The Effects of Cured Dentin Bonding Agents on Secretion of Pro-Inflammatory Cytokines, IL-1\(\beta\) and TNF-\(\alpha\), by Human Monocytes

Jamshid Hadjati\(^1\), Alaviye Vahid\(^2\), Hamid Kermanshah\(^3\), and Shohre Ghabraei\(^2\)

\(^1\)Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
\(^2\)Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
\(^3\)Department of Operative, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

Received: 24 December 2007; Received in revised form: 4 February 2008; Accepted: 2 March 2008

ABSTRACT

Dentin Bonding Agents (DBA) have been used as root-end filling materials. Present study evaluated the effects of polymerized DBA on secretion of pro-inflammatory cytokines by normal human monocytes.

In this study, monocytes were directly isolated from human peripheral blood, and exposed to cured Scotch Bond 1 (single bond) and Prime and Bond for 36 and 72 hours. Secretion of IL-1\(\beta\) and TNF-\(\alpha\) in the presence of lipopolysaccharide was evaluated in supernatants of monocyte culture.

DBAs significantly caused reduction of cytokine production by human monocytes after 36 and 72 hours. Prime and bond exposure caused more prominent decrease in TNF-\(\alpha\) production after 72 hours.

We conclude that DBA in polymerized form can alter normal function of human monocytes.

Key words: Dentin bonding agents; IL-1\(\beta\); Monocytes; TNF-\(\alpha\)

INTRODUCTION

The purpose of placing a retrograde filling is to provide a tight, biocompatible apical seal, which prevents the leakage of potential irritants from the root canal into the periapical tissues.\(^1\)

Dentin bonding agents (DBAs) in combination with composite resins have been shown to inhibit apical leakage. The ability of such material to bond chemically to dentin may significantly decrease leakage.\(^2\) Dentin bonding agents contain resins, some of which remain in unpolymerized form even after curing.\(^3\) The biocompatibility of DBAs has not been completely defined. However unpolymerized monomeric resins of DBAs are known as cellular toxins.\(^4\),\(^5\)

Macrophages in large numbers line submucosae, perivascular spaces and bony trabeculae. Their responses to inflammatory stimuli include the secretion of some 100 substances, ranging in molecular mass from 32 (super oxide anion) to 440,000 (fibronectin), and in biologic activity from induction of cell growth to cell death. Thus, through their abundance, distribution, motility, and versatility, macrophages can influence almost every aspect of the immune and inflammatory responses, and healing processes.\(^6\),\(^7\)

Macrophages mediate death of stromal, parenchymal and other immune cells by engaging the
death program, resulting in apoptosis. In addition, they induce destruction of matrix and extra cellular structures. However, there is another side to the inflammatory macrophages. Evidence is provided that these cells at the same sites possess the ability to aid cell proliferation, secrete and stabilize new matrix components and induce resident cells to secrete matrix components themselves. IL-1β and TNF-α are among the most important secretory products of macrophages that mediate similar functions in innate immunity and inflammatory responses. Biologic effect of these cytokines is dose dependent. At low concentration their principal function is as mediator of local inflammation and in larger quantities, they cause systemic effects. Both cytokines stimulate osteoclastic bone resorption and IL-1β is the most potent mediator of bone resorption.

Previous studies on the effects of monomeric and unpolymerized forms of DBAs on different cell types including fibroblasts, and monocytic cell lines showed that monomeric components of these materials can interfere with cell viability and functions such as cytokine production. However, little is known about the effects of polymerized form of these materials on human normal cells.

In the present study, we attempted to clarify the effects of polymerized form of two DBAs, Scotch Bond I (Single Bond) and Prime and Bond, on cytokine production of human monocytes. Secretion of IL-1β and TNF-α as two important pro-inflammatory cytokines were evaluated, which both are involved in inflammatory, immune and healing processes.

MATERIALS AND METHODS

Monocyte Culture

Peripheral human blood monocytes were separated from heparinized blood samples as described by Epstein et al. Briefly, blood samples were layered over Ficoll Hypaque (Biotest Diagnostic, Dreieich, Germany) and centrifuged at 400g for 20 min at 20°C. The mononuclear cell interface was removed and washed by repeated centrifugations. Mononuclear cells were counted and adjusted to 2.5×10⁶ cell/ml and suspended into 24 well microplates. The monocytes were isolated by their adherent capacity, followed by 1-hour incubation at 37°C in humidified 95% O₂ and 5% CO₂. Isolated monocytes were cultured in RPMI-1640 (Sigma Chemical) supplemented with 10% pooled human AB serum, 100 units/ml penicillin (Sigma Chemical), 100μg/ml streptomycin (Sigma Chemical), and 2 mmol/L glutamine (Sigma Chemical).

DBA Preparation

Two commercially available DBAs, Scotch Bond (Single Bond) (3M Dental Products, St. Paul, Minn) and Prime & Bond (Dentsply International, Milford, Del), were selected for this study. The material samples were placed on sterile plastic discs (6mm diameter) with a dispenser and light cured (Coltolux 75, Coltene/Whaledent, Mahwah, NJ). To protect from the effect of oxygen, the surface of the DBA was covered with glycerin and light cured again for 20 seconds. Then the discs were washed in sterile deionized water. The control discs were prepared without DBA. Preliminary experiments showed that there was not any difference between negative (test without disc) and control disc. The prepared discs (test and control) were transferred to the wells of microplates and incubated at 37°C for 36 and 72 hours in the presence of human monocytes.

Cytokine Production by Monocytes

IL-1β and TNF-α production by cultured monocytes were measured in supernatants in two different conditions, with or without the presence of 10μg/ml LPS (Sigma Chemical), using enzyme-linked immunosorbent assay kits (DRG Diagnostics, Marburg, Germany). LPS was added to the wells 12 hours before supernatant collection. Results are expressed as the mean of three wells from three individual experiments.

Statistical Analysis

The results were analyzed with ANOVA (confidence level 95%) and Bonferroni post hoc tests.

RESULTS

Polymerized forms of Scotch Bond and Prime & Bond suppressed IL-1β and TNF-α secretion from human monocytes. Both agents caused a significant (P<0.001) decrease in TNF- secretion from stimulated monocytes after 36 hours (Figure1 A). There was not any significant difference between two DBAs in this effect. After 72 hours, Prime and Bond and Scotch Bond suppressed TNF-α secretion, although the effect of Prime and bond was more prominent (Figure1 B).

There was also a significant decrease of TNF-α secretion in response to both DBAs after 72 hours compare to 36 hours. IL-1β secretion from stimulated monocytes was completely suppressed after 36 hours in response to DBAs. The same effect is observed after 72 hours, although cytokine production at 72 hours by control group was significantly higher than 36 hours culture (Figure 2). Inhibitory effect of both DBAs on IL-1β secretion was more prominent than TNF-α.
Dentin Bonding Materials and Proinflammatory Cytokines

![Graph A](image)  
**Figure 1.** Tumor necrosis factor-α concentration in supernatant of monocytes culture after (A) 36 hours and (B) 72 hours exposure to two different kinds of dentin bonding materials, (SB) Scotch Bond and (PB) Prime and Bond in the presence (+) and absence (-) of Lipopolysaccharide (LPS).

![Graph B](image)

**DISCUSSION**

IL-1 and TNF-α are pro-inflammatory cytokines playing important roles in antimicrobial defence, wound healing and host antitumor response. Release of these cytokines during inflammatory processes can stimulate bone resorption. Furthermore, it has been reported that these cytokines play an important role in the pathogenesis of human apical periodontitis. Recent investigations suggest that these cytokines play different functional roles in early versus late phases of periodontal wound healing. Short-term blockade of these cytokines may facilitate periodontal wound healing, whereas prolonged blockade may have adverse effect.

Studies have shown that monomers of dental resins are actually cytotoxic, but little is known on their long-term effects at sub lethal concentration. In this study, we investigated the effects of the polymerized form of dentin bonding agents, Scotch Bond and Prime and Bond, on LPS stimulated pro-inflammatory cytokine production by human normal monocytes after 36 and 72 hours exposure.

Both DBAs suppressed TNF-α secretion at different time intervals. According to our earlier report, the main part of polymerized DBA effect on monocytes viability occurs after 36 hours. However, as shown in figure 1, exposure to DBAs for 72 hours caused decrease in TNF secretion up to 95% compared to about 50% decrease after 36 hours. IL-1β secretion was completely suppressed after 36 and 72 hours.

The results of other studies examining the effect of DBAs on human monocyte cell line consistently show that monomeric components of DBAs can suppress TNF-α and IL-1β secretions. Study to assess pulpal response on direct pulp capping with Clearfil SE Bond, Prompt-L-Pop, Etch and Prime3.0 and Singel Bond in periods of 7, 21 and 65 days resulted on unacceptable dentin-pulp complex and wound healing with tertiary dentin bridge formation.

Matsumoto et al. have shown that IL-1β produced by macrophages is responsible for activation of the osteoclastic bone resorption and its excessive...
production may be involved in the inhibition of healing processes of periapical lesions. They have suggested suppression of IL-1β production and its secretion, using a combination of antibacterial agents and IL-1β inhibitors, which could activate periapical healing mechanisms.22

Use of IL-1 and TNF antagonists or their soluble receptors in non-human primate or other experimental models appeared to be efficacious in prevention of inflammatory response, and connective tissue loss.26 Thus, it seems that IL-1 and TNF have a detrimental effect in the early phase of periodontal wound healing, and short-term suppression of these cytokines after surgical procedures and inflammatory condition may be beneficial. However, long-term blockade of pro-inflammatory cytokines, up to fourteen days, may reverse the effects.21

Although the results of the current study are closer to normal condition than other studies on human cell lines or experimental conditions, it needs to be extended to in vivo evaluation considering the impact of these materials on physiological and pathological processes in periapical tissues after surgical procedures.

ACKNOWLEDGEMENTS

This study was financially supported by the Vice Chancellor for research at Tehran University of Medical Sciences.

REFERENCES