LTA (L.4015 – lot 104H4039; Sigma Chemical Co., St. Louis, MO, USA) from samples of *Streptococcus faecalis* was prepared at a concentration of 150 µg/ml with bi-distilled water and stored in sterile 1-mm flasks in a sterile environment (5,12).

Teflon tubes (5 mm long and 1 mm in diameter), sterilized by gamma radiation, were used. A Teflon

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tube filled with fibrin foam (Fibrinol, Lab. Baldacchi S.A., São Paulo, Brazil) was implanted in the subcutaneous tissue of the dorsal region in 8 control rats and a Teflon tube filled with Fibrinol and soaked in LTA was implanted in 8 experimental rats. The animals were divided into 4 groups with 4 animals each, and 2 control group and 2 experimental group animals were observed at 2, 7, 14 and 30 days.

Inhalation of ethyl-ether anesthesia was used for the surgical procedure. After initial disinfection on the dorsal region of the animal, trichotomy was made from the anterior to the posterior region. The skin area was sponged with chlorhexidine (Clorohex, Ceras Johnson's, Rio de Janeiro, RJ, Brazil). Two 10-mm long incisions through the skin were made. The cutaneous tissue was raised exposing the subcutaneous tissue by divulsion. The Teflon tubes were placed 2 to 3 cm from the incision and the incisions were sutured with 3-0 silk (Ethicon, Johnson's & Johnson's, São José dos Campos, SP, Brazil).

The animals were sacrificed at 2, 7, 14, and 30 days by inhalation of sulfuric ether. The whole dorsal flap of each animal was raised, after divulsion where the implants were identified and removed with surrounding tissue. Each implant was placed into 10% formaldehyde for 72 h for fixation before the specimens were processed for paraffin embedding. Each block was oriented so that the sections would go through the longitudinal direction of the tubes, in the area of the largest diameter of the tube, with a thickness of 6 μm. The histological analysis was carried out at both ends of the tubes.

The tissue near each end of the tube was divided into 3 areas (I, II, III), and analyzed using a light microscope (Zeiss Photomicroscope) at 100X and 400X magnification. The area which had the densest cell concentration was chosen to be the representative area for each group. The cells were classified as polymorphonuclear leukocytes, mononuclear, giant and blood cells. The vessel conditions were also observed.

RESULTS

In the control group, at day 2, there was edema and fibrils around the tube ends, with a prevalence of dispersed mononuclear cells and few neutrophil polymorphonuclear cells.

At day 7, the tissue had discretely invaginated into the lumen of the tubes; inflammatory cells, dispersed mononuclear cells, and a large amount of fibrils were seen. Some vessels in formation were observed farther from the ends of the tube. Adipose tissue outlined the inflammatory reaction.

At day 14, collagen fibers were irregularly placed at both ends of the tubes. Tissue invagination was intense and there was a narrow area of inflammatory

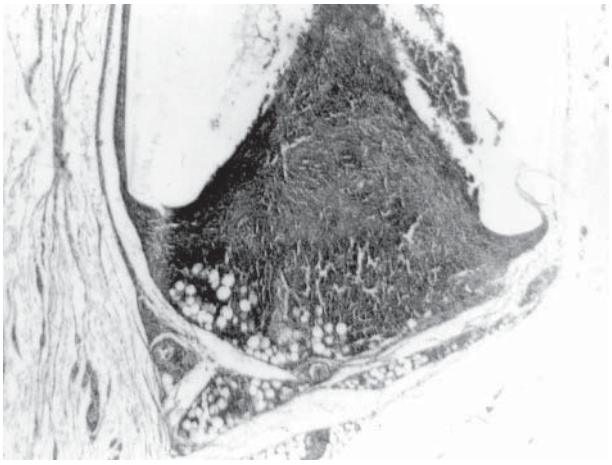

Figure 1. Control group - 14 days. Collagen fibers were irregularly placed at both ends of the tubes. Tissue invagination was intense and there was a narrow area of inflammatory cells, with an abundance of new blood vessels. The tissue showed organization and the repair process was under way. Original magnification 40X; H&E.

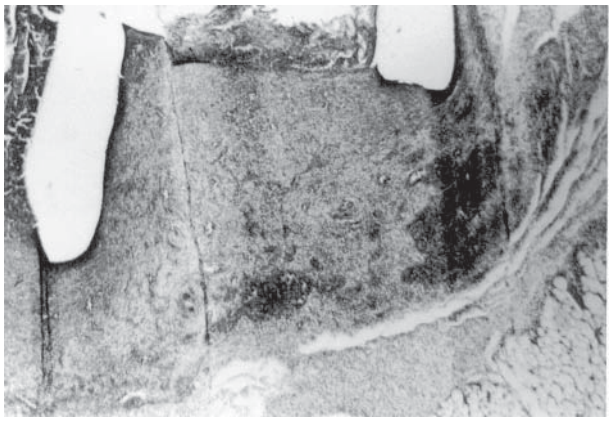

Figure 2. Experimental group - 14 days. The inflammatory reaction around the ends of the tubes was extensive, showing mononuclear cells and bleeding points. Polymorphonuclear leukocytes were observed in the area in contact with the invaginated tissue with Fibrinol inside the tube. A large amount of collagen fibers was also observed with dispersed inflammatory cells and hyperemic vessels. Original magnification 40X; H&E.

cells, with an abundance of new blood vessels. The tissue showed organization and the repair process was under way (Figure 1).

On day 30, light inflammatory reaction was observed, with the presence of mononuclear cells, collagen fibrils and vessels in proliferation located at the ends of the tubes, being surrounded by adipose and soft connective tissues.

In the experimental group, at day 2, close to the tube ends, fibrin accumulation, mononuclear and polymorphonuclear cells were observed, and the edema was abundant and extensive.

On day 7, the tissue reaction of the experimental group was disorganized, with areas of bleeding and an intense amount of blood vessels. The edema was still extensive, showing dispersed inflammatory mononuclear cells and a fibrous net.

On day 14, the inflammatory reaction around the ends of the tubes was extensive, showing mononuclear cells and bleeding points. Polymorphonuclear leukocytes were observed in the area in contact with the invaginated tissue with Fibrinol inside the tube. A large amount of collagen fibers was also observed with dispersed inflammatory cells and hyperemic vessels (Figure 2).

On day 30, chronic inflammation was present at the ends of the tubes, with no invaginated tissue. Polymorphonuclear leukocytes were present in the area in contact with the material with Fibrinol, at the end of the tube. In the periphery of the inflammatory reaction, macrophages with material in the cytoplasm were seen. In some areas, giant cells and foreign body type cells were present. Surrounding the inflammatory reaction, there was a thick collagen fiber layer, disposed irregularly, occupying an extensive area, with few mononuclear cells and blood vessels.

DISCUSSION

In recent years, bacteria or its byproducts have been identified as the major cause of pulp and periapical tissue lesions. Research reports that even bacterial fragments are able to cause tissue lesions (2,4,13,14).

The model used in this study is similar to the tooth delivering bacterial byproducts to the periapical area, initiating an inflammatory reaction at surrounding tissues (4,6,13). Samples of the LTA from *Streptococcus faecalis* were used because this microorganism is present in human infected root canals. This microorganism is one of the most resistant bacteria and remains viable even after cleaning and disinfection procedures. Additionally, there are indications that single species of Gram-positive facultative anaerobic organisms play a significant role in the pathogenesis of persistent periapical lesions in previously endodontically treated teeth (3,15,16).

In the control group of this study, the tissue invagination observed in the inflammatory reaction around the tube ends is in agreement with previous reports (17; see Figure 1). The chronic inflammatory reaction was observed only in the invaginated tissue. In the experimental group, the inflammatory reaction was more intense than in the control group. The structural feature looks like an apical granuloma, including fibrous tissue around the lesion. The absence of invaginated tissue confirms the irritating action of LTA (Figure 2). The reaction to the connective tissue indicates that LTA inside the tube penetrates the adjacent area by diffusion and is able to stimulate a chronic tissue response. It is an important factor for the outgrowth of the chronic inflammatory process, as found at 14 days (6). According to the findings, it is possible to speculate that similar reactions may occur in humans because of the nature of the connective tissue reaction to the bacteria or their byproducts.

No collagen fiber capsule was observed in the control group. The tissue reaction was surrounded by connective tissue, sometimes identified as adipose, with no inflammatory cells. This evidence suggests that the LTA diffusion into the tissue becomes more diluted by tissue fluids when it is far from the tube, giving conditions for a high collagen fiber production, as described by Fish (18) and confirmed by Pinero et al. (12).

Hausmann et al. (9) verified similar responses in bone resorption for LTA and LPS, with LTA being the less potent. In the root canal system, a continuous production of LTA derived from microflora is likely to occur; thus, LTA may contribute to the initiation and continuation of a periapical lesion, with harmful consequences to the host.

RESUMO

O ácido lipoteicóico (LTA), presente em microrganismos Gram-positivos, tem características físico-químicas que favorecem sua ação como imunógeno. Devido a característica polimicrobiana da infecção dos canais radiculares, o LTA pode participar no

desenvolvimento da patologia periapical. Reação do tecido subcutâneo do rato ao implante de tubo de Teflon, preenchido com Fibrinol embebido em solução de ácido lipoteicóico à 150 µg/ml, foi observada. O LTA provocou reação tissular.

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Accepted September 8, 2002