ARCHIVES OF ORAL BIOLOGY 60 (2015) 789–798

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Oral administration of 5-hydroxytryptophan aggravated periodontitis-induced alveolar bone loss in rats

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ARTICLE INFO

Article history:
Accepted 31 January 2015

Keywords:
5-Hydroxytryptophan
Serotonin
Periodontitis
Alveolar bone loss
Osteoclast
Osteocyte

ABSTRACT

Objective: 5-Hydroxytryptophan (5-HTP) is the precursor of serotonin and 5-HTP has been widely used as a dietary supplement to raise serotonin level. Serotonin has recently been discovered to be a novel and important player in bone metabolism. As peripheral serotonin negatively regulates bone, the regular take of 5-HTP may affect the alveolar bone metabolism and therefore influence the alveolar bone loss induced by periodontitis. The aim of this study was to investigate the effect of 5-HTP on alveolar bone destruction in periodontitis.
Design: Male Sprague-Dawley rats were randomly divided into the following four groups: (1) the control group (without ligature); (2) the 5-HTP group (5-HTP at 25 mg/kg/day without ligature); (3) the L group (ligature + saline placebo); and (4) the L + 5-HTP group (ligature + 5-HTP at 25 mg/kg/day). Serum serotonin levels were determined by ELISA. The alveolar bones were evaluated with micro-computed tomography and histology. Tartrate-resistant acid phosphatase staining was used to assess osteoclastogenesis. The receptor activator of NF-kB ligand (RANKL) and osteoprotegerin (OPG) expression in the periodontium as well as the interleukin-6 positive osteocytes were analysed immunohistochemically.
Results: 5-HTP significantly increased serum serotonin levels. In rats with experimental periodontitis, 5-HTP increased alveolar bone resorption and worsened the micro-structural destruction of the alveolar bone. 5-HTP also stimulated osteoclastogenesis and increased RANKL/OPG ratio and the number of IL-6 positive osteocytes. However, 5-HTP treatment alone did not cause alveolar bone loss in healthy rats.
Conclusion: The present study showed that 5-HTP aggravated alveolar bone loss, deteriorated alveolar bone micro-structure in the presence of periodontitis, which suggests 5-HTP administration may increase the severity of periodontitis.

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http://dx.doi.org/10.1016/j.archoralbio.2015.01.015
0003–9969/

1. Introduction

Periodontitis-induced alveolar bone loss is the most prevalent form of bone pathology[1] and a major cause of tooth loss in adults. Although bacterial infections are the primary trigger of periodontitis, alveolar bone loss is mainly caused by the uncoupling of the normally balanced remodelling processes of bone resorption and bone formation.[2] Systematic and local factors that modulate bone remodelling can greatly influence the occurrence and severity of alveolar bone loss.

5-Hydroxytryptophan (5-HTP) is the precursor of serotonin (i.e., 5-HT). 5-HT has been known as a neurotransmitter that regulates mood and behaviour in the central nervous system for decades. Recently, growing evidence has shed light on the important role of 5-HT in the regulation of bone metabolism,[3] although debate about this issue still exists.[4] Peripheral 5-HT down regulates osteoblast proliferation and mediates the effect of low-density lipoprotein receptor-related protein 5 (LRP5).[5,6] In contrast, 5-HT up regulates osteoclast formation and deficient osteoclastogenesis has been observed in 5-HT-depleted mice.[7,8] In our previous in vitro study, 5-HT was found to activate ERK pathway and stimulate the secretion of interlukin-6 (i.e., IL-6),[9] which is an important inflammatory cytokine in periodontitis and a multifunctional regulator of bone remodelling,[10] by osteocytes. The pharmacologic inhibition of peripheral 5-HT genesis prevents the bone loss caused by ovariectomy.[5,11] In contrast, the widely used SSRI antidepressants (i.e., inhibitors of 5-HT transporters) which elevate 5-HT level, have been found to decrease bone mineral density and increase the risk of bone fracture.[12–15]

Because alveolar bone is a part of the skeletal system, if 5-HT is confirmed to regulate bone metabolism, it might also influence the progress of alveolar bone loss in periodontitis. Notably, a gene polymorphism for the 5-HT transporter was recently been found associated with aggressive periodontitis.[16] However, we still have a very limited understanding of the role of 5-HT in periodontitis. Two recent studies investigated the effects of SSRIs on periodontitis and produced conflicting results. One study by Branco de Almeida et al.[17] suggested a therapeutic effect of fluoxetine, whereas the other study by Carvalho et al.[18] reported a deteriorative effect of venlafaxine on periodontal bone loss.

As the precursor of 5-HT, 5-HTP has been widely used clinically as a 5-HT supplement for decades.[19] The aromatic amino acid decarboxylase (AAAD) exclusively and freely converts 5-HTP to 5-HT without any biochemical feedback inhibition.[20] If 5-HT can regulate bone and influence the alveolar bone loss induced by periodontitis, it is reasonable to assume the regular administration of 5-HTP would have the same effect. This potential effect is particularly important because of the large number of people who take 5-HTP as a dietary supplement to improve depression, obesity or headaches. In the present study, we tested the hypothesis that the use of 5-HTP would increase circulating 5-HT levels and thus influence the progression of alveolar bone loss in a rat model of experimental periodontitis.

2. Materials and methods

2.1. Animals

Forty male Sprague-Dawley rats between the ages of 6 and 8 weeks with body weights that ranged from 180 to 220 g were purchased from the Laboratory Animal Centre of Sichuan University. The rats were housed in standard conditions (12-h light/dark cycle and temperature 22–25 °C) with free access to food and water. All of the protocols described in this study were approved by the Institutional Ethics Committee of the State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University.

2.2. Experimental groups and the induction of experimental periodontitis (EPD)

The animals were randomly assigned into the following four experimental groups ($n = 10$ animals/group) after 7 days of acclimatization: (1) the control group (without ligature); (2) the 5-HTP group (5-HTP at 25 mg/kg/day without ligature); (3) the L group (ligature + saline placebo); and (4) the L + 5-HTP group (ligature + 5-HTP at 25 mg/kg/day). The 5-HTP was dissolved in saline solution. All treatments (saline or 5-HTP) were given orally (via gavage) 1 h before ligature attachment and daily during the experimental periods.

To induce periodontitis, rats were general anesthetized and a ligature was put in a subgingival position around the cervixes of the right maxillary first molars. The upper molars were chosen as periodontitis can be induced by ligature more rapidly in the upper molar than the lower molar.[21] The rats were killed under general anaesthesia 14 days after the attachment of the ligature. The maxillae were collected and fixed in 10% neutral formalin for 24 h and then submitted to micro-CT.

2.3. Serum 5-HT determination

Serum was harvested from blood collected from the tail vein on days 7 and 14. Serum 5-HT was measured immediately after the blood collection with an ELISA kit (CUSABIO BIOTECH, Wuhan, China) according to manufacturer's instructions. Briefly, a monoclonal antibody specific to 5-HT has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin-labelled 5-HT and unlabeled 5-HT (standards and samples) with the pre-coated antibody specific to 5-HT. After incubation (37 °C, 1 h), the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated (37 °C, 30 min) before washing. The amount of bound HRP conjugate is reverse proportional to the concentration of 5-HT in the sample. The substrate solution is added and incubated (avoid of light, 37 °C, 20 min). After adding the stop solution, run the microplate reader and conduct measurement at 450 nm immediately. The sensitivity of the ELISA kit for serotonin was 0.4 ng/ml.

2.4. Micro-CT analysis

After fixation, the maxillae were transferred to 70% ethanol for micro-CT scanning (VivaCT 40, Scanco Medical, Basserdorf,