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Differential effects of natural Curcumin and chemically modified curcumin on inflammation and bone resorption in model of experimental periodontitis

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ABSTRACT

Objective: The purpose of this study was to compare the effects of the oral administration of natural curcumin and a chemically modified curcumin (CMC2.24) on osteoclast-mediated bone resorption, apoptosis, and inflammation in a murine model of experimental periodontal disease.

Design: Fifty male rats were distributed among the following treatment groups: (i) 2% carboxymethylcellulose, (ii) CMC2.24 30 mg/kg body weight, (iii) Curcumin 100 mg/kg body weight and (iv) no treatment. Compounds were administered daily by oral intubation over a 15-day period of time. Periodontal disease was induced by injections of LPS (lipopolysaccharide) into the gingival tissues three times per week. Contralateral sides were injected with the same volume of PBS (phosphate buffered saline) vehicle. After 15 days, hemimaxillae and gingival tissues were harvested. Bone resorption was assessed by μCT (microcomputer tomography). Formalin-fixed, paraffin embedded histological sections were stained with haematoxylin/eosin (H/E) for the assessment of cellular infiltrate or subjected to immunohistochemistry for detecting TRAP (tartrate-resistant acid phosphatase)-positive cells and caspase-3. Apoptosis was assessed in the gingival tissues by DNA fragmentation.

Results: CMC2.24 and curcumin caused a significant reduction of the inflammatory cell infiltrate, however μCT analysis showed that only CMC2.24 reduced bone resorption and the number of TRAP-positive multinucleated cells (osteoclasts). Curcumin, but not CMC2.24, significantly reduced the number of apoptotic cells in the gingival tissues and of osteocytes in the alveolar bone crest.

Conclusions: The results suggest that CMC2.24 and curcumin inhibit inflammation by different mechanisms, but only CMC2.24 was capable of reducing alveolar bone resorption in the LPS-induced model of periodontitis.

1. Introduction

Natural curcumin (diferuloylmethane) is a hydrophobic polyphenol composed of a mixture of three curcuminoids: curcumin, demethoxycurcumin and bisdemethoxycurcumin with various biological activities reported (Shehzad, Park, Lee, & Lee, 2013). Diverse studies report on anti-inflammatory, anti-microbial and anti-neoplastic properties of curcumin in diverse conditions such as diabetes, cancer, auto-immune conditions and chronic inflammatory conditions including Crohn's disease and rheumatoid arthritis. Despite the promising perspectives (Di Martino et al., 2017; Kumar, Ahuja, Ali, & Baboota, 2010), clinical use of curcumin is limited because of its poor absorption in the gastrointestinal tract, short plasma half-life and low bioavailability after oral administration (Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Shoba et al., 1998).

Based on the reports of potent biological activities and on studies indicating its safety and virtual absence of unwanted side effects (Lao et al., 2006; Vernillo, Ramamurthy, Golub, & Rifkin, 1994), there is great interest in developing synthetic analogues, with a defined and consistent chemical composition and improved pharmacological properties. The so-called chemically modified curcumins (CMC) combine low toxicity and potent inhibitory activity of matrix metalloproteinases (MMPs) (Zhang, Golub, Johnson, & Wishnia, 2012), properties that justify an assessment in vivo models. CMCs are part of a class of

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polyenolic Zinc-binding inhibitors able to inhibit both MMPs and cytokines. These compounds were originally developed as 1,3-bis ketone synthetic analogues, based on the fundamental role of β-ketone as a zinc-binding region (Golub, Suomalainen, & Sorsa, 1992). The natural and some early chemically-modified curcumins are di-ketonic; however the compound tested in this and our more recent studies (Elburki, Rossa et al., 2017; Elburki, Moore et al., 2017; Elburki et al., 2014; Zhang et al., 2012) are tri-ketonic with stronger Zn^{++} binding and MMP-inhibitory properties. This tri-ketonic CMC has a phenyl-aminocarbonyl group at carbon 4 and demonstrates higher water solubility than the natural compound (unmodified natural curcumin). The potency of different formulations of newer CMCs as inhibitors of MMPs was evaluated in vitro and CMC2.24, in particular, had the lowest IC50 values (Zhang et al., 2012). In an in vitro model of inflammation-mediated cartilage destruction, CMC2.24 inhibited cartilage degradation by 96% in comparison with the vehicle control, whereas natural (unmodified) curcumin had no effect (Elburki, Rossa et al., 2017).

Evidence reported in the literature and studies by our research group using natural curcumin and CMC2.24 demonstrate a significant effect in the inhibition of inflammation and bone resorption (Correa et al., 2017; Elburki, Rossa et al., 2017; Elburki, Moore et al., 2017; Elburki et al., 2014; Guimaraes et al., 2011; Guimaraes et al., 2012; Zhou et al., 2013), which are hallmark characteristics of experimental models of periodontal disease and also of the clinical condition in humans (Dentino, Lee, Mailhot, & Hefti, 2013; Graves, Kang, Andriankaja, Wada, & Rossa, 2012; Hajishengallis, Lamont, & Graves, 2015). In spite of a significant inhibition of the inflammatory infiltrate and potent reduction of various inflammatory mediators, oral administration of curcumin had no effect on inflammatory bone resorption (Guimaraes et al., 2012), a critical and usually irreversible feature of destructive periodontal disease. Even studies that report significant curcumin-induced inhibition of bone resorption in periodontal disease models, the decrease in bone resorption severity was less than 10% in comparison with the vehicle control group (Correa et al., 2017; Zhou et al., 2013). On the other hand, an in vivo study showed that systemic administration of CMC2.24 reduced LPS-induced bone resorption in rats by more than 20% (Elburki, Moore et al., 2017). However, these studies were performed independently and there is no direct comparison between natural curcumin and CMC2.24 in a simultaneously executed experiment.

Based on data suggesting increased biological potency and superior pharmacological properties of CMC2.24 in comparison with natural curcumin, this study now describes and compares, for the first time in parallel experiments carried out simultaneously, the effects of the oral administration of natural curcumin and CMC2.24 in a murine model of LPS-induced periodontal disease. The outcomes of interest were: bone resorption, inflammation, osteoclastogenesis and apoptosis.

2. Materials and methods

2.1. Experimental design

The experimental protocol was approved by the Ethical Committee for Animal Use (CEUA) of the School of Dentistry at Araraquara – UNESP (license number 12/2011) and performed in accordance with the guidelines from the Brazilian College for Animal Experimentation (COBEA). Fifty male Holtzman rats (*Rattus norvegicus albinus, Holtzman*) of 10 to 14 weeks of age and weighing between 150 and 200 g were used in this study. Animals were kept in polypropylene cages in a room with controlled temperature (21 ± 1C) and humidity (65–70%) and a 12 h light–dark cycle. The rats were fed standard rat chow (Labina/Purina) and water *ad libitum*. All animals were submitted to injections on the palatal aspect of the first molars bilaterally: LPS was injected on left side and PBS on the right side (Elburki, Rossa et al., 2017; Elburki, Moore et al., 2017; Elburki et al., 2014; Guimaraes et al., 2012). After inhalation anaesthesia, 30 μg of lipopolysaccharide from *Escherichia coli* (strain O55:B5; Sigma Chemical Co., St Louis, MO, USA) diluted in PBS were injected into the palatal gingiva (3 uL volume per injection) using a 10 μL Hamilton-type microsyringe (Agilent). Control sides were injected with the same volume of PBS vehicle. Injections were performed three times per week for 15 days between the upper first and second molars using syringes and needles dedicated for either LPS or PBS. The animals were randomly assigned to the following four experimental groups (n = 10 animals/group) according to the compound administered systemically: (i) 2% carboxymethylcellulose (CMC2.24 vehicle control), (ii) CMC2.24 30 mg/kg body weight, (iii) Curcumin 100 mg/kg body weight and (iv) no treatment. CMC2.24 was synthesized at the laboratories of the Chemistry Department, State University of New York (SUNY) at Stony Brook (Stony Brook, NY, USA) and natural curcumin was obtained commercially (Sigma-Aldrich Co. cat# C1386, Lot# 081M1611 V). The doses of 30 mg/kg CMC2.24 and 100 mg/kg curcumin were based in our previous studies showing that both doses are effective in reducing inflammatory mediators production and inflammatory infiltrate in gingival tissue of rats with experimentally-induced periodontitis (Elburki, Rossa et al., 2017; Elburki, Moore et al., 2017; Elburki et al., 2014; Guimaraes et al., 2012; Guimaraes et al., 2011). A group treated only with corn oil (curcumin vehicle control) was not included in this study because data from our previous studies showed no effect of lipid vehicle on bone loss and inflammation (Guimaraes et al., 2011; Correa et al., 2017). Compounds were administered daily during 15 days by oral intubation beginning 24 h after the start of local PBS and LPS injections in the protocol for induction of experimental periodontal disease. Animals were euthanized by cervical dislocation under inhalation anaesthesia and the hemi-maxillae were carefully dissected. Gingival soft tissue (approximately 1.5 mm in the frontal plane × 3 mm in the sagittal plane) adjacent to the upper first molars were carefully dissected from 5 samples from each experimental condition, immediately flash-frozen in liquid nitrogen and subsequently stored at −80C until the moment of total protein extraction. The other 5 samples from each experimental condition were subjected to 10% buffered formalin fixation and subsequent histological processing for paraffin embedding (as described below) and used in histological and immunohistochemical analysis.

2.2. Microcomputer tomography (uCT)

All hemi-maxillae (with and without soft tissues) were scanned on a microcomputer tomograph (Skyscan 1176, SkyScan Aartselaar, Belgium) using 18 μm slices. Digital radiographic images of each sample were reconstructed into a three dimensional model and a standardized gray scale value was set to distinguish mineralized from non-mineralized tissues. These three dimensional images were re-oriented on the sagittal, coronal and transversal planes in a standardized manner using anatomical landmarks on molar teeth. A standardized region of interest of 9.72 mm^3 was defined, including the first molar, the anterior half of the second molar and extending medially (towards the center of the palate) approximately 1 mm from the most palatal aspect of the crown of the first molar. All image reconstruction, re-orientation and analysis of bone volume/total volume (BV/TV fraction) in the region of interest was performed using the software package of the scanning equipment (NRecon/Dataviewer/CTan/CTvo, Skyscan, Aartselaar, Belgium) by a trained examiner who was not aware of the experimental conditions of each sample.

2.3. Stereometric and morphometric analysis

The hemi-maxillae with preserved soft tissues were immersed in 10% buffered formalin fixative solution for 24 h, washed in running water decalcified in tetrasodium-EDTA aqueous solution (0.5 M, pH 8.0) for 2–3 months, under agitation at room temperature. Each specimen consisted of a section containing the three upper molars and the surrounding alveolar process and soft tissues. After inclusion in