Involvement of 11β-HSD1 in metabolic syndrome and periodontal disease

Masanori Shiraishi¹, Hirofumi Sawai², Yutaka Nagano³, Masatoshi Ueda⁴ and Makoto Umeda⁵

¹Graduate School of Dentistry (Periodontology), ²Department of Internal Medicine and ³Department of Periodontology, Osaka Dental University, 8-1 Kuzuhahanazono-cho, Hirakata-shi, Osaka 573-1121, Japan

It has been suggested that 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), a glucocorticoid-activating enzyme, plays an important role in metabolic syndrome. Although the relationship between periodontal disease and metabolic syndrome has been established, the involvement of 11β-HSD1 in periodontal disease has not been reported. This study was performed to elucidate the role of 11β-HSD1 as a new therapeutic target in periodontal disease. Periodontal tissue was obtained from patients diagnosed as having periodontal disease for the experimental group and from patients whose teeth were extracted for orthodontic treatment as the control group. The expression levels of RANKL, RANK, osteoprotegerin and 11β-HSD1 mRNA were examined by reverse transcription-PCR. There was no apparent difference in RANKL, RANK or osteoprotegerin mRNA expression between the control group and the periodontal disease group, whereas a statistically significant increase of 11β-HSD1 mRNA expression was detected in the periodontal disease group compared with the controls. Furthermore, the depth of periodontal pockets significantly correlated with the increased expression of 11β-HSD1 mRNA. These results suggest that the increased expression of 11β-HSD1 may play a role in the progression of periodontal disease. (J Osaka Dent Univ 2013; 47: 7-10)

Key words: Metabolic syndrome; Periodontal disease; 11β-HSD1; Glucocorticoid

INTRODUCTION

Glucocorticoids have various effects on metabolism and immune responses.¹² Cortisol is the major glucocorticoid hormone in humans.² 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an enzyme converting hormonally inactive cortisone to active cortisol, and has been recently suggested to be involved in tissue-specific regulation of glucocorticoid action.³⁻⁵ 11β-HSD1 is abundantly expressed in adipose tissue and liver,⁶ and recent investigations have revealed that 11β-HSD1 plays an important role in metabolic syndrome. It has been reported that the expression of 11β-HSD1 is increased in adipocytes, especially in the visceral fat.⁷⁻⁸ Transgenic mice overexpressing 11β-HSD1 selectively in adipose tissue have shown phenotypes similar to metabolic syndrome including visceral fat obesity, insulin resistance, dyslipidemia and hypertension.⁹⁻¹⁰ On the other hand, 11β-HSD1-deficient mice have been reported to be protected against metabolic diseases under overnutrition.¹¹ Since inhibitors of 11β-HSD1 ameliorated metabolic syndrome and prevented atherosclerosis in mice, it is postulated that inhibitors of 11β-HSD1 may be applied as novel therapeutics for metabolic syndrome in humans.¹²⁻¹³

The relationship between metabolic syndrome and periodontal disease has been reported.¹⁴⁻¹⁷ Although a recent report has shown that oral fibroblasts and keratinocytes express 11β-HSD1,¹⁸ the involvement of 11β-HSD1 in periodontal disease has not been reported thus far. In this study we investigated the expression levels of 11β-HSD1 in periodontal tissue from patients with periodontal disease.

MATERIALS AND METHODS

Subjects

Periodontal tissue was obtained from patients diagnosed as having periodontal disease (n = 15), while teeth extracted from patients for orthodontic treatment...
were used as controls (n = 10). All subjects understood the study and provided written informed consent. This study was approved by the ethics committee at Osaka Dental University.

RNA extraction
Total RNA was extracted from each sample of periodontal tissue using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. Briefly, each tissue sample of 50–100 mg was homogenized in 1 mL TRIzol Reagent, to which 0.2 mL chloroform was added. After centrifugation at 12,000 × g for 15 min at 4°C, the aqueous phase was transferred to a new tube. Then, 0.5 mL isopropyl alcohol was added and mixed. After centrifugation at 10,000 × g for 10 min at 4°C, the supernatant was removed and the RNA precipitate was washed with 1 mL of 75% alcohol and centrifuged at 7,500 × g for 5 min at 4°C. After the supernatant was removed, the RNA pellet was air-dried and dissolved in RNase-free water. The quantity of RNA was calculated with the absorbance at 260 nm, and the purity of RNA was assessed by the spectrometric absorbance ratio at 260 nm/280 nm.

Real-time reverse transcription-PCR
Reverse transcription-PCR was performed using TaqMan® RNA-to-C™ 1-Step Kit and StepOnePlus™ Real Time PCR System (Applied Biosystems, Foster City, CA, USA). TaqMan® Gene Expression Assays for RANKL (Hs00243522), RANK (Hs00187192), OPG (Hs01170168), 11β-HSD1 (Hs01547870) and GAPDH (Hs02758991) were purchased from Applied Biosystems. According to the manufacturer’s protocol, 1 μL of each Gene Expression Assay was mixed with 0.5 μL of RT Enzyme Mix, 10 μL of RT-PCR Mix, template (10 ng RNA) and Nuclease-free water (total volume: 20 μL). The RNA was reverse transcribed at 48°C for 15 min followed by 95°C for 10 min. PCR was performed up to 45 cycles at 95°C for 15 sec for denaturing and 60°C for 1 min for annealing/extension. The expression levels of RANK, RANKL, osteoprotegerin (OPG), and 11β-HSD1 mRNA relative to GAPDH mRNA were analyzed.

Statistical analysis
For comparison of the expression levels of mRNA between the control group and the periodontal disease group, the Wilcoxon-Mann-Whitney U-test was performed using Kaleida Graph (Synergy Software, Reading, PA, USA). Significance testing of Spearman’s correlation coefficient between the level of 11β-HSD1 mRNA and the depth of periodontal pockets was performed using SPSS software (IBM, Armonk, NY, USA).

RESULTS
As shown in Fig. 1, the expression levels of RANK, RANKL, and OPG mRNA relative to GAPDH mRNA were 0.001575 ± 0.000749, 0.000211 ± 0.000229, and 0.000665 ± 0.000777 in the control group, and 0.001653 ± 0.002726, 0.000303 ± 0.000428, and 0.00041 ± 0.000466 in the periodontal disease group, respectively. There was no statistically significant difference in the expression levels of RANK, RANKL and OPG mRNA between the control group and the periodontal disease group. On the other hand, the expression levels of 11β-HSD1 mRNA were 0.000815 ± 0.001413 in the control group and 0.003028 ± 0.004149 in the periodontal disease group. Thus, a statistically significant (p < 0.05) increase of 11β-HSD1 mRNA expression was detected in the periodontal disease group compared with the controls.

![Graph](https://example.com/graph.png)

**Fig. 1** Comparison of the levels of RANK, RANKL, OPG and 11β-HSD1 mRNA in the periodontal tissue between the periodontal disease group and the controls. The ratios of the four factors are shown relative to GAPDH mRNA. Bars indicate one SD. *p < 0.05.
Furthermore, we investigated the relationship between the depth of the periodontal pockets and the expression level of 11β-HSD1 mRNA (Fig. 2). Spearman's correlation coefficient between the depth of periodontal pockets and the expression level of 11β-HSD1 mRNA was 0.518, which was statistically significant (p<0.01). These results suggest that the increased expression of 11β-HSD1 may play a role in the progression of periodontal disease.

**DISCUSSION**

Although the relationship between metabolic syndrome and periodontal disease has been reported, the precise mechanism by which periodontal disease is associated with metabolic disease remains to be determined. The involvement of 11β-HSD1, a glucocorticoid-activating enzyme, in metabolic syndrome has been recently established. In this paper we showed that the expression level of 11β-HSD1 mRNA was significantly increased in the periodontal tissue of patients with periodontal disease. Furthermore, the expression level of 11β-HSD1 mRNA was positively correlated with the depth of periodontal pockets. These results suggest that the increased expression of 11β-HSD1 may play a role in the progression of periodontal disease.

To the best of our knowledge, this is the first report investigating the involvement of 11β-HSD1 in periodontal disease. A report showed that saliva levels of cortisol increased in periodontitis, possibly due to increased expression of 11β-HSD1 in periodontal tissue. Although the relationship between metabolic syndrome and periodontal disease is well known, the mechanism connecting the two diseases remains unclear. In this report we found an increased expression of 11β-HSD1 in periodontal disease. Since the increased expression of 11β-HSD1 in adipocytes plays a crucial role in metabolic syndrome, our data suggest that 11β-HSD1 may be the factor connecting metabolic syndrome with periodontal disease.

Metabolic syndrome is regarded as chronic inflammation of adipocytes. Involvement of 11β-HSD1 in other chronic inflammatory diseases including rheumatoid arthritis and inflammatory bowel diseases has been reported. Periodontal disease is associated with the chronic inflammation of periodontal tissue, suggesting that 11β-HSD1 may be commonly involved in chronic inflammation. The mechanism by which 11β-HSD1 induces chronic inflammation remains to be elucidated.

In conclusion, we demonstrated that the expression of 11β-HSD1 is increased in periodontal disease, and concluded that this increased expression may be involved in progression of the disease. Further investigation will be needed to elucidate the precise role of 11β-HSD1 in periodontal disease.

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