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Systemic treatment with resveratrol and/or curcumin reduces the progression of experimental periodontitis in rats

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Background and Objective: Periodontitis is a chronic inflammatory disease of periodontal tissues that leads to the destruction of bone and other connective tissues. Resveratrol and curcumin are plant-derived substances with biological properties that may have immunomodulatory properties. This study investigated the effect of continuous administration of resveratrol and curcumin and the association of resveratrol and curcumin on the progression of experimental periodontitis in rats.

Material and Methods: Forty Wistar rats were assigned randomly to the following groups: group 1, experimental periodontitis + placebo (PL) (n = 10); group 2, experimental periodontitis + resveratrol (RSV) (n = 10); group 3, experimental periodontitis + curcumin (C) (n = 10); and group 4, experimental periodontitis + resveratrol + curcumin (COMBI) (n = 10). Periodontitis was induced in rats by tying a silk suture, as a ligature, around one of the first molars. Daily administration of the placebo solution, 10 mg/kg of resveratrol, 100 mg/kg of curcumin or 10 mg/kg of resveratrol plus 100 mg/kg of curcumin was carried out from day 0 to day 30. At the end of the relevant experimental periods, rats were killed and the specimens obtained were processed for morphometric analysis of bone loss. Gingival tissues surrounding the first molar were collected for quantification of interleukin (IL)-1β, IL-4, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) using a Luminex/MAGPIX assay.

Results: Intergroup comparisons of the morphometric outcomes revealed higher bone-loss values in the PL group ($p < 0.05$) when compared with RSV, C and COMBI groups. There was no difference in bone-loss values among RSV, C and COMBI groups ($p > 0.05$). The immunoenzymatic assay of the gingival tissue showed a lower concentration of IL-1β in the COMBI group in comparison with the PL group ($p < 0.05$). Higher values of IL-4 were demonstrated in groups RSV, C and COMBI in comparison with the PL group ($p < 0.05$). Only RSV caused a reduction in the levels of IFN-γ ($p < 0.05$). There was no difference in the concentration of TNF-α amongst the four groups ($p > 0.05$).

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Conclusion: Resveratrol and curcumin are capable of reducing alveolar bone loss in an animal model of periodontitis. This occurred when these agents were added singly or in combination with one another, but there did not appear to be either synergistic or additive effects.

Periodontitis is a chronic inflammatory condition, caused by bacteria, that leads to the destruction of supporting tissues around teeth, resulting ultimately in significant morbidity as a result of infection, pain and tooth loss. In fact, periodontitis is the single most important cause for loss of teeth in the adult population (1–4). So-called periodontal pathogenic bacteria are considered to be necessary, but not sufficient, to cause periodontitis. Inflammatory cell infiltration occurs in periodontitis and the cells brought into the region include T lymphocytes, macrophages and, most importantly, polymorphonuclear neutrophils, which constitute the majority of the inflammatory cell infiltrate. Polymorphonuclear neutrophil-mediated production of proteases and reactive oxygen species (ROS) leads to progressive tissue destruction around teeth (5–8), and ROS production leads to recruitment of osteoclasts that resorb the surrounding bone (9–11). Periodontal inflammation is also mediated by several cytokines that play a major role in bone resorption (8,12). Treatment of periodontitis by targeting the periodontal pathogenic bacteria has in general led to good results in stopping the progression of the disease, but not in all cases and probably not as robustly as might be preferred. Consequently, various investigators have focused on the notion that down-regulation of the inflammatory response could lead to even more improvement in patients with periodontitis. These approaches have included, but are not limited to, the use of nonsteroidal anti-inflammatory drugs (13) to reduce production of the proinflammatory mediator prostaglandin E, and the down-regulation of matrix metalloproteinases (MMPs) to prevent connective tissue breakdown (14,15) and to neutralize ROS, which in turn also inhibits MMP production/activity, thereby reducing MMP-mediated connective tissue breakdown even more (16). More recently, host modulation or immunodulation with the use of natural plant-derived products has been explored. Two plant-derived substances – curcumin and resveratrol – are of particular interest (17–22).

Resveratrol (3,4′,5-trihydroxystilbene) is a nonflavonoid polyphenol antifungal present in at least 72 plants and also in foods that are found commonly in the human diet, including grapes, cranberries and peanuts (23). It is also found in red wine and is believed to explain, at least in part, the French paradox (24–27). Resveratrol is a pleiotropic molecule and as such has attracted great attention because it possesses several biological properties. These include improvement of metabolic control in diabetes (28) and anticancer activity (29). It has also been shown to be a potent antioxidant (28,30), and importantly it not only neutralizes ROS but, probably as a result of the fact that it is an antagonist of the aryl hydrocarbon receptor in bone and other tissue (31–34), it actually inhibits the synthesis of CYP450, one of the enzymes responsible for producing ROS (35). These properties alone could explain how resveratrol might protect against damage caused by inflammation and associated ROS. Moreover, there is also information indicating that it induces osteoblastogenesis, a quality that would act against the bone loss seen in periodontitis (36,37). The ability of resveratrol to reduce periodontal disease progression was recently demonstrated in rats (19).

Curcumin, from the root of the turmeric plant *Curcuma longa*, is an extended pseudosymmetric polyphenol (diferuloylmethane) (38,39). In recent years, *in vitro* and *in vivo* research has suggested that the substance has anticarcinogenic, antiviral, antioxidant and anti-inflammatory effects (40–42). Zhou *et al*. (43) demonstrated that curcumin prevents bone loss in an experimental periodontitis model. However, these findings have not been confirmed by others (44). In association with studies that do demonstrate an effect on prevention of bone loss, these investigations have shown that curcumin impacts on inflammation profoundly by inducing marked reductions in the development of an inflammatory infiltrate within the periodontal lesion while also stimulating an increase in the content of collagen as well as greater numbers of fibroblastic cells within the periodontium and associated lesions when curcumin was administered daily to rats with experimentally induced periodontitis (43,44).

It has been shown that the combination of resveratrol and curcumin might have additive, or possibly even synergistic, effects with respect to host modulation (i.e. down-regulation of inflammation). Combined use of these two agents has been shown to reduce the levels of proinflammatory cytokines (45,46), and also to lead to increased antioxidant activity (47), as well as anti-cancer activity (22,48–52). In fact, in a recent study, it was shown that a combination of resveratrol and curcumin inhibits carcinogenesis *in vivo* (53). Similarly, the combination of resveratrol and curcumin has demonstrable synergistic cardioprotective activity (54).

Considering the evidence above, this study investigated the effects of continuous systemic administration of both resveratrol and/or curcumin on the development and progression of bone and attachment loss around teeth in a rat model of experimental periodontitis. In addition to studying morphologically determined loss in the bone and soft tissues surrounding affected teeth, changes in the levels of pro- and anti-inflammatory cytokines

in response to curcumin and/or resveratrol were also measured. The general hypothesis is that the use of these compounds (i.e. resveratrol and/or curcumin) on their own should down-regulate biomarkers of inflammation that are otherwise up-regulated in periodontal tissues affected by periodontitis. It is hypothesized further that when these compounds are used in combination, there will actually be additive, and possibly synergistic, inhibition of inflammation and associated destruction of periodontal tissues.

Material and methods

Animals

Forty adult (10 wk of age) male Wistar rats (of 200–300 g) were used. The rats were acclimatized for 15 d before use and they were kept in temperature-controlled cages, exposed to a 12-h light/12-h dark cycle, and had free access to water and food *ad libitum* (Labina; Purina, Paulinia, São Paulo, Brazil) in the Bioterium (accredited Animal Facility) of Paulista University. The experimental procedure was approved by the Paulista University Institutional Animal Care and Use Committee (205/13 CEP/ICS/UNIP).

Experimental design

Treatment groups—The rats were assigned randomly to one of the following treatment groups: group 1, experimental periodontitis + placebo (PL) (n= 10); group 2, experimental periodontitis + resveratrol (RSV) (n= 10); group 3, experimental periodontitis + curcumin (C) (n= 10); and group 4, experimental periodontitis + resveratrol + curcumin (COMBI) (n= 10). Group 1 received a placebo solution; group 2 received 10 mg/kg of resveratrol (19); group 3 received 100 mg/kg of curcumin (43); and group 4 received both 10 mg/kg of resveratrol and 100 mg/kg of curcumin: the therapies were administered daily, via gavage, for 30 d (i.e. from day 0 to day 30). A stock solution of resveratrol (R5010-500MG; Sigma-Aldrich, São Paulo, São Paulo, Brazil) (molecular weight = 228.2) was prepared in 100 mL of Tween-80 (P4780; Sigma-Aldrich) and a stock solution of curcumin (C1386-50G; Sigma-Aldrich) was prepared in 9% ethanol (absolute ethyl alcohol; Merck KGaA, Darmstadt, Germany) and further dilutions of both were made in water to obtain the concentrations required for this investigation. The placebo solution was composed of the same quantities of Tween-80 (Dopalen; Agribrands, Paulınia, São Paulo, Brazil) and water, as used in the preparation of resveratrol. The rats were evaluated daily throughout the experiment to check for possible clinical or toxicological symptoms.

Rat periodontitis model—To induce experimental periodontitis, one of the mandibular first molars of each rat was assigned randomly to receive a cotton ligature (Corrente Algodão 10; Coats Corrente, São Paulo, São Paulo, Brazil) in a cervical position that was then tied submarginally. The ligatures were kept in position to allow biofilm accumulation over 30 d. So that the contralateral tooth could be used as an internal control, it was not ligated. This procedure was performed under general anesthesia by intramuscular administration of ketamine hydrochloride (Dopalen; Agribrands) (0.5 mL/kg) and xylazine hydrochloride (Rompun, Bayer, São Paulo, São Paulo, Brazil) (10 mg/kg).

Euthanasia and collection of specimens—Thirty days after the induction of experimental periodontitis, the rats were killed by CO_2 inhalation. The mandibles were excised for morphometric analysis. Three millimeters of gingival tissue from the area surrounding the lower first molar affected by experimental periodontitis, as well as tissue from the control site, were also collected for analysis and quantification of immune-inflammatory mediators using the Luminex/MAGPIX assay (MAGPIX; Luminex via MiraiBio, Alameda, CA, USA).

Measurement of alveolar bone loss—After gingival dissection, the mandibles were defleshed by immersion in 8% sodium hypochlorite for 4 h. The specimens were washed in running water and dried immediately with compressed air. To distinguish the cemento–enamel junction, 1% aqueous methylene blue solution (MAGPIX; Luminex via MiraiBio) was applied to the specimens for 1 min, which were then washed in running water. Photographs were taken with a 6.1-megapixel digital camera (EOS 40D; Canon, New York, NY, USA) that was placed on a tripod to keep the camera parallel to the ground at the minimal focal distance. The specimens were fixed in wax with their occlusal planes parallel to the ground and long axes perpendicular to the camera. Photographs of the buccal aspects were taken of both test and control sides. To validate measurement conversions, all specimens were photographed alongside a ruler (unit: millimeters) (19). Alveolar bone loss was assessed on the buccal surface of the lower first molars by measuring the distance of the cemento–enamel junction from the alveolar bone crest at three sites equidistant from one another (Fig. 1). The average alveolar bone height of each tooth was calculated. A single examiner, who was unaware of the experimental manipulations, carried out morphometric measurements of alveolar bone loss. The measurements were performed after intraexaminer calibration by evaluating 10 images, not taken for this study, that show alveolar bone loss similar to that in the present study. The examiner took the linear measurements of all photographs twice within 24 h. The intraclass correlation showed 95.2% reproducibility.

Immunoenzymatic assay—The collected tissues were placed in sterile tubes containing 400 μL of phosphate-buffered saline and 0.05% Tween-20. All samples were stored at −20°C. Next, the tissue was weighed, then cut into small pieces (1–2 mm^3) using scissors, solubilized in phosphate-buffered saline to a final concentration of 100 mg of tissue/mL and mixed for 10 min using a vortex mixer. Then, each sample was centrifuged at 370 ***g*** for 5 min, and the supernatant was collected, divided into aliquots (2 aliquots of 100 mL) and stored at

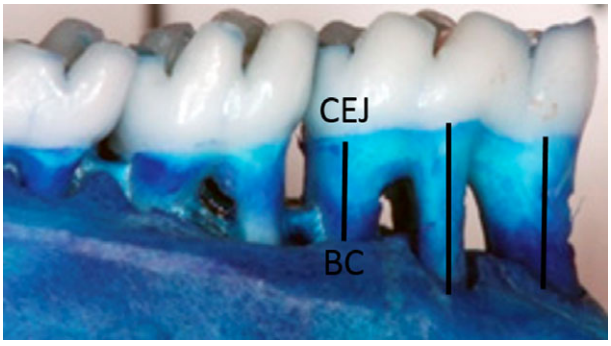

Fig. 1. Diagram representing the morphometric measurements. Vertical lines on the roots show the linear measurement from the cemento–enamel junction (CEJ) to the bone crest (BC).

70°C until required for use. To minimize protease activity, the procedures were carried out at 4°C. The levels of interleukin (IL)-1β, IL-4, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) were measured using the Luminex/MAGPIX system and commercially available kits for the various analytes (RCYTOMAG-80K; Millipore, Billerica, MA, USA). In all cases, the manufacturers' instructions were followed. Samples were diluted in the diluents of the kits. The dilution was taken into consideration when calculating the concentration of each substance with reference to a standard curve, prepared using the standard proteins in the kit. The standard curve range used for measurement of IL-1β was 2.4–10.000 pg/mL, for IL-4 it was 4.9–20.000 pg/mL, for IFN-γ it was 14.6–60.000 pg/mL and for TNF-α it was 2.4–10.000 pg/mL.

Statistical analysis

To test the null hypothesis that resveratrol or curcumin (alone or in combination) had no influence on alveolar bone loss or on interleukin levels, intergroup analysis was performed using an ANOVA (after adjustment of the data to normal distribution – $\log_{10}$ – for the Shapiro–Wilk test) followed by Tukey's test. In addition, a paired Student's *t*-test was used for intragroup comparisons between ligated and unligated teeth. The significance level established for all analyses was 5% [Statistical Analysis System (SAS) 9.3, Cary, NC, USA].

Results

Clinical analysis

The rats showed no signs of systemic illness, nor did they lose weight, throughout the experimental period. No deaths occurred. Clinical examination performed at the time of death revealed signs of gingival inflammation, including color/volume changes and bleeding around the ligated teeth of all groups; in contrast, there were no signs of inflammation around the nonligated sites (contralateral teeth).

Morphometric results

Using intragroup analysis, significant differences in alveolar bone loss between unligated and ligated teeth were demonstrated in all groups, such that 0.15 mm (average value; Table 1) more bone was lost from ligated sites than from nonligated sites ($p < 0.05$). Intergroup comparisons of the ligated mandibular molars revealed significantly higher bone-loss values in the PL group compared with the RSV, C and COMBI groups (the RSV group had 0.1 mm less bone loss, the C group had 0.08 mm less bone loss and the COMBI group had 0.09 mm less bone loss, respectively, than the PL group; Table 1) ($p < 0.05$). However there were no significant differences between any of the groups treated with resveratrol or curcumin, either alone or in combination ($p > 0.05$). Considering intergroup comparisons of alveolar bone loss in unligated teeth, no statistically significant differences were noted ($p > 0.05$). The morphometric findings are shown in Table 1 and Fig. 2.

Table 1. Alveolar bone loss (in mm) for ligated and unligated teeth

Group	Ligated		Unligated	
PL	1.41	0.07	1.19	0.12
RSV	1.31	0.06*	1.21	0.08†
C	1.33	0.05*	1.18	0.17†
COMBI	1.32	0.04*	1.18	0.09†

Values are given as mean SD.
*Significant difference from placebo.
†Significant difference from the ligated side (two-way ANOVA/Tukey test; $p < 0.05$).
C, experimental periodontitis + curcumin; COMBI, experimental periodontitis + resveratrol + curcumin; PL, experimental periodontitis + placebo; RSV, experimental periodontitis + resveratrol.

Gingival tissue IL levels

The concentrations of interleukin-1β, interleukin-4, interferon-c and tumor necrosis factor-a in gingival tissue obtained from rats in each group are shown in Table 2; treatment with the combination of resveratrol and curcumin resulted in a significant reduction in the levels of IL-1β ($p < 0.05$), compared with placebo, in both ligated and unligated sides (Table 2). Intragroup comparison showed a significant difference between the levels of IL-1β on ligated and unligated sides ($p < 0.05$). Although there did not appear to be either additive or synergistic effects between resveratrol and curcumin with regard to the levels of IL-4 ($p < 0.05$) (Table 2), lower levels of this cytokine were observed in the PL group in comparison with the other experimental groups ($p < 0.05$) on the ligated and unligated sides (Table 2). The levels of IFN-γ were reduced significantly in RSV-treated rats compared with placebo rats in the ligated side ($p < 0.05$) (Table 2). Intragroup comparison showed that there was a statistically significant difference between the levels of IFN-γ on ligated and unligated sides ($p < 0.05$). Regarding TNF-α, there were no significant differences between any of the groups for ligated and unligated sides ($p > 0.05$) (Table 2).

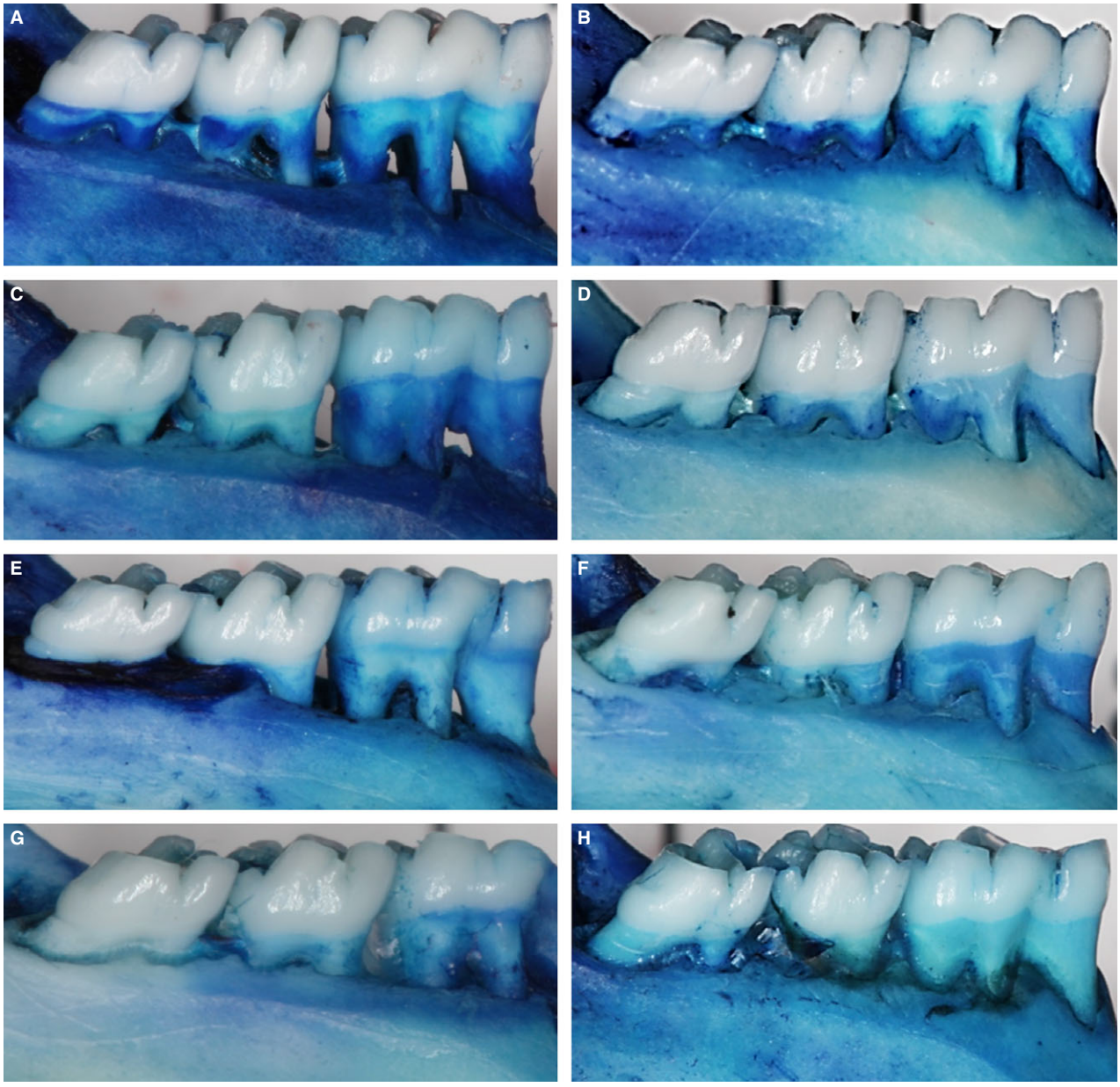

Fig. 2. Representative photographs illustrating the morphometric findings of the groups. (A) Experimental periodontitis + placebo (PL) group: ligated teeth; (B) PL group: unligated teeth; (C) Experimental periodontitis + resveratrol (RSV) group: ligated teeth; (D) RSV group: unligated teeth; (E) Experimental periodontitis + curcumin (C) group: ligated teeth; (F) C group: unligated teeth; (G) Experimental periodontitis + resveratrol + curcumin (COMBI) group: ligated teeth; (H) COMBI group: unligated teeth.

Discussion

Plant-derived substances have been used as treatment alternatives in immunomodulatory therapy. Resveratrol and curcumin are polyphenols, which may modulate the host response in the presence of experimental periodontitis. Some studies have shown the effect of these substances alone, but there is no information available on the effects of the combination of resveratrol and curcumin on the progression of experimental periodontitis. In this investigation, it was not possible to demonstrate either additive or synergistic effects of resveratrol and curcumin, when delivered concurrently, on alveolar bone loss caused by experimental periodontitis; this was despite some additive effects on the production of IL-1β, an important proinflammatory modulator. The administration of resveratrol and curcumin, alone or in combination, appeared to enhance the levels of IL-4, whereas resveratrol alone, and not curcumin, reduced the levels of IFN-γ. It is noteworthy that although no significant difference was found in the amounts of IL-1β when resveratrol and curcumin were used alone, a trend toward lower levels of IL-1β in these groups was observed when compared with the control group.

Table 2. Concentrations of interleukin (IL)-1β, IL-4, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) (in pg/mL), as measured using the multiplex assay

	IL-1β				IL-4				IFN-γ				TNF-α			
Group	Ligated		Unligated		Ligated		Unligated		Ligated		Unligated		Ligated		Unligated	
PL	1.22	0.31	0.83	0.39†	0.02	0.01	0.04	0.02	406.83	540.32	124.05	84.78†	0.04	0.03	0.03	0.01
RSV	0.87	0.57	0.66	0.53	0.26	0.22*	0.14	0.24*	81.03	117.62*	174.48	84.90	0.05	0.02	0.04	0.03
C	0.94	0.45	0.63	0.72	0.15	0.20*	0.18	0.18*	196.37	164.36	132.43	149.84	0.04	0.03	0.07	0.06
COMBI	0.52	0.30*	0.33	0.37*	0.13	0.09*	0.22	0.08*	191.45	134.58	122.36	93.11	0.04	0.01	0.06	0.04

Values are given as mean SD.

*Significant difference from placebo.

†Significant difference from the ligated side (two-way ANOVA/Tukey test – after adjustment of the data to a normal distribution – $\log_{10}$ for Shapiro–Wilk test; $p < 0.05$).

C, experimental periodontitis + curcumin; COMBI, experimental periodontitis + resveratrol + curcumin; PL, experimental periodontitis + placebo; RSV, experimental periodontitis + resveratrol.

Resveratrol administered alone led to reduced loss of alveolar bone in experimental periodontitis when compared with placebo. These results are in agreement with previous studies carried out in our laboratory (19). Similarly, it has also been shown, using a *Porphyromonas gingivalis*-ligature-induced periodontitis model in diabetic mice, that resveratrol caused decreases in alveolar bone loss and also reductions in the levels of IL-1β, IL-6, IL-8, TNF-α and toll-like receptor compared with the control (55). In addition, resveratrol significantly downregulating TLR4 expression and TLR4 downstream signaling activation. In agreement with our findings, Tamaki *et al*. (56) observed that resveratrol intake reduced alveolar bone resorption and reduced the levels of *IL1β*, *IL6* and *TNFα* mRNAs. An *in vitro* study (57), using human periodontal ligament cells stimulated with lipopolysaccharide of *P. gingivalis*, showed that treatment with resveratrol reduced the production of proinflammatory cytokines and nitric oxide (a cytotoxic substance produced by bacteria). In the current study, resveratrol administered alone modulated the production of IFN-γ, a proinflammatory cytokine related to the severity of periodontitis (58).

In addition, treatment with curcumin during experimental periodontitis has been shown by others to suppress the expression of RANKL/RANK/osteoprotegerin (43), as well as to reduce the concentrations of IL-6 and TNF-a in gingival tissues (59) – actions that would suppress the formation of osteoclasts. The biological mechanisms underlying the effects of curcumin seem to involve regulation of various molecular targets, including transcription factors (such as nuclear factor-κB), growth factors (such as vascular endothelial growth factor), cytokines (such as TNF-α, IL-1 and IL-6), protein kinases and other enzymes (such as cyclooxygenase-2 and 5-lipoxygenase). Curcumin has also been shown to reduce the formation of inflammatory mediators, such as IL-1β, in articular chondrocytes (60) and has antagonist activity against proinflammatory cytokines (61). In any case, given the effects of curcumin, noted above, this should conceivably lead to reductions in osteoclastogenesis, and this effect on osteoclasts should be analyzed in future investigations that focus on the generation and regulation of the levels of reactive oxygen species, given that periodontitis is a disease largely mediated by oxidative stress effects on RANKL-induced osteoclastogenesis, whilst both curcumin and resveratrol, in particular, have potent antioxidant activity (7,8,35,62,63). Accordingly, as observed in this study, although there were no additive effects when resveratrol and curcumin were administered concurrently, the administration of curcumin alone did lead to reductions in bone loss when compared with placebo. Zhou *et al*. (43) also observed reduced alveolar bone loss with curcumin treatment using a ligature-induced periodontitis model in rats and hypothesized that these effects were mediated by suppression of the expression of RANKL/RANK/osteoprotegerin and the reduction of proinflammatory cytokines (TNF-α and IL-6). Another study verified the influence of curcumin in a lipopolysaccharide-induced periodontitis model (59). The results show that curcumin totally blocked expression of prostaglandin E2 and produced a dose-dependent inhibition of IL-6 and TNF-α in the gingival tissues. However, in the present study, curcumin administered alone did not modulate the expression of any cytokine.

This is the first study to evaluate whether or not the combination of resveratrol and curcumin interacted in some way with regard to periodontitis: neither synergistic nor additive effects on reduction of the alveolar bone loss in experimental periodontitis was demonstrated following treatment with these two agents. Some *in vitro* studies have demonstrated synergistic effects in relation to reduction of the levels of proinflammatory cytokines (45,46), as well as synergistic antioxidant activities (47). Other investigations have also demonstrated that resveratrol and curcumin have synergistic anti-cancer activity (22,49–53,64), as well as cardioprotective activity (54). In fact, with respect to carcinogenesis, Malhotra *et al*. (53) conducted an *in vivo* study to verify the effects of curcumin and resveratrol in lung cancer. Their results showed that chemopreventive synergism

occurred and involved modulation of p53 hyperphosphorylation and regulation of caspases and other enzymes related to cellular metabolism. In our study, experimental periodontal disease led to an increase in the levels of IL-1β in the PL group. However, the use of resveratrol and curcumin alone modulated the levels of IL-1β in the ligated side, resulting in no differences being found between ligated and unligated teeth. Notably, the most impressive results were observed when resveratrol and curcumin were combined, leading to reductions in the levels of IL-1β on both ligated and unligated sides compared with the PL group. Although these findings differ from those reported in other laboratories, our data do parallel the findings reported by Csaki *et al.* (45) and Shakibaei *et al.* (46) on the synergistic effects of resveratrol and curcumin with regard to inhibition of nuclear factor-κB leading to inhibition of proteasome activity (by resveratrol) and/or inhibition of inhibitor of NF-κB - nuclear factor κB-kinase activation (by curcumin). Inhibition of nuclear factor-κB suppresses matrix-degrading gene products (MMP-3 and MMP-9), angiogenesis and inflammation-related gene products (vascular endothelial growth factor and cyclooxygenase-2). Even with only some evidence about the putative synergistic effects of resveratrol and curcumin, it might still be hypothesized that the absence of modulation of the other cytokines tested here, in the presence of experimental periodontitis, is a consequence of the similarities in the mechanistic pathways of resveratrol and curcumin, suggesting similar mechanisms of action.

In any event, it can be concluded that resveratrol and curcumin do not have synergistic effects on the inhibition of the progression of experimentally induced periodontitis. However, and very importantly, both agents caused significant reduction in inflammation-mediated destruction of periodontal soft tissues and bone. Given what is already known about the combined effects of these molecules, longer term studies in models of experimental periodontitis might be required to elucidate their effects more clearly. This could lead to the development of more effective prevention approaches for periodontitis in humans.

Conflict of interest

There is no conflict of interest to declare.

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