Methodologies for Preliminary Assessment of the Biocompatibility of Endodontic Materials in Connective Tissue: a review

Metodologias de Avaliação Preliminar da Biocompatibilidade de Cimentos Endodônticos em Tecido Conjuntivo: uma revisão de literatura

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Abstract

This literature review discusses the objectives, importance and limitations of the experimental models and evaluation criteria used in preliminary studies of the biological compatibility of endodontic materials. It was ascertained that this is an important stage in determining the biocompatibility of materials and one which, within certain limits, allows the reaction of tissues directly involved in endodontic treatment to be predicted. However, the methodology used by these studies has not been standardized. The type of tissues investigated, the experimental periods observed and the methods used to analyze inflammatory response are considered fundamental requirements for designing experiments and understanding their results.

Keywords: Endodontics; Biocompatible materials; Root canal filling materials; Inflammatory response.

Resumo

Esta revisão de literatura discorre acerca da compreensão dos objetivos, importância e limitações de modelos experimentais e de critérios de avaliação de estudos secundários de compatibilidade biológica de cimentos endodônticos. Constatou-se que esta é uma etapa importante da determinação da biocompatibilidade de materiais, permitindo, dentro de determinados limites, a projeção da reação dos tecidos diretamente envolvidos no tratamento endodôntico. Entretanto, não há padronização metodológica nos estudos. Consideram-se requisitos fundamentais para o delineamento experimental e compreensão dos resultados o tipo de tecido avaliado, os períodos experimentais observados e o tipo de análise da resposta inflamatória.

Palavras-Chave: Endodontia; Cimentos endodônticos; Biocompatibilidade; Resposta Inflamatória.

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Introduction

Despite the importance of determining the biological compatibility of materials, there are many related issues on which consensus is lacking. Definitions of biocompatibility should involve the expected response to the treatment resulting from interactions between the material and biological systems, and include the desired responses (BROWNE, 1988; WILLIAMS, 2003; WILLIANS, 2008).

The concept of biocompatibility is related to the definition of appropriate methodologies for its evaluation. It is recommended that initial tests be carried out (cytotoxicity, mutagenicity and systemic toxicity when taken orally), followed by other preliminary tests (subcutaneous, muscular and osseous implants and sensitization and irritation tests) and then preclinical animal usage studies. Only then should tests with humans be carried out (INTERNATIONAL STANDARDS ORGANIZATION,1991; INTERNATIONAL STANDARDS ORGANIZATION, 1997).

Secondary tissues tests are intended to identify products or components of products with the potential to cause injury. Since they do not allow therapeutic responses in specific applications, they are not alone sufficient to establish biocompatibility. However, they make it possible to assess tissue reactions in animal models, making them a stage that is indispensable to completing materials assessment (INTERNATIONAL STANDARDS ORGANIZATION,1991; INTERNATIONAL STANDARDS ORGANIZATION, 1997)).

Preliminary tests of endodontic materials fall into this category and are carried out frequently (SOUSA et al., 2006; BATISTA et al., 2007; ZAFALON et al., 2007; SCARPARO; GRECCA; FACHIN, 2009; SCARPARO et al., 2010), as a result of their practicality and the relevance of their results, which allow the behavior of materials with relation to tissues to be investigated, thereby making it possible, within certain limits, to predict their effects on the tissues in the apical and periapical regions. Despite many attempts to standardize experimental models and the means of evaluating responses, there is still a great diversity of methodology, which makes comparison between studies difficult and allows for contradictory results. This being so, the objective of this paper is to discuss the methodology of secondary studies of the biocompatibility of endodontic materials.

Experimental models

Animals studied and methods used to implant materials

Many secondary endodontic materials evaluation studies employ rodents, and Wistar rats are the standard model (KAPLAN et al., 2003; ZMENER, 2004; ZMENER; BANEGAS; PAMEIJER, 2005; BATISTA et al., 2007). According to Mittal, Chandra e Chandra (1995) testing on rats is an economical method for investigating the biological effects of materials. This model is based on their similarities with humans, observed after the greater part of the rat genome had been mapped (KOLA, 2004). Some studies also use animals such as guinea pigs (YESILSOY et al., 1988; SOUSA et al., 2006) and rabbits (PHILLIPS, 1967). Using these animals, however, is unjustifiable in view of the technical difficulties and costs involved, without accruing any true benefit.

The principal methodologies involve direct injection of the material into tissues or the implantation of tubes – made of polyethylene (YALTIRICK et al., 2004; SHAHI et al., 2006), dentine, (HOLLAND et al., 1999) teflon (KOLOKOURIS et al., 1998; SOUSA et al., 2006) or silicone (ZMENER; BANEGAS; PAMEIJER, 2005), – containing the experimental materials. Implantation of tubes containing the test materials offers the advantage of standardizing the material/tissue interface, which reduces the risk of large quantities of material increasing the inflammatory reaction (and not thereby reproducing clinical conditions). Furthermore, the technique facilitates biopsies (because the tubes indicate where incisions should be made) and analysis of results (since it makes it easier to observe reactions in more strictly defined zones) (ONYCK, 1970; YALTIRICK et al., 2004).

Directly injecting materials into tissues reduces interference by surgical factors with the inflammatory response, since the procedure is less traumatic (YESILSOY et al., 1988). This concern appears to be relevant to shorter experimental periods, where reactions to the surgical procedure could mask the response to the materials. Some authors also defend this option because it avoids interference with the results due to the presence of the tubes. This justification does not appear plausible since polyethylene and teflon tubes are widely used and their acceptability has been proven (TORNECK, 1966; PHILLIPS, 1967; BATISTA et al., 2007).

Tissues assessed in secondary tests of endodontic materials

The area chosen to carry out the surgical procedure should bring the materials into contact with tissues that are similar to those in which they will be applied clinically. Therefore, the correct tissues for testing materials are bone tissue (PERTOT et al., 1992; ZMENER; BANEGAS; PAMEIJER, 2005; SOUSA et al., 2006) and subcutaneous connective tissue (ZMENER, 2004; BATISTA et al., 2007; ZAFALON et al., 2007), being complementary methodologies.

During root canal treatment of vital teeth, the obturation material primarily comes into contact with the tissues in the vicinity of the pulp stump. This is basically mature connective tissue that is poor in cells, but rich in fibers and other structural elements. The application of sealers to the subcutaneous connective tissue of animals is aimed to reproduce the reactions of this area. In contrast, where there is pulp necrosis, changes take place in the periapical tissues (HOLLAND et al., 1983). These mean that the obturation material comes into contact with bone tissue, justifying its evaluation. Bone tissues can also be affected if the sealer leaks.

Definition of experimental periods

When the intention is to observe the intensity of events related to acute inflammation, short experimental periods should be chosen (24-48h). This is because, after the aggression, the complement is activated, with production of vasoactive compounds, recognition and phagocytosis by resident macrophages and secretion of chemical mediators. These lead to vascular changes and cell migration (especially of neutrophils) (COTRAN; KUMAR; COLLINS, 2004).

Although the importance of determining immediate reactions is recognized, it is also known that, over shorter periods, characteristics of the inflammatory action produced by materials may be being masked by the response induced by the act of surgery, and this aspect should be taken into consideration (KALLUS; EKLUND, 1983). On the other hand, even if the decision is taken not to assess short periods, one should be aware of the fact that characteristics of acute inflammation that remain for prolonged periods indicate more aggressive material. There are situations in which neutrophils may continue to be present in large numbers for longer periods (COTRAN; KUMAR; COLLINS, 2004).

Over a longer experimental periods one would expect to observe a chronic inflammatory reaction, with variable intensity and duration. During this phase there is cellular immunoresponse, with lymphocytes and their products. Additionally, macrophages can be observed, which, in addition to fighting the agent of the aggression, may induce destruction of the parenchyma. Humoral immunoresponse can also be observed (with production of antibodies by plasmacytes), vascular remodeling and stimulation of fibroblasts, which begin to produce collagen, characterizing the repair process. Therefore, chronic inflammatory response exhibits periods of tissue destruction alternating with periods of repair (COTRAN; KUMAR; COLLINS, 2004).

Histopathological analysis of the response to materials should, therefore, be based on the characteristics of the tissue reactions produced over time. Over periods of 24-48 hours, the immediate response to the presence of the material is assessed; the intermediate response is observed after periods of 7, 15 and 30 days (demonstrating the development of the reaction to the materials); response after longer periods, such as 60 days, indicates tissue repair mechanisms. Some authors (YALTIRICK et al., 2004) recommend even longer periods, 90 or 120 days, which they justify by the fact that tissue healing, depending on the materials, could take longer.

Analysis of results

The attempt to classify degrees of inflammatory reaction is clearly evident in the studies reviewed here. Nevertheless, there is an absence of standardization of the assessment methodology, even though attempts have been made to establish criteria (AMERICAN NATIONAL STANDARDS INSTITUTE, 1979; FÉDERATION DENTAIRE INTERNATIONAL, 1980).

In 1979, the American Dental Association (ADA) developed a protocol intended to regulate secondary tests of endodontic obturation materials. This protocol suggests that the material to be tested should be implanted in subcutaneous rat tissue after filling teflon tubes and that responses should be evaluated after periods of 2 and 12 weeks.

These responses are then classified in the following manner: (*1*) absent or minimal: presence of well-organized tissue, with no greater inflammatory response at the ends of the tube than at the center; (*2*) moderate response: after 2 weeks, presence of some inflammatory cells at the extremities of the tube that are not present at the center. Tissue adjacent to the tube has preserved its structure, with small numbers of leukocytes, lymphocytes, plasmacytes, macrophages and giant cells. Fibrous condensation surrounding the tube can also be observed. (*3*) severe: after 2 weeks the normal structure of the connective tissue can be seen to be damaged, with infiltrate made up of neutrophils and lymphocytes. At twelve weeks, intense response can be observed at the ends of the tube, with fibrous condensation around its center. Although the connective tissue structure has recovered, lymphocytes, plasmacytes, macrophages and giant cells can be observed to have accumulated, and there may still be neutrophils, which indicate tissue degeneration.

The Federation Dentaire International (FDI) also offers qualitative analysis criteria, presenting the following classification: (*1*) inflammation absent or mild – thickness of reaction zone is similar at tube opening as that observed along the tube with no or few inflammatory cells; (*2*) moderate – increased inflammatory reaction with plasmacytes and macrophages present; (*3*) severe– large reaction zone with macrophages and plasmacytes present and occasional foci of neutrophils, lymphocytes or both.

The FDI also suggests criteria for interpreting the results, defining the reaction as acceptable when the material produces: (*1*) mild reaction over periods of 2 and 12 weeks; or (*2*) moderate over 2 weeks reducing to mild by 12 weeks. In contrast, the reaction is considered unacceptable if the material provokes: (*1*) mild reaction at 2 weeks increasing to moderate or severe at periods close to 12 weeks; or (*2*) moderate reaction from one period to the next; or (*3*) severe reaction at any point.

Although these protocols provide observations of features that are important for defining and qualifying the type of response, based on knowledge of the biological events that characterize inflammation and the repair process, they do not make it possible to make precise comparisons of the degree of inflammatory response, presenting objectivity deficits. Studies like those published by Görduysus, Etikän e Gökös (1998) and Kolokouris et al. (1998) came close to the type of assessment suggested and used descriptive analysis.

In response to these difficulties, attempts have been made to quantify the inflammatory response in order to allow for comparisons between study groups by means of statistical tests. Authors like Yesilsoy et al. (1988) have assessed tissue response using inflammatory cell counts. In their study, reactions were observed after periods of 6, 15 and 80 days, based on the criterion of number of cells counted per 45x magnification field. The authors defined inflammation as absent (level 0) when no inflammatory cells were observed; mild (level 1) when a mean of up to 25 cells per field were observed; moderate (level 2) when the mean number of cells per field was between 25 and 124; and severe (level 3) when the mean was greater than or equal to 125 cells.

Although objective, this method does not identify specific characteristics of the inflammatory process in its different phases and fails to indicate factors that are important for assessing materials acceptability, such as cell types, fibrous condensation and necrosis. For the reasons given, the assessment criteria used by Figueiredo et al. (2001) appear interesting since they make it possible to combine descriptive analysis with objective analysis of results.

The cellular components (neutrophils, lymphocytes and plasmacytes, eosinophils, macrophages and giant cells) are classified according to the following scores: (*1*) Absent (inflammatory cells absent or within blood vessels); (*2*) Mild (cells present, but sparse or in reduced clusters); (*3*) Moderate (cells present, but do not dominate the microscopic field); (*4*) Intense (cells present in the form of infiltrate close to the material).

Fibrous condensation is classified using the following scale: (*1*) Absence of collagen fibers surrounding the area containing the test material; (*2*) Presence of a thin layer of collagen fibers surrounding the area containing the test material; (*3*) Presence of a thick layer of collagen fibers surrounding the area containing the test material.

Abscess formation is classified as follows: (*1*) Absence of abscess; (*2*) Presence of abscess in contact with the location where the material had been, (*3*) Presence of abscess areas distant from the location where the material had been.

With relation to inflammatory cells, it is of relevance to mention which cells can be used to indicate the acceptability characteristics of materials. Neutrophils appear with greater intensity during shorter experimental periods. They have functions in the acute inflammatory response and are mobile cells with phagocytic action. They may be involved in chronic processes in smaller numbers, but in suppurative processes, even when chronic, they can play a leading role. In contrast, lymphocytes and plasmacytes are normally the most abundant cells after experimental periods longer than 2 days, since they act during the chronic phase of inflammation and are related to cell-based immune responses and synthesis of antibodies (COTRAN; KUMAR; COLLINS, 2004).

Macrophages should also be assessed since they are cells that participate in elimination of the agent of aggression, and are capable of provoking tissue destruction by release of oxygenated radicals, enzymes and cytokines. Multi-nuclear giant cells, in common with macrophages, represent a foreign body reaction, which characterizes the presence of material that is difficult for the body to break down (COTRAN; KUMAR; COLLINS, 2004). Hemosiderin pigmentation can also indicate difficulties breaking down material when observed after long experimental periods. Hemosiderin is a pigment that indicates excess iron is present in tissues as a result of breaking down red blood cells, normally eliminated by macrophages during the initial phases of inflammation.

Eosinophils are related to hypersensitivity reactions and possess a histamine-blocking action, playing a role in the later phases of certain inflammations, especially with materials with allergenic potential (COTRAN; KUMAR; COLLINS, 2004).

Another feature that is sometimes neglected in assessment is formation of the fibrous capsule. This is a criteria for acceptability, because it indicates an immune response that renders foreign bodies that have been recognized inoffensive to the body (CATANZARO-GUIMARÃES; PERCINOTO, 1984; NASSRI; LIA; BOMBANA, 2003). Fibrosis varies in density and organization and may be arranged by chance or exhibit capsular characteristics.

It has also been proposed that the extent of the response be assessed by means of histomorphometry. Kaplan et al. (2003) investigated the volume of inflammatory reaction, defining materials as more aggressive when they caused inflammatory reactions covering larger areas. The difficulty with this methodology lies in standardizing sectioning angles when processing specimens for slides. Due to these difficulties, we considered fibrous condensation to be a more reliable criterion, since the presence of the fibrous capsule limits the presence of infiltrate, which is also a criterion that makes it possible to observe the extent of the reaction.

In addition to the features already mentioned, analysis of results should also include observations on the formation of abscesses provoked by contact with materials, which would also characterize aggression against tissues (KOLOKOURIS et al., 1998; PERTOT et al., 1992; SOUSA et al., 2006).

Final comments

Carrying out secondary tissue reaction testing with endodontic materials is an important step towards determining the biocompatibility of these materials, allowing, within certain limits, predictions to be made of how tissues directly involved in the endodontic treatment will react. Nevertheless, it is important to point out that this stage does not dispense with the need to carry out tests in which the endodontics materials are applied in the actual clinical situations for which they are designed, first with animals and later with humans.

The experimental design of secondary biocompatibility studies should include meeting objectives such as the types of tissue, experimental periods and methodology used to analyze tissue response. The implantation of tubes in rats seems to be an appropriated experimental model. Knowledge of the characteristics of the inflammatory process is of fundamental importance to interpretation of the results, in this sense the assessment criteria used by Figueiredo et al. (2001) appear interesting since they make it possible to combine descriptive analysis with objective analysis of results.

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