Program Book

8th International Dental Scientific Meeting
Dentistry Faculty, Hasanuddin University

June 20th-22nd, 2014 - Grand Clarion Hotel & Convention - Makassar, Indonesia

“Comprehensive Dentistry In The International Community “

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Effect of Locally Delivered Minocycline on Gingival Crevicular Fluid MMP-9 and IL-10 Levels in Endodontic Treatment

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Abstract
Healing of Chronic Apical Periodontitis (CAP) may take up to 4 years after primary root canal treatment. Activated macrophages is assumed to be involved in the prolong repair of CAP by releasing mediators of inflammation. Minocycline has been known to have an anti-inflammatory activities in addition to anti bacterial properties. The aim of this clinical study was to examine the anti inflammatory effect of locally delivered minocycline on gingival crevicular fluid Matrix Metalloproteinase-9 (MMP-9) and Interleukin-10 (IL-10) levels following non-surgical root canal treatment and prior to obturation by enzyme-linked immunosorbent assay (ELISA). Gingival crevicular fluid was obtained from the buccal aspects of sixteen upper anterior treated teeth from 12 patients before and one week after locally delivered minocycline on apical root surface. Data was analyzed using Wilcoxon test. Both levels of MMP-9 and IL-10 remained almost unchanged (from 10.18 ± 2.66 ng to 10.89 ± 1.30 ng for MMP-9, and from 0.74 ± 0.40 pg to 0.62 ± 0.15 pg for IL-10), which showed no significant differences before and after locally delivered minocycline. It can be concluded that minocycline had no anti inflammatory effect either in suppressing MMP-9 level or increasing IL-10 level in endodontic treatment.

Keywords: minocycline, MMP-9, IL-10, gingival crevicular fluid, endodontic treatment.
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INTRODUCTION

Endodontic treatment is aimed to eradicate bacteria from the infected root canal system that will later be sealed to prevent recontamination1. The elimination of bacteria that evoked the periapical inflammation, the periapical lesion should resolve and repair should take place. Healing of the lesion may take time which may not allow earlier treatment plan to provide final restorations of the treated teeth. The prolonged healing process of periapical lesions is assumed that the activated cells in the lesion may maintain their state of activation long after the initial cause of their activation has been eliminated. Macrophages are known to persist in tissues for months and when their activation persists, they may inhibit the fibroblasts, maintain osteoclastic activity, therefore preventing both soft connective tissue and bone repair2,3.

Assuming that such inhibitory mechanisms are involved in the prolonged repair of periapical lesions, pharmacological modulations has been proposed to block the process4. Tetracycline may be used to inhibit cytokine secretion by activated macrophages and may also
inhibit bone resorption, which is mediated by inhibition of connective tissue metalloproteases, that are unrelated to their antimicrobial activities.⁵

Minocycline is a second generation, semi synthetic tetracycline that more recently has been described to exert a variety of biological actions beyond its antimicrobial activity, including anti inflammatory and anti apoptotic, inhibition for osteolysis, as well as suppression of angiogenesis and tumor metastasis which have been confirmed in different experimental models of non-infectious diseases.⁶

Healing process is a complex biologic process that included inflammation, chemotaxis, mitosis, vascular cells, synthesis of matrix extracell protein, and remodelling which involved cytokines, growth factors, proteases and hormones. When imbalance occurred, chronic infection persists therefore the treatment should be directed to create a condition to allow the normal function of those molecules and healing might occurred⁷.

Matrix metalloproteinases (MMPs) is type of endopeptidases that have an essential role in the process of degradation of almost all components of extracellular matrix. MMPs produced by monocytes are involved in the migration of these cells through degradation of basement membrane and connective tissues in chronic inflammation lesions. Expression of MMPs is very low in normal conditions and increased under the stimulation of proinflammatory cytokines, hormones and growth factors. MMP-9 are gelatinases that have the ability to degrade denatured collagen type I,II, III and have been detected in periapical lesions. MMP-9 has been reported to show high activities in periapical granulomas which has been related to the higher chronic infiltrates. MMP -9 could be used as one of candidates predictor of early success in root canal treatment with periapical lesions.⁸
Interleukin-10 (IL-10) is a multifunctional cytokine with diverse effects on most hemopoietic cell types that has the ability to inhibit activation and effector function of T cells, monocytes, and macrophages, therefore limit and ultimately terminate inflammatory responses. IL-10 inhibited production of prostaglandin E2 (PGE2), through downregulation of cyclooxygenase 2 (COX-2) expression. This also affected expression of matrix metalloproteinases. Consequently, IL-10 inhibited the ability of monocytes/macrophages to modulate extracellular matrix turnover through its inhibitory effects on the production of gelatinase and collagenase (MMP2/MMP9), and also its ability to enhance production of tissue inhibitor of metalloproteinases (TIMP) and hyaluronectin.9

Gingival crevicular fluid (GCF) has long been developed and used as indicator of periodontal disease.10 Lately, Gingival crevicular fluid has also been used in endodontic therapy to evaluate gelatinolytic with periapical lesions.11 Uptake of gingival crevicular fluid is relatively simple and non-invasive, which is an additional value to be used to evaluate the status of apical inflammation in addition to clinical criteria and radiography.12 However, clinical studies are still scarce in using gingival crevicular fluid to evaluate healing of peripical lesion following root canal treatment menggunakan CKG untuk menilai kesembuhan periapikal setelah perawatan saluran akar

This study was aimed to examine the level of MMP-9 and IL-10 of gingival crevicular fluid in the process of healing following root canal treatment with the benefit of locally application of minocycline.
MATERIAL and METHODS

Patient selection

Sixteen patients consulting at the clinic of Endodontic Department, Oral and Dental Hospital, Hasanuddin University, were included when they had clinical diagnosis of Chronic Apical Periodontitis (CAP). The age of patients ranged from 16-38 years. Diagnostic criteria included the presence of apical lesion detected by periapical radiography in upper anterior teeth with clinical determination of non-vital pulp, and had indication of endodontic treatment. Exclusion criteria included marginal periodontal diseases, defined by the absence of clinical attachment loss (≥ 2 mm), increased probing depths (≥ 3 mm), systemic illness or previous antibiotics or non-steroid anti-inflammatory treatment during the 3-month period prior to the study. All the protocols and procedures were approved by the Ethics Commitee of Medical Faculty (0892/H4.8.4.5.31/PP36-KOMETIK/2014), Hasanuddin University, and informed consents were obtained from all individuals.

Clinical procedures

Prior to endodontic treatment, gingival crevicular fluid was taken at buccal/labial surfaces of pretreated teeth and contralateral teeth as control for MMP-9 and IL-10 expression. Before canal preparation, a sterile paper point (size 20; Dochem, China) was introduced into the full length of the canal, and retained in position for 60 seconds for bacterial sampling (S1). All the paper point were placed in each screw-cap container containing Stuart-transport medium and were directly sent to the microbiology laboratory for microbial cultivation.

The root canal was prepared by using hand K-files with back-and-forth alternated rotation motion (size 15/40 and 45/80; FKG Dentaire, Switzerland). The working length was established
with an apex locator and confirmed by radiographs. Master apical files ranged from 30-40, depending on both root anatomy and initial apical files. Chemomechanical preparation was completed at the same appointment in all cases. The root canals were irrigated with 3 mL of 2.5% sodium hypochlorite (local medical supplies) using a 27-gauge needle after each instrument size. The canals were then flushed with 2 mL of sterile aquadest solution, dried with sterile paper point (size 20; Dochem, China). Gingival crevicular fluid was taken at buccal/labial surfaces of treated teeth and contralateral teeth as control for MMP-9 and IL-10 expression. The canal was medicated with calcium hydroxide paste (Ultradent, Utah, USA) which was placed by means of lentulo spiral fillers and packed with a cotton pellet at the level of canal entrance. A radiograph was taken to ensure proper placement of the calcium hydroxide paste in the canal. Access cavity was filled with a temporary filling, Cavit-G (3M, ESPE, USA). When the canal is sterile (no symptoms, no exudates and negative to percussion), gingival crevicular fluid was taken again at buccal/labial surfaces of treated teeth and contralateral healthy teeth as control for MMP-9 and IL-10 expression.

Minocycline preparation

Minocycline was removed from a capsule, mixed with propylene glycol and macrogol with the same proportions, to become a paste.

- Choose a gutta percha which suits the prepared canal, apply with minocycline paste on the tip of gutta-percha, inserted into the apical of the canal and left it, the cavity was temporized. One week following the medication, gingival crevicular fluid was collected again at buccal/labial surfaces of treated teeth and contralateral healthy teeth as control for MMP-9 and IL-10 expression. The same procedures were repeated two
weeks following obturation. Points contaminated with blood or saliva was thrown away. Points were then placed into eppendorf for ELISA evaluation.

Paper strip was inserted into the crevicular gingival to collect the fluid

**Isolating and detection of species**

Each paper point was taken out from the container and inserted into the other container filled with enhancement medium of Brain-Heart Infusion Broth (BHIB), vortexed for 60 seconds to remove all bacteria and spread on BHIB. One mL of BHIB was added with 9 mL NaCl 0.9%, diluted and repeated in 3 series until it reached $10^3$ CFU bacteria. One mL was swabbed on a sterile petri dish contained Blood Agar (BA) and incubated anaerobically for 24 h at 37°C in gas vac. Another 1 mL was swabbed on a sterile petri dish contained Nutrient Agar (NA) to detect facultative anaerob bacteria. Appeared colonies were counted visually.
RESULTS

Tabel 5.9. Kadar MMP-9 setelah pemberian CaOH (1), setelah pemberian minosiklin (2), dua minggu setelah pengisian (3), dan satu bulan setelah pengisian (4) pada kelompok yang diberikan minosiklin dan kelompok kontrol negatif

<table>
<thead>
<tr>
<th>Kelompok</th>
<th>MMP-9</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Mino</td>
<td>11.34±0.65</td>
<td>11.57±0.42</td>
</tr>
<tr>
<td>KN</td>
<td>10.37±1.54</td>
<td>10.48±1.32</td>
</tr>
<tr>
<td>p-value*</td>
<td>0.58</td>
<td>0.12</td>
</tr>
</tbody>
</table>

No significant difference of MMP-9 level were noted before and after application of Minocycline although their level after 2 and four weeks were lower (p>0.05), while negative control showed no changes along the study.
Table 5.10. Kadar IL-10 setelah pemberian CaOH (1), setelah pemberian minosiklin (2), dua minggu setelah pengisian (3), dan satu bulan setelah pengisian (4) pada kelompok yang diberikan minosiklin dan kelompok kontrol negatif

<table>
<thead>
<tr>
<th>Kelompok</th>
<th>IL-10</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Mino</td>
<td>0.65±0,10</td>
<td>0.67±0,22</td>
</tr>
<tr>
<td>KN</td>
<td>0.65±0,10</td>
<td>0.62±0,05</td>
</tr>
<tr>
<td>p-value†</td>
<td>0.41</td>
<td>0.95</td>
</tr>
</tbody>
</table>

No significant difference of IL-10 level were noted before and after application of Minocycline although their level after 2 and four weeks were higher (p>0.05), while negative control also showed higher IL-10 level but has no significant difference.

Discussion

MMP-9 (gelatinase B) showed important role in normal healing process of the tissues which showed higher expression for a while and then decrease as healing. The persistence expression has been related to chronic disease. Molecular study reported 46% of samples were free of bacteria following chemomechanical preparation and irrigation with NaOCl 2.5%, and 62.5% samples were free of bacteria following medication with CaOH. Neither irrigation nor medication of root canal may able to eliminate all the bacteria in the root canal systems. High pH of calcium hydroxide could be neutralized by certain bacteria and the action of dentine buffer. The presence of different bacteria following medication might show the effectiveness of medicament used. Failure of treatment is associated with the presence of bacteria in the apical part of root canal system that have endured or evaded antimicrobial treatment, survived in the filled canal and are capable of inflaming the periapical tissue. This might explained the result of this study which showed the presence of bacteria either medication with calcium hydroxide or following application with minocycline (unpublished data).
IL-10 is produced in low quantity to match the mechanism of immune stimulation. IL-10 and TGF-β may suppress the immune response of Th1 and Th17. On the other hand, study in an experimental model of autoimmune disease showed that IL-10 together with IL-17 have protective function to limit the inflammation and tissue destruction.

The application with minocycline showed lack of effectiveness on the decrease of MMP-9 level in the root canal treatment with chronic apical periodontitis. The same result for IL-10 level which has less effect in the increase of IL-10 level. Minosiklin with sub anti bacterial dose has no effect on non-anti bacterial activities in the root canal treatment with chronic apical periodontitis.

**CONCLUSION**

Under the limitation of this study, it can be concluded that local application with minocycline has no anti inflammatory effect in endodontic therapy of chronic apical periodontitis.

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