

# The role of Interleukin-6 and Interleukin-10 in human pulpal inflammation

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국문초록

## 성인 치수염에서의 INTERLEUKIN-6와 INTERLEUKIN-10의 작용에 관한 연구

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### 연구목적

Cytokine은 유해 미생물에 대한 숙주의 방어기전으로서의 염증반응에서 숙주세포 상호간의 작용을 매개해 주는 역할을 하며, 치수조직에서도 그 존재가 확인된 바 있다. Interleukin-6와 Interleukin-10은 염증의 초기에 작용하는 cytokine으로 알려져 있으나, 치수 및 치근단 질환에서의 역할과 상호작용에 대해서는 잘 알려져 있지 않다. 본 연구에서는 성인의 치수염이 있는 치아에서 Interleukin-6와 Interleukin-10의 농도를 측정하고 이를 정상 치수와 비교함으로써 이들의 치수염에서의 작용을 연구하는 것을 목적으로 하였다.

### 방법

총 60개의 성인 치아들을 대상으로 하였다. 치수염으로 진단된 치아들을 실험군으로 하였고, 정상 치수를 가진 치아들을 대조군으로 하였다. 발치한 치아에서 치수조직을 적출하였다. ELISA를 사용하여 적출된 치수조직 내의 Interleukin-6와 Interleukin-10의 양을 측정하였으며, 그 결과를 Mann-Whitney rank sum test를 사용하여 통계학적 유의성을 검증하였다. 조직학적 검사를 위해서는 발치된 치아에서 치수조직을 적출하여 헤마톡실린-에오신 염색을 시행한 후 관찰하였다.

### 결과

1. Interleukin-6의 농도는 실험군에서 대조군보다 유의하게 높게 나타났다( $p < 0.05$ ).
2. Interleukin-10의 농도는 실험군에서 대조군보다 유의하게 높게 나타났다( $p < 0.05$ ).
3. 조직학적 관찰 결과 실험군에서 림프구의 침윤과 부분적인 조직의 괴사 등 염증반응의 양상을 관찰할 수 있었다.

**주요어** : 치수염, Interleukin-6, Interleukin-10, ELISA, 조직학적 관찰

## I. Introduction

Pulpal and periradicular tissues react to bacterial infections as do other connective tissues elsewhere in the body. The degree of pulpal and periapical response to bacterial irritants varies from slight tissue inflammation to complete pulpal necrosis or acute periradicular osteomyelitis with

systemic signs and symptoms of severe infection. As a result of presence of microorganisms in the dentin, a variety of immunocompetent cells can be recruited to the dental pulp. The pulp is initially infiltrated by chronic inflammatory cells, such as macrophages, lymphocytes, and plasma cells. Pulp studies have shown the presence of immunocompetent cells and cells that recognize foreign

antigens<sup>11</sup>. As a results of the interaction of microorganisms and their by-products, various mediators of inflammation, such as neuropeptides, vasoactive amines, kinins, complement component, and arachidonic acid metabolites, are released<sup>29</sup>.

It was shown that cytokines play important roles and regulate the intensity and duration of the immune response against potentially pathogenic agents. The occurrence of Interleukin(IL)-1 and IL-1 producing cells has been demonstrated in human inflamed pulps<sup>30</sup>. The roles of IL-2 and IL-6 have also been studied in healthy and inflamed dental pulps<sup>1,31</sup>.

In one study on periodontal disease, the amount of IL-6 and IL-10 were significantly higher in the inflamed gingival tissues than in the peripheal blood from the healthy subjects<sup>6</sup>. IL-6 was detected in human pulps, periapical lesions<sup>5</sup> and odontogenic cysts<sup>7</sup>.

Interleukin-6 is produced by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to IL-1 and TNF<sup>8</sup>. One of the best described actions of IL-6 is on B lymphocytes. IL-6 serves as a growth factor for activated B lymphocytes late in the sequence of B cell differentiation. Activated B lymphocytes differentiate into plasma cells and produce antibody against antigens or infective microorganisms. IL-6 may serve as a cofactor of T lymphocytes and thymocytes activation. Activated T lymphocytes can produce cytokines including IL-10 that mediate inflammatory reactions. IL-6 also acts as a cofactor with other cytokines for the growth of early bone marrow hematopoietic stem cells. In previous studies of pulpal and periapical pathology, IL-6 is known to be one of pro-inflammatory cytokines<sup>9,10</sup>.

Interleukin-10 is produced by the TH2 subset of CD4<sup>+</sup> helper cells<sup>11</sup>. One of the major activities of IL-10 is to inhibit cytokine (i.e., TNF $\alpha$ , IL-1, chemokine, and IL-12) production by macrophages. It inhibits the production of IFN- $\gamma$  which contribute to pathologic bone resorption in periapical lesion<sup>12</sup>. And It also suppresses the production of IL-6 by T-lymphocytes<sup>13</sup>. The effect

of these actions is to inhibit T cell-mediated immune response. In addition to its inhibitory effects on macrophages, IL-10 has stimulatory actions on B lymphocytes.

Takehashi et al. have shown that pathogenesis of pulpal and periapical lesion is closely related to microorganisms<sup>14</sup>. In their study, dental pulps of conventional and germ-free rats were exposed to their own flora. Pulpal and periradicular lesions developed in conventional rats but failed to develop in germ-free rats.

The purpose of this study was to determine IL-6 and IL-10 in human pulpal inflammation and to investigate their roles in the progress of pulpal inflammation.

## II . Materials and Methods.

### 1. Preparation of tissue samples and Protein assay

Total 60 teeth were extracted. The experimental group consist of 30 teeth with inflamed pulps. The control group consist of 30 teeth with healthy pulps. The extracted teeth were removed from the liquid nitrogen tank, and allowed to thaw for 10 minutes. After the teeth were cracked open, pulp tissues were carefully removed from the teeth. Half of the pulp tissue from one tooth was used for detection of IL-6 and the other half was used for IL-10. Pulp tissues were homogenized in buffer(0.1 M potassium chloride, 0.02 M TRIS: pH = 7.6) in glass homogenizer and centrifuged(2000 RPM, 4C, 10 min). Supernatants were collected. The concentrations of protein in tissue sample were measured by protein assay kit. The concentrations were measured in g/ml tissue sample(BCA protein assay kit, Pierce, USA).

### 2. ELISA

The concentrations of IL-6 and IL-10 were measured using ELISA kits (R & D System Inc., USA).

### 3. Statistical Analysis

Results from ELISA were analyzed using Mann Whitney rank sum test and presented as P values. Values less than .05 were considered to be significant.

### 4. Histological Study

Teeth were extracted, and pulp tissues were carefully removed from the teeth. The pulp tissues were longitudinally sectioned and stained by Hematoxylin and Eosin. The specimens were examined under light-microscope (x 400).

## III. Results

### 1. ELISA

The mean concentrations of the cytokines were illustrated in table 1. The mean concentration of IL-6 was 43.62 in the experimental group, and 24.41 in the control group. The mean concentration of IL-10 was 1.21 in the experimental group, and 0.42 in the control group (Table 1).

### 2. Histological study

In the histological study, the dental pulp tissues in the experimental group showed intense infiltration of polymorphonuclear leukocytes (Fig. 1a, 1b). The dental pulp tissues in the control group

showed no infiltration of polymorphonuclear leukocytes (Fig. 2a, 2b).

## IV. Discussion

The concentrations of IL-6 and IL-10 were higher in the experimental group than those in the control group. This findings may suggest that IL-6 and IL-10 might be involved in developing pulpal inflammation stimulated by specific bacteria. Matsushima et al demonstrated that Gram-negative bacteria, such as *L. casei*, from carious lesions, might be involved in developing pulpitis through the stimulation of IL-6 production<sup>15</sup>.

The results of this study were comparable to the study of Barkhordar's<sup>5</sup>, which examined the level of interleukin-6 in inflamed human dental pulps and periapical lesion. In their study, the inflamed pulpal tissues exhibited significantly higher levels of IL-6. In the study of Nakanishi<sup>16</sup>, differences between normal and inflamed pulp were found in the levels of IL-6, but the difference were not statistically significant.

One investigator<sup>17</sup> examined the production of various cytokines including IL-6 and IL-10 in murine periapical inflammation. The production of both cytokines increased beginning on day 7 and increased to day 14. This results indicate that a cytokine network is activated in response to bacterial irritation and IL-6 and IL-10 played a role in the progress of periapical pathogenesis.

Another study<sup>18</sup> have shown that expression of IL-6 mRNA was significantly higher in diseased periodontal tissues compared to healthy controls. They also have shown the correlation between extent of tissue damage and bone destruction. These results were comparable to our study and support the findings that IL-6 and IL-10 was produced and released to have a role in the process of pulp inflammation.

There are a few studies on the role of IL-6 and IL-10 in pulpal inflammation. But the the role of IL-6 and IL-10 in the development of pulpal and periapical pathogenesis were not clearly identified.

A study of Opal SM et al., have shown that IL-

**Table 1.** Mean concentrations of IL-6 and IL-10

	experimental group	control group
IL-6	43.62±3.83*	24.41±3.25*
IL-10	1.25±0.16*	0.42±0.08*

\* : statistically significant difference (p<0.05) between the experimental group and the control group

S.D. : Standard Deviation

6 is one of anti-inflammatory cytokine<sup>19</sup>. On the contrary, in the study of Panichi et al. IL-6 was known to be one of three pro-inflammatory cytokines<sup>9</sup>. IL-10 is known to be anti-inflammatory cytokine that suppresses the production of IL-6.

In one study it was suggested that IL-6 produced by dental pulp cells is involved in the metabolism of extracellular matrix and the destruction of dental pulp tissue<sup>15</sup>. Ishimi et al have shown that IL-6 induces bone resorption both alone and in concert with other bone-resorbing agents<sup>20</sup>. Although Lowik et al have shown that IL-6 may be a mediator in PTH-stimulated osteoclastic bone resorption<sup>21</sup>.

At the early stage of pulpal inflammation, IL-6 was produced and it stimulated B lymphocyte to produce antibody and activate T lymphocyte to produce their own cytokines including IL-10. And in the advanced stage of pulpal inflammation, IL-10 was produced and inhibited the production of cytokines, including IL-1 and IL-6<sup>12</sup>.

Further studies are necessary to elucidate the roles of IL-6 and IL-10 in developing irreversible inflammation in the dental pulp.

## V. Conclusion

Total 60 teeth were extracted. Pulp tissues were carefully removed from the teeth. The concentrations of IL-6 and IL-10 were measured using ELISA kits. Pulp tissues were stained and examined under light-microscope.

The results were as follows:

1. The concentrations of interleukin-6 in the experimental group were higher than those in the control group (P<0.05).
2. The concentrations of interleukin-10 in the experimental group were higher than those in the control group (P<0.05).
3. In the histological study, the dental pulp tissues in the experimental group showed intense infiltration of polymorphonuclear leukocytes.

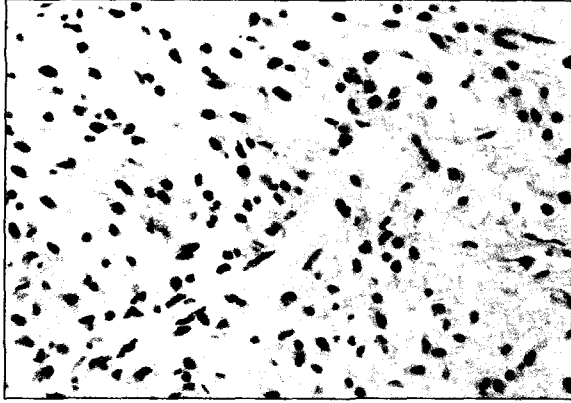
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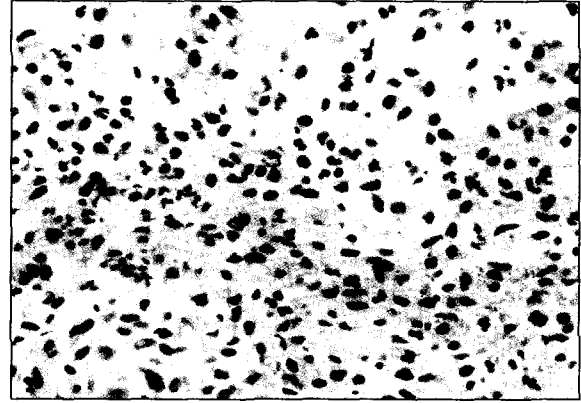
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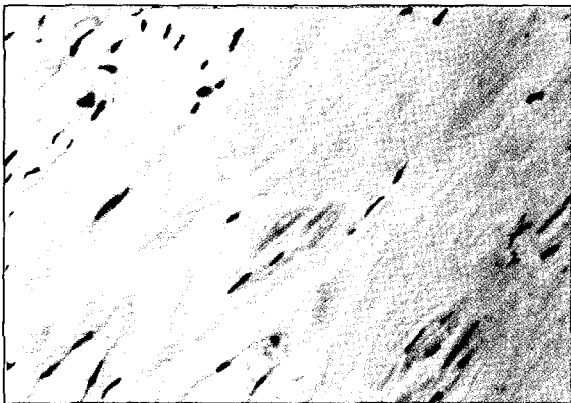
Explanation of Figures



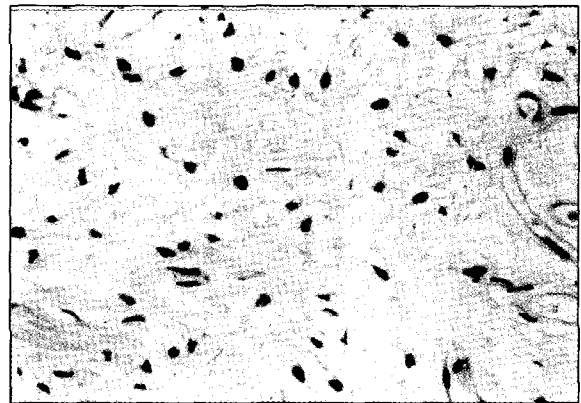
**Fig. 1a.** Pulp tissue(experimental group)



**Fig. 1b.** Pulp tissue(experimental group)



**Fig. 2a.** Pulp tissue(control group)



**Fig. 2b.** Pulp tissue(control group)