Inflammatory Response to Calcium Hydroxide Based Root Canal Sealers

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This study evaluated the inflammatory response to Sealapex, CRCS, Apexit, and Sealer 26 in the subcutaneous tissue and in peritoneal cavity of Balb/c mice. The inflammatory response of subcutaneous tissue was analyzed after 2, 4, 8, and 16 days. Intense neutrophilia was seen in response to all sealers during the initial periods. Differences among them related to the presence of necrosis and the number of inflammatory cells. In the intermediate phase marked differentiation of cells of the mononucleate phagocytic system into macrophages, epithelioid cells and multinucleate giant cells were observed with Sealapex. This response was less intense with CRCS and Apexit. Tissue necrosis was observed only at tissue sealer interfaces and only during the initial period with Sealapex but was seen throughout the experiment with all other sealers.

The animals were injected in the peritoneal cavity with solutions containing the sealers and five mice from each group were killed 6 and 24 h, and 5 and 15 days later. During the initial periods (6 and 24 h) there was an intense migration of polymorphonuclear leukocytes to the peritoneal cavity in response to all sealers compared to the control. This migration was more intense for Sealer 26 and Apexit. An increase in mononucleate cell number was observed after 6 and 24 h and 5 days for all sealers and no differences were observed in relation to the control after 15 days.

During the operative phases of endodontic treatment the apical limit of the filling and the nature of the sealing material used are of fundamental importance for an ideal biological response, i.e. closure of the foramen by deposition of mineralized tissue (1, 2). At the end of endodontic treatment the repair process starts. It is characterized by cell proliferation and formation of an organic matrix resulting in root apex sealing when the process is not interrupted. During this initial phase the irritant potential of the sealer may retard or even prevent repair because, taken together with the irritation due to the pathological process itself and with the aggression of the operative steps preceding the sealing procedure, it may lead to variable losses of connective tissue. Thus, material that is not harmful to apical and periapical tissues should be used.

However, many studies have revealed that most commercially available sealers irritate the apical tissues (3, 4). Thus, it has been proposed that zinc oxide-eugenol sealers, extensively used over the last decades, be replaced with calcium hydroxide sealers to obtain adequate biological sealing, because calcium hydroxide is considered to be biocompatible with tissues and to induce the formation of mineralized tissue. Few reports are available in the literature about biological tests of root canal filling materials based on calcium hydroxide, their toxic side effects, and the possible infiltration of alveolar bone by their components in cases of overfillings. Because controversial data have been reported about these products and their physicochemical and biological properties, the objective of the present study was to evaluate the inflammatory response induced by Sealapex, CRCS, Sealer 26, and Apexit when injected into the subcutaneous tissue and peritoneal cavity of mice.

MATERIALS AND METHODS

Subcutaneous Tissue

Eighty isogenic female BALB/c mice aged 6 to 8 weeks and weighing 15 to 20 g were divided into four groups, each injected with one of the four root canal sealers to be tested: group 1—Sealapex (Sybron/Kerr, Indústria e Comércio Ltda., Guarulhos, SP, Brazil); group 2—CRCS (The Hygenic Corporation, Ohio, EUA); group 3—Sealer 26 (Dentsply Indústria e Comércio Ltda., Petrópolis, RJ, Brazil); and group 4—Apexit (Schaan/Liechtenstein).

After manipulation and setting, the sealers were ground with a mortar and pestle and sifted through a #200 strainer. A 0.1 ml suspension of each sealer at 1 mg/ml dilution in PBS (7.2 g sodium chloride, Merck; 1.38 g monobasic sodium phosphate, Merck; 6.96 g dibasic potassium phosphate, Merck) was injected subcutaneously into the animals. Five animals selected at random from each group were sacrificed with excess sulfuric ether 2, 4, 8, and 16 days after the injection and the lesions obtained at the site of inoculation were excised and fixed in phosphate buffered formalin for 16 h. The tissues were dehydrated in increasing ethanol concentrations, infiltrated into a GMA monomer, and embedded in Technovit
7.200. The tissues were embedded in methacrylate resin, cut into 2-μm sections with a Sorvall JBH-A-A microtome, stretched in distilled water at room temperature, and mounted on glass slides heated to 55–60°C until the section adhered to the glass.

The slides were then covered with a few drops of staining solution A (1 g toluidine blue, 1 g sodium tetraborate, 100 ml distilled water) for 3 to 5 min on a plate heated to 55–60°C, and excess stain was removed with running water. After drying on the heated plate, the sections were stained with a few drops of stain B (1 g basic fuchsin powder plus 100 ml 50% ethanol) for 1 min at room temperature, abundantly rinsed in running water, dried on a heated plate and mounted with Entellan (Merck 7961) by the method of Abreu et al. (5).

Data were analyzed statistically by analysis of variance and by the Student’s T test, with the level of significance set at \( p < 0.05 \).

**Peritoneal Cavity**

A total of 100 isogenic female BALB/c mice aged 6 to 8 weeks and weighing 15 to 20 g were divided into four groups, each injected with one of the four root canal sealers to be tested: group 1—Sealapex (Sybron/Kerr, Indústria e Comércio Ltda., Guarulhos, SP, Brazil); group 2—CRCS (The Hygienic Corporation, Ohio, EUA); group 3—Sealer 26 (Dentsply Indústria e Comércio Ltda., Petrópolis, RJ, Brazil); group 4—Apexit (Schaan/Liechtenstein). The control group (group 5) was injected with PBS alone. The animals received a 1 ml intraperitoneal injection of each of the above solutions.

Five animals from each group were sacrificed 6 and 24 hours and 5 and 15 days after injection and their peritoneal cavity was washed with 2 ml PBS containing 0.5% sodium citrate. Twenty μl of peritoneal wash from the animals injected with the sealer suspensions and 40 μl of peritoneal wash from the controls (PBS) were used to prepare smears on slides using a Cytospin 3 (Shandon) cytocentrifuge operating at 900 rpm for 3 min. The slides were dried and stained with Rosenfeld stain (0.97 g Giemsa, Merck; 0.53 g May-Grunwald, Merck; 1000 ml methanol p.a., Merck) for 5 min and running water was added dropwise to each slide for 5 min until the slide was fully covered. The slides were then washed in running water and dried at room temperature (6).

Differential counts of mononuclear cells (macrophages and lymphocytes) and polymorphonuclear neutrophils were then performed using a Zeiss light microscope equipped with an immersion objective (1000×).

**RESULTS**

**Inflammatory Reaction in Subcutaneous Tissue**

The inflammatory reaction observed in the subcutaneous tissue of animals injected with the sealers was characterized by intense cell infiltration (Fig. IA, B, C, and D). The cell kinetics presented well-defined evolving stages for each sealer, which were classified as initial phase (2 and 4 days), intermediate phase (8 days) and late phase (16 days).

During the initial phase, large amounts of polymorphonuclear leucocytes were observed in response to all sealers, the phenomenon being more intense in response to CRCS (Fig. 2) and Apexit. In some cases the neutrophil infiltrate was accompanied by tissue necrosis, especially in response to Sealer 26 and Sealapex. The necrosis was usually located in the central part of the lesion and surrounded by mononuclear cells, suggesting intense migration of these cells during this phase. CRCS and Apexit also induced
Inflammatory response observed 4 days after the subcutaneous inoculation of CRCS. The lesion was composed of sheets of immature macrophages within the area of necrosis. 250× (basic fuchsin).

Inflammatory response observed 4 days after the subcutaneous inoculation of Sealapex. The lesion was characterized by a granulomatous reaction composed of mature macrophages and epithelioid cells. 400× (basic fuchsin).

Inflammatory response observed 16 days after subcutaneous injection of Sealer 26. The lesion was characterized by a granulomatous reaction with giant cells and an area of necrosis is seen. 400× (basic fuchsin).

Inflammatory response observed 16 days after the subcutaneous inoculation of Apexit. The lesion presents a central area composed of extensive areas of necrosis. 250× (basic fuchsin).

necrosis, which, however, was scattered diffusely among the inflammatory cells.

During the intermediate phase there was a sharp reduction in the number of these polymorphonuclear cells, which was observed more in response to Sealapex (Fig. 3), followed by CRCS, Apexit, and Sealer 26. A progressive increase in mononuclear cells (monocytes, macrophages, and epithelioid cells) also occurred during this phase, varying according to the sealer tested.

During the late phase the inflammatory response was characterized by the presence of few neutrophils and by an intense granulomatous reaction with a predominance of epithelioid cells and multinucleate giant cells (except in response to Apexit). The multinucleate giant cells presented phagocytic material in their cytoplasm, and the presence of necrosis was also observed in response to Sealer 26 (Fig. 4) and CRCS. Marked necrosis was also observed in response to Apexit (Fig. 5) with low cellularity and a small degree of cell differentiation.

Cell Migration to the Peritoneal Cavity

All four root canal sealers induced a significant increase in neutrophil numbers in the peritoneal cavity characterized by an increase not only in polymorphonuclear but also in mononuclear cells (Fig. 6A, B).

Six h after injection, all four sealers tested induced a significant increase in neutrophil numbers in the peritoneal cavity compared to the control group (p < 0.05). Apexit induced the largest number of neutrophils, followed by Sealer 26. Sealapex and CRCS induced the smallest number of neutrophils and were statistically identical to each other. Twenty-four h after injection, Sealapex, CRCS, and Apexit induced similar neutrophilia, which was lower than that induced by Sealer 26. All sealers induced significant neutrophilia compared to the PBS control (p < 0.05). Five days after injection there was a marked decrease in neutrophil number in response to Sealapex and CRCS, with no significant difference from the control (p > 0.05). In animals injected with Sealer 26 and Apexit, the neutrophilia continued to be elevated but did not differ significantly from that observed in the control and in the groups injected with Sealapex and CRCS. Fifteen days after injection, neutrophil numbers returned to control levels in all groups.

No significant difference in mononuclear cell numbers was detected among sealers at any of the times tested (p > 0.05). When the four sealers were compared to the control group, a significant difference in mononuclear cell number was observed only at 6 and 24 h after injection (p < 0.05).
possibly explained by the low solubility of the agent and its high cell differentiation was observed in response to Sealapex, a fact circumscribe and prevent the diffusion of the inducing agent and is Ca\(^{2+}\) formation of a granulomatous lesion (8) whose function is to phase, in which the decrease in neutrophils was accompanied by an increase and differentiation of mononuclear cells at the site of injection. Cell differentiation at the inflammatory site results in the exudative type and acted as a stimulus to constant migration of polymorphonuclear leucocytes to the inflammatory site. This re-

DISCUSSION

During the initial periods after injection the irritating property of the four sealers was characterized by a significant increase in polymorphonuclear leucocytes compared to the PBS control (p < 0.05). When compared to each other, Sealapex and CRCS presented lower neutrophilia than Sealer 26 and Apexit (p < 0.05). These results are in contrast to a report of progressive reduction of the inflammatory cell infiltrate induced by Apexit in subcutaneous tissue of mice after 30 days and no reduction of the inflammatory infiltrate in response to Sealapex (7).

A clear difference was also observed during the intermediate phase, in which the decrease in neutrophils was accompanied by an increase and differentiation of mononuclear cells at the site of injection. Cell differentiation at the inflammatory site results in the formation of a granulomatous lesion (8) whose function is to circumscribe and prevent the diffusion of the inducing agent and is correlated to certain physicochemical characteristics of the latter such as concentration, particle size, and solubility (9). The highest cell differentiation was observed in response to Sealapex, a fact possibly explained by the low solubility of the agent and its high Ca\(^{2+}\) concentration. This is in contrast to CRCS, which did not release Ca\(^{2+}\), as has also been observed by others (10, 11) and was confirmed by us in a comparative study of the four sealers (unpublished data).

The introduction of calcium hydroxide into root canal sealers is based on the fact that ionic Ca\(^{2+}\) acts on the process of cell differentiation and on macrophage activation. In the present experiment, the highest cell differentiation associated with intense macrophage activity was observed in response to Sealapex as was also reported by others (12) in a study comparing CRCS and Sealapex.

In addition to cell differentiation we observed areas of necrosis of variable extent and duration to the four sealers. In animals injected with Sealapex, this necrosis was observed only during the initial phase of the response and was progressively reduced with time, whereas in the animals injected with the remaining sealers it persisted throughout the experiment.

Few studies have been published about the biological properties of Apexit and Sealer 26. The unsatisfactory results obtained here with Sealer 26 may be compared with those obtained with AH26, a sealer having the same chemical composition as Sealer 26 but without silver, which induces severe inflammation and degeneration (13). Our data, however, disagree with those reported by Spanberg and Langeland (14) who observed a low toxic response to AH26. The unsatisfactory results obtained here with CRCS agree with those reported by others (15) who stated that different formulations of calcium hydroxide sealers lead to different tissue responses depending on the substances incorporated into these sealers. They also agree with data reported by Tronstad et al. (12) who stated that CRCS is essentially a standard zinc oxide-eugenol sealer containing eucalyptol and eugenol, components considered to be toxic (13, 16). Eugenol may act directly on the respiratory chain of cells (17), inducing irreversible cell damage and necrosis.

In the present study, necrosis was frequent and was accompanied by low cell numbers and differentiation, in contrast to data reported by others (16, 19) who detected normal tissue 90 days after CRCS implantation. The necrosis caused by Apexit was of the exudative type and acted as a stimulus to constant migration of polymorphonuclear leucocytes to the inflammatory site. This re-

Fig 6. Migration of neutrophils (A) and mononuclear cells (B) in the peritoneal cavity induced by PBS (●), Sealer 26 (●), CRCS (♣), Sealapex (○), and Apexit (□). The columns represent the mean ± SEM for the animal (p < 0.05).

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Pathologic gambling is now a recognized disease with studies showing that a "high," perhaps related to increased CNS release of norepinephrine, is involved. Self-destructive gambling has always been with us—indeed, Dostoyevsky’s famous 1866 novel, The Gambler, was written as part of the author’s attempts to pay off his own gambling debts. It is estimated that in 1992, $330,000,000,000 was wagered in the USA alone.

About 5% of the U.S. population is affected with this personality and family destroying illness at some time in their lives (Stat Manual of Mental Disorders, 4th ed). Treatment is said to meet with some success. But don’t bet on it.

Wallace Sturr