The Effect of the Bleaching Agent Sodium Perborate on Macrophage Adhesion in Vitro: Implications in External Cervical Root Resorption

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The purpose of this study was to investigate the in vitro effect of sodium perborate, which is used as a bleaching agent in the treatment of discolored pulpless teeth, on substrate adherence capacity of macrophages. Inflammatory macrophages were obtained from Wistar rats and resuspended in RPMI-1640 medium. As a test of macrophage adherence, 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 sodium perborate decreased in a dose-dependent manner and decreased significantly (p < 0.05) the adherence index of rat peritoneal macrophages. Sodium perborate was less potent than sodium hypochlorite and eugenol in inhibiting macrophage adhesion. The inhibitory effect of sodium perborate on macrophage adhesion further supports the concept that this agent is not implicated in external cervical root resorption associated with intracoronal bleaching.

Discoloration of nonvital teeth is an aesthetic deficiency frequently requiring treatment. The most common cause of tooth discoloration is intracoronal blood decomposition (1). Intracoronal bleaching is commonly used for the treatment of discolored endodontically treated teeth due to its efficacy, simplicity, and relatively low cost (2). Several bleaching materials have been used for treating discolored nonvital teeth. Traditionally, an aqueous solution of 30 to 35% hydrogen peroxide and heat were used for this purpose. Later, hydrogen peroxide was used instead of water to obtain synergism and enhance the bleaching efficacy (3). Salvas (4) reported successful results using sodium perborate as a bleaching agent. Spasser (5) bleached teeth successfully without hydrogen peroxide or heat using a paste of sodium perborate and water. Sodium perborate is frequently used as a bleaching agent in the walking bleach technique and mixed with hydrogen peroxide in the thermocatalytic method (6).

The development of external cervical root resorption after internal bleaching of discolored pulpless teeth is a serious sequela that has been reported in the literature. External cervical root resorption has been associated with the use of hydrogen peroxide, and a role for sodium perborate could not be excluded (7). One explanation given for this phenomenon is that it results from an inflammatory process initiated by the presence of bleaching agents in the attachment apparatus (8).

On the other hand, it has been demonstrated that inflamed periodontal tissues contain a variety of immunocompetent cells, with macrophages predominating (9). Several studies suggest that macrophages and osteoclasts (macrophage-derived cells) play a main role in the pathogenesis of external cervical root resorption. Macrophages stimulate osteoclastic bone resorption, and dentin and cementum destruction by the activation of lytic processes in periodontal tissues (10–12). Moreover, macrophage-derived inflammatory mediator—such as prostaglandin E₂, tumor necrosis factor-alpha, and interleukin-1β—were found to be associated with periodontal destruction and cervical root resorption (13).

Although molecular mechanisms involved in macrophage function are poorly defined (14), it is well known that adherence is the first step in the phagocytic process of inflammatory macrophages (15). The effect of sodium perborate on macrophage phagocytic function has not been studied. In this study, the in vitro effect of sodium perborate on substrate adherence capacity of rat inflammatory macrophages was examined.

MATERIALS AND METHODS

Chemicals

Sodium perborate was obtained from Oral-B Laboratorios (Amosan) (C/Caleruega 102, Madrid, Spain), and RPMI-1640 medium was obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade.

Collection of Rat Peritoneal Macrophages

The protocol was approved by our experimentation committee. 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 0.9% NaCl by centrifugation, resuspended in RPMI-1640 medium, counted, adjusted in the same medium at 2–4 × 10⁶ macrophages/ml and used immediately for experiments. Mean cells per rat varied from 20–30 × 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 >95%.

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 that resemble the adherence to tissues as reported by Noga et al. (18). Amosan (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 (19). 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 non-adherent macrophages/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

Sodium perborate inhibited substrate adherence capacity of macrophages in all conditions tested. The inhibitory effect of sodium perborate was a time- and dose-dependent phenomenon. The AIs obtained in control peritoneal macrophages and incubated with different dilutions of sodium perborate (1:1000, 1:100, and 1:10) for 5, 15, and 30 min are shown in Fig. 1. As can be seen, the substrate adherence capacity of control macrophages increased progressively, reaching a maximum between 15 and 30 min. When 1:1000 dilution of sodium perborate was added to the incubation medium, no significative changes were found (p < 0.05). However, higher sodium perborate concentrations (1:100 and 1:10 dilutions) decreased the AI significantly at all times tested (p < 0.05 and p < 0.01, respectively). In subsequent experiments, incubation of cells was performed at 37°C for 15 min in a humidified atmosphere of 5% CO₂ to provide a maximal inhibitory effect of sodium perborate.

The effect of sodium perborate on the AI of macrophages was compared with the effect of sodium hypochlorite, another bleaching agent, and eugenol (Fig. 2). As can be seen, when sodium perborate was added to the incubation medium at a final dilution of 1:1000, a light decrease of AI was found (4.57%) (p > 0.05). However, lower sodium perborate dilutions (1:100 and 1:10) decreased strongly and significantly the AI by 16% (p < 0.05) and 42.7%, respectively (p < 0.05). A 1:100 dilution of eugenol and sodium hypochlorite decreased significantly (p < 0.05) the AI of macrophages with more potency than sodium perborate. Eugenol and sodium hypochlorite, at 1:10 dilution, virtually abolish the AI of macrophages.

DISCUSSION

In the present study, we demonstrated that sodium perborate inhibits in vitro substrate adherence capacity of rat peritoneal macrophages. As far as we know, this is the first time that an effect of sodium perborate on macrophages has been described.

The sensitivity of cells to a sodium perborate dilution as high as 1:100, a concentration of sodium perborate lower than that used in the treatment of periodontal disease, and the potent inhibitory effect of the lower dilution (1:10)—which is a sodium perborate concentration similar to that which could be found in gingival tissues—suggest that its inhibitory effect on macrophage substrate adherence capacity may have physiological significance in vivo at the level of periodontal tissues.

The phagocytic cell adherence to a smooth plastic surface is comparable with that taking place in animal tissues (17, 18). Taking into account that adhesion is the first step in the phagocytic
Oxide leakage during the bleaching of pulpless teeth depends, most of the reported cases of bleaching-related root resorption are associated with the use of Superenox and heat during bleaching (2). These reports suggest that bleaching-related resorption is provoked by highly concentrated hydrogen peroxide. On the other hand, Holmstrup et al. (6) did not find any signs indicating cervical root resorption in teeth bleached with sodium perborate and water after a 3-yr follow-up.

It is recognized that the bleaching efficacy of sodium perborate is enhanced by the addition of 30% hydrogen peroxide (3). However, an equal bleaching result may be accomplished without hydrogen peroxide by increasing the number of treatments (2). Therefore, it is recommended that sodium perborate be used mixed with water rather than with hydrogen peroxide to prevent the occurrence of bleaching-associated external root resorption.

We conclude that, taking into account that adhesion is the first step in the phagocytic function of macrophages and that osteoclasts are macrophage-derived cells, the inhibitory effect of sodium perborate on macrophage adhesion could explain, at least in part, the infrequent cases of external cervical root resorption that occur when bleaching is performed only with sodium perborate. Whenever indicated, bleaching with sodium perborate should be the treatment of choice.

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References


