Comparative Effects of Two Endodontic Irrigants, Chlorhexidine Digluconate and Sodium Hypochlorite, on Macrophage Adhesion to Plastic Surfaces

Juan José Segura, DDS, MD, PhD, Alicia Jiménez-Rubio, Juan Miguel Guerrero, and Juan Ramón Calvo, MD, PhD

This study was designed to compare the “in vitro” effect of chlorhexidine digluconate, proposed as a new irrigant solution, with sodium hypochlorite, the currently irrigant solution used to disinfect the root canal system before obturation of the canal, on substrate adherence capacity of macrophages. Inflammatory macrophages were obtained from Wistar rats and resuspended in RPMI-1640 medium. The adherence capacity of macrophages to a plastic surface was determined. Assays were conducted in Eppendorf tubes for 15 min of incubation at 37°C in an humidified atmosphere of 5% CO₂ in air. The adherence index was calculated. Chlorhexidine digluconate inhibited substrate adherence capacity of macrophages in all conditions tested. Half-maximal inhibition (IC₅₀) was observed at 1:5.6 dilution. Chlorhexidine digluconate was less potent than 5.25% sodium hypochlorite (IC₅₀ = 1:24.1 dilution) in inhibiting substrate adherence capacity of macrophages. Taking into account that substrate adherence is the first step in the phagocytic process of macrophages, chlorhexidine digluconate could inhibit macrophage function and modulate inflammatory reactions at the level of inflamed periapical tissues.

Bacteria and their products play an essential role in the pathogenesis of pulpo-periapical diseases (1). A long-standing endodontic infection allows bacteria to propagate to the entire root canal system, including ramifications, isthmuses, apical deltas, and dentinal tubules. Therefore, a major objective in endodontic therapy is the disinfection of the root canal system before obturation of the canal. A solution of 5.25% sodium hypochlorite is the current irrigant of choice (2). Recently, 2.0% chlorhexidine gluconate irrigant has been shown to possess in vitro antimicrobial activity equivalent to that of 5.25% sodium hypochlorite (3) and to instill substantive antimicrobial activity when used as an endodontic irrigant (4). Moreover, in a clinical and laboratory study, 0.12% chlorhexidine gluconate has been shown to reduce the endodontic microbiota (5).

Macrophages are the most dominating immunocompetent cells during all stages of experimentally induced periapical lesions (6). They are known to have several mediator and regulatory functions, and are involved in the entire spectrum of defense reactions (7). Macrophages interact with other cells and components of the extracellular environment by means of adhesion receptors. Adhesion is the first step in the phagocytic process of macrophages (8). Scavenger receptors have been reported to mediate macrophage adhesion to serum-coated plastic surfaces (9), and adhesion assays exploiting this property have been developed (10). Using these assays, sodium hypochlorite, as well as glutaraldehyde (11), calcium hydroxide (12), and EDTA (13), have been shown to decrease “in vitro” substrate adherence capacity of macrophages. Subsequently, it has been argued that leakage of sodium hypochlorite through the apical foramen during root canal treatment could alter macrophage functions modulating reparative mechanisms and decreasing inflammatory reactions in periapical tissues (11).

Several studies indicate that chlorhexidine has toxic effects on human cells and granulation tissue. Concentrations of chlorhexidine well below those used in clinical dentistry have been reported to cause cell injury, cell death, and inhibition of protein synthesis in human fibroblasts cultures, and HeLa cell cultures (14). Thus, a chlorhexidine digluconate (CHX) leak to periapical tissues could alter repair mechanisms.

In this study, the effects of 5.25% sodium hypochlorite and 0.12% CHX on macrophage adhesion to plastic surfaces are compared.

MATERIALS AND METHODS

Collection of Inflammatory Macrophages

Peritoneal macrophages were elicited from Wistar rats by the method described previously (15) (Fig. 1). Briefly, each rat was
**RESULTS**

CHX inhibited substrate adherence capacity of macrophages in all conditions tested. The inhibitory effect of CHX was a time- and dose-dependent phenomenon. The AIs obtained in control peritoneal macrophages and incubated with different CHX dilutions for 5, 15, and 30 min are shown in Fig. 2. As can be seen, the substrate adherence capacity of control macrophages increased progressively, reaching a maximum (69.0 ± 3.4) at 30 min. When a 1:1000 CHX dilution was added to the incubation medium, no significant changes were found (p > 0.05). Lower CHX dilutions (1:100, and 1:10) decreased the AI significantly (p < 0.05) at all times tested. In subsequent experiments, incubation of cells was performed at 37°C for 15 min in a humidified atmosphere of 5% CO₂ in air.

The dilution-effect curve for the effects of 0.12% CHX on the AI of macrophages is shown in Fig. 3. When 0.12% CHX at 1:1000 dilution was added to the incubation medium, a slight decrease of AI was found (5.96%) (p > 0.05). The highest dilution of CHX that caused a significant inhibition of AI was 1:100 (p < 0.05). CHX at 1:50 and 1:10 dilution decrease strongly and significantly the AI by 23.6% (p < 0.05) and 29.9% (p < 0.05), respectively. Half-maximal inhibition (IC₅₀) was obtained with 1:5.6 CHX dilution.

The effect of 0.12% CHX on macrophage AI was compared with that of 5.25% sodium hypochlorite (fig. 4). Sodium hypochlorite was more potent than 0.12% CHX in inhibiting substrate adherence capacity of macrophages. As can be seen, when 5.25% sodium hypochlorite was added to the incubation medium at a final dilution of 1:1000, a significant decrease of AI were found (27.1%) (p < 0.05). Lower sodium hypochlorite dilutions (1:500, 1:100, 1:50, and 1:10) decreased strongly the AI by 39.1% (p < 0.05), 48% (p < 0.05), 62.7% (p < 0.01), and 76.8% (p < 0.01), respectively. IC₅₀ was obtained with 1:24.1 dilution of 5.25% sodium hypochlorite.

**Statistical Analysis**

All values were expressed as the mean ± SD of five separate experiments performed in triplicate. Data were evaluated statistically with the ANOVA test. A value of p < 0.05 was considered statistically significant.

**Assay of Adhesion to Plastic Surfaces**

The quantification of substrate adherence capacity was performed according to the technique described previously by De la Fuente et al. (16), with minor modifications. Aliquots of 180 μl of cell suspension were dispensed in Eppendorf tubes, which simulates the adherence to tissues as reported by De la Fuente et al. (16) and Noga et al. (17). 0.12% CHX or 5.25% sodium hypochlorite (20 μl) were dissolved directly in RPMI-1640 medium to a final dilution of 1:10, 1:100, or 1:1000 in the incubation medium. RPMI-1640 medium (20 μl) alone was added to the control samples. Adherence assays were performed after 5, 15, or 30 min of incubation at 37°C in a humidified atmosphere of 5% CO₂ in air. 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 was observed. The adherence index (AI) was calculated according to the following equation:

\[
AI = 100 - \left( \frac{\text{Nonadherent macrophages/ml}}{\text{Initial macrophages/ml}} \right) \times 100.
\]

**Fig 1. Collection of rat peritoneal macrophages (15). RBC, red blood cell.**

**Fig 2. Time course of 0.12% CHX inhibition of the AI of macrophages. Macrophages were incubated in the absence (●) or presence of different dilutions of CHX (1:1000 (○), 1:100 (□), and 1:10 (▲)). After 5, 15, or 30 min, the reaction was stopped and the AI of macrophages calculated. Each point is the mean of five separate experiments performed in duplicate.**
In the present study, we demonstrated that 0.12% CHX decreases "in vitro" substrate adherence capacity of rat peritoneal macrophages. The sensitivity of cells to a CHX dilution is as low as 1:100, a concentration of CHX 100-fold lower than those used in endodontics (3–5). This suggests that this inhibitory effect on macrophage adhesion may have physiological significance in vivo at the level of periradicular tissues. If sufficient chlorhexidine were able to come into contact with the periradicular tissues during the initial stages of healing, one could rationalize that the drug, inhibiting macrophage adhesion and modulating neutrophil chemotaxis (18), could affect inflammatory and immune responses at the level of inflamed periradicular tissues.

Although 5.25% sodium hypochlorite was more potent than 0.12% CHX in inhibiting macrophage adhesion, the sodium hypochlorite concentration that caused IC_{50} (= 1:24.1 dilution of 5.25% sodium hypochlorite (i.e. 0.22% sodium hypochlorite)) was higher than CHX concentration that caused IC_{50} of AI (= 1:5.6 dilution of 0.12% CHX (i.e. 0.021% CHX)).

Our results are in agreement with those of Goldschmidt et al. (14). These authors tested CHX as a cytopathological agent. The concentration of chlorhexidine that resulted in impaired cellular function and/or cell death for human cells was 0.004%. In related studies, Neiders and Weiss (19) demonstrated that chlorhexidine inhibits Ehrlich ascites cell division.

Several studies have shown that macrophages are the most dominating immunocompetent cells during all stages of experimentally induced periradicular lesions (6, 20). Macrophages play an essential role in the immune response of the host to inflammatory and infectious processes (7, 8). At the level of periradicular tissues macrophages, with phagocytosis and antigen presentation, as well as with the secretion of several proinflammatory cytokines (interleukin-1β and tumor necrosis factor-α) and prostaglandins (PGE$_2$), have a central function in the regulation of host response during chronic inflammation and repair.

Therefore, we conclude that chemomechanical preparation of root canals using 5.25% sodium hypochlorite or 0.12% CHX as irrigant solutions should be performed carefully to avoid their leakage, because they could reduce macrophage adhesion modulating the repair mechanisms and inflammatory reactions in periradicular tissues.

This study was supported by grants from Dirección General de Investigación Científica y Técnica (DGICYT, PB94-1434 and PM95-0159) (Ministry of Education and Culture of Spain).

This work was carried out at the Department of Dental Pathology and Therapeutics, the University of Seville, School of Dentistry, Seville, Spain. Address requests for reprints to Dr. Juan J. Segura, c/Mallen, 5, 1 D, 41018 Seville, Spain.

**References**

6. Kawashima N, Oki T, Kosaka T, Suda H. Kinetics of macrophages and lymphoid cells during development of experimentally induced periradical le-

You Might Be Interested

It has been said that all males will eventually develop cancer of the prostate if they live long enough, so prognosis is of keen interest to at least 50% of the population. A recent 15-year follow-up study of men with tumors localized to the prostate and who chose not to be treated has been concluded (JAMA 277:467). Of 223 subjects, 25 had died of metastatic disease in 15 years. Of the 57 who were still alive (others died from nonprostate related causes), only 2 had evidence of metastases.

These data should be compared with survival rates for surgerized patients at a similar time interval so informed choices can be made.

William Cornelius