Effect in vitro of Tifell (formocresol-eugenol) on macrophage adhesion

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Summary
The purpose of this study was to investigate the effect in vitro on macrophage adhesion of Tifell (formocresol-eugenol), used as an intracanal medicament and in therapeutic pulpotomies in primary teeth. Macrophages were obtained from Wistar rats. As a test of macrophage phagocytic function the adherence capacity of macrophages to a plastic surface was determined. Assays were carried out in Eppendorf tubes after 15 min of incubation at 37°C in a humidified atmosphere of 5% carbon dioxide. The adherence index (AI) was calculated. Results showed that Tifell decreased significantly (P < 0.05) the AI of macrophages. Half maximal inhibition of AI was obtained at 1:334.5 Tifell dilution (AI = 27.75; P < 0.05). Taking into account that substrate adherence is the first step in the phagocytic action of macrophages and in antigen presentation, Tifell could inhibit macrophage function and modulate immune and inflammatory responses in dental pulp and periapical tissues.

Keywords: dental pulp, immunology, pharmacology, pulpotomy.

Introduction
Formocresol (Buckley’s formula) consists of 19% formaldehyde and 35% cresol in a vehicle of 15% glycerin in water (American Dental Association 1984). It has been advocated for pulpotomy procedures in primary teeth (Morawa et al. 1975, McDonald & Avery 1990) and it is used as an intracanal medicament in root canal treatment to neutralize the infected and necrotic contents of the root canal (Wesley et al. 1970, Walton & Torabinejad 1990). Eugenol is an ingredient in many over-the-counter ‘toothache’ drops, temporary filling materials, restorative materials, bases, cements, and in sealers for root canal obturation (Jendresen & Phillips 1969, Osetek 1988, Brännström et al. 1981). Tifell (Bucca Laboratories, Madrid, Spain) consists of 20% formaldehyde, 20% tricresol, and 20% eugenol. Tifell is widely used by Spanish dentists as an intracanal medicament and in pulpotomy procedures.

Formocresol is highly toxic to cells (Seltzer & Bender 1990). Polyvinyl sponge implants containing full-strength formocresol caused fixation of fibroblasts and adjacent cells (Stratton & Han 1970). A 1:5 dilution of formocresol markedly suppressed lactic dehydrogenase activity (Cunningham et al. 1982). At weaker concentrations formocresol does not fix the tissue, but may originate cellular degeneration (Langeland et al. 1971).

Human clinical studies have shown that formocresol treatment caused severe inflammatory reactions or necrosis of the dental pulp (Rölling et al. 1976). In the light of the foregoing discussion, the use of formaldehyde-containing pastes on pulp tissues is controversial (Walton & Torabinejad 1990, Seltzer & Bender 1990). It has been claimed that eugenol is not irritating to the pulp if applied to dentine (Weiss & Bjovant 1970). However, when eugenol is placed on an exposed pulp, a marked inflammatory reaction occurs (Brännström & Nyborg 1976, Webb & Bussell 1981, Brännström et al. 1981).

It has been demonstrated that inflamed pulp contains a variety of immunocompetent cells, with macrophages predominating (Trowbridge 1990). Moreover, macrophages are the dominant immunocompetent cells during all stages of experimentally induced periapical lesions (Marton & Kiss 1993, Kawashima et al. 1996). They are known to have several mediator and regulatory
functions, and are involved in the entire spectrum of defence reactions (Unanue & Allen 1987). Besides phagocytosing foreign objects, they produce several biologically active substances, such as enzymes, prostaglandins and cytokines. It is well known that adherence is the first step in the phagocytic action of inflammatory macrophages (Doherty et al. 1987, Male et al. 1989). No studies have been performed that evaluate the effect of Tifell on the phagocytic mechanism of the macrophages. The purpose of this study was to examine the effect of Tifell on substrate adherence capacity of rat inflammatory macrophages in vitro.

Materials and methods

Collection of inflammatory macrophages

Peritoneal macrophages were elicited from Wistar rats by a method described previously (Segura et al. 1993). 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% sodium chloride. 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% sodium chloride. The remaining cells were then washed with 0.9% sodium chloride by centrifugation, resuspended in RPMI-1640 medium (Sigma, St Louis, MO, USA), counted, adjusted in the same medium at 2–4 \( \times 10^6 \) macrophages/mL and immediately used for the experiments. The mean number of cells per rat ranged from 20–30 \( \times 10^6 \), of which 85–95% were macrophages by morphological criteria using Giemsa and Papanicolaou staining techniques. Viability, as determined by trypan-blue exclusion, was always greater than 95%.

Assay of substrate adherence capacity

The quantification of substrate adherence capacity was carried out according to the technique described previously by De la Fuente et al. (1991), with minor modifications. Aliquots of 180 \( \mu \)L of cell suspension were dispensed in Eppendorf tubes, which simulate the adherence to tissues as reported by Noga et al. (1974) and De la Fuente et al. (1991). Tifell (20 \( \mu \)L) was 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 \( \mu \)L) 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% carbon dioxide. After gentle removal (5 s in the vortex in position 5) of nonadherent cells, aliquots of 10 \( \mu \)L from each sample were taken and the number of nonadherent macrophages per millilitre was counted in Neubauer chambers. No agglutination of macrophages was observed. The adherence index (AI) was calculated according to the following equation:

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AI = 100 - \frac{\text{Nonadherent macrophages/mL}}{\text{Initial macrophages/mL}} \times 100.
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Statistical analysis

All values were expressed as the mean ± standard deviation of five separate experiments performed in triplicate. The data were evaluated statistically with the ANOVA test. A value of \( P < 0.05 \) was considered statistically significant.

Results

Tifell inhibited substrate adherence capacity of macrophages in all conditions tested. The inhibitory effect of Tifell was a time-and dose-dependent phenomenon. The adherence indexes obtained in control peritoneal macrophages and incubated with different dilutions of Tifell (1:1000, 1:100, and 1:10) for 5, 15, and 30 min are shown in Fig. 1. The substrate adherence capacity of control macrophages increased with time, reaching a maximum between 15 and 30 min. When a 1:1000 dilution of Tifell was added to the incubation medium no significant changes were found.
(P > 0.05). However, higher Tifell concentrations (1:100 and 1:10 dilutions) decreased the adherence significantly at all times tested.

The concentration–effect curve for the effect of Tifell on the adherence index of macrophages is shown in Fig. 2. When Tifell was added to the incubation medium at a final dilution of 1:1000, a slight decrease in adherence index was found (12.0%) (P > 0.05). However, the 1:100 dilution of Tifell significantly decreased the adherence index by 59.5% (P < 0.05). Half maximal inhibition of the adherence index was obtained at 1:334.5 Tifell dilution (AI = 27.75; P < 0.05). The 1:10 dilution of Tifell decreased the adherence index strongly and very significantly by 94.3% (P < 0.01).

Discussion

In the present study it has been demonstrated that Tifell decreases the substrate adherence capacity of rat peritoneal macrophages in vitro.

The sensitivity of cells to a Tifell dilution as high as 1:1000, the lowest Tifell concentration tested, suggests that the inhibitory effect of macrophage adherence induced may have physiological significance in vivo after formocresol–eugenol pulpotomy if this substance leaked through the apical foramina to invade the periradicular tissues.

Other chemicals used in endodontics also have been found to inhibit macrophage adhesion; they include glucocorticoids (Rhinehart et al. 1974), zinc oxide–eugenol root canal sealer (Maseki et al. 1996), EDTA (Segura et al. 1997), calcium hydroxide (Segura et al. 1997), glutaraldehyde, sodium hypochlorite (Jiménez-Rubio et al. 1997), parachlorophenol and camphorated parachlorophenol (Llamas et al., in press) and could also alter inflammatory and immune responses in periapical tissues.

Formocresol–eugenol and other formaldehyde-containing pastes have been advocated for pulpotomy procedures in both primary (Morawa et al. 1975, McDonald and Avery 1990) and permanent teeth (Muñiz et al. 1983). Full-strength formocresol caused fixation of pulpal tissues (Stratton et al. 1970, Morawa et al. 1975, Muñiz et al. 1983). A 1:5 dilution of formocresol also fixed the tissues markedly (Cunningham et al. 1982). At weaker concentrations formocresol does not fix the tissues but the connective tissue cells show signs of degeneration (Powell et al. 1973) and varying degrees of inflammation are induced in the subjacent tissues (Langeland et al. 1971). In pulpotomies performed in monkey teeth treated with N2, another formaldehyde-containing preparation, histological examination revealed the presence of particles of this substance in the residual pulp and periodontal ligament. Particles were located in vessels, macrophages, and multinucleated foreign body giant-cells (Horsted et al. 1982). These findings demonstrated that formocresol–eugenol could leak through the apical foramen into the periradicular tissues.

Eugenol, when loosely mixed with zinc oxide and applied to dentine as a temporary filling material or as a liner under other restorative materials, has been considered as the safest, from a biological standpoint, of all the filling materials (Seltzer & Bender 1990). Tissue culture studies showed, however, that zinc oxide–eugenol is toxic to pulp (Brännström & Nyborg 1976, Das 1981, Seltzer & Bender 1990). Several studies on cytotoxicity of zinc oxide–eugenol root canal sealer reported that the cytotoxicity of this sealer might be mainly caused by the eugenol component (Rappaport et al. 1964, Spangberg & Langeland 1973, Antrim 1976, Yesilsoy & Feigal 1985, Nakamura et al. 1986, Araki et al. 1993).

Adhesion is the first step in the phagocytic action of macrophages (Doherty et al. 1987, Male et al. 1989). The inhibitory effect of Tifell on macrophage adhesion shown in this paper suggests that Tifell inhibits macrophage phagocytosis. The present results are in agreement with a previous report by Maseki et al. (1996) who demonstrated that Canals, a eugenol-containing sealer, inhibits the rate of macrophage phagocytosis.

Macrophages play an essential part in the immune response of the host to inflammatory and infectious processes, as well as in the repair process. At the level of the periradicular tissues, macrophages, by means of phagocytosis and antigen presentation, have a central role in the immune response.
function in the repair of chronic apical periodontitis (Trowbridge 1990, Kawashima et al. 1996, Toriya et al. 1997). In experimental apical periodontitis, the influx of macrophages into the periapical tissues was most evident between 0 and 3 days after the pulp exposure (Kawashima et al., 1996). This indicates that the periapical tissues are highly responsive to pulpal injury and begin to work rapidly as a second line of local defense to eliminate noxious stimuli invading the pulp (Marton et al. 1993, Kawashima et al. 1996). Thus, if formocresol–eugenol leaks into the periapical tissues it could inhibit macrophage function and delay the repair processes.

Formocresol alone is widely used by American clinicians for pulpotomy procedures (McDonald & Avery 1990). In Spain and other European countries the formocresol formulation most frequently used in therapeutic pulpotomies and as an intracanal medicament contains eugenol. The results of the present study, together with previous findings, suggest that a formocresol formulation without eugenol could be less toxic to macrophages if leakage occurred into the periapical tissues. Further studies must be performed to validate this assertion, comparing the in vitro effects of formocresol alone and formocresol–eugenol on substrate adherence capacity of rat inflammatory macrophages with the goal of developing intracanal medicaments that are tolerated by the periapical tissues.

It is concluded that Tifell, when used in pulpotomy procedures or during root canal therapy, could modify the macrophage functions that modulate repair mechanisms and inflammatory reactions in pulpal and periapical tissues.

Acknowledgements

This investigation was supported by grants from DGCYT (PB94-1434 and PM95-0159) (Ministry of Education and Culture of Spain).

References


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