Effects of Enamel Matrix Derivative on the Viability, Cytokine Secretion, and Phagocytic Activity of Human Monocytes

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Abstract

Introduction: There is some controversy about the effect of enamel matrix derivative (EMD) on inflammation and resorption. The aim of this study was to investigate the effect of EMD on the inflammatory response of monocytes and their phagocytic activity *in vitro*. **Methods:** Human monocytes were incubated in complete medium (CM) and exposed to 50, 100, and 200 μg/mL EMD for different time points (12, 24, 48, and72 hours). Untreated monocytes were considered as controls. Cellular viability was evaluated through a 3-(4, 5 dimethylthiazol-2-yl) 2, 5-diphenyl-2 tetrazolium bromide assay. For cytokine measurements, the cells were treated simultaneously with 50, 100, or 200 μg/mL EMD and 10 μg/mL *Escherichia coli* lipopolysaccharide. Cell-free supernatants were collected after 12, 24, 48, and 72 hours of incubation. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) concentrations were measured by an enzyme-linked immunosorbent assay kit. Phagocytic activity of the cells was assayed using the PHAGOTEST kit (Glycotope Biotechnology, Heidelberg, Germany) according to the manufacturer's instructions. **Results:** The viability of cells exposed to 50, 100, and 200 μg/mL EMD for 12, 24, 48, and 72 hours were similar to the controls. There was no significant differences in the production of TNF-α and IL-1β among samples with various concentrations (50, 100, and 200 μg/mL) of EMD and control (EMD = 0) at 12, 24, 48, and 72 hours. Phagocytic activity of monocytic cells increased significantly after 72 hours compared with 12 hours. **Conclusions:** Based on the results of this study, EMD does not promote releasing of the two studied proinflammatory and resorbing cytokines, TNF-α and IL-1β. By increasing the phagocytic activity of monocytic cells, EMD might accelerate wound healing. *(J Endod 2010;36:1000–1003)*

Key Words

Emdogain, inflammation, interleukin-1β, phagocytic activity

Enamel matrix derivative (EMD) contains amelogenin and other enamel matrix proteins. Several researches have confirmed the efficiency of EMD in promoting the formation of acellular cementum as well as strengthening the periodontal ligament and accelerating tissue regeneration (1–4). Moreover, EMD has been suggested as a pulp tissue regenerative agent in vital pulp therapy (5, 6). In the Sabbarini et al study (5), EMD, as a pulpotomy agent, showed better radiographic results compared with formocresol. Min et al (6) suggested that EMD as a pulp capping agent may facilitate the formation of hard tissue on the exposed pulp.

In some clinical studies, the application of EMD on the root surfaces and into the socket of traumatized teeth has eliminated inflammatory root resorption and prevented ankylosis of these teeth (3, 4). In the study by Sato et al (7), LPS-stimulated monocytes exposed to EMD exhibited a significant decrease in proinflammatory tumor necrosis factor-α (TNF-α) production. Also, prostaglandin E-2–mediated inhibition of TNF-α production was enhanced in the presence of EMD. In contrast, St George et al (8) showed that external inflammatory root resorption occurred after the application of EMD into infrabony defects. On the other hand, two clinical studies showed that EMD had no significant effect on systemic secretion of interleukin-1β (IL-1β) (9, 10). TNF-α and IL-1β are two important proinflammatory cytokines mainly produced by monocytes/macrophages. These cytokines are strong stimulators of osteoclastic or odontoclastic activity in the resorption process (11). Considering the controversy about the effect of EMD on inflammation and resorption, these two cytokines were chosen to be studied.

The aim of this study was to investigate the viability of human monocytes exposed to different concentrations of EMD, its influence on the release of two monocytic inflammatory cytokines (TNF-α and IL-1 β), and to assay the phagocytic function of monocytic cells exposed to EMD at various time points.

Materials and Methods

Preparation of Emdogain

Emdogain gel (Straumann, Basel, Switzerland) was dissolved in 0.1% acetic acid (pH = 5.7) to provide a soluble form of EMD (30 mg/mL). Then, it was diluted to working strength dilutions (50, 100, and 200 μg/mL) in complete medium (CM) containing RPMI-1640 (Gibco; Life Technologies, Carlsbad, CA) supplemented with 2 mmol/L L-glutamine, 100 μg/mL streptomycin, 100 U/mL penicillin, and 10% fetal bovine serum (Gibco).

Isolation of Monocytes

Peripheral blood mononuclear cells were separated from fresh buffy coats (Tehran Blood Transfusion Center, Tehran, Iran) containing human white blood cells. The

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Supported by Tehran University of Medical Sciences and Health Services, Tehran, Iran (grant no. 132/6570)

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0099-2399/$0 - see front matter

doi:10.1016/j.joen.2010.02.032

buffy coats were obtained from the healthy volunteers without any known viral or chronic infectious diseases.

Briefly, blood samples was layered over Ficoll Histoprep (BAG Healthcare, Lich, Germany) 1.077 and centrifuged at 3,000 rpm for 20 minutes at 22°C .The mononuclear cell interface was removed and washed twice with prewarmed RPMI to omit ficoll and platelets. The remaining peripheral blood mononuclear cells were cultured into 96-well microplates (10^6 cells/well) in CM for 2 hours. Then, microplates were washed three times to remove nonadherent mononuclear cells such as T and B lymphocytes. The monocytes were isolated by their adherent capacity. Therefore, the experiment was performed on monocytes, which adhered to the microplates.

Viability Assay

The viability of monocytes was assayed using 3-(4, 5 dimethylthiazol-2-yl) 2, 5-diphenyl-2 tetrazolium bromide (MTT) (Sigma, St. Louis, MO) (5 mg/mL) according to the manufacturer's instructions.

One hundred thousand monocytes were seeded in 96-well microplates containing 200 μL CM and exposed to 50, 100, and 200 μg/mL EMD. Some wells without EMD treatment were considered as controls.

The experiment was performed in triplicate, and cells were incubated at 37°C in 5% CO_2/95% O_2 for 12, 24, 48, and 72 hours. Four hours before completing the incubation time, 20 μL MTT was added to the each well. The microplates were centrifuged at 2,000 rpm for 5 minutes, and then the upper medium was discarded. This assay is based on the cellular conversion of MTT into a blue formazan product that can be read by placing 96-microplates into spectrophotometer. Crystalline formazan deposits were dissolved 1 hour after the addition of 100 μL acidic alcohol isopropanol to each well. Optical density at 570 nm absorbance is directly related to the number of viable cells in culture.

TNF-α and IL-1β Detection Assay

Human monocytes were cultured in 96-well microplates at a density of 10^5 cell/well in triplicate. Then, cells were treated simultaneously with 50, 100, or 200 μg/mL EMD and 10 μg/mL *Escherichia coli* LPS. Cultures without LPS did not produce sufficient TNF-α or IL-1β to be detected by enzyme-linked immunosorbent assay (ELISA). Therefore, monocytes treated with 10 μg/mL LPS without EMD were considered as the control. Cell-free supernatants were collected for cytokine measurement after 12, 24, 48, and 72 hours of incubation. TNF-α and IL-1β concentrations were measured by an ELISA kit (U-Cytech Biosciences, Utrecht, The Netherlands) according to the manufacturer's recommendations. The detection limit of these two cytokines in the ELISA system used in this study was 5 pg/mL.

THP-1 Phagocytosis Assay

In order to get the appropriate amounts of cells, human acute monocytic leukemia cell line (THP-1 cells) was cultured for 10 days. Then cells were incubated in 6-well plates containing CM for 6, 12, and 72 hours. Three wells were considered for EMD treatment and the remaining wells as controls. Phagocytic activity of the cells was assayed using the PHAGOTEST kit (Glycotope Biotechnology, Heidelberg, Germany) according to the manufacturer's instructions. Briefly, 5×10^5 cells were used per test. Then, 20 μL precooled FITC-labeled *E. coli* bacteria was added to the cells in 5-mL flowcytometery tubes. All tubes were vortexed and incubated for 2 hours at 37°C. The control tubes were remained on ice. Phagocytosis was then terminated by removing all samples simultaneously and placing them in iced water. Quenching solution was added to the samples in order to suppress the fluorescence of adhering bacteria not being phagocytosed. All tubes were vortexed immediately after the addition of the quenching solution and washed twice with washing solution. Each tube received 2 mL lysing solution afterwards and was vortexed and incubated for 20 minutes at room temperature. The samples were centrifuged, and the supernatant was discarded .After the final washing, 200 μL DNA staining solution was added to each tube. The tubes were vortexed and incubated for 10 minutes in iced water. The samples were analyzed within 30 minutes by a flowcytometer (Partec).

Statistical Evaluation

The results were analyzed using two-way analysis of variance, Tukey HSD, and Tamhane post hoc tests. A p value of <0.05 was considered significant.

Results

Viability Analysis

The viability of cells exposed to 50, 100, and 200 μg/mL EMD for 12, 24, and 72 hours were similar to the controls. A partial nonsignificant increase in cell viability was observed after a 72-hour incubation time with 200 μg/mL concentration of EMD (Fig. 1*A*).

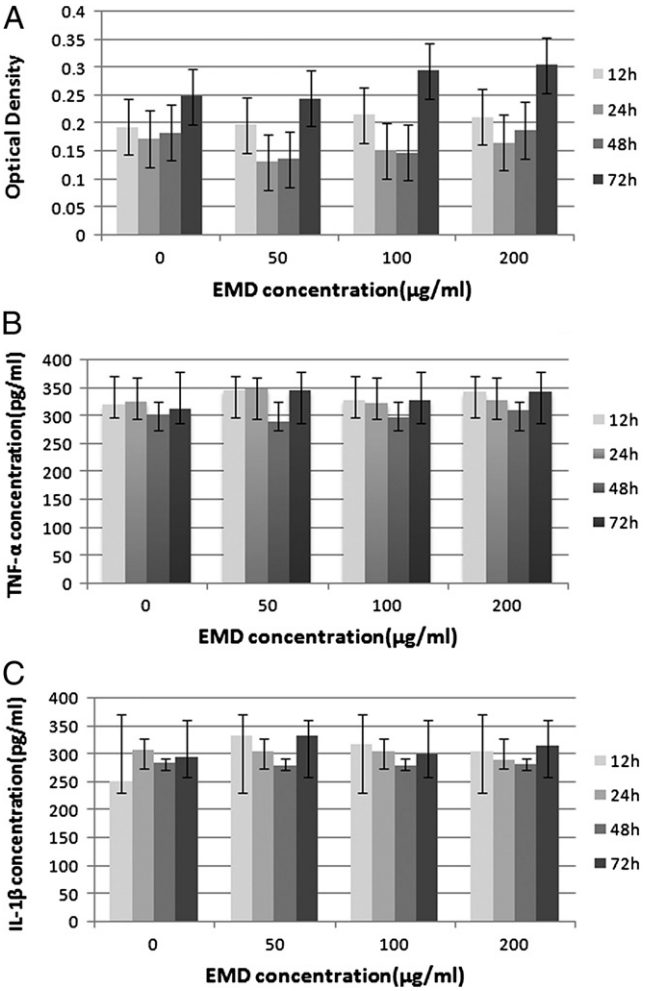

Figure 1. The effect of different concentrations of EMD (0, 50, 100, and 200 μg/mL) on (*A*) viability and the release of (*B*) TNF-α and (*C*) IL-1β of human monocytes.