A-24

Butyric acid in the gingival crevice induced HEME accumulation leading to mitochondrial dysfunction

Marni E. Cueno, Kenichi Imai, Noriko Matsukawa, Takamitsu Tsukahara and Kuniyasu Ochiai

Department of Microbiology, Dental Research Center, Nihon University School of Dentistry, Tokyo, Japan

Abstract

Periodontopathic bacterial accumulation in the gingival crevice is age-related which suggests that periodontopathic bacterial products, such as butyric acid, increase concurrently with the age of the host. Butyric acid is an extracellular metabolite that is commonly produced by certain periodontopathic bacteria and is deposited in the gingival crevice. In addition, butyric acid accumulation has been associated with mitochondrial dysfunction attributable to mitochondrial oxidative stress (MOS) which would suggest that mitochondrial products, such as HEME, play a role in MOS generation. HEME is a biomolecule produced in mammalian mitochondria and serves as a subunit in several enzymes involved in varying cellular processes, including stress induction. In this study, we establish the significance of HEME in butyric acid-induced MOS leading to mitochondrial dysfunction. Throughout the study, we used blood obtained from rat models injected with butyric acid in the gingival tissue. We measured butyric acid amounts in the blood at 0, 15, 60, and 180 min after injection to establish butyric acid retention in the gingival tissue. Blood serum and mitochondria (extracted from whole blood cells) were isolated from all blood samples obtained at each timeframe. BCA protein assay was performed in all the samples to standardize protein amounts. HEME, superoxide dismutase (SOD), catalase (CAT) and hydrogen peroxide (H_2O_2) assays were measured in blood serum and mitochondria. We established that butyric acid has a higher tendency to be retained in the gingival tissue which, in turn, would imply that butyric acid accumulation would have adverse effects on gingival cells. Noticeably, butyric acid levels peaked at 60 min and, subsequently, decreased at 180 min. This suggests that butyric acid infiltration into gingival cells is highest at 60 min and eventually decreased at 180 min possibly due to blood diffusion. In blood serum samples, we observed that after butyric acid injection, all assay levels immediately decreased after 15 min and gradually normalized after 180 min. We attributed the sudden decrease in all assay levels at 15 min to the primary reaction of whole blood cells towards butyric acid presence. Likewise, we associated

the gradual normalization of all assay levels to butyric acid diffusion in the blood stream. In blood mitochondria, we found that all assays were higher at 180 min as compared to 0 min which would suggest that butyric acid has a sustained effect in blood cells, particularly blood mitochondria, and similarly induces MOS. We correlated butyric acid-induced MOS with HEME accumulation. Our results suggest that butyric acid has sustained effects in blood cells and could cause an increase in HEME, SOD, CAT and H_2O_2 levels in the blood mitochondria. Moreover, we suspect that increased HEME levels favor H_2O_2 accumulation over CAT activity which, in turn, leads to MOS and, consequentially, mitochondrial dysfunction.