New Investigator Award

Chairman: Chung-Moon Um(Seoul National University) 09:00- 10:00 Auditorium

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IL-1 and TNF-α release in human polymorphonuclear leukocytes after exposure to *P. endodontalis* LPS

Chan-Je Park, Sung-Sam Lim
Department of Conservative Dentistry, College of Dentistry, Seoul National University

Bacterial lipopolysaccharide (LPS) plays a major role in the development of periapical bone resorption. IL-1 and TNF-α are known to stimulate bone resorption and inhibit bone formation. Recent evidence has indicated that polymorphonuclear leukocytes (PMNs) have the ability to release IL-1 and TNF-α Calcium hydroxide is an effective medicament in root canal infections, reducing the microbial titre within the canal. It has been proposed that the therapeutic effect of $Ca(OH)_2$ may also be the result of direct inactivation of LPS.

The purpose of this study was to investigate whether treatment of P. endodontalis LPS with calcium hydroxide alters its biological action as measured by human PMN secretion of IL-1 and TNF-α. P. endodontalis ATCC 35406 was cultured, and LPS was extracted using the hot-phenol water extraction method and purified. Purchased E. coli LPS was also purified. 100μg/ml of each LPS in pyrogen free water were incubated with 25mg/ml $Ca(OH)_2$ at 37℃ for 7 days. The supernatants were subjected to ultrafiltration, and the isolates were lyophilized and weighed. PMNs were obtained from peripheral blood by centrifugation layered over Lymphoprep. The cells were resuspended (4×10^6 cells/ml) in RPMI 1640 followed by LPS treatment with various concentrations (0, 0.1, 1, 10μg/ml) for 24 hours at 37℃ in 5% CO_2. The cell supernatants were collected and the levels of IL-1α, IL-1β and TNF-α were measured by ELISA. The results were as follows;

1. The levels of all three cytokines released from PMN stimulated with calcium hydroxide treated each LPS were significantly lower than those released from PMN stimulated with untreated each LPS ($p<0.05$), while they were not different from that of the control unstimulated PMN ($p>0.05$).
2. The levels of secretion for all three cytokines were affected in a dose-dependent manner in PMN stimulated with each LPS ($p<0.05$), but not in PMN stimulated with calcium hydroxide treated each LPS ($p>0.05$).
3. The levels of all three cytokines released from PMN stimulated with P. endodontalis LPS were significantly lower than those released from PMN stimulated with E. coli LPS ($p<0.05$).

These findings demonstrated that calcium hydroxide is able to eliminate the ability of a P. endodontalis LPS to stimulate cytokine production in PMN.