

# MMP - 3

. . .

## I.

, fibronectin, laminin proteoglycan core protein 60% , 90%

proteinase metal, serine, cysteine, aspartic . metal 가 proteinase matrix metalloproteinase (MMP) interstitial collagenase (MMP - 1, - 8, - 13), gelatinase (MMP - 2, - 9), stromelysin (MMP - 3, - 10, - 11) 1,3).

1-3),

MMP , ,

. Viella 4)

MMP , ,

, 가

1,3). MMP, tissue inhibitor of metalloproteinase (TIMP)

Woolly 5)

가

7-

9). Meikle 10-12)

. Gangbar 6)

MMP - 1, - 2, - 3, TIMP .

가

가

가

가

interleukin - 1 (IL - 1), tumor  
necrosis factor - cytokine  
transforming growth factor -

가  
가  
Minocycline - HCl

IL - 1  
14 - 17). IL -  
IL - 1  
IL - 1 IL - 1 15  
18 - 20). IL - 1

25,32),  
가  
26,32),

prostaglandin  
가  
10).

가 , 가

가  
IL - 1  
MMP - 3  
가 , IL - 1 MMP - 3

## II.

### 1.

25 - 27).  
Tetracycline - HCl

(1)

28,29) ,  
30), ,  
31)

HBSS(Hanke's buffered salt solution,  
GIBCO/BRL, USA) 5ml 4

Doxycycline - HCl tetracycline - HCl

6 - 8  
HBSS 3 1x1x1mm

10 - 15  
(NUNC, Netherland) 20% fetal  
bovine serum(FBS, Gibco, USA) 10  
unit/ml penicillin, 100µg/ml strepto -

mycin DMEM (Dulbecco's - 20  
 Modified Eagle's Medium) , 37 , Tetracycline -  
 100% , 5% CO<sub>2</sub> (Sanyo, HCl(Sigma, USA), doxycycline - HCl(Sigma,  
 Japan) 가 USA), minocycline - HCl(Sigma, USA) 3  
 - 20 가  
 (confluent)가 ,  
 (NUNC, Netherland) 5 - 6 2.  
 5 - 10 .  
 (2) IL - 1 tetracycline (1) IL - 1 가  
 IL - 1 (R & D Systems, Minneapolis, MN) 6 well IL - 1 24  
 dimethyl sulfoxide(Sigma, USA)

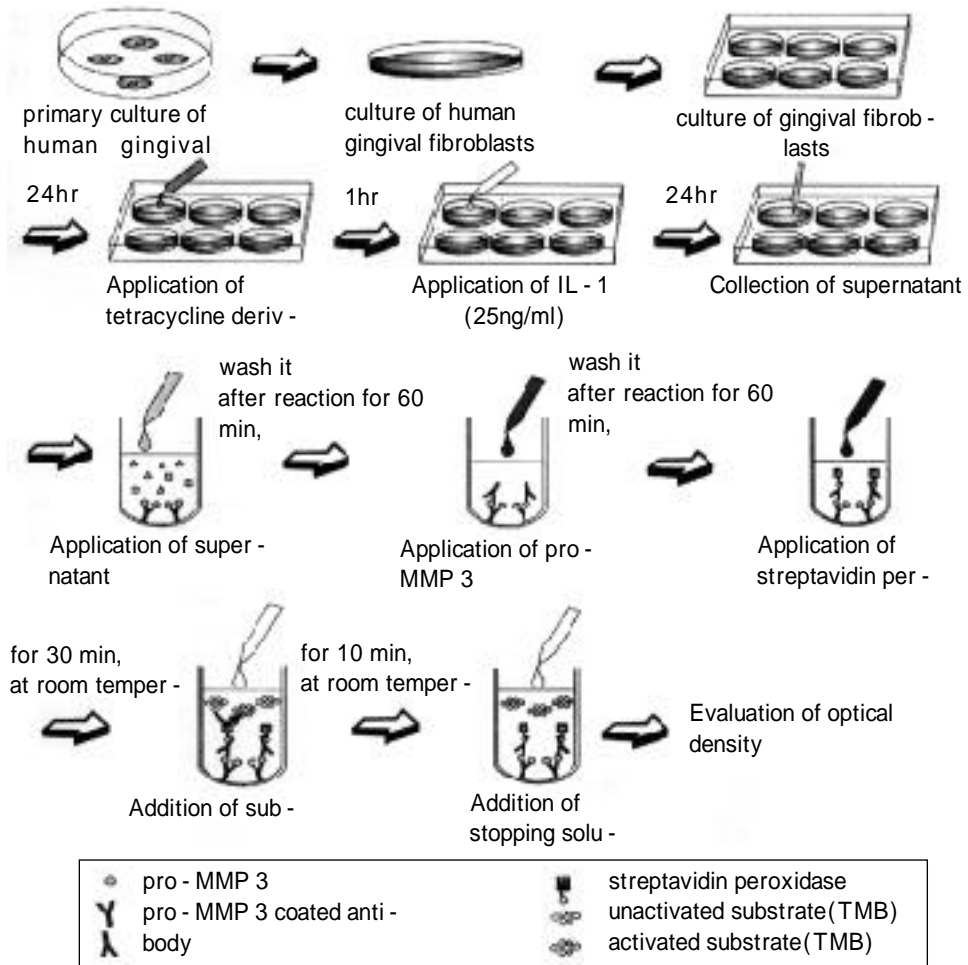


Figure 1. A Schematic illustration of the experimental procedure

0.1% FBS가 DMEM buffer 3, 100 μl biotiny -  
. 24 lated antibody well 60  
IL - 1 가 wash buffer 3  
5, 10, 25, 50, . Streptavidin peroxidase  
100ng/ml IL - 1 1ml 0.1% FBS가 well 100μl 30  
DMEM 1ml 24 wash buffer 3  
ELISA well 3,3', 5,5' - tetramethylbenzidine  
MMP - 3 가 100μl 10  
MMP - 3 IL - 1 100μl stopping solution  
. stopping  
(2) ELISA MMP - 3 reaction 30 microwell  
(Figure 1) plate reader (Bio - Tek instrument, USA)  
450nm well (optical densi -  
multiwell culture plate ty)  
proMMP - 3 ELISA kit(The MMP - 3  
Binding site, San Diego. CA) 96 well plate (relative activity) 가  
well triplicate  
60 well wash (3) Tetracycline

Table 1. The activity of MMP - 3 according to the concentrations of IL - 1

Concentration(ng/ml)	Number	Optical density(Mean ±S.D)	Relative activity(Mean(%) ± S.D)
0(control)	6	0.30 ± 0.26	100.00
5	6	1.17 ± 0.60	395.05 ± 202.87
10	6	0.99 ± 0.52	336.06 ± 174.50
25	6	2.01 ± 0.83*	680.94 ± 280.89*
50	6	1.96 ± 0.73*	664.00 ± 247.48*

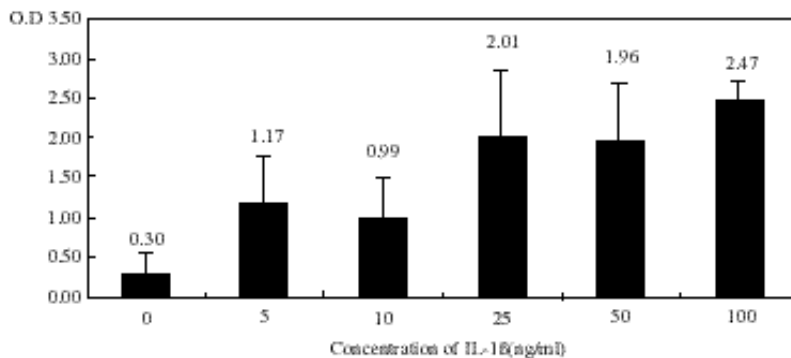


Figure 2. The difference of the optical density(O.D) of MMP - 3 according to the concentrations of

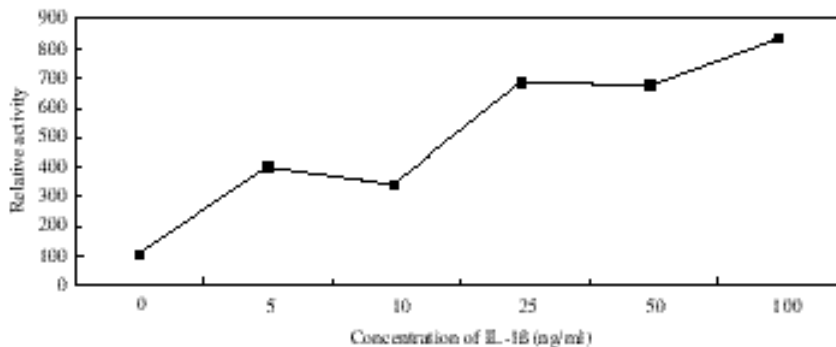


Figure 3. The difference of the relative activity of MMP - 3 according to the concentrations of IL - 1

10, 25, 50, 100, 200µg/ml tetracycline - HCl, doxycycline - HCl, minocycline - HCl 1ml 0.1% FBS DMEM 1ml 가 (4) IL - 1 가 25ng/ml 24 IL - 1 MMP - 3 tetracycline

proMMP - 3 ELISA kit  
MMP - 3

Table 2. The activity of MMP - 3 according to the concentrations of tetracycline - HCl

Concentration(ng/ml)	Number	Optical density(Mean ± S.D)	Relative activity(Mean(%) ± S.D)
0(control)	6	1.11 ± 0.09	100.00
10	6	0.56 ± 0.12	51.18 ± 13.48
25	6	0.73 ± 0.25	67.48 ± 26.50
50	6	1.00 ± 0.40	92.31 ± 41.18
100	6	1.37 ± 0.34	125.40 ± 37.46

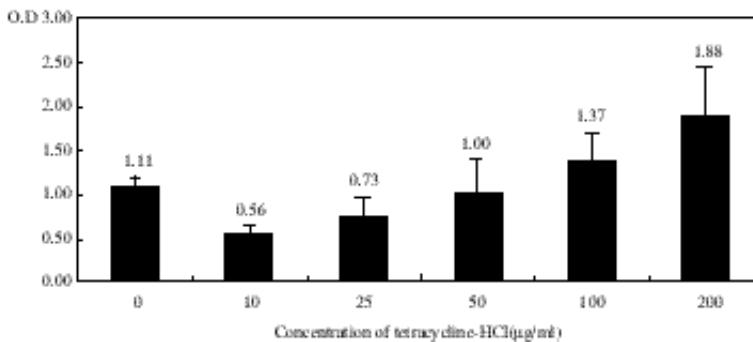


Figure 4. The difference of the optical density(O.D) of MMP - 3 according to the concentrations of

III.

(one - way ANOVA)

Turkey test

1. IL - 1

MMP - 3

SPSS/PC+ , p<0.05

5, 10, 25, 50, 100ng/ml

Table 3. The activity of MMP - 3 according to the concentrations of doxycycline - HCl

Concentration( $\mu\text{g/ml}$ )	Number	Optical density (Mean $\pm$ S.D)	Relative activity (Mean (%) $\pm$ S.D)
0(control)	6	1.26 $\pm$ 0.12	100.00
10	6	0.52 $\pm$ 0.03*	41.77 $\pm$ 3.60*
25	6	0.67 $\pm$ 0.06*	53.06 $\pm$ 2.25*
50	6	0.73 $\pm$ 0.09*	58.09 $\pm$ 3.70*
100	6	1.00 $\pm$ 0.05*	80.03 $\pm$ 10.34*

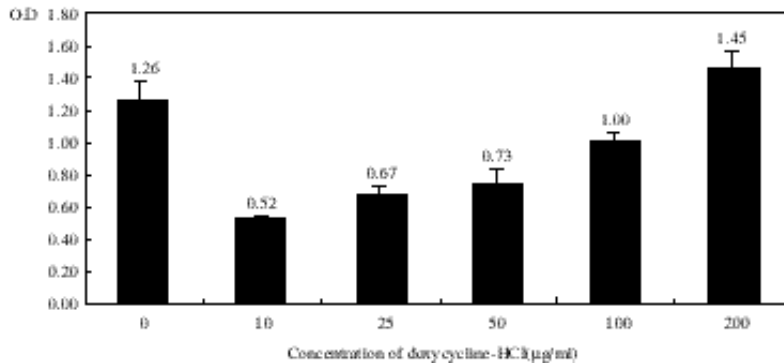


Figure 5. The difference of the optical density(O.D) of MMP - 3 according to the concentrations of

Table 4. The activity of MMP - 3 according to the concentrations of minocycline - HCl

Concentration( $\mu\text{g/ml}$ )	Number	Optical density (Mean $\pm$ S.D)	Relative activity (Mean (%) $\pm$ S.D)
0(control)	6	2.69 $\pm$ 0.12	100.00
10	6	0.89 $\pm$ 0.18*	33.05 $\pm$ 6.53*
25	6	1.06 $\pm$ 0.22*	39.00 $\pm$ 7.93*
50	6	1.64 $\pm$ 0.30*	60.71 $\pm$ 10.98*
100	6	1.89 $\pm$ 0.27*	69.98 $\pm$ 10.36*

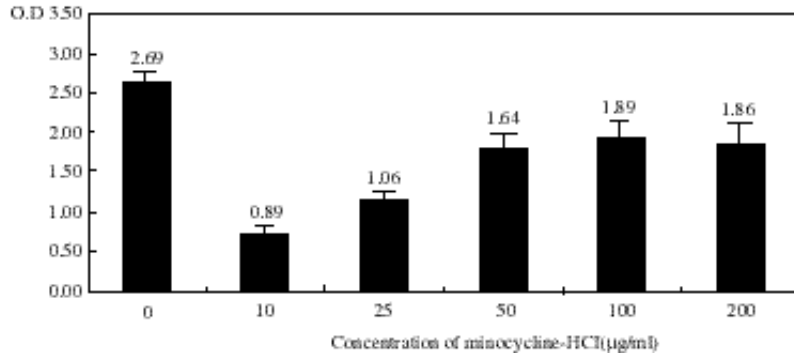


Figure 6. The difference of the optical density(O.D) of MMP - 3 according to the concentrations of

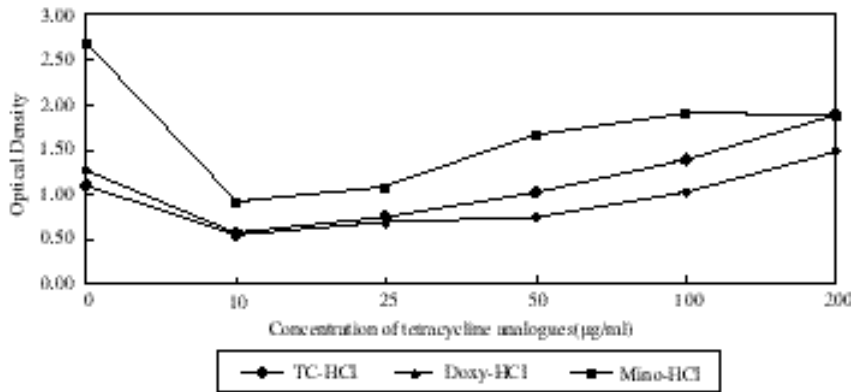


Figure 7. The difference of the optical density of MMP - 3 according to the concentrations of 3

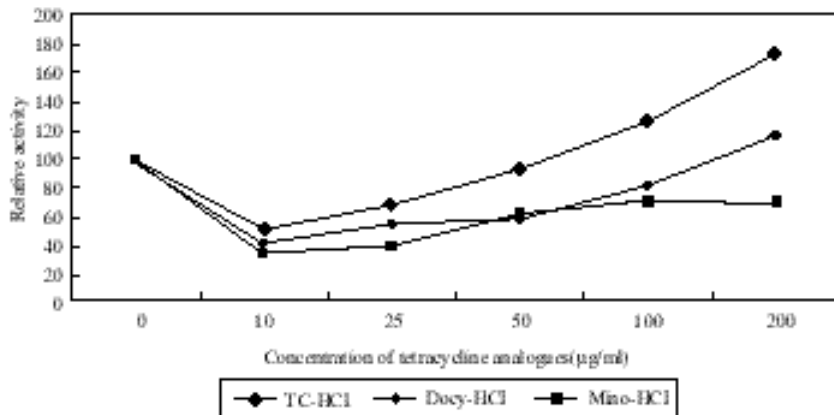


Figure 8. The difference of the relative activity of MMP - 3 according to the concentrations of 3

IL - 1 24  
 IL - 1 가 MMP - 3  
 25ng/ml

(p<0.05, Table 4, Figure 6).

5. Tetracycline MMP - 3

MMP - 3 가 (p<0.05,  
 Table 1, Figure 2, Figure 3).

3 tetracycline

MMP - 3 IL - 1  
 25ng/ml

MMP - 3  
 (Figure 7, Figure 8).

2. Tetracycline - HCl  
 MMP - 3

IV.

Tetracycline - HCl 가 MMP - 3  
 tetracy -  
 cline - HCl  
 , 10µg/ml  
 tetracycline - HCl 가

cytokine

MMP - 3 , 200µg/ml

MMP 8,9,33 -  
 35). MMP

tetracycline - HCl  
 MMP - 3 가  
 (p<0.05, Table 2, Figure 4).

3. Doxycycline - HCl  
 MMP - 3

MMP TIMP  
 1,12,14,36 - 39).

Doxycycline - HCl  
 10, 25, 50, 100µg/ml doxy -  
 cycline - HCl MMP - 3  
 가 , 200µg/ml  
 MMP - 3 가  
 (p<0.05, Table 3,  
 Figure 5).

interstitial collagenase(MMP - 8)가  
 40,41),

interstitial collagenase (MMP - 1)가  
 1).

4. Minocycline - HCl MMP - 3

MMP - 8  
 MMP - 1

4,42,43).

Minocycline - HCl  
 10µg/ml  
 200µg/ml

MMP - 8, MMP - 9 doxycycline chemi -  
 cally modified non - antimicrobial tetracy -  
 clines(CMTs)

43,44).

MMP - 3 가

MMP MMP -



3(stromelysin - 1) gelatin, proteoglycans, laminin, fibronectin type IV IX collagen

San Diego. CA)  
MMP - 3

45 - 47).

MMP - 3

MMP - 3

IL - 1

IL - 1 5, 10, 25, 50, 100ng/ml  
25ng/ml

MMP - 1

MMP - 3 가

48,49).

5,000 - 15,000

25ng/ml . Stashenko <sup>54)</sup>

IL - 1 <sup>20)</sup>, proinflammatory cytokine MMP

IL - 1 가

MMP - 3 IL - 1

6.2 ± 4.6ng/ml,  
12.6 ± 2.5ng/ml,  
28.4 ± 7.2ng/ml

IL - 1 MMP - 3가

49).

25ng/ml IL - 1

IL - 1 가 MMP - 3

Tetracycline IL - 1

tetracycline MMP - 3

MMP - 3  
MMP Zn<sup>2+</sup>

tetracycline - HCl,

<sup>30,44)</sup>, tetracycline - HCl

doxycycline - HCl, minocycline - HCl

가 4 - 8µg/ml

tetracycline

1

30µg/ml

IL - 1 24

Gordon <sup>55)</sup>, doxycycline - HCl

MMP - 3

48

MMP MMP

가 3 - 10µg/ml

MMP inhibitor

Pascale <sup>15)</sup>, minocycline - HCl

<sup>1,50 - 53)</sup>, IL - 1

4µg/ml Ciancio <sup>26,32)</sup>

MMP - 3 . MMP - 3

tetracycline

chromatogra -

10, 25, 50, 100, 200µg/ml

phy, reverse transcription - polymerase chain reaction(RT - PCR). immunocyto - chemistry, ELISA

MMP - 3

tetracy -

cline - HCl 25µg/ml

proMMP - 3 ELISA kit(The Binding site,

MMP - 3 가

200 $\mu$ g/ml 가 HCl

MMP - 3 (p<0.05), tetracycline - 가 Ingman 24 100 $\mu$ g/ml 58) minocycline - HCl 2 $\mu$ g/ml , 50 $\mu$ g/ml 14,38) , 100 $\mu$ g/ml Doxycycline - HCl 100 $\mu$ g/ml 가 (p<0.05), tetracycline doxycycline(15  $\mu$  M) tetracycline(350  $\mu$  M) Zn<sup>2+</sup> Burns 56) doxycycline - HCl 20mg 가

MMP - 3 (p<0.05), 200 $\mu$ g/ml minocycline(190  $\mu$  M) IC<sub>50</sub> minocycline - HCl tetracycline IL - 1 MMP - 3 MMP - 3 가 3 가 20mg doxycycline 3 가 MMP - 3 IL - 1 가 MMP - 3 Golub 30,44) doxycycline IL - 1 가

Minocycline - HCl 57) Somerman 50 $\mu$ g/ml 가 minocycline - HCl MMP - 3 MMP - 3 가 tetracycline 가 가

MMP - 8  
MMP - 1      MMP - 3  
, doxycycline

MMP - 8  
가      가

MMP - 3      가  
(p<0.05).

3. Minocycline - HCl      10 - 200µg/ml  
MMP - 3      가  
(p<0.05).

tetracycline

IL - 1      MMP - 3  
가

VI.

59) ,

가  
V.

Tetracycline      MMP - 3  
가  
MMP - 3

IL - 1 ,  
tetracycline - HCl, doxycycline - HCl,  
minocycline - HCl (10, 25, 50, 100,  
200µg/ml) , 1 25ng/ml  
IL - 1      24  
ELISA

1. Tetracycline - HCl      25µg/ml  
MMP - 3      가  
, 200µg/ml  
MMP - 3      가  
(p<0.05).

2. Doxycycline - HCl      100µg/ml  
MMP - 3      가  
(p<0.05), 200µg/ml

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- Abstract -

## Effect of Tetracycline Analogues on The Activity of Matrix Metalloproteinase - 3 in Gingival Fibroblasts

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Extracellular matrix component is degraded by enzymes of the matrix metalloproteinases (MMPs). MMPs are produced by both hemopoietic and structural cells. Increased activity of MMP - 3 in periodontium is strongly associated with inflammatory periodontal disease.

The purpose of the present study was to determine the effect of tetracycline analogues on the activity of MMP - 3. Tetracycline - HCl, doxycycline - HCl, and minocycline - HCl were applied to human gingival fibroblasts at various concentrations of 10, 25, 50, 100, 200  $\mu\text{g}/\text{ml}$ , and 1 hour later IL - 1 of 25  $\text{ng}/\text{ml}$  was added. After incubation for 24 hours the cells were reacted by enzyme - linked immunosorbent assay using proMMP - 3 ELISA kit. The optical density was measured by microwell plate reader at 450 nm. The relative activity of MMP - 3 was calculated as the percentage of the optical density of each experimental group to that of the control. The difference of the optical density and the

relative activity of MMP - 3 between the experimental groups and the control was statistically analyzed by one way ANOVA.

The results were as follows:

1. Tetracycline - HCl showed the tendency to inhibit the activity of MMP - 3 at the concentration lower than 25  $\mu\text{g}/\text{ml}$ , but increased significantly the activity of MMP - 3 at the concentration of 200  $\mu\text{g}/\text{ml}$  ( $p < 0.05$ ).
2. Doxycycline - HCl inhibited significantly the activity of MMP - 3 at the concentration lower than 100  $\mu\text{g}/\text{ml}$ , but increased significantly the activity of MMP - 3 at the concentration of 200  $\mu\text{g}/\text{ml}$  ( $p < 0.05$ ).
3. Minocycline - HCl inhibited the activity of MMP - 3 at the concentration in the range of 10 to 200  $\mu\text{g}/\text{ml}$ .

Within the limit of the present study, the above results suggested that the low concentration of tetracycline analogues could inhibit the activity of MMP - 3 induced by IL - 1 in human gingival fibroblasts.

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Key words ; tetracycline analogues, Interleukin - 1 , matrix metalloproteinase - 3 human gingival fibroblasts.