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Amyloid beta (A4) precursor protein expression in human periodontitis-affected gingival tissues

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ABSTRACT

Objective: Periodontitis involves periodontal tissue destruction and is associated with chronic inflammation and ageing. Periodontitis has recently been recognised as a risk factor for Alzheimer's disease (AD). We showed upregulation of molecules in the AD pathway including amyloid beta (A4) precursor protein (APP), a key gene in AD, interleukin-1 beta (IL-1β), and complement component 1 (q subcomponent, A chain) (C1QA) in periodontitis compared to healthy tissues. Here, we quantitatively analysed the expression levels of APP, IL-1β, and C1QA and determined the localisation of APP in gingival tissues.
Design: Fourteen chronic periodontitis patients and 14 healthy participants were enrolled. Six samples of total RNA from two distinct sites of healthy and periodontitis-affected gingival tissues from three randomly selected patients were used for microarray analyses, and significant biological pathways in periodontitis were identified. Differential gene expression of APP, IL-1β, and C1QA, which belong to the AD pathway, were analysed with quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR) using samples from these 14 chronic periodontitis patients and 14 healthy controls. APP localisation was analysed with immunohistochemistry.
Results: APP, IL-1β, and C1QA mRNA levels were significantly upregulated in periodontitis-affected gingival tissues. APP was mainly localised in macrophages in gingival connective tissues underneath the epithelial layers.
Conclusions: An association between AD and periodontitis was detected with microarray and computer-aided data mining analyses. qRT-PCR identified differential gene expression in periodontitis-affected gingival tissue that may be related to AD pathogenesis. Elevated APP, IL-1β, and C1QA transcripts and APP-expressing macrophages in periodontitis-affected gingival tissues were observed, suggesting a relationship between periodontitis and AD pathogenesis.

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1. Introduction

Periodontal diseases such as periodontitis are caused by infections of 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.[1–4] Few studies have examined the relationship between oral health in early life and Alzheimer's disease (AD) late in life.[5–7] Inflammation is a crucial process in atherosclerosis and cardiovascular disease, and may also play a major role in AD and periodontitis.[8–11] Chronic inflammation, as measured by serum inflammatory markers including interleukin (IL)-1, IL-6, IL-10, tumour necrosis factor (TNF)-α, C-reactive protein, antichymotrypsin, intracellular adhesion molecule-1, and vascular cell adhesion molecule-1, is associated with an increased risk of cognitive decline[12–14] and dementia,[15] as well as periodontitis.[16] However, whether periodontal disease is a factor preceding dementia is not clear. Also, little biological evidence is available regarding the relationship between periodontitis and AD.

We previously reported comprehensive in situ gene expression analyses in periodontitis-affected gingival tissues and found 15 significantly increased and four significantly decreased biological pathways compared to healthy control gingival tissues.[17] Using microarray pathway frequency analyses, we found that components of the AD pathway are significantly elevated in inflamed human gingival tissues obtained from patients with generalised chronic periodontitis including amyloid beta (A4) precursor protein (APP), a key gene in AD. Therefore, to confirm the biological pathway data, further quantitative measurements of mRNA expression of AD-related genes including APP, interleukin-1 beta (IL-1β), and complement component 1 (q subcomponent, A chain) (C1QA), which were identified in our previous study are needed. Furthermore, as far as we are aware, APP mRNA and protein expression in gingival tissue has never been reported. Evaluation of the immunohistochemical localisation of APP and the cells producing APP in inflamed periodontitis-affected gingival tissues is also needed.

2. Materials and methods

2.1. Participants

The study was approved by the regional ethics committee of the Faculty of Dentistry, Niigata University, and all participants provided written informed consent prior to participating in the study. A total of 28 individuals were recruited from patients attending Niigata University Medical & Dental Hospital, Niigata, Japan. All participants were systemically healthy Japanese, possessed a minimum of 20 teeth with good oral hygiene, 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 46.4% of participants. Fourteen patients with generalised severe chronic periodontitis who had received conventional periodontal treatment more than 4–8 weeks before the study were selected (Group P). Two distinct gingival samples including healthy and periodontitis-affected gingiva were taken from three randomly selected patients in Group P and were used for microarray analysis (Table 1). Another 14 individuals who were clinically periodontally healthy and who had no history of periodontal disease, impacted teeth, or severe dental caries were also enrolled (Group H) (Table 2).

2.2. Collection of gingival tissue samples

Gingival tissue sampling for microarray analyses and qRT-PCR were done as previously reported.[17] A total of 14 periodontitis and 14 clinically healthy gingival tissue samples were harvested. Diseased sites showed bleeding on probing (BOP), a gingival index of ≥2, and had a probing pocket depth (PPD) and clinical attachment level (CAL) of ≥5 mm. Healthy sites had a PPD of ≤2 mm with neither CAL nor gingival inflammation. The periodontitis-affected and healthy gingival (connective and epithelial) tissue samples were obtained during periodontal flap surgery and tooth extraction, as described previously.[18]

2.3. RNA extraction

The tissue samples were immediately submerged in 500 μl of RNA stabilisation reagent (RNA later®, QIAGEN, Valencia, CA, USA) and stored overnight at 4 °C. The samples were homogenised thoroughly using a tissue homogeniser, and total RNA was isolated using the RNAiso® reagent (TaKaRa Bio Inc., Otsu, Japan). Quality control and quantitation of total RNA were determined using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) prior to the microarray experiments.

2.4. Microarray analyses

Microarray analyses and pathway frequency analyses were performed as previously described.[17] In brief, 3 μg total RNA was reverse transcribed using a GeneChip® T7-Oligo (dt) Promoter Primer Kit (Affymetrix, Santa Clara, CA, USA) and cDNA Synthesis Kit (M-MLV version, TaKaRa Bio Inc.). Synthesis of biotinylated cRNA was performed using a GeneChip® IVT Labelling Kit (Affymetrix) for in vitro transcription. Following fragmentation, 10 μg cRNA was hybridised at 45 °C for 16 h on a GeneChip® Human Genome U133 Plus 2.0 Array® (Affymetrix). GeneChips were washed and stained in the GeneChip® Fluidics Station 450 (Affymetrix). Fluorescence intensities of chips were captured with a GeneChip Scanner 3000 7G (Affymetrix) and calculated using Microarray Suite version 5.0 (MAS5.0) (Affymetrix) with the Affymetrix default settings and global scaling as the normalisation method. The data were filtered to ensure both statistical and biological significance. Values were normalised to the median signal values for each array. Genes that showed a statistically significant gap compared with the control group were selected using a paired *t*-test ($P < 0.05$). Bonferroni adjustment for multiple tests was performed. The data sets presented in the study have been deposited in the GEO database (accession no. 252252).