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Short communication

Periodontal disease level-butyric acid putatively contributes to the ageing blood: A proposed link between periodontal diseases and the ageing process

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ABSTRACT

Periodontal diseases are partly attributable to periodontopathic bacteria found in the host, whereas, butyric acid (BA) is a common secondary metabolite produced by periodontopathic bacterial pathogens. BA has been linked to oxidative stress induction while oxidative stress has long been associated with the ageing process. However, the possible link between BA-induced oxidative stress and the ageing process has never been elucidated. Here, we attempted to show the possible role of periodontal diseaselevel-BA (PDL-BA) in influencing the rat blood ageing process. We injected PDL-BA into the young rat gingiva and, after 24 h, heart blood extraction was performed. Blood obtained from PDL-BA-treated young rats was compared to untreated young and middle-aged rats. We found that cytosolic, but not mitochondrial, heme was affected 24 h post-injection. In addition, we observed that PDL-BA treatment altered blood NOX activation, NADPH-related oxidative stress components (H_2O_2 and GR), calcium homeostasis, cell death signals (CASP3 and CASP1), and age-related markers (SIRT1 and mTOR) in young rats, with some components more closely mimicking levels found in middle-aged rats. In this regard, we propose that PDL-BA may play a role in contributing to the rat blood ageing process.

1. Introduction

Periodontopathic bacteria produce secondary metabolites which are detected in high concentrations in the saliva of patients with severe periodontal disease (Yu et al., 2014) and, subsequently, are linked to periodontal disease development (Kurita-Ochiai and Ochiai, 2010). Periodontal diseases are partly attributable to periodontopathic bacteria found in the host (Cekici et al., 2014). In addition, periodontal diseases have been suggested to affect overall health and well-being, including the development of systemic diseases often found among the elderly (Seymour et al., 2007).

Butyric acid (BA) is a common secondary metabolite produced by periodontopathic bacterial pathogens and, likewise, bacterial pathogens utilize BA as a virulence factor (Kurita-Ochiai and Ochiai, 2010) wherein higher BA levels increase periodontopathic bacterial pathogenicity (Cueno et al., 2014b). Similarly, BA has previously been linked to oxidative stress induction (Cueno et al., 2013a, 2014a), whereas, oxidative stress has long been associated to the ageing process (Romano et al., 2010). This would highlight the significance of oxidative stress to the ageing process. Previous studies have shown that the blood (or certain blood components) play a significant role in the ageing process. Evidence suggests that the blood of young mice transfused to an older mice rejuvenated the tissues and organs of the older mice while the blood of older mice transfused to younger mice caused tissue and organ deficits (Scudellari, 2015). In a periodontal disease scenario when periodontal disease level-BA (PDL-BA) levels are sustained in the gingival tissue and gradually enters the bloodstream (Cueno et al., 2013a, 2014a), we hypothesize that PDL-BA may contribute to the blood ageing process. Here we begin to explore this hypothesis. A better understanding of the possible consequences of gingival PDL-BA on the host blood ageing process would highlight the possible role of periodontopathic bacteria in the host ageing process and, likewise, could shed light on the correlation of periodontopathic bacteria and certain age-related diseases.

2. Materials and methods

2.1. Animal handling and treatment

Throughout this study, we used 20 wk-old (young) and 40 wk-old (middle-age) Sprague-Dawley male rats (Nippon CLRA,

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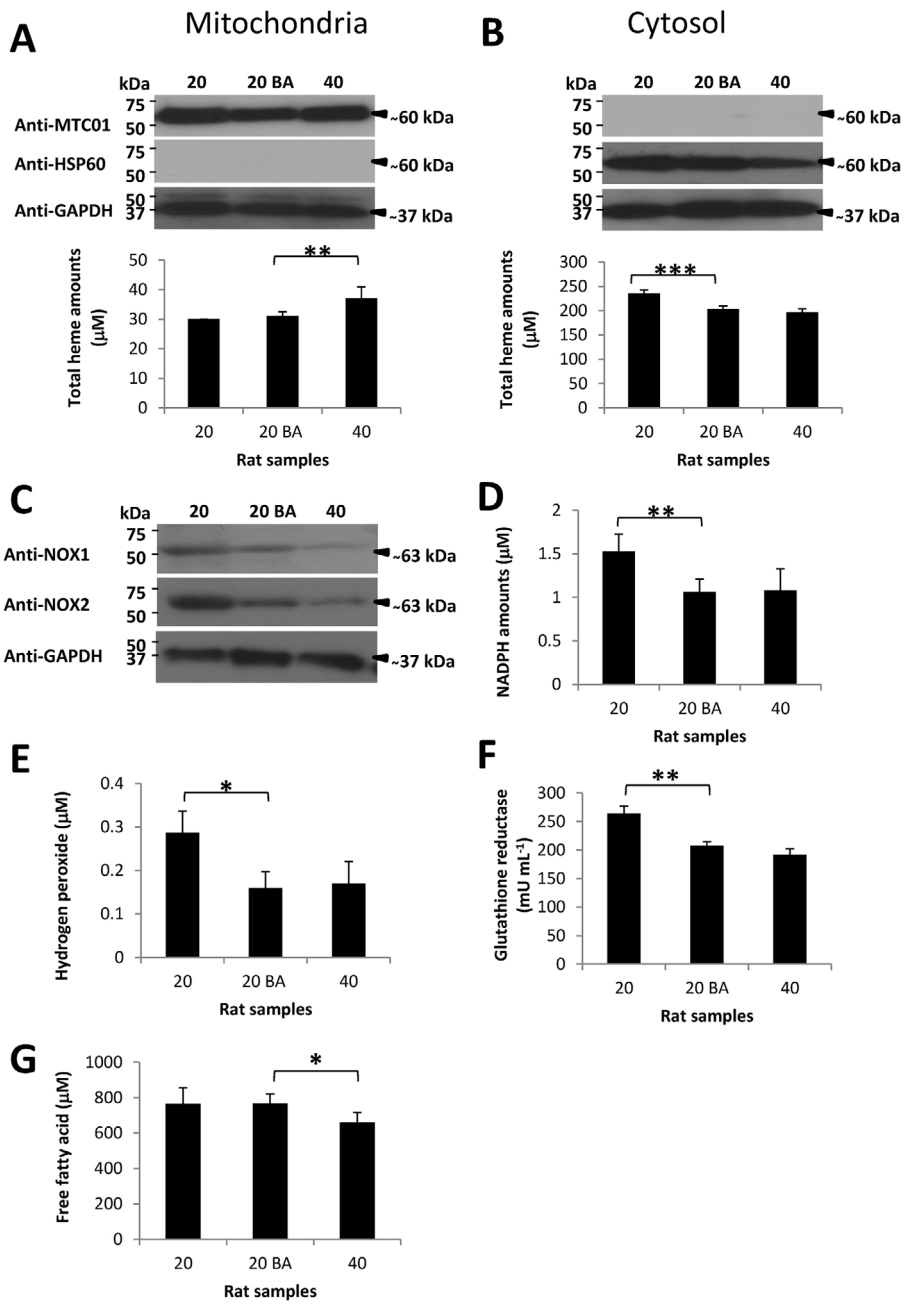

Fig. 1. Blood cytosolic heme and NADPH-associated oxidative stress components are affected among PDL-BA-treated young rats. Quantification of (A) mitochondrial and (B) cytosolic total heme amounts. (*Upper panels*) Cell fraction purity. Antibodies used to detect mitochondria-specific antibody (MTC01), cytosol-specific heat-shock protein 60 (HSP60), and the housekeeping glyceraldehydes-3-phosphate (GAPDH) are indicated. (*Lower panels*) Total heme amounts measured in the rat blood mitochondria and cytosol. (C) NADPH oxidase (NOX) detection. Antibodies used to detect blood cytosolic NOX1, NOX2, and the housekeeping glyceraldehydes-3-phosphate (GAPDH) are shown. Assay measurements of (D) NADPH, (E) hydrogen peroxide, (F) glutathione reductase, and (G) free fatty acid amounts are presented. Young rat (20), PDL-BA-treated young rat (20BA), and middle-aged rat (40) are indicated. Results shown are mean ± SE utilizing independent samples of Sprague-Dawley male rats (untreated young: n = 5; PDL-BA-treated young: n = 3; untreated middle-age: n = 7). Statistical analyses were performed using Anderson-Darling normality test and, if passed ($p > 0.05$), Student's *t* test ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

Shizuoka, Japan). All rats (young treated: n = 3, young controls: n = 5, middle-aged untreated: n = 7) were handled as previously described (Cueno et al., 2014a, 2013b) and in accordance with the guidelines for animal studies of Nihon University (AP10D023). Briefly, rats were housed in individual stainless steel cages and placed in a room under controlled temperature (23–25° C), relative humidity (40–60%), and lighting (12 h). Rats consumed 20–50 mL water and 2–5 briquettes daily which are both readily accessible. Heart blood was collected using a 25Gx1" needle while rats were under an intraperitoneal anesthesia (Pentobarbital sodium).

BA treatment simulating periodontal disease was performed as previously described (Cueno et al., 2014a, 2013b). Only young rats (n = 3) were treated. Briefly, PDL-BA (5 mM) concentration was achieved by injecting 1 M sodium butyrate (Wako) into the rat gingival tissue at a ratio of 10 μL 1 M BA per 300 g rat. Lowest possible non-lethal BA amount was determined based on the rat body weight and heart blood was collected using a 25Gx1" needle 24 h post-injection and while rats were under an intraperitoneal anesthesia (Pentobarbital sodium). For the purpose of this study, we