The relationship between rheumatoid arthritis and periodontal disease

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Directed by Professor Soo-Kon Lee

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Chuan-Xin Su

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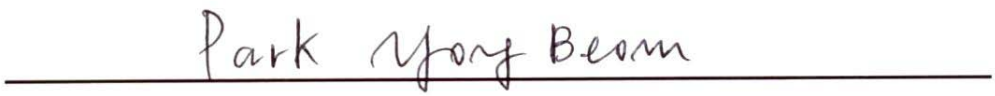
This certifies that the Master's Thesis of

Chuan-Xin Su is approved.

Thesis Supervisor: Soo-Kon Lee



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The time of my master course has elapsed shortly soon and it’s time to complete my course. Although it’s not so long period of my life to study about research, I have had much more knowledge and experience about research as other people. On future reflection it seems that I have not realized my various thought for my master course, but I’m so happy to meet many nice persons. All of them helped me to improve my studies and I’m so grateful to their help.

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ABSTRACT

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Background: Peridontitis has many similar characteristics of synovitis in rheumatoid arthritis (RA). Recent reports have suggested a significant relationship between RA and periodontal disease (PD) in RA patients. The aim of this study was to investigate the relationship between RA and PD and to evaluate the treatment response

in RA and PD in animal model.

Method: Twenty-five male DBA/1 mice 8 weeks old were divided into five groups. Group 1 (CONTROL) and Group 2 (CONTROL+TNFi) were the control groups without collagen injection. The other three groups which were experimental groups were given a subcutaneous injection of bovine type II collagen (100μg/mice) emulsified in complete Freund's adjuvant (Arthrogen-CIA, Redmond, WA) (1:1, w/v) into the base of tail. Three weeks later, the mice were given a booster subcutaneous injection of bovine type II collagen (100μg/mice) in incomplete Freund's adjuvant (1:1, v/v) into the base of tail. After the development of arthritis (2 weeks after the booster collagen injection), Group 3 (CIA+TNFi) was given the anti-TNF-α (5.5mg/kg), and the Group 4 (CIA+MTX) was given the intraperitoneal injection of methotrexate (MTX) (35mg/kg) twice a week for five weeks. At the same time the Group 5 (CIA) and the Group 1 (CONTROL) were untreated while Group 2 (CONTROL+TNFi) was

injected with anti-TNF-α. Arthritis score and paw thickness were measured and histopathological assessment of joint sections was performed. The expression of proinflammatory cytokines and enzymes was evaluated by immunohistochemical staining. Jaws were scanned and reconstructed into a 2 and 3-dimensional structure with micro-CT. The average exposure areas of teeth were measured to calculate the severity of PD.

Results: The anti-TNF-α therapy and MTX attenuated the severity of arthritis and histopathological findings in CIA mice. Micro CT confirmed that the periodontal disease existed in CIA mice. There were significant differences in the average area measurements (CONTROL 0.0177 vs CONTROL+TNFi 0.0229 CIA 0.02205 $p<0.05$) between Group 1 and Group 2 or Group 5. The average exposed area was significantly lower in Group 5 than in Group 3 or Group 4 (CIA 0.02205 vs CIA+TNFi 0.02466, CIA+MTX 0.0254, $p<0.05$).

Conclusions: Collagen induced arthritis was associated with the occurrence of periodontitis. The MTX/TNFi drugs didn’t affect PD while they treated CIA. Further studies will be necessary to investigate the cause of these differences.

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Ⅰ. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks the joint architecture and consequent disability[1,2]. RA can also make diffuse inflammation in the lungs,

pericardium, pleura, and sclera, and also nodular lesions, most common in subcutaneous tissue under the skin. Environmental factors such as age, gender, smoking as well infections play a role in the pathogenesis of RA[3]. Although bacterial infections and oral pathogens have been related to the pathogenesis of RA[4-8], the involved pathologic mechanisms are unknown; autoimmunity plays a pivotal role in its chronicity and progression.

Periodontal disease (PD) refers to a number of inflammatory diseases affecting the tissues that surround and support the teeth. PD is caused by the bacteria which adhere to and grow on the tooth's surfaces. A diagnosis of periodontitis is established by inspecting the soft gum tissues around the teeth with a probe and radiographs by visual analysis, to determine the amount of bone loss around the teeth. Periodontal ligament (PDL) resides between the cementum of the roots of the teeth and the alveolar bone. In this location, PDL-derived cells are uniquely situated to maintain the overall

integrity of the PDL. PDL-derived cells produce various inflammatory mediators to recognize the somatic components including lipopolysaccharide (LPS) [9].

The oral cavity harbours a large reservoir of pathogenic organisms that may contribute to chronic bacteremia and potential damage of distant organs, including the joints[10]. Also, there is evidence of a specific antibody response against anaerobic oral bacteria as well as the presence of oral bacterial DNA in the synovial fluid of RA patients [11].

The purpose of the study was to illustrate the relationship between RA and PD. I hypothesized that CIA was a risk factor for the development of PD. The hypothesis was that if we treat collagen induced arthritis with drugs such as methotrexate or anti-TNF-α agent periodontal disease might be relieved.

Ⅱ. MATERIALS AND METHODS

1. Induction of collagen induced arthritis

All animals were treated in accordance with the guidelines and regulations for the use and care of animals of Yonsei University, Seoul, Korea. Firstly, twenty five male DBA/1 mice at 8 weeks of age (SLC, Shizoka, Japan) were divided into five groups. Group 1 (CONTROL) and Group 2 (CONTROL+TNFi) were the control groups without collagen injection. The other three groups which were experimental groups were given an subcutaneous injection of bovine type II collagen(100μg/mice) emulsified in complete Freund's adjuvant (Arthrogen-CIA, Redmond, WA) (1:1, w/v) to the base of the tail. Three weeks later, the mice were given a booster subcutaneous injection of bovine type II collagen (100μg/mice) in incomplete Freund's adjuvant (1:1, v/v) to the base of the tail. After development of arthritis (2 weeks after the booster

collagen injection), Group 3 (CIA+TNFi) was given the anti-TNF-α (5.5mg/kg twice a week, Wyeth, Pfizer Inc). The Group 4 (CIA+MTX) was given the intraperitoneal injection of methotrexate (MTX) (35mg/kg, Yuhan Co. Ltd) twice a week for five weeks. At the same time the Group 5 (CIA) and the Group 1 (CONTROL) were untreated while Group 2 (CONTROL+TNFi) which was normal was treated by anti-TNF-α.

2. Assessment of arthritis severity

Mice were observed twice a week for 70 days after primary immunization. Arthritis severity was evaluated by visual inspection. All 4 legs of the mice were evaluated and scored from 0 to 4 according to the following scale: 0 = no signs of arthritis, 1= swelling and/or redness of the paw or 1 digit, 2 = 2 joints involved, 3 = more than 2 joints involved, and 4 = severe arthritis of the entire paw and all digits.

Paw thickness was measured with a Vernier caliper. Arthritis scoring and paw thickness measurement were performed by 2 independent observers.

3. Histopathological and immunohistochemical study

Mice were anesthetized and sacrificed on day 70, and joints and jaws were removed for histopathological examination after routine fixation, decalcification, and paraffin embedding of tissue. Tissue sections were prepared and stained with hematoxylin and eosin (H&E). Sections were sequentially incubated with specific antibodies directed against murine TNF-α (Hycult Biotechnology, Uden, The Netherlands), IL-1β, and iNOS, (Santa Cruz Biotechnology, Santa Cruz, CA) followed by the appropriate secondary antibodies (ISU Abxis, Seoul, Korea). All tissue samples were counterstained with hematoxylin. After immunohistochemical staining, expression of the different markers in the periodontal tissue of paw and knee joints was scored semiquantitatively on a 4-point scale independently and blindly by 2 individual

pathologists, and the average of their scores were calculated. A score of 0 represented minimal expression, 1 represented mild expression, and 2 represented moderate expression whereas 3 represented abundant expression of a marker. Minor differences between observers were resolved by mutual agreement.

4. Micro-CT imaging

Mice were observed twice a week for 70 days after primary collagen injection, euthanized, and their jaws were excised and fixed in 4% formalin for 2 days. Left mandibules were analyzed by Ultrasound Computerized Tomography (UCT) (skysan; SCANCO USA, Inc.)[12]. Using 2 and 3-dimenional image reconstructions, the cementoenamel junction (CEJ) and marginal bone crest (MBC) were manually drawn. Alveolar bone resorption was determined as the distance and exposed root surface area between CEJ and MBC were measured.

5. Statistical analysis

All statistical analyses were conducted using SPSS package for Windows, version 17 (SPSS Inc., Chicago, IL). The representative values were the means of those obtained from CIA mice, and all values in the experimental groups were compared to controls. All results and measurements were expressed as the mean ± standard deviation. Statistical comparisons between the 2 groups were evaluated by Mann-Whitney U test and t-test, and correlations between parameters measured by micro-CT were calculated using Spearman's correlation coefficient. The level of significance was set at 0.05.

Ⅲ. RESULTS

1. The severity of arthritis in mice

Group 3, 4, 5 showed definite evidence of arthritis such as redness and swelling in the joints after collagen injected. After the treatment of TNFi and MTX, arthritis score improved significantly in Group 3 and Group 4, although Group 5 showed continued arthritis with sacrificed. (Figure 1)

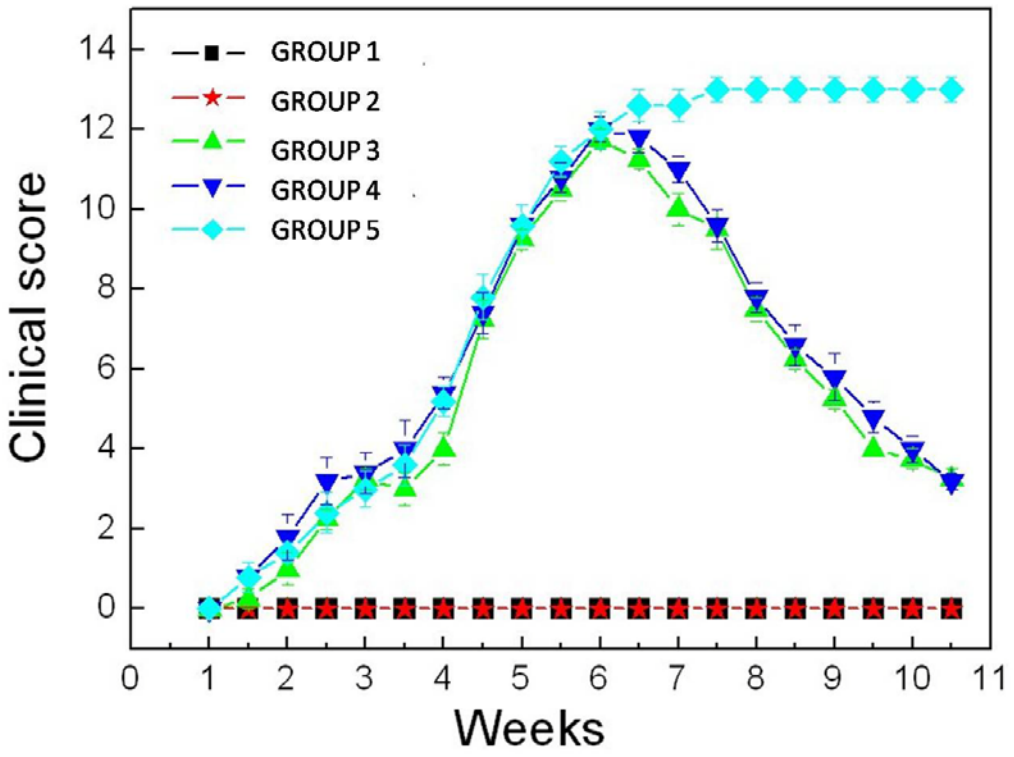

Figure 1. The severity of arthritis in mice. CIA mice model were treated by collagen at the 1st week and the 4th week. As the developing of the disease, CONTROL+TNFi, CIA+TNFi and CIA+MTX groups were treated by TNFi and MTX with others untreated at 6th week. The improvement of arthritis was remarkable in the treated CIA mice model groups while others stable.

2. The histopathological finding and the immunohistochemical staining for TNF-α, IL-1ß and iNOS in joints

The histopahtological evaluation showed that the joint sections were not inflamed in Group 1, Group 2, Group 3 and Group 4 which showed the intact bone structure. However, the severe inflammations were found in Group 5. Immnunohistochemical analysis of paws and knee joints tissues obtained from Group 5 exhibited markedly positive staining for TNF-α, IL-1ß and iNOS. However, TNF-α, IL-1ß and iNOS were not expressed in treated groups even Group 2 and Group 1 (Figure 2 and 3).

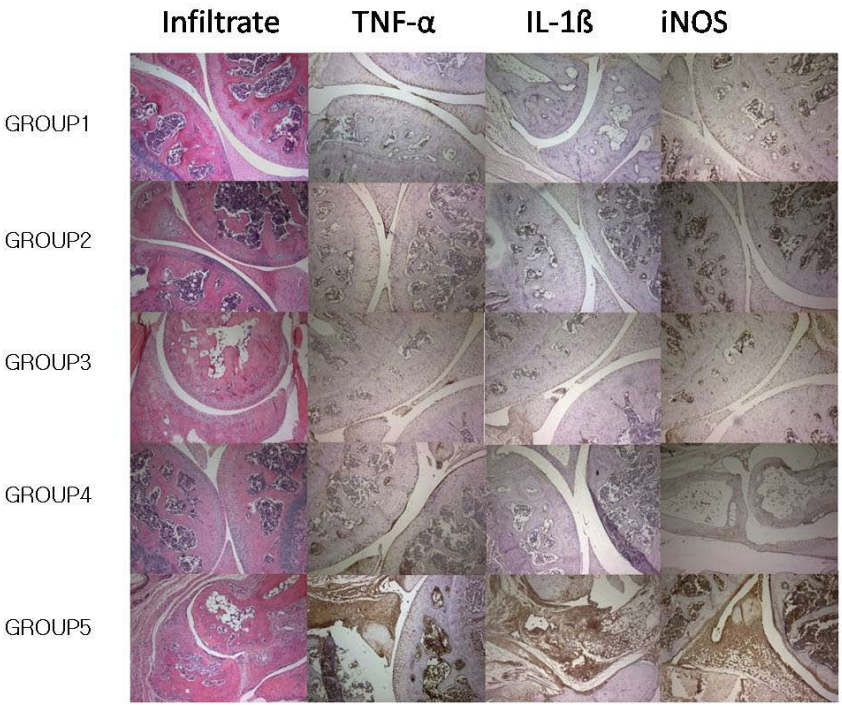

Figure 2. Histopathological finding and immunohistochemical staining for TNF-α, IL-1ß and iNOS in joint tissues. Histopathological evaluation revealed severe inflammation in the joint sections of untreated CIA mice. In contrast, the extent of arthritis and bone destruction was significantly reduced in the joints of mice be treated. TNF-α, IL-1β and iNOS were showed markedly positive in CIA group. In CONTROL group and the treated group, immunohistochemical staining were normal. And CIA group expressed significant cytokines around joints.

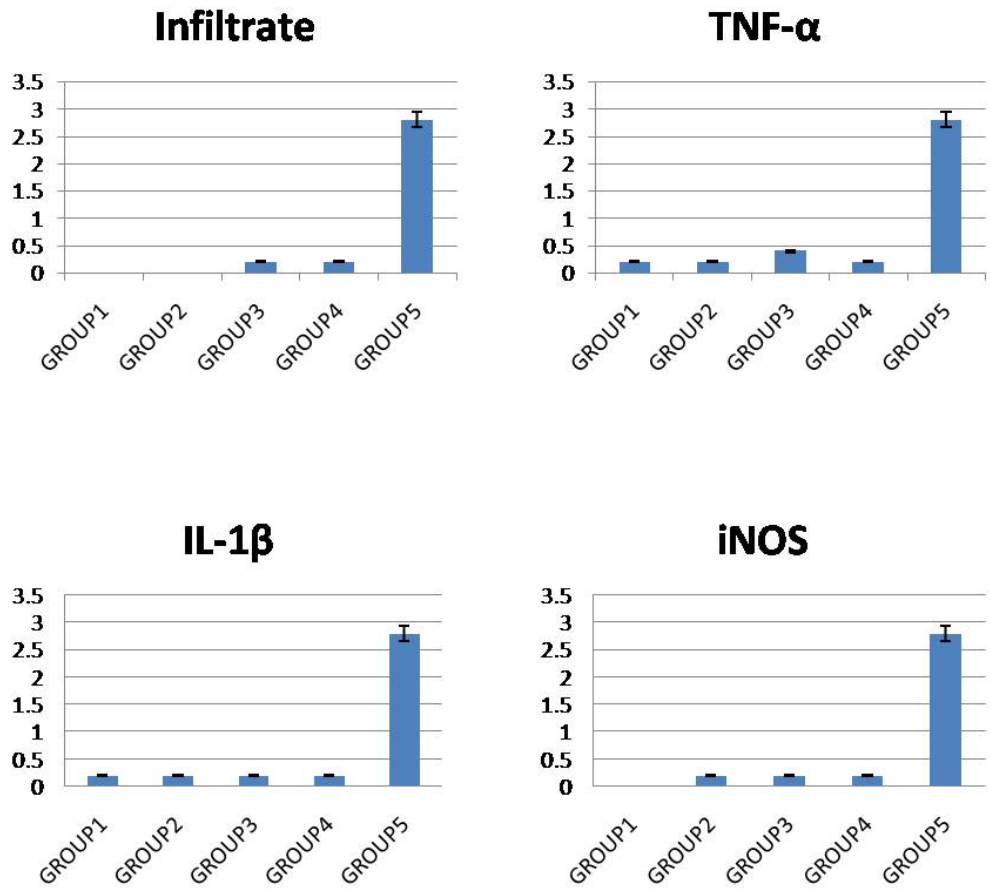

Figure 3. The scores of histopathological finding and immunohistochemical staining.

When we compared the values between the untreated and each treated group, cytokines were showed markedly positive in CIA group ($p<0.05$).

3. Micro-CT findings

CEJ-bone crest distance was measured for the severity of periodontal disease and the extent of the periodontal tissue breakdown was compared among the groups to investigate the difference. The area of exposed root surface and the length of exposed root (mesial and distal aspect) were measured by image program (Figure 4 and 5).

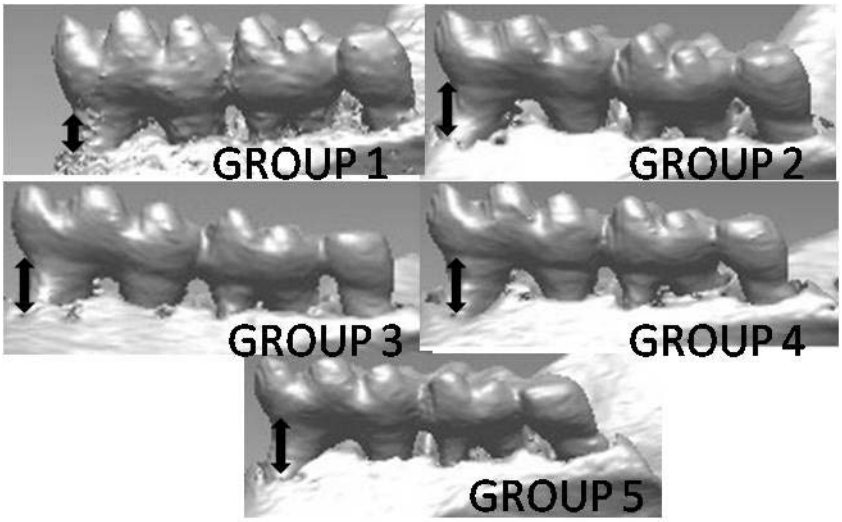

Figure 4. Micro-CT imaging pictures. The distance between the cementoenamel junction and the alveolar bone rest were obviously increased in CONTROL+TNFi group, CIA+TNFi group, CIA+MTX group and CIA group than in CONTROL group.

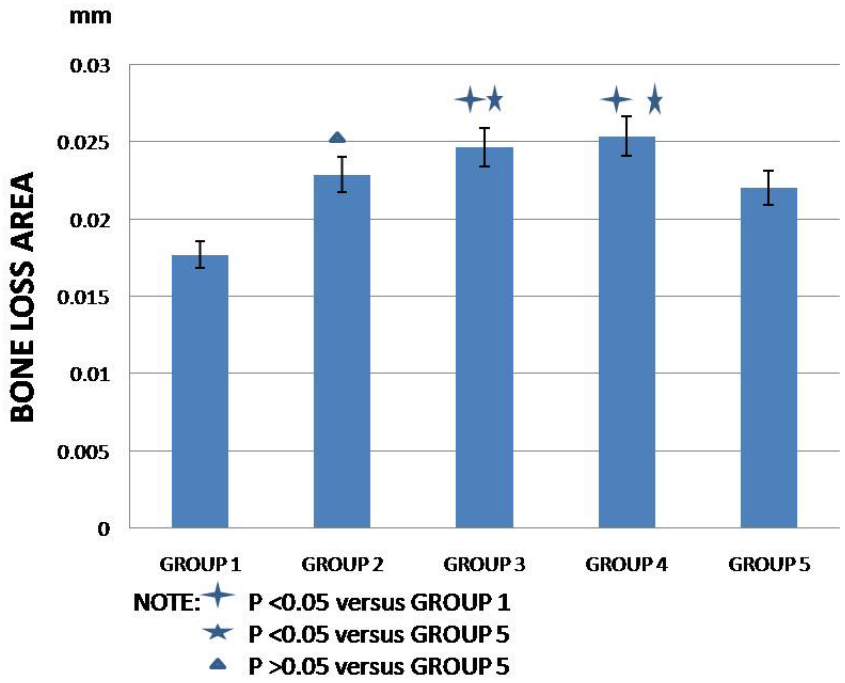

Figure 5. Micro-CT measurement results proved the severity of PD in mice. It was easy to see that the mice in Group 1 were normal than other groups. Even the mice in Group 2, Group 3 and Group 4 also got PD. And the Group 3 and Group 4 showed heavier PD than others.

4. The histopathological finding and the immunohistochemical staining for TNF-α, IL-1ß and iNOS in jaws

All groups without CONTROL group showed the damage to the alveolar bone. The epithelium became spongiotic and hyperplastic and the underlying stroma was densely infiltrated with lymphocytes and plasma cells. Immnuno-histochemical analysis of jaws obtained from CIA group exhibited markedly positive staining for TNF-α, IL-1ß and iNOS. However, TNF-α, IL-1ß and iNOS were also expressed in treated groups even CONTROL+TNFi group in periodontal tissues. There was no cytokines expressed in CONTROL group (Figure 6 and 7).

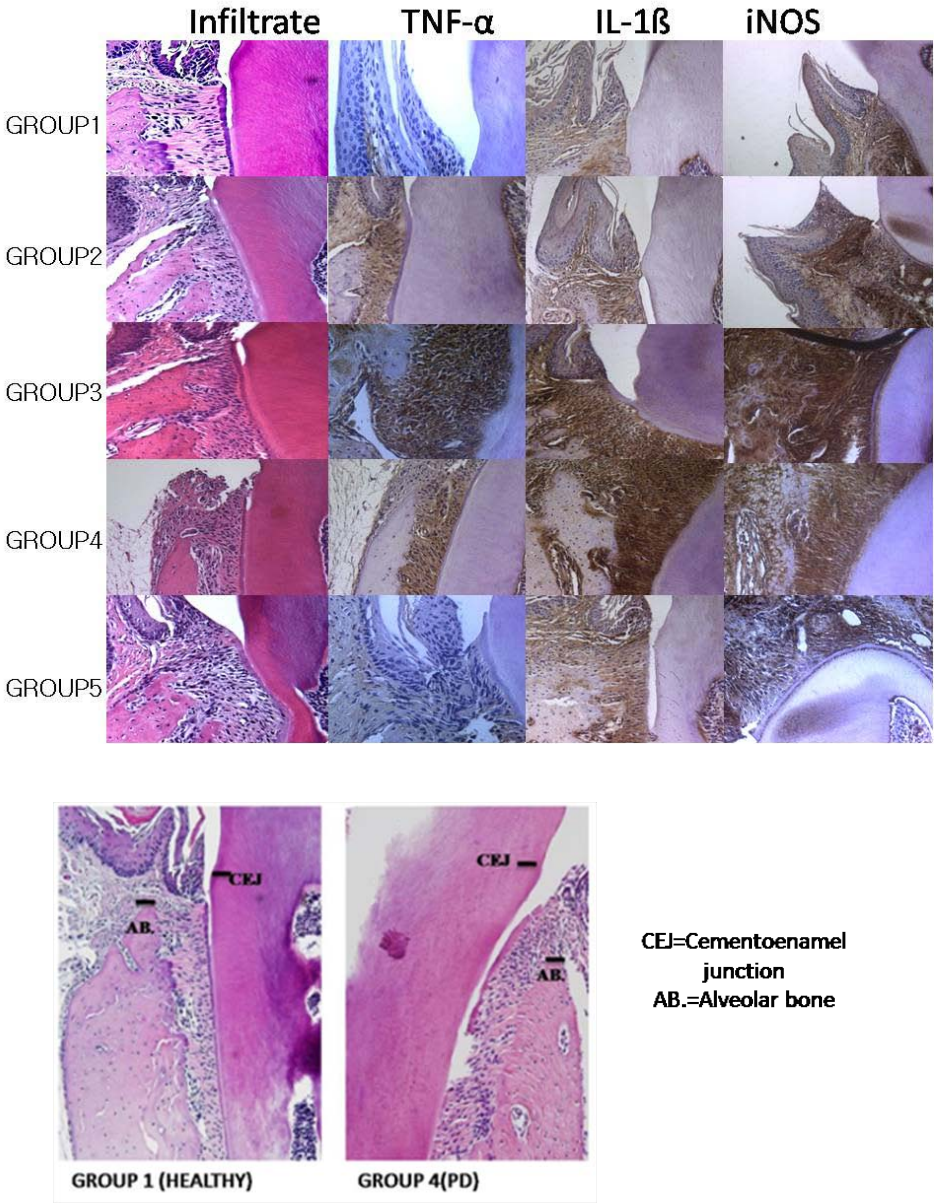

Figure 6. Histopathological finding and immunohistochemical staining for TNF-α, IL-1ß and iNOS in periodontal tissue. In Group 1(CONTROL), the junctional epithelium

was thin cell layer with some rete ridge extending into the underlying gingival fibrous tissue. However, in Group 5(CIA) and other groups, detachment of gingiva from tooth surface and downward displace of epithelial attachment. The levels of TNF- α, IL- 1β and iNOS in the periodontal tissues were significantly higher than that in Group 1. The healthy tissue compared with the inflammated periodontal tissue showed that detachment of gingival from tooth surface and downward displacement of epithelial attachment. Gums separated from the teeth, forming pockets. Gum tissue and bone were destroyed.

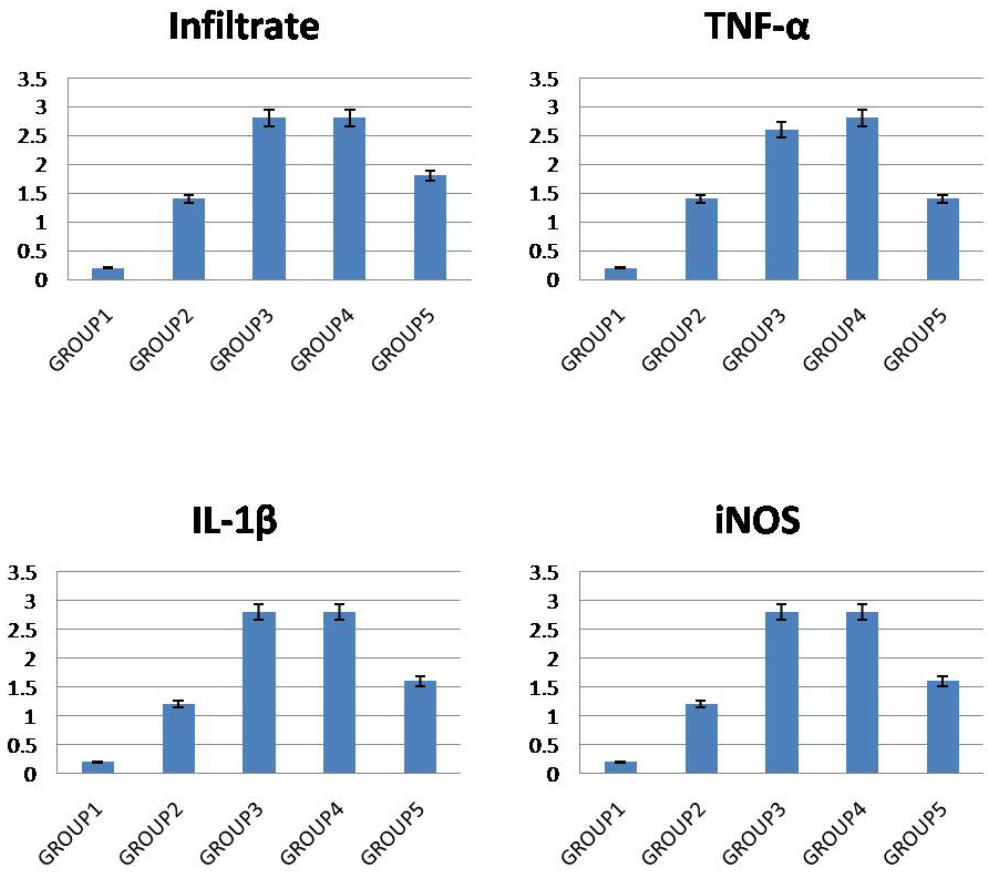

Figure 7. The scores of histopathological finding and immunohistochemical staining.

When we compared the values among the groups, cytokines were markedly positive in CONTROL+TNFi group, CIA+TNFi group, CIA+MTX group and CIA group. ($p<0.05$)

Ⅳ. DISCUSSION

In this study, I found that CIA was associated with the occurrence of periodontal disease. The anti-inflammatory drugs didn't affect on PD while they treated CIA. PD was getting worse by the treatment of MTX and anti-TNF-α in CIA group while CIA was improved by it. The clinical score and the histopathological and immunohistochemical study for mice showed the arthritis was improved by the treatment of TNFi/MTX while the CONTROL group, CONTROL+TNFi group and CIA group stable. The results of histopathological and immunohistochemical study and the Micro-CT image showed the PD existed in all groups except the CONTROL group. There were significant differences in the average area measurements (CONTROL 0.0177 vs CIA 0.02205 CONTROL+TNFi 0.0229 $p<0.05$) between CONTROL group and CONTROL+TNFi or CIA group. The average exposed area was significantly lower in CIA group than in CIA+TNFi or CIA+MTX group (CIA 0.02205 vs

CIA+TNFi 0.02466, CIA+MTX 0.0254, $p<0.05$).

TNF-α and IL-1β were all potent inducers of the production of acute phase reactants by hepatocytes and thereby mediate the acute phase response seen in CIA and PD. INOS, which was mainly associated with the periodontal inflammation[13], enabled its participation in anti-microbial and anti-tumor activities in order to take part in the oxidative burst of macrophages. I found that the clinical score of mice models showed that the mice in CONTROL group and CONTROL+TNFi group were normal while the mice in CIA+TNFi group and CIA+MTX group got arthritis and be healing after treated and the mice in CIA group were abnormal. All mice in CIA group got RA while others didn't. And the immuohistochemical results of joints tissue also proved TNF-α, IL-1β and iNOS were significantly positive in CIA group. However, the immuohistochemical results of jaws tissue proved that the TNF-α, IL-1β and iNOS were positive in all groups except CONTROL group . And the cytokines were marked

positive in CIA+TNFi group and CIA+MTX group.

MTX was introduced more than 50 years ago to treat cancer because of its anti-proliferative effects. Since the early 1980s, it has become the disease-modifying anti-rheumatic drug (DMARD) of choice in the treatment of RA and was used in many other rheumatic diseases as well. It was known that MTX has a side effect of bone marrow suppression. There were also ulcerative mucositis, decreased hepatic and renal function as side effects of MTX. However, according to the recent research, MTX caused progressed periodontitis because of the chronic long-term neutropenia and the change of microflora[14,15]. And the dose of MTX in our experiment was high. We were not sure whether the low dose MTX also caused the serious periodontitis. That might explain the progressed PD in CIA+MTX group but could not explain the severity of periodontitis.

Rheumatoid arthritis treated by anti-TNF-α was based on the recognition of the

role on TNF-α of the inflammatory response in many organ systems. However, the paper showed that anti-TNF-α make excessive bone resorption resulting in bone loss[16,17]. In my research, it was clear that the TNF-α had significantly expression in the groups which were being treated by TNFi not only the CIA+TNFi group but also CONTROL+TNFi group in periodontal tissues while they were normal in the same group of joint tissues. From the results of Micro-CT, it was easily to see that the exposure area was the largest in CIA+TNFi group and CIA+MTX group. And it was lager in CONTROL+TNFi group and CIA group than in CONTROL group.

There were many researchers hypothesized the relationship between RA and PD[18-20], and there were some researchers who test the association between them using clinical patients [21-25]. They demonstrate that RA may be associated with PD.

Despite I proved there was association between CIA and PD, I still need further research. The further research must be proved the details of the PD's occurrence

progress in CIA models. Mice might be observed at the regular intervals for the effect of the drugs in periodontal tissues.

In conclusion, based on the findings of the present study, a role of RA in the progression of periodontal disease was proposed. However, the drug's affection was not proposed. Further, studies were needed to confirm the findings of our study in the future.

Ⅴ. CONCLUSION

CIA associated with the occurrence of PD. The DMARD didn’t improved PD while they treated CIA. Further studies will be necessary to investigate the cause of these differential effects.

REFERENCES

1. Fox DA, Etiology and pathogenesis of rheumatoid arthritis. In: Koopman WJ. Arthritis and allied conditions. A textbook of rheumatology. 1st ed. Lippincott, Williams & Williams, Baltimore; 2001. p. 1085-102.

2. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001; 15 358(9285): 903-11.

3. Alamanos Y, Drosos A A. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev 2005; 4: 130-6.

4. Cai XY, Yang C, Zhang ZY, Qiu WL, Ha Q, Zhu M. A murine model for septic arthritis of the temporomandibular joint. J Oral Maxillofac Surg 2008; 66(5): 864-9.

5. Smith RL, Merchant TC, Schurman DJ. In vitro cartilage degradation by Escherichia coli and Staphylococcus aureus. Arthritis Rheum 1982; 25(4): 441-6.

6. Jasin HE. Bacterial lipopolysaccharides induce in vitro degradation of cartilage matrix through chondrocyte activation. J Clin Invest 1983; 72(6): 2014-9.

7. Rosenstein ED, Greenwald RA, Kushner LJ, Weissmann G. Hypothesis: The humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. Inflammation 2004; 28(6): 311-8.

8. Hara Y, Kaneko T, Yoshimura A, Kato I. Serum rheumatoid factor induced by intraperitoneal administration of periodontopathic bacterial lipopolysaccharide in mice. J Periodont Res 1996; 31(7): 502-7.

9. Kasamatsu A, Uzawa K, Shimada K, Shiiba M, Otsuka Y, Seki N, et al. Elevation of galectin-9 as an inflammatory response in the periodontal ligament cells exposed to Porphylomonas gingivalis lipopolysaccharide in vitro and in vivo. Int J Biochem Cell Biol 2005; 37(2): 397-408.

10. LaPorte DM, Waldman BJ, Mont MA, Hungerford DS. Infections associated with dental procedures in total hip arthroplasty. J Bone Joint Surg Br 1999; 81(4): 56-9.

11. Trombone AP, Claudino M, Colavite P, de Assis GF, Avila-Campos MJ, Silva JS, et al. Periodontitis and arthritis interaction in mice involves a shared hyper-inflammatory genotype and functional immunological interferences. Genes Immun 2010; 29. [Epub ahead of print]

12. Waarsing JH, Day JS, Weinans H. An improved segmentation method for in vivo microCT imaging. J Bone Miner Res 2004; 19(10): 1640-50.

13. Popkov VL, Fil'chukova IA, Lapina NV, Galenko-Yaroshevskii VP, Dukhanin AS. Activity of nitric oxide synthase and concentration of nitric oxide end metabolites in the gingiva under experimental pathological conditions. Bull Exp Biol Med 2005; 140(4): 391-3.

14. Yoshinari N, Kameyama Y. Aoyama Y. Effect of long-term methotrexate-induced neutropenia on experimental periodontal lesion in rats. J Periodontal Res 1994; 29(6): 393-400.

15. Wu JZ. Chemosensitivity testing of adenocystic tongue and gingival cancer cell lines. Zhonghua Kou Qiang Yi Xue Za Zhi 1992; 27(2): 107-8, 28.

16. Lerner UH. Inflammation-induced bone remodeling in periodontal disease and the influence of post-menopausal osteoporosis. J Dent Res 2006; 85(7): 596-607.

17. Pers JO, Saraux A, Pierre R, Youinou P. Anti-TNF-α Immunotherpy is associated with increased gingival inflammation without clinical attachment loss in subjects with rheumatoid arthritis. J Periodontal 2008; 79(9): 1645-51.

18. Liao F, Li Z, Wang Y, Shi B, Gong Z, Cheng X. Porphyromonas gingivalis may play an important role in the pathogenesis of periodontitis-associated rheumatoid arthritis. Med Hypotheses 2009; 72(6): 732-5.

19. Mercado F, Marshall RI, Klestov AC, Bartold PM. Is there a relationship between rheumatoid arthritis and periodontal disease? J Clin Periodontol 2000; 27(4): 267-72.

20. Zhang DZ, Zhong DY, Deng J, Wang JB. Relationship between periodontal disease and rheumatoid arthritis. Hua Xi Kou Qiang Yi Xue Za Zhi 2005; 23(6): 498-501.

21. De Pablo P, Dietrich T, McAlindon TE. Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. J Rheumatol 2008; 35(1): 70-6.

22. Dissick A, Redman RS, Jones M, Rangan BV, Reimold A, Griffiths GR, ET AL. Association of periodontitis with rheumatoid arthritis: a pilot study. J Periodontol 2010; 81(2): 223-30.

23. Laurell L, Hugoson A, Hakansson J, Pettersson B, Sjostrom L, Berglof FE, et al. General oral status in adults with rheumatoid arthritis. Community Dent Oral Epidemiol 1989; 17(5): 230-3.

24. Sjostrom L, Laurell L, Hugoson A, Hakansson JP. Periodontal conditions in

adults with rheumatoid arthritis. Community Dent Oral Epidemiol 1989; 17(5): 234-6.

25. Kobayashi T, Murasawa A, Komatsu Y, Yokoyama T, Ishida K, Abe A, et al. Serum cytokine and periodontal profiles in relation to disease activity of rheumatoid arthritis in Japanese adults. J Periodontol 2010; 81(5): 650-7.

ABSTRACT (IN KOREAN)

류마티스 관절염과 치주질환의 관계

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소전신

배경: 치주질환(PD)은 류마티스 관절염(RA)의 활막염과 유사한 특성을 가지고 있다. 최근 보고에서는 관절염환자에서 류마티스 관절염과 치주질환은 중요한 관계를 가지고 있다고 하지만 collagen-induced arthritis 모델에서의 치주질환에 대한 연구는 아직 없는것으로 알려져 있다. 이 연구의 목적은 RA 와 PD 의 관계를 밝히고 동물 모델에서 RA 와 PD 의

치료 반응을 관찰 하는 것이다.

방법: 8 주된 수컷, 25 마리의 DBA/1 mice 를 5 개 그룹으로 나누었다. 그중 3 개 그룹(CIA+TNFi, CIA+MTX and CIA)은 type II 콜라겐을 주사하였고 3 주후 booster injection 을 실행하였다. type II 콜라겐을 주사한 그룹 (CIA+TNFi)중에서 한그룹은 anti-tumor necrosis factor (TNF) therapy 를 실행하였고 다른 한 그룹은 methotrexate (MTX)(CIA+MTX)를 주사하였다. Collagen 을 injection 하지 않은 그룹(CONTROL)들 중에서 한 그룹은 anti-TNF-α therapy (CONTROL+TNFi) 를 실행하였다. Arthritis score 와 paw thickness 를 측정하였고 관절 절편의 조직병리학 평가도 진행하였다. Proinflammatory cytokine 와 효소들의 발현은 면역조직화학적 방법으로 평가하였다. Jaw 를 micro-CT 스캔 하였고 2D 와 3D 구조를 복원하였다. 평균 노출면적과 치아의 길이를 측정하여 PD 의 중증도을 검증하였다.

결과: anti-TNF-α치료와 MTX 는 관절염을 호전시켰고 CIA mice 의

조직병리학 결과를 호전시켰다. CIA mice 에 치근막염이 발생 하였다는 것은 micro CT 결과로 확인되였다. 치아의 평균면적의 측정결과는 CONTROL 그룹에서 CIA 그룹 혹은 CONTROL+TNFi 그룹에(0.0177 vs 0.02205 vs 0.0229 $p<0.05$) 비해 의미있게 낮았고, CIA 그룹이 CIA+MTX 그룹 혹은 CIA+TNFi 그룹에(0.02205 vs 0.02466 vs 0.0254, $p<0.05$)비해 의미있게 낮았다.

결론: 류마티스 관절염과 치근막염의 발병은 연관이 있다. 그러나 DMARD 약물은 관절염를 호전시키나 PD 는 호전시키지 않을 뿐만 아니라 악화시켰다. 향후 이 차이들의 원인을 찾아내기 위해서는 더 많은 연구가 필요할것이다.