The increased ratio of 11β-hydroxysteroid dehydrogenase type 1 versus 11β-hydroxysteroid dehydrogenase type 2 in chronic periodontitis irrespective of obesity

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Abstract
11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which converts inactive cortisone to active cortisol, has been reported to play an important role in metabolic diseases as well as chronic inflammatory diseases. The involvement of 11β-HSD1 in chronic periodontitis was investigated in the present study. The relationship between the levels of 11β-HSD1, chronic periodontitis, and body mass index (BMI) was analyzed. The expression of 11β-HSD1 mRNA was significantly higher in the chronic periodontitis group than in the control group. Since the expression of 11β-HSD2, which converts active cortisol to inactive cortisone, was slightly lower in the chronic periodontitis group than in the controls, the ratio of 11β-HSD1 versus 11β-HSD2 was significantly higher in the chronic periodontitis group than in the controls. A correlation was not observed between BMI and the level of 11β-HSD1 or between BMI and the ratio of 11β-HSD1 versus 11β-HSD2. These results suggested that an increase in the ratio of 11β-HSD1 versus 11β-HSD2 was associated with chronic periodontitis irrespective of obesity.

Keywords: 11β-Hydroxysteroid dehydrogenase type 1, 11β-Hydroxysteroid dehydrogenase type 2, Chronic periodontitis, Cortisol, Obesity

Background
Cortisol, a major glucocorticoid hormone in humans, is known to have various effects on the metabolism of carbohydrates, fat, and proteins as well as immune responses: briefly, it promotes the conversion of proteins and lipids into glucose, and limits and resolves the inflammatory process (Rhen and Cidlowski 2005; Gross and Cidlowski 2008). It has been reported that salivary cortisol levels were positively associated with the extent and severity of chronic periodontitis (Genco et al.1998; Hilgert et al. 2006; Ishisaka et al. 2007; Rosania et al. 2009; Rai et al. 2011; Haririan et al. 2012; Mesa et al. 2014). With regard to serum cortisol levels, a report showed no significant differences between the chronic periodontitis patients and the controls (Mengel et al. 2002), whereas another report demonstrated that serum cortisol levels were significantly higher in patients with severe periodontitis than the controls in older Japanese adults who had never smoked (Ishisaka et al. 2008). Furthermore, it was reported that ligature-induced experimental periodontitis was more severe in Fischer 344 rats, which mount a strong hypothalamic–pituitary–adrenal (HPA) axis response leading to the increased levels of corticosterone (a major glucocorticoid in rodents), than in MHC-identical but HPA low-responding Lewis rats, suggesting the causal relationship between the increased glucocorticoid levels and severity of periodontitis (Breivik et al. 2000, 2001).

Among the many enzymes involved in the biosynthesis and catabolism of cortisol, 11β-hydroxysteroid
dehydrogenase (11β-HSD) catalyzes the conversion between active cortisol and inactive cortisone, and is involved in the tissue-specific intracellular regulation of the actions of glucocorticoids; 11β-HSD type 1 (11β-HSD1) converts inactive cortisone to active cortisol, whereas 11β-HSD type 2 (11β-HSD2) catalyzes the opposite reaction (Livingstone et al. 2000; Seckl and Walker 2001; Tomlinson et al. 2004; Chapman et al. 2013). 11β-HSD1 is known to be abundantly expressed in adipose tissue and the liver (Tomlinson et al. 2004). The expression of 11β-HSD1 was previously reported to be increased in the adipocytes, especially those in visceral fat, of patients with metabolic diseases (Bujalska et al. 1997; Rask et al. 2001; Paulsen et al. 2007). Furthermore, transgenic mice selectively overexpressing 11β-HSD1 in adipose tissue showed phenotypes similar to metabolic diseases including visceral fat obesity, insulin resistance, dyslipidemia, and hypertension (Masuzaki et al. 2001, 2003). In contrast, 11β-HSD1-deficient mice were protected against metabolic diseases with overnutrition (Kotelevtsev et al. 1997). Moreover, inhibitors of 11β-HSD1 have been shown to ameliorate metabolic diseases and prevent atherosclerosis in mice (Hermanowski-Vosatk et al. 2005; Nuotio-Antar et al. 2007). These findings suggest that 11β-HSD1 plays a crucial role in metabolic diseases, to which inhibitors of 11β-HSD1 may be applied as novel therapeutics. Indeed, several highly potent and selective 11β-HSD1 inhibitors have been developed for the treatment of metabolic diseases including type 2 diabetes mellitus (Rosenstock et al. 2010; Feig et al. 2011; Anil et al. 2014; Okazaki et al. 2014; Hamilton et al. 2015).

A relationship is known to exist between metabolic diseases and chronic periodontitis (Saito et al. 1998; Shimazaki et al. 2007; D’Aiuto et al. 2008; Morita et al. 2010). Although the expression of 11β-HSD1 was recently detected in oral fibroblasts and keratinocytes (Cirillo et al. 2011), the involvement of 11β-HSD1 in chronic periodontitis has not yet been examined. We recently reported preliminary results that the expression level of 11β-HSD1 in gingival tissues from patients with chronic periodontitis was higher than that in the controls (Shiraishi et al. 2013). In the present study, we further investigated the expression of 11β-HSD1 as well as 11β-HSD2, and analyzed the relationships between the expression of 11β-HSD1/2, chronic periodontitis, and obesity.

Methods

Participants

Periodontal tissues were obtained from patients diagnosed with chronic periodontitis (n = 38), while those surrounding teeth extracted from patients for orthodontic treatments were used as controls (n = 14). Informed consent was obtained from all individual participants (ages between 30 and 70 years) included in this study. This study was approved by the Ethics Committee at Osaka Dental University on September 16, 2009 (approval number: 90921).

Clinical periodontal examination

Chronic periodontitis was defined as probing depth of ≥3 mm, bleeding on probing, and radiographically determined alveolar bone resorption of more than one-third of the root of the tooth, whereas control group patients did not have chronic periodontitis. The dentists who performed the clinical periodontal examination were the members of the Department of Periodontology, Osaka Dental University, and therefore they used the same criteria for the diagnosis of chronic periodontitis. Periodontal tissues, including both epitheliums and connective tissues, were surgically removed when probing depth after the basic periodontal treatment (including plaque control, scaling, and root planing) was ≥4 mm. When the tooth mobility was class III in Miller classification and alveolar bone resorption was more than two-thirds of the root of the tooth at the first examination, the tooth was extracted without the basic periodontal treatment and periodontal tissues surrounding the extracted tooth were used for experiments.

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 of the 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. A total of 0.5 ml isopropyl alcohol was then 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 7500 × 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 according to 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 (RT)-polymerase chain reaction (PCR)

RT-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 11β-HSD1 (#Hs01547870),
11β-HSD2 (11β-HSD2) was also performed. The expression levels of RANKL, RANK, and OPG mRNA were also examined, since these molecules are involved in osteoclast differentiation and it was reported that the expression of RANKL was higher and that of OPG was lower in the periodontitis group than healthy control (Vernal et al. 2006; Bostanci et al. 2007; Wara-aswapati et al. 2007). As shown in Fig. 1, the values of 11β-HSD1 (Fig. 1a) and RANKL (Fig. 1c) were significantly (P = 0.005 and P = 0.014, respectively) higher in the periodontitis group than in the control group. Furthermore, the values of 11β-HSD2 (Fig. 1b) were slightly lower in the periodontitis group than in the control group. Thus, the ratio of 11β-HSD1 versus 11β-HSD2 was significantly (P = 0.001) higher in the periodontitis group than in the control group (Fig. 2). No significant differences were observed in the expression levels of RANK (Fig. 1d) and OPG (Fig. 1e) between the two groups.

Since correlations have already been reported between 11β-HSD1 expression and metabolic diseases (Bujalska et al. 1997; Rask et al. 2001; Paulsen et al. 2007) and between metabolic diseases and chronic periodontitis (Saito et al. 1998; Shimazaki et al. 2007; D’Aiuto et al. 2008; Morita et al. 2010), the relationship between the expression of 11β-HSD1 in gingival tissues and BMI as an index of obesity was analyzed in this study. Since significance testing of Spearman’s correlation coefficient between BMI and the level of 11β-HSD1 mRNA indicated P = 0.539 (Fig. 3a), and that between BMI and the ratio of 11β-HSD1 versus 11β-HSD2 indicated P = 0.656 (Fig. 3b), a correlation was not observed between BMI and the expression of 11β-HSD1 or between BMI and the ratio of 11β-HSD1 versus 11β-HSD2. Furthermore, no significant differences were observed in the level of 11β-HSD1 mRNA (Fig. 3c) or in the ratio of 11β-HSD1 versus 11β-HSD2 (Fig. 3d) between obese (BMI ≥ 25, n = 9) and non-obese (BMI < 25, n = 43) participants (P = 0.171 and P = 0.887, respectively).

Discussion
In the present study, we investigated the relationship between 11β-HSD1/2 and chronic periodontitis, and demonstrated that the expression of 11β-HSD1 as well as the ratio of 11β-HSD1 versus 11β-HSD2 was significantly increased in chronic periodontitis. To the best of our knowledge, this is the first study to investigate the involvement of 11β-HSD1/2 in chronic periodontitis. Since it has been reported that salivary and serum cortisol levels were positively associated with the extent and severity of periodontitis (Genco et al. 1998; Hilgert et al. 2006; Ishisaka et al. 2007; Rosania et al. 2009; Rai et al.

**Table 1** Characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 11)</th>
<th>Periodontitis group (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of females/males</td>
<td>6/5</td>
<td>18/20</td>
<td>0.68*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.1 ± 12.6</td>
<td>52.3 ± 11.6</td>
<td>0.43†</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.5 ± 6.3</td>
<td>163.8 ± 9.6</td>
<td>0.91†</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.0 ± 13.0</td>
<td>60.4 ± 13.7</td>
<td>0.47†</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.1 ± 3.3</td>
<td>22.3 ± 3.3</td>
<td>0.30†</td>
</tr>
</tbody>
</table>

*Except where otherwise indicated, values are the mean ± SD
*χ² test
† t test
2011; Haririan et al. 2012; Mesa et al. 2014; Mengel et al. 2002), the increased expression of 11β-HSD1 as well as the increased ratio of 11β-HSD1 versus 11β-HSD2 would result in an increase in intracellular cortisol levels, which might play a role in the pathophysiology of chronic periodontitis.

Although a correlation has already been reported between metabolic diseases and chronic periodontitis (Saito et al. 1998; Shimazaki et al. 2007; D’Aiuto et al. 2008; Morita et al. 2010), the mechanism connecting the diseases remains unclear. Although we demonstrated that the expression of 11β-HSD1 as well as the ratio of 11β-HSD1 versus 11β-HSD2 was increased in chronic periodontitis, a correlation was not observed between BMI and the expression of 11β-HSD1 or the ratio of 11β-HSD1 versus 11β-HSD2, suggesting that 11β-HSD1 might play a role in chronic periodontitis irrespective of obesity. In accordance with these results, the involvement of 11β-HSD1 in other chronic inflammatory diseases including inflammatory bowel diseases and rheumatoid arthritis has been reported (Zbankova et al. 2007; Hardy et al. 2008; Stegk et al. 2009). Thus, these results may implicate 11β-HSD1 in the pathophysiology of chronic inflammation.

Since it has been known that stress is associated with concurrent activation of the HPA axis and that there is a relationship between stress and periodontitis, the relationships between stress, cortisol and periodontitis have been investigated in several studies (Genco et al. 1998; Hilgert et al. 2006; Rosania et al. 2009; Rai et al. 2011; Haririan et al. 2012; Mesa et al. 2014; Mengel et al. 2002). Since 11β-HSD1 converts inactive cortisone to active...
cortisol intracellularly, the increased expression of 11β-HSD1 in chronic periodontitis seems to be independent of the HPA axis. However, further studies are required to determine the relationship between stress and the expression of 11β-HSD1 in chronic periodontitis.

The increased expression of RANKL in chronic periodontitis in the present study is consistent with previous findings (Vernal et al. 2006; Bostanci et al. 2007; Wara-aswapati et al. 2007). Although the expression of OPG was slightly increased in chronic periodontitis in the present study, it was reduced in two previous studies (Bostanci et al. 2007; Wara-aswapati et al. 2007). Thus, the role for OPG in chronic periodontitis remains to be determined.

There are limitations related to this study since it is a case–control study. Moreover, there are limitations of β error in this study due to a relatively small number of controls as well as obese participants.

Conclusions
We demonstrated that the increased expression of 11β-HSD1 as well as the increased ratio of 11β-HSD1 versus 11β-HSD2 was associated with chronic periodontitis. Since a correlation was not observed between body mass index and 11β-HSD1 expression or 11β-HSD1/2 ratio, the increased 11β-HSD1 expression or 11β-HSD1/2 ratio might play a role in the pathophysiology of chronic periodontitis irrespective of obesity. Further investigations will be needed to elucidate the precise role of 11β-HSD1/2 in chronic periodontitis.

Authors’ contributions
TN and AF carried out the RT-PCR experiments. HY and KI participated in the design of the study and collected samples. MU and HM participated in the design of the study and performed the statistical analysis. HS participated in the design of the study, performed the statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript.
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Competing interests
The authors declare that they have no competing interests.

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