In Vitro Study of the Effect of Sodium Hypochlorite and Glutaraldehyde on Substrate Adherence Capacity of Macrophages

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The purpose of this study was to investigate the in vitro effect of two irrigation solutions used in endodontics (5.25% sodium hypochlorite and 1% glutaraldehyde) on substrate adherence capacity of macrophages to determine if these substances can alter macrophage function. Inflammatory macrophages were obtained from Wistar rats and resuspended in RPMI-1640 medium. Substrate adherence capacity assays were carried out in Eppendorf tubes for 15 min of incubation at 37°C in a humidified atmosphere of 5% CO₂. The adherence index was calculated. Results showed that both sodium hypochlorite and glutaraldehyde significantly decreased the substrate adherence capacity of inflammatory macrophages. To take into account that adhesion is the first step in the phagocytic process of macrophages and in antigen presentation, sodium hypochlorite and glutaraldehyde could inhibit macrophage function and reduce inflammatory reactions in periapical tissues when they are used in root-canal therapy.

The use of irrigation solutions such as sodium hypochlorite (1, 2) and glutaraldehyde (3, 4) during endodontic therapy to facilitate debridement, clearing, and shaping of root canals is accepted. Moreover, the extrusion of sodium hypochlorite through the apical foramen into periapical tissues has been suggested in the case of teeth with necrotic pulp and evidence of chronic apical periodontitis (5).

On the other hand, it has been demonstrated that inflamed periapical tissues contain a variety of immunocompetent cells, with macrophages predominating (6, 7). Macrophages are implicated in bone resorption (8) and play an essential role in the pathogenesis of human periapical pathosis (9). Although molecular mechanisms involved in macrophage function are poorly defined (10), it is well known that adherence is the first step in the phagocytic process of inflammatory macrophages (11).

Recently it has been showed that EDTA, another irrigant and chelating agent used in root canal therapy, can alter macrophage function (12, 13).

In this study the effect of sodium hypochlorite and glutaraldehyde on substrate adherence capacity of inflammatory macrophages was studied to determine if these irrigation solutions could modulate macrophage function.

MATERIALS AND METHODS

Sodium hypochlorite, glutaraldehyde, disodium salt of ethylenediamine tetraacetate (EDTA), and RPMI-1640 medium were obtained from Sigma (St. Louis, MO). All other chemicals were reagent grade.

Peritoneal macrophages were elicited from Wistar rats by the method described previously (13). Briefly, each rat was injected intraperitoneally with 5 ml of sterile 6% sodium caseinate. Animals were killed after 4 days by decapitation and the peritoneal cavity was washed with 10 ml of cold 0.9% NaCl. After a 2-min massage, the cell exudate was removed with a syringe and centrifuged for 10 min at 250 × g at 4°C. The contaminating red blood cells were lysed with cold 0.2% NaCl. The remaining cells were then washed with 0.9% NaCl by centrifugation, resuspended in RPMI-1640 medium, counted, adjusted in the same medium at 2–4 × 10⁶ macrophages/ml, and, immediately, used for experiments. Mean cells per rat varied from 20 to 30 × 10⁶, of which 85% to 95% were macrophages by morphological criteria in Giemsa and Papanicolaou staining techniques. Viability, as determined by trypan-blue exclusion, was always greater than 95%.

The quantification of substrate adherence capacity was carried out according to the technique described previously by De la Fuente et al. (14) with minor modifications. Aliquots of 180 µl of cell suspension were dispensed in Eppendorf tubes, which resemble the adherence to tissues as reported by Noga et al. (15) and De la Fuente et al. (14). Sodium hypochlorite (5.25%), 1% glutaraldehyde, or 1% EDTA (20 µl) was added to a final dilution of 1:10, 1:100, or 1:1000 in the incubation medium. RPMI-1640 medium (20 µl) was added to control samples. Adherence assays were performed at 15 min of incubation at 37°C in a humidified atmosphere of 5% CO₂ to provide a maximal adherence index (16).
After gentle removal (5 s in the vortex in position 5) of nonadherent cells, aliquots of 10 μl from each sample were taken and the number of nonadherent macrophages/ml was counted in Neubauer chambers. No agglutination of macrophages were observed. When two substances were tested simultaneously, 10 μl each were added together with 180 μl of cell suspension to the Eppendorf tubes, to a final volume of 200 μl. The adherence index (AI) was calculated according to the following equation:

\[ AI = 100 \times \frac{\text{Nonadherent macrophages/ml}}{\text{Initial macrophages/ml}} \times 100 \]

All values were expressed as the mean ± SEM of the number of experiments, performed in duplicate, as indicated in the corresponding figures. The data were evaluated statistically by Student's t-test. A value of \( p < 0.05 \) (two tailed) was considered statistically significant.

RESULTS

Sodium hypochlorite inhibited substrate adherence capacity of macrophages in a dose-dependent manner. The adherence indexes obtained in control peritoneal macrophages and incubated with different sodium hypochlorite dilutions are shown in Figure 1. As can be seen, when sodium hypochlorite was added to the incubation medium at a final dilution of 1:1000, a significant decrease of adherence index was found (27.1%) \( (p < 0.05) \). Lower sodium hypochlorite dilutions (1:100 and 1:10) decreased the AI by 48% and 76.8%, respectively \( (p < 0.01) \).

Glutaraldehyde also inhibited substrate adherence capacity of macrophages. The dilution-effect curve for the effects of glutaraldehyde on the adherence index of macrophages is shown in Figure 2. Final dilutions of glutaraldehyde (1:100, 1:10) decreased the adherence index by 31% \( (p < 0.05) \), 57.1% \( (p < 0.01) \), and 97.7% \( (p < 0.001) \), respectively.

Finally, the effect of sodium hypochlorite and glutaraldehyde on macrophage adhesion was compared with that of 1% EDTA (Fig. 3), another irrigant that decreased adherence index of macrophages \( (13) \). As can be seen, both sodium hypochlorite and glutaraldehyde were less potent than EDTA in inhibiting substrate adherence capacity of macrophages.

DISCUSSION

In the present study we demonstrated that sodium hypochlorite and glutaraldehyde, two irrigation solutions used in endodontics during root canals treatment \( (1-4) \), decrease in vitro substrate adherence capacity of rat peritoneal macrophages.

The sensitivity of cells to a sodium hypochlorite or glutaraldehyde dilution as high as 1:1000, concentration of sodium hypochlorite or glutaraldehyde lower than those used in endodontics, and the potent inhibitory effect of lower dilutions of both irrigation solutions (1:100, 1:10), which are very similar to that used in root canals preparation and could be found in periapical tissues, suggest that their inhibitory effect on macrophage adherence may have physiological significance in vivo at the level of periapical tissues. Moreover, irrigant concentration found in periapical tissues after leakage during root canal treatment may correspond to the concentration of sodium hypochlorite and glutaraldehyde that caused maximal inhibition of AI.
However, sodium hypochlorite and glutaraldehyde inhibited adherence index with less potency than EDTA (13).

Macrophages play an essential role in the immune response of the host to inflammatory and infectious processes, as well as in the reparative process, but the molecular mechanisms involved are poorly defined (10). At the level of periapical tissues macrophages, with phagocytosis and antigen presentation, have a central function in the repair of chronic apical periodontitis (6–9, 17). The phagocytic cell adherence to a smooth plastic surface is comparable to that taking place in animal tissues (14–16). However, since adherence is the first step in the phagocytic process and essential for macrophage function (11), the inhibitory effect produced by the irrigation solutions studied in this article on substrate adherence capacity suggests that they could inhibit phagocytosis in macrophages.

We conclude that the apical extrusion of sodium hypochlorite and glutaraldehyde during the root canal therapy could modify macrophage functions modulating reparative mechanisms and decreasing inflammatory reactions in periapical tissues.

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References


