In Vitro Effect of Parachlorophenol and Camphorated Parachlorophenol on Macrophages

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The purpose of this study was to investigate the "in vitro" effect of parachlorophenol and camphorated parachlorophenol, used in endodontics for the disinfection of root canals, on the substrate adherence capacity of macrophages. Inflammatory macrophages were obtained from Wistar rats and resuspended in RPMI-1640 medium. As a test of macrophage phagocytic function, 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 a humidified atmosphere of 5% CO₂. The adherence index was calculated. Results showed that parachlorophenol and camphorated parachlorophenol significantly decreased the substrate adherence capacity of inflammatory macrophages. Taking into account that adhesion is the first step in the phagocytic process of inflammatory macrophages and in antigen presentation, parachlorophenol and camphorated parachlorophenol could inhibit macrophage function and modulate immune and inflammatory reactions in periapical tissues.

Peritoneal macrophages were elicited from Wistar rats by the method described previously (16). 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 0.2% NaCl by centrifugation, resuspended in RPMI-1640 medium, counted, adjusted in the same medium at 2 to 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 Papainel staining techniques. Viability, as determined by trypan-blue exclusion, was always >94%.
Assay of Substrate Adherence Capacity

The quantification of substrate adherence capacity was conducted according to the technique described previously by De la Fuente et al. (17), with minor modifications. Aliquots of 180 μl of cell suspension were dispensed in Eppendorf tubes, which resemble the adherence to tissues as reported by De la Fuente et al. (17) and Noga et al. (18). Parachlorophenol and CMCP (20 μl) were directly dissolved 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 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 non-adherent cells, aliquots of 10 μl from each sample were taken, and the number of nonadherent macrophages per milliliter was counted in Neubauer chambers. No agglutination of macrophages was observed. When two substances were tested simultaneously, 10 μl of 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:

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

Statistical Analysis

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

RESULTS

Parachlorophenol and CMCP inhibited substrate adherence capacity of macrophages in a dose-dependent manner. The adherence indexes obtained in control peritoneal macrophages and incubated with different parachlorophenol dilutions are shown in Fig. 1. As can be seen, when parachlorophenol was added to the incubation medium at a final dilution of 1:1000, a significant decrease of adherence index was found (29.3%) (p < 0.05). Lower dilutions (1:100 and 1:10) strongly decreased the adherence index by 47.6% and 86.7%, respectively (p < 0.01).

CMCP also inhibited the substrate adherence capacity of macrophages. The dilution-effect curve for the effects of CMCP on the adherence index of macrophages is shown in Fig. 2. Final dilutions of CMCP (1:1000, 1:100, and 1:10) decreased the adherence index by 40.4% (p < 0.05), 49.5% (p < 0.05), and 92.4% (p < 0.001), respectively.

DISCUSSION

In the present study, we demonstrated that parachlorophenol and CMCP, two derivatives of phenol used in endodontic treatment as antiseptics and sedatives, decrease "in vitro" substrate adherence capacity of rat peritoneal macrophages. CMCP was more potent than parachlorophenol in inhibiting macrophage adhesion.

The sensitivity of cells to parachlorophenol and CMCP dilutions as high as 1:1000, which are similar to that which could be found in periapical tissues, suggests that their inhibitory effect on macrophage adherence may have physiological significance in vivo at the level of periapical tissues.

Although phenolic compounds are antiseptic, it has been shown that they also are toxic to mammalian cells (19) and to dental pulp cell cultures (20). Our results show that camphorating increases the inhibitory effect of parachlorophenol on macrophage adhesion, a result that is in good agreement with that of Soekanto et al. (20), who found that camphorated phenol and CMCP were more toxic for dental pulp cell cultures than their respective uncamphorated compounds.

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 level of periapical tissues, macrophages—by phagocytosis and antigen presentation—have a central function in the repair of chronic apical periodontitis (8–11, 14). In experimental apical periodontitis, the influx of macrophages...
into the periapical tissues was most evident between 0 and 3 days after the pulp exposure (11). 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 pulp (11). If parachlorophenol or CMCP are put into root canal, they could inhibit macrophage function and delay the reparative processes. Parachlorophenol and CMCP, perhaps should only be used in endodontic treatment of teeth with a necrotic pulp.

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