Effects of formocresol alone vs. formocresol with eugenol on macrophage adhesion to plastic surfaces

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Abstract

Purpose: *The purpose of this study was to compare the in-vitro effects of a European-based formocresol formulation that incorporates eugenol with formocresol alone on the adhesion of macrophages to plastic surfaces.*

Methods: *Macrophages were obtained from Wistar™ rats. The adherence capacity of macrophages to a plastic surface was determined. Assays were carried out in Eppendorf® tubes incubated for 15 min at 37° C in a humidified atmosphere of 5% CO_2. The adherence index was calculated.*

Results: *Results showed that both formocresol/eugenol and formocresol alone significantly decreased the adherence index of macrophages. The formocresol formulation that incorporated eugenol was more potent in inhibiting macrophage adhesion than formocresol alone.*

Conclusions: *Taking into account that adherence to a substrate is the first step in the phagocytic process of macrophages and in antigen presentation, both formocresol formulations could inhibit macrophage function and modulate immune and inflammatory responses in dental pulp and periapical tissues (Pediatr Dent 20:3 177–80, 1998).*

Formocresol (Buckley's formula) consists of 19% formaldehyde and 35% cresol in a vehicle of 15% glycerin in water.[1] This formocresol formulation, as well as modifications that contain different proportions of formaldehyde and cresol, are widely used by American dentists for pulpotomy procedures in primary teeth[2, 3] and as an endodontic drug to neutralize the septic and necrotic contents of root canal.[4, 5]

Formocresol is highly toxic to cells.[6, 7] Tissue changes in dog livers and kidneys induced by the absorption of formocresol from pulpotomy sites have been shown.[8] Polyvinyl sponge implants containing full-strength formocresol cause fixation of fibroblasts in adjacent cells.[9] A 1:5 dilution of formocresol markedly supressed lactic dehydrogenase activity.[10] At weaker concentrations, formocresol does not fix the tissue, but might create signs of cellular degeneration.[11] Human clinical studies have shown that formocresol treatment causes severe inflammatory reactions or necrosis of the pulp.[12]

However, the amount of formocresol that could be absorbed from pulpotomy sites is minimal.[13] In the light of the preceding discussion, the use of formaldehyde-containing pastes on pulp tissues is controversial.[5, 9]

In Europe, therapeutic pulpotomies of primary teeth are performed using a formulation of formocresol which incorporates eugenol (20% formaldehyde, 20% tricresol, and 20% eugenol). Eugenol is an ingredient in many over-the-counter toothache drops, temporary filling materials, restorative materials, bases, cements, intracanal medicaments, and in pastes used in endodontics as a sealer for root canal fillings.[14-16] It has been claimed that eugenol does not irritate the pulp if applied to dentin. In contrast, Glass and Zander[17] showed that eugenol induced chronic inflammation when placed on an exposed pulp. Moreover, Brännström and Nyborg[18] and Webb and Bussell[19] also demonstrated that eugenol produces a marked inflammatory reaction in pulp.

On the other hand, it has been shown that inflamed pulp and periapical tissues contain a variety of immunocompetent cells, with macrophages predominating.[20] Moreover, macrophages are the most dominant immunocompetent cells through all stages of induced periapical lesions.[21, 22] Macrophages are implicated in chronic inflammation and repair of pulpal and periapical tissues.[23] They are known to have several mediator and regulatory functions, and are involved in the entire spectrum of the defense reactions.[24] It is well documented that adherence is the first step in the phagocytic process of inflammatory macrophages.[25]

The in-vitro effects of formocresol alone and a formocresol formulation that incorporates eugenol on the substrate adherence capacity of rat inflammatory macrophages are compared in this study.

Methods

Formocresol/eugenol (20% formaldehyde, 20% tricresol, and 20% eugenol) was obtained from Laboratono Bucca (Juan Alvarez Mendizabal, Madrid). Formocresol (42.1% cresol, 42.1% formaldehyde, 15.8% ethilic alcohol) was obtained from J. Bird

Moyer. RPMI-1640 medium was obtained from Sigma (St. Louis, MO).

The protocol was approved by our experimentation committee. Peritoneal macrophages were elicited from Wistar rats.[26] 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 x 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 x 10^6 macrophages/mL and immediately used for experiments. Mean cells per rat varied from 20–30 x 10^6, of which 85–95% were macrophages by morphological criteria in Giemsa and Papanicolaou staining techniques. Viability, as determined by trypan-blue exclusion, was always greater than 94%.

The quantification of substrate-adherence capacity was carried out according to the technique described previously by De la Fuente et al.[27] with minor modifications. Aliquots of 180 μL of cell suspension were dispensed in Eppendorf tubes, which mimic the adherence characteristics to tissues as reported by Noga et al.[28] Formocresol alone or formocresol/eugenol (20 μL) 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 μL) was added to control samples. Adherence assays were performed with 15 min of incubation at 37°C in a humidified atmosphere of 5% CO_2 to provide a maximal adherence index.[21] After gentle centrifugation to remove nonadherent cells (3 s in the vortex in position 5), 10-μL aliquots were taken from each sample and the number of nonadherent macrophages/mL were counted in Neubauer chambers. No agglutination of macrophages was observed. The adherence index (AI) was calculated according to the following equation:

AI = 100 – Nonadherent macrophages/mL ÷
Initial macrophages/mL x 100

All values were expressed as the mean ± standard deviation of five separate experiments performed in triplicate, as indicated in the corresponding figure. The data were statistically evaluated by ANOVA. A value of $P < 0.05$ was considered statistically significant.

Results

Both formocresol alone and formocresol/eugenol inhibited the substrate-adherence capacity of macrophages in a dose-dependent manner (Fig 1). When added to the incubation medium at a final dilution of 1:1000, formocresol/eugenol decreased the AI by 12% ($P < 0.05$). Moreover, the 1:100 dilution of formocresol/eugenol significantly decreased the AI by 59% ($P < 0.05$). The 1:10 dilution of formocresol/eugenol strongly and very significantly decreased the AI by 94% ($P < 0.01$). Inhibition (half of the maximal) of AI (IC_{50}) was obtained at 1:327.3 formocresol/eugenol dilution.

Formocresol alone at a final dilution of 1:1000 did not significantly decreased the AI of macrophages ($P > 0.05$). However, the 1:100 dilution of formocresol significantly decreased the AI by 26% ($P < 0.05$). The 1:10 dilution of formocresol decreased the AI of macrophages by 78% ($P < 0.05$). Inhibition (half of the maximal) of AI (IC_{50}) was obtained at 1:48.5 formocresol dilution.

Discussion

In our study we demonstrate that both formocresol alone and formocresol/eugenol decrease the in-vitro substrate-adherence capacity of rat peritoneal macrophages to plastics surfaces. Although it has been shown that the adherence of macrophages to plastic requires cell surface characteristics different from adherence to collagen, fibronectin, or other cells,[29] the macrophage adhesion to a smooth plastic surface is comparable to that taking place in animal tissues.[26–28, 30, 31]

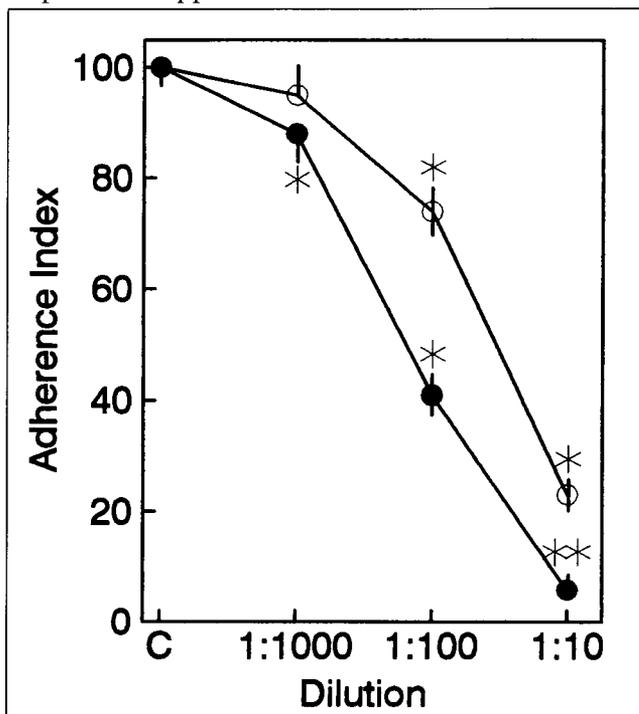The sensitivity of cells to formocresol alone and formocresol/eugenol dilutions as high as 1:100, which

Fig 1. Dilution-effect curve for the effects of formocresol alone and formocresol/eugenol on AI of rat peritoneal macrophages. Macrophages (2–4 x 106/inl) were incubated at 37°C in humidified, 5 % CO_2 in the absence (C) or presence of decreasing dilutions (increasing concentrations) of formocresol alone (C) and formocresol/eugenol (e). After 15 mm reaction was stopped and the AI calculated. Each point is the mean of five experiments performed in triplicate. * $P < 0.05$ vs. control (C). ** $P < 0.01$ vs. control (C).

are similar to those found in periapical tissues, suggests that their inhibitory effect on macrophage adhesion may have physiological signficance at the level of periapical tissues following formocresol/eugenol pulpotomy if this substance leaks through the apical foramen and invades the periapical zone.

Formocresol alone was less potent in inhibiting macrophage adhesion than formocresol formulations containing eugenol. The increased inhibitory effect of formocresol/eugenol on the substrate-adherence capacity of macrophages could be due to the chelating effect of eugenol on calcium ions.[32] The substrate adherence capacity of macrophages requires the presence of calcium ions in the medium.[33] Thus, the chelating action of eugenol on calcium ions could decrease macrophage adhesion.

Conclusions

Formocresol alone is widely used by American clinicians for pulpotomy procedures.[2] In Europe, on the contrary, a formocresol formulation incorporating eugenol is frequently used. Our results demonstrate that formocresol alone is less toxic to macrophage adhesion than formocresol/eugenol.

Macrophages play an essential role in the immune response of the host to inflammatory and infectious processes, as well as in the reparative process. At the periapical-tissue level, macrophages, with phagocytosis and antigen presentation, have a central function in the repair of chronic apical periodontitis.[21, 23]

The influx of macrophages into the inflamed periapical tissues was most evident between 0 and 3 days after the pulp exposure.[21] 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 pulp.[22] Then, when formocresol, alone or combined with eugenol, is used in pulpotomy procedures or during endodontic therapy, macrophage function could be modified and the reparative mechanisms and inflammatory reactions could be altered at the pulpal and periapical-tissue level.

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