JOURNAL OF APPLIED ORAL SCIENCE
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J Appl Oral Sci *2004; 12(2): 93-8*

IMPORTANCE OF BACTERIAL ENDOTOXIN (LPS) IN ENDODONTICS

A IMPORTÂNCIA DA ENDOTOXINA BACTERIANA (LPS) NA ENDODONTIA ATUAL

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Received: March 01, 2004 - Returned for modification: April 22, 2004 - Accepted: May 12, 2004

ABSTRACT

New knowledge of the structure and biological activity of endotoxins (LPS) has revolutionized concepts concerning their mechanisms of action and forms of inactivation. Since the 1980's, technological advances in microbiological culture and identification have shown that anaerobic microorganisms, especially Gram-negative, predominate in root canals of teeth with pulp necrosis and radiographically visible chronic periapical lesions. Gram-negative bacteria not only have different factors of virulence and generate sub-products that are toxic to apical and periapical tissues, as also contain endotoxin (LPS) on their cell wall. This is especially important because endotoxin is released during multiplication or bacterial death, causing a series of biological effects that lead to an inflammatory reaction and resorption of mineralized tissues. Thus, due to the role of endotoxin in the pathogenesis of periapical lesions, we reviewed the literature concerning the biological activity of endotoxin and the relevance of its inactivation during treatment of teeth with pulp necrosis and chronic periapical lesion.
UNITERMS: Bacterial endotoxin (LPS); Gram-negative bacteria, Calcium hydroxide.

RESUMO

O conhecimento mais aprofundado sobre a estrutura e atividade biológica das endotoxinas (LPS) revolucionou os conceitos sobre seu mecanismo de ação e formas de inativação. A partir da década de 80, os avanços tecnológicos na cultura e identificação microbiológica demonstraram que, em canais radiculares de dentes portadores de necrose pulpar e lesão periapical crônica, visível radiograficamente, predominam microrganismos anaeróbios, particularmente os gram-negativos. Como se sabe, os microrganismos gram-negativos, além de possuírem diferentes fatores de virulência e gerarem produtos e sub-produtos tóxicos aos tecidos apicais e periapicais, contêm endotoxina em sua parede celular. Esse conhecimento é particularmente importante, uma vez que a endotoxina é liberada durante a multiplicação ou morte bacteriana, exercendo uma série de efeitos biológicos relevantes, que conduzem a uma reação inflamatória e à reabsorção dos tecidos mineralizados. Tendo em vista o papel da endotoxina na patogênese das lesões periapicais, os autores realizaram uma revisão da literatura específica, abordando suas atividades biológicas e a importância de sua inativação durante o tratamento de dentes portadores de necrose pulpar e lesão periapical.
UNITERMOS: Endotoxina bacteriana; Gram-negativos; Hidróxido de cálcio.

INTRODUCTION

Bacterial endotoxin (LPS) has been amply studied. In fact, interest in knowledge concerning the structure of bacterial endotoxin, its mechanism of action, and forms of inactivation in both the clinical and laboratory studies is obvious by the fact that in the past 10 years, a total of 28.100 articles have been reported on Medline (http://

www.ncbi.nln.nih.gov/PubMed). In Dentistry, much research using different *in vivo* and *in vitro* methodologies has emphasized the importance of anaerobic bacteria and endotoxin in the etiology of chronic periapical lesions[3,9,13,19,21,25,36,42,52,57,63]. However, only few articles have evaluated the effect of the presence of LPS in root canals on apical and periapical tissues[8,10,32,37,43,55] and some articles have reported the inactivation of LPS toxic properties after endodontic procedures both *in vivo* and *in vitro*[1,4,6,23,37,42,48,49,55,61,68].

ROLE OF GRAM-NEGATIVE MICROORGANISMS AND ENDOTOXIN ON ETIOLOGY OF PERIAPICAL LESIONS

When dental pulp is exposed to the oral cavity due to caries or trauma, it is initially contaminated by predominantly aerobic and facultative microorganisms. Due mainly to the existing nutritional relationships between microorganisms together with the slow decrease of oxygen tension in root canals, a microbial shift takes place leading to a predominance of anaerobic microogranisms[60]. Since the 1980's, technical advances in microbiological culture and identification have shown that anaerobic microorganisms predominate in root canals of teeth with pulp necrosis and radiographically visible chronic periapical lesion[29,30,51,60], especially Gram-negative[4]. This polymicrobial infection is located not only in the lumen of the root canal and dentinal tubules, but also in apical craters and the entire root canal system[29,30,58].

Gram-negative microorganisms have different virulence factors[26] and form products and sub-products that are toxic to apical and periapical tissues. They also contain endotoxin in their cell wall[33].

Endotoxin, present on all Gram-negative bacteria, is composed of polysaccharides (polymerized sugars), lipids (complexes containing fatty acids) and proteins. Endotoxin can be named lipopolysaccharide (LPS), emphasizing its chemical structure[46,65]. Lipid A is the region of the endotoxin molecule responsible for its toxic effects[11,27,33,34,65]. In 1993, Raetz[45] published a short review about the synthesis of lipid A and classified the endotoxins as extraordinary lipids.

Besides the chemical structure, much has been studied about the mechanism of action of endotoxins. When free to act, endotoxins do not cause cell or tissue lesions directly, but they stimulate competent cells to release chemical mediators. Researches showed that macrophages are the main target of endotoxins. Thus, endotoxins are not intrinsically venoms. Their effects depend on the host's response, as reported by Lewis Thomas, in *The Lives of a Cell*: "This oppressive uncontrolled and autodestructive behavior of the host is what makes endotoxin a venom." Furthermore, the same autor wrote: "Endotoxins are read by our tissues as the worst of news. When in contact with an endotoxin, our organism places all of its defenses at disposal with the idea to bombard, block and destroy all the tissue in the area. This appears to generate panic"[46].

During endodontic treatment this is particularly significant because endotoxin (LPS) is released during multiplication or bacterial death causing a series of biological effects[4,33], which lead to an inflammatory reaction[46] and periapical bone resorption[59,67].

Even though the bacterial etiology of periapical lesions has already been proven since the classic study of Kakehashi, et al.[24], few investigations have evaluated the isolated effect of LPS in contact with apical and periapical tissues[8,10,32,37,43,55].

Among all animals, humans are the most sensitive to the effects of endotoxins[66], which makes the knowledge of their biological effects on tissues fundamentally important. Endotoxins from vital or nonvital, whole or fragmented bacteria act on macrophages[46], neutrophils[35] and fibroblasts[9], leading to the release of a large number of bioactive or cytokine chemical inflammatory mediators[33], such as tumor necrosis factor (TNF)[5,33,68], interleukin-1 (IL-1)[31,33,68], IL-5[31], IL-8[31], alpha-interferon[33] and prostaglandins[7]. Furthermore, LPS is cytotoxic[20] and acts as a potent stimulator of nitric oxide production[5,68].

LPS also activates Hageman factor (factor XII of coagulation), has a lethal effect on animals[33], induces fever[21], activates the complement system[7,21,34], thus acting in inflammatory response reactions by increasing vascular permeability, neutrophil and macrophage chemotaxis, lysozyme and lymphokine release[34], activation of the metabolic cycle of arachidonic acid[7,33] being mitogenic for B lymphocytes[33] and causing mastocyte degranulation[18]. In infected root canals, endotoxin can contribute to an increased release of vasoactive neurotransmitter substances in the region of the nerve endings in periapical tissues, causing pain[51].

According to Torabinejad, et al.[62], the products of arachidonic acid metabolism and the activation of the complement system play an important role in bone resorption that is associated with periapical lesions in human teeth.

Besides causing an inflammatory reaction, LPS adheres irreversibly to mineralized tissues acting as a potent stimulator of bone resorption[52,67], acting on the synthesis and release of cytokines that activate osteoclasts[22,23], such as IL-1 and TNF, and stimulates the release of prostaglandin-E2 that also influences osteoclasts[48,64]. In tissue culture, Nair, et al.[36] observed stimulation of bone resorption by endotoxin, confirming the role of LPS in the pathogenesis of periapical lesions seen by others[4,10,32,52,59,67].

Considering the discussed above, the major objective of the dental professional during treatment of root canals of permanent teeth with pulp necrosis and chronic periapical lesion should not be only bacterial death, but also the inactivation of lipid A, which is the toxic portion of endotoxin. This objective is not reached by using root canal antibacterial dressings, which only kill the bacteria remaining in the root canal system after biomechanical preparation.

Medical and dental literature have published studies that have attempted to obtain a medication or substance that inactivates bacterial endotoxin, eliminating its biologically toxic potential. Caustic soda[10,39], polymyxin B[44], neutrophilic enzymes[35], lysozyme[41], formocresol[50], 1.25% chlorhexidine[1],

and sodium hypochlorite[6] have been tested, with no significant results. Many of these products present inherent limitations due to their high toxicity causing damaging effects when in contact with vital tissues. Thus, their routine clinical use is limited. The action of laser on periapical bacterial biofilm has also been tested[2], however, its use is limited by the fact that there is no free access to the sites where the endotoxin is present, the root canal system of infected teeth, except when apical surgery is performed.

ROLE OF CALCIUM HYDROXIDE IN THE INACTIVATION OF BACTERIAL ENDOTOXIN

The first reference[40] to the introduction of calcium hydroxide in dentistry was in 1838. However, its clinical use progressed only after the studies by Hermann[16] in 1920. Calcium hydroxide, which has a highly alkaline pH, has been used in numerous different clinical situations, i.e., direct pulp protection, pulpotomy in permanent or deciduous teeth, root canal dressing in the treatment of permanent teeth with incomplete rhizogenesis, filling sealer in root canals, root perforations, dental resorptions, and antiseptic intracanal dressing[56]. This ample use has been attributed to its antibacterial activity[12,29,30], biocompatibility[15,17,38,54], hygroscopic property, ability to reduce periapical exudate[16], and its capacity to induce mineralization[28,54] and to dissolve necrotic tissue remnants after biomechanical preparation that can act as bacterial substrate[14] leading to the stimulation of apical and periapical repair of teeth with chronic lesions.

Currently, one of the concerns of Endodontics is the treatment of teeth with pulp necrosis and periapical lesion because treatment failure is higher than in cases without periapical lesion. In teeth with chronic periapical lesion, there is a greater prevalence of Gram-negative anaerobic bacteria disseminated throughout the root canal system (dentinal tubules, apical craters and cementum lacunae), including apical bacterial biofilm. Because these areas are not reached by instrumentation, the use of an rootcanal dressing is recommended to aid in the elimination of these bacteria and increase the possibility of clinical success[25,28,38,63].

According to Leonardo, et al.[30], teeth with and without radiographically visible periapical lesion are different pathological entities requiring different treatment. In the first case, they recommend the use of an root canal dressing between treatment sessions, because the success of treatment in cases with a periapical lesion is directly related to the elimination of bacteria, products and subproducts from the root canal system. The procedures and medicaments used in root canal treatment should not only lead to bacterial death, but also to the inactivation of bacterial endotoxin.

Because of the lack of information concerning the effect of intracanal dressings on residual LPS that may adhere to mineralized tissues[8], Safavi and Nichols[48] evaluated *in vitro* the effect of calcium hydroxide on bacterial LPS. They concluded that calcium hydroxide hydrolyzes the highly toxic lipid A molecule that is responsible for the damaging effects of endotoxin. In a later study, Safavi and Nichols[49] concluded that calcium hydroxide transforms lipid A into fatty acids and amino sugars which are atoxic components. These results were confirmed in recent studies by Barthel, et al.[4] and Olsen, et al.[42] who reported that calcium hydroxide detoxifies bacterial LPS *in vitro*.

In 2002, Nelson-Filho, et al.[37] carried out an *in vivo* study to evaluate radiographically the effect of endotoxin plus calcium hydroxide on apical and periapical tissues of dog's teeth. They observed that the endotoxin caused the formation of periapical lesions after 30 days and that calcium hydroxide inactivated bacterial LPS. Silva, et al.[55] analyzed histopathologically apical and periapical tissues of dog teeth in which the root canals were filled with bacterial LPS and calcium hydroxide. They reported that LPS caused the formation of periapical lesions and that calcium hydroxide detoxified this endotoxin *in vivo*.

More recently, Tanomaru, et al.[61] evaluated the effect of biomechanical preparation using different irrigating solutions and a calcium hydroxide-based root canal dressing in dog teeth containing endotoxin. Biomechanical preparation with only irrigating solutions did not inactivate the endotoxin, however, the same treatment associated with the use of the calcium hydroxide root canal dressing (Calen®, SS White Artigos Dentários Ltda – RJ - Brasil) was effective in the inactivation of the toxic effects of this endotoxin. With the objective of evaluating the production of TNF-α, IL-1 and nitrite in cultures of human monocytes incubated with different concentrations of LPS and associated with the calcium hydroxide-based paste (Calen®) or pure calcium hydroxide, Zuccolotto[68] showed that calcium hydroxide was capable of inactivating LPS.

Jiang, et al.[23] also evaluated the direct effects of LPS on osteoclastogenesis and the capacity of calcium hydroxide to inhibit the formation of osteoclasts stimulated by endotoxin. They reported that calcium hydroxide significantly reduced osteoclast differentiation.

This new knowledge has revolutionized concepts about root canal dressings, indicating calcium hydroxide as not only the medicament most indicated, but fundamentally the only one currently capable of inactivating the endotoxin present in the root canal system of teeth with pulp necrosis and chronic periapical lesion.

CONCLUSIONS

- Bacterial endotoxin (LPS), which is a component of Gram-negative cell wall, is present in all teeth with pulp necrosis and radiographically visible chronic periapical lesion. It plays fundamental role in the genesis and maintenance of periapical lesions due to the induction of inflammation and bone resorption;

- Calcium hydroxide inactivates the toxic effects of bacterial endotoxin, *in vitro* and *in vivo*, and is currently the only clinically effective medicament for inactivation of endotoxin.

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