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Research paper

Expression of neprilysin in periodontitis-affected gingival tissues

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ABSTRACT

Objective: Although the pathogeneses of Alzheimer's disease (AD) and periodontal diseases have overlapping features, including ageing and chronic inflammation, the association between AD and periodontitis remains unclear. To explore the pathogenesis of periodontitis, a comprehensive gene expression/transcriptome analysis in periodontitis-affected gingival tissues found that the AD pathway was significantly up-regulated in periodontitis-affected gingival tissues. AD-related genes, amyloid beta precursor protein (APP), interleukin-1 beta and compliment 1QA, were significantly elevated in periodontitis. In the present study, balance between mRNA expression of APP and a potent amyloid degradation enzyme, neprilysin (NEP), as well as protein localisation of APP and NEP were analysed.
Design: Eighteen periodontitis-affected and 18 clinically healthy control gingival tissues were taken from patients with severe chronic periodontitis or undergoing tooth extraction. Total RNA was purified and used for quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR). The localisation of APP and NEP was analysed by immunohistochemistry (IHC).
Results: Both APP and NEP genes were up-regulated in periodontitis-affected gingival tissues. APP-expressing macrophages and NEP-expressing neutrophils and fibroblasts, reflecting inflammatory stages, were detected in inflamed gingival tissues by IHC.
Conclusion: The up-regulation of APP and NEP mRNA levels in periodontitis-affected gingival tissues compared with healthy controls was confirmed by qRT-PCR analyses. Since NEP is one of the primary enzymes that degrades amyloid beta, increased NEP mRNA levels in periodontitis may act as an inhibitor of amyloid beta accumulation in gingival tissues, balancing increased APP mRNA expression. However, NEP has several effects including degradation of vasoactive substances; therefore, further sresearch is needed.

1. Introduction

Periodontal diseases such as periodontitis are caused by infection with periodontopathic bacteria. Repeated infection and the host immune response result in periodontal tissue destruction that is also associated with an increased risk of vascular diseases and mortality (Beck, Garcia, Heiss, Vokonas, & Offenbacher, 1996; DeStefano, Anda, Kahn, Williamson, & Russell, 1993; Grau et al., 2004; Wu et al., 2000). According to the World Health Organization, approximately 5–20% of older adults (aged ≥65 years) suffer from severe forms of periodontal disease, which if untreated can result in tooth loss (Petersen, Bourgeois, Ogawa, Estupinan-Day, & Ndiaye, 2005). Patients with Alzheimer's disease (AD) and those with periodontal diseases often share common characteristics, including advanced age and chronic inflammation, though few studies have examined the relationship between oral health in early life and AD late in life (Gatz et al., 2006; Kamer et al., 2008; Kondo, Niino, & Shido, 1994; Stein, Desrosiers, Donegan, Yepes, & Kryscio, 2007). AD is a neurodegenerative disorder that causes degenerative change, including descent of cognitive function and memory impairment, and is the most common type of dementia. It was reported that the estimated prevalence of dementia was 5.2% and the number of people with dementia was 46.78 million in the world. The prevalence of AD was estimated

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around 60–70% of dementia, which was shown to increase with aging. Moreover, the population of dementia is expected to increase exponentially in future (Alzheimer's Disease International, 2015). AD is characterized by a variety of pathological features, such as extracellular senile plaques mainly composed of amyloid beta peptide (Aβ), intracellular neurofibrillary tangles, synaptic loss and brain atrophy (Forman, Trojanowski, & Lee, 2004; Hardy & Selkoe, 2002; Selkoe, 2001). Inflammation is a crucial process in atherosclerosis and cardiovascular disease and believed to play a major role in both AD and periodontitis (Kamer et al., 2008; Rogers, 2008; Watts et al., 2008). Chronic inflammation, as measured by serum inflammatory markers including interleukin (IL)-1, IL-6, IL-10, tumour necrosis factor (TNF)-α, C-reactive protein, alpha 1-antichymotrypsin, intracellular adhesion molecule-1 and vascular cell adhesion molecule-1, is associated with an increased risk of cognitive decline (Weaver et al., 2002; Yaffe et al., 2003) and dementia (Tan et al., 2007) as well as periodontitis (Nakajima & Yamazaki, 2009). The relationship between periodontitis and AD was suggested that periodontitis is a potential risk of reading AD. The pathogens and virulence factors of periodontitis and inflammatory mediators may cause persistent inflammation in the brain resulting neurological disorder (Gaur & Agnihotri, 2015; Watts et al., 2008). In animal experiment, tooth loss due to periodontitis cause mastication disorders and malnutrition, which accelerates brain memory impairment (Yamazaki, Wakabayashi, Kobayashi, & Suzuki, 2008). In contrast, the onset and progression of AD cause a decrease in manual dexterity and difficulty of oral self-plaque control leading to poor sanitation. AD patients also have a difficulty in consultation of dental clinic and reception of dental care. These are able to be contribution factors of the cause of periodontitis (Gaur & Agnihotri, 2015; Stein et al., 2007; Watts et al., 2008). Very recently, a systematic review with meta-analysis revealed that the presence of periodontal disease was epidemiologically associated with the presence of AD (Odds ratio: OR 1.69, 95% CI 1.21–2.35), when only severe form of periodontitis was evaluated, it was more significantly associated (OR 2.98, 95% CI 1.58–5.62) (Leira et al., 2017). Thus, periodontitis and AD could be related bidirectionally from the reasons above. However, whether periodontal disease is a factor preceding dementia is not clear. In addition, little biological evidence is available regarding the relationship between periodontitis and AD.

We previously reported differential gene expression profiles in periodontitis-affected gingival tissues compared with healthy gingival tissues using microarray analyses (Abe et al., 2011). Fifteen significantly increased pathways, including the AD pathway, and four significantly decreased pathways were found (Abe et al., 2011). Using microarray pathway frequency analyses and quantitative real-time reverse transcription polymerase chain reaction analysis (qRT-PCR), we found that components of the AD pathway were significantly elevated in inflamed human gingival tissues obtained from patients with generalized chronic periodontitis, including amyloid beta (A4) precursor protein (APP), a key gene in AD (Kubota et al., 2014).

Aβ accumulation, which is produced by amyloidogenic APP processing, is considered to be one of the principal causes of AD. An Aβ degradation enzyme, neprilysin (NEP), plays a key role in regulation of Aβ. NEP is an 85–110 kDa zinc-dependent membrane metalloprotease (also known as enkephalinase, neutral endopeptidase, common acute lymphoblastic leukaemia antigen and CD10) (Brown, Greaves, Lister, Rapson, & Papamichael, 1974; Letarte et al., 1988; Schwartz et al., 1980). NEP is reported to be widely expressed in several tissues and cells including human buccal mucosal epithelium, skin and lung fibroblasts and neutrophils in blood (Braun, Martin, Ledbetter, & Hansen, 1983; Connelly, Skidgel, Schulz, Johnson, & Erdös, 1985; Johnson, Ashton, Schulz, & Erdös, 1985; Kinoshita, Awano, Yoshida, Soh, & Ansai, 2013). We re-analysed our previously reported microarray data (Abe et al., 2011) and found a tendency of increased NEP transcription levels in periodontitis-affected gingival tissues. To our knowledge, no quantitative analysis of NEP in periodontitis-affected gingival tissues has been reported. In addition, the cell type responsible for NEP expression in gingival tissues has not been determined.

The aim of the present study is to analyse the mRNA levels of APP and NEP using qRT-PCR and characterise the localisation of NEP protein in periodontitis-affected gingival tissues using immunohistochemistry (IHC). Simultaneous analysis of APP and NEP mRNA levels is valuable for consideration of their regional functions in gingival tissues.

2. Materials and methods

2.1. Participants

The study was approved by the regional ethics committee of the Faculty of Dentistry, Niigata University (27-R9-6-11), and all participants provided written informed consent prior to participating in the study. A total of 36 individuals were recruited from patients attending Niigata University Medical & Dental Hospital, Niigata, Japan. All participants were systemically healthy Japanese individuals, did not have diabetes, were not pregnant, were not current smokers and had taken no systemic antibiotics or anti-inflammatory drugs within the previous 6 months. Women accounted for 41.7% of participants. Eighteen patients with generalised severe chronic periodontitis who had received conventional periodontal treatment more than 4–8 weeks before the study were selected (group P). Another 18 individuals who were clinically periodontally healthy and had no history of periodontal disease, impacted teeth or severe dental caries were also enrolled (group H) (Table 1). The classification of American Academy of Periodontology was used as the definition to classify periodontal patients in this study (Armitage, 1999).

2.2. Collection of gingival tissue samples

Gingival tissue sampling for qRT-PCR and IHC analyses was performed as previously described (Kubota, Nomura, Takahashi, & Hara, 1996; Kubota, Matsuki, Nomura, & Hara, 1997; Nakasone et al., 2009). A total of 18 periodontitis and 18 clinically healthy gingival tissue samples were harvested. Diseased sites showed bleeding on probing, a gingival index of ≥2, a probing pocket depth

Table 1
Clinical characteristics of study participants.

	Group H (n = 18)	Group P (n = 18)
Gender (M:F)	11:7	10:8
Age (years)	27.5 ± 4.3	62.9 ± 11.0
GI	0.0 ± 0.0	2.0 ± 0.0
BOP	–	+
mean percentage of sites with BOP (%)	0.0	100.0
PPD		
mean ± SD (mm)	2.2 ± 0.8	7.1 ± 2.2
5 mm (n/%)	0/0.0	2/11.1
6 mm (n/%)	0/0.0	8/44.4
≥7 mm (n/%)	0/0.0	8/44.4
CAL		
mean ± SD (mm)	2.2 ± 0.8	7.4 ± 2.8
5 mm (n/%)	0/0.0	2/11.1
6 mm (n/%)	0/0.0	4/22.2
≥7 mm (n/%)	0/0.0	9/50.0

GI: gingival index, BOP: bleeding on probing, PPD: probing pocket depth, CAL: clinical attachment level, Values are presented as mean ± standard deviation.