Journal of
PERIODONTAL RESEARCH

J Periodont Res 2011; 46: 697–703

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2011.01392.x

Histone deacetylase inhibitors and periodontal bone loss

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Cantley MD, Bartold PM, Marino V, Fairlie DP, Le GT, Lucke AJ, Haynes DR. Histone deacetylase inhibitors and periodontal bone loss. J Periodont Res 2011; 46: 697–703. © 2011 John Wiley & Sons A/S

Background and Objective: Bone loss caused by enhanced osteoclast activity is a significant feature of periodontitis. Histone deacetylase inhibitors (HDACi) can suppress osteoclast-mediated bone loss *in vitro* and *in vivo*. This study investigated whether HDACi can suppress bone loss in experimental periodontitis.

Material and methods: Experimental periodontitis was induced in mice by oral inoculation with *Porphyromonas gingivalis* bacteria. Mice were treated orally with olive oil alone, with olive oil and a novel compound – 1179.4b – which targets both Class I and Class II histone deacetylases (HDACs) or with olive oil and MS-275, which targets Class I HDACs. Micro-computed tomography scans of live mice, stereo imaging and histological analyses were used to detect changes in bone.

Results: In the absence of treatment there was a 13.2% increase in bone volume in controls compared with a 7.4% decrease in *P. gingivalis*-inoculated mice. 1179.4b significantly reduced bone loss, with a 3.4% increase in bone volume ($p < 0.01$). MS-275 did not have a significant effect on *P. gingivalis*-induced bone loss. Histological analysis revealed that 1179.4b reduced bone loss despite having no effect on inflammation.

Conclusion: HDACi were found to effectively suppress bone loss in the mouse model of periodontitis. 1179.4b – the inhibitor of Class I and Class II HDACs – was more effective at suppressing bone loss than MS-275, which targets Class I HDACs only. These compounds may therefore have the potential to be used for the management of periodontitis.

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Key words: histone deacetylase inhibitor; periodontitis; osteoclasts; bone resorption

Accepted for publication May 24, 2011

A hallmark of periodontitis is pathological bone loss that, if left untreated, can ultimately result in tooth loss. This bone loss is attributed to enhanced osteoclast formation and activity (1). During the inflammatory process of periodontitis, a large array of cytokines, including interleukins 1, 6, 11 and 17, and tumor necrosis factor-α (TNF-α), are produced, which are reported to up-regulate the expression of RANKL by fibroblasts and lymphocytes (2,3). Elevated RANKL leads to enhanced production of osteoclasts, which degrade the alveolar bone. Enhanced osteoclast numbers have been reported in human and animal models of periodontitis and are associated with increased RANKL expression and corresponding low levels of its natural inhibitor, osteoprotegerin (OPG) (4–6). Enhanced degradation of bone by osteoclasts is becoming an important target to treat pathological bone loss. Denosumab, a monoclonal antibody to RANKL, has recently been approved by the US Food and Drugs Administration (FDA) for use in postmenopausal women at risk of osteoporosis (7).

Many current treatments for periodontitis target only the infection and inflammation. Despite recent advances in understanding the cellular and molecular processes of bone destruction, there are few effective treatments that directly target the alveolar bone destruction. Hence, therapeutics directly targeting osteoclastic bone

destruction may be useful adjuncts to current treatments. One potential treatment to directly target bone destruction, and possibly inflammation, in periodontitis is histone deacetylase inhibitors (HDACi) (1).

Histone deacetylases (HDACs) play a role in the regulation of histone and nonhistone proteins. There are two main classes of HDACs, Class I and Class II. Class I includes HDACs 1, 2, 3 and 8, which are primarily found in the nucleus. Class II includes HDACs 4, 5, 7 and 9 that belong to class IIa HDACs and HDACs 6 and 10 belonging to class II b HDACs, which are able to shuttle between the nucleus and the cytoplasm. These enzymes remove acetyl groups, originally added to lysine amino acids by histone acetyltransferases, causing the chromatin to become condensed, resulting in the repression of genes, including inflammatory genes and cell-cycle genes, along with numerous others that can affect a variety of cellular functions. Although this is the major function of HDACs, lysine deacetylation is now known to be a significant post-translational modification of many nonhistone proteins, such as nuclear factor-κB (NF-κB) (8); (9–11). NF-κB can be reversibly acetylated, which has positive and negative regulatory effects on its action (9). This highlights the diverse role that post-translational modifications can play in the regulation of many important proteins.

Inhibition of HDACs has been shown to up-regulate cell-cycle inhibitors, down-regulate immune stimulators and repress inflammatory cytokines, such as TNF-α (12), a major cytokine associated with periodontitis (13); and a range of HDACi [suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA) and depsipeptide (FR901228)] have also demonstrated osteoclast suppression *in vitro* (14–16). HDACi have also recently been reported to have potential applications for use in restorative dentistry (17).

Interestingly, inhibitors of HDACs have also been shown to stimulate osteoblast maturation (18,19). Using cultures of primary osteoblasts (MC3T3-E1), HDACi TSA was shown to accelerate mineralization and increase expression of osteoblast genes, including type I collagen, osteopontin, bone sialoprotein and osteocalcin (18). This property of HDACi could potentially aid in the repair of mineralized tissue in the periodontium.

Direct suppression of osteoclasts by HDACi [SAHA, TSA and depsipeptide (FR901228)] *in vitro* has been demonstrated via suppression of osteoclast factors, including c-Fos, nuclear factor of activated T cells c1 (NFATc1) and by the induction of inhibitory factors, including interferon-β (14,20,21). We have recently investigated the effects of a novel HDACi (1179.4b), which targets Class I and Class II HDACs, and the effects of MS-275, which targets Class I HDACs, on the formation and activity of human osteoclasts *in vitro*. 1179.4b was found to reduce human osteoclast formation and activity *in vitro* with a half-maximal (50%) inhibitory concentration (IC_{50}) of < 0.16 nM (20). This appeared to occur via the suppression of TNF receptor-associated factor 6 (TRAF-6), which resulted in reduced expression of nuclear factor of activated T cells 1 (NFATc1) and osteoclast associated receptor (OSCAR) during the terminal stages of osteoclast formation. However, MS-275, which targets Class I HDACs, was 100–1000 times less effective at inhibiting osteoclast formation than 1179.4b. These results show that the inhibitor of Class I and Class II HDACs suppresses resorption *in vitro* but its effects in an animal model of pathogenic bone loss are not known.

As other HDACi compounds, including TSA, have been shown to suppress osteoclast bone resorption in animal models of rheumatoid arthritis (RA) (14,22,23), it is possible that HDACi will have similar effects on the bone loss seen in periodontitis. Therefore, based on this thought and on the results of our *in vitro* studies we hypothesized that the novel HDACi, 1179.4b, which targets both classes of HDACs would inhibit bone resorption in a mouse model of periodontitis. The aim of this study was to compare the effects of 1179.4b and MS-275, which target Class I HDACs, on bone loss and inflammation in this mouse model (24) by assessing changes in the alveolar bone and soft tissues using micro-computed tomography (micro-CT) scans, histological analysis and stereo imaging.

Material and methods

Figure 1 shows the chemical structures of the HDACi compounds evaluated. 1179.4b is a synthetic compound, developed in our laboratories, which targets Class I and Class II HDACs [Compound 52 in (25)]. MS-275 is a selective inhibitor of Class I HDACs that has been shown to induce hyperacetylation of nuclear histones in various tumor cell lines (26).

Mouse model of periodontitis

Experimental periodontitis was induced in 6- to 8-wk-old female BALB/c mice

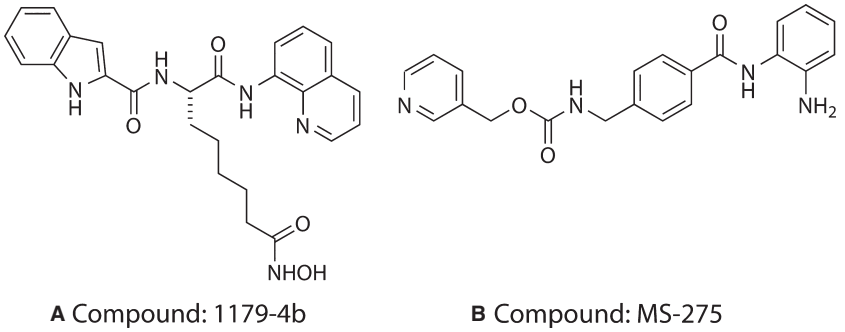

Fig. 1. Chemical structures of: (A) Histone deacetylase inhibitor (HDACi) 1179.4b which targets Class I and Class II HDACs [(S)-N8-hydroxy-2-(1H-indole-2-carboxamido)-N1-(quinolin-8-yl)octanediamide; Compound 52 in (25)] and (B) HDACi MS-275 which targets Class I HDACs [N-(2-aminophenol)-4-(N-(pyridine-3-yl-methoxycarbonyl) aminomethyl) benzamide) (39)].

($n = 18$) over a period of 81 d, as described previously (24,27). Mice were housed in the Institute of Medical and Veterinary Science Animal House, Adelaide, Australia. Ethical approval was received from the IMVS Animal Ethics Committee (Project no. 148/06).

Mice were initially treated with 1 mg/mL of kanamycin (Sigma-Aldrich, St Louis, MO, USA) in water for 7 d to reduce the native flora and to support colonization of *Porphyromonas gingivalis* (*P. gingivalis*), as previously described (24,27,28). Three days after antibiotic treatment ceased, periodontitis was induced by a monoinfection with *P. gingivalis* bacteria, as also previously described (24,28). Briefly, cultures of *P. gingivalis* (strain W50) were grown anaerobically on blood agar plates and stored for 3 d at 37°C, in an atmosphere of $N_2/CO_2/H_2$ (90 : 5 : 5), before harvesting. The bacterial viable count was found to be 24.3×10^{10} colony-forming units/mL with a dry weight of 12.5 mg/mL. *P. gingivalis* was suspended in 2 mL of carboxymethyl cellulose (2% CMC in phosphate-buffered saline) (Sigma-Aldrich), as previously described (24,27,28). A 0.1-mL sample of the bacterial suspension (i.e. 0.1 mL in CMC) was directly swabbed around the molars using a small brush. Control mice were swabbed with CMC alone. Following each inoculation, mice were kept without food or water for 1 h. The first sequence consisted of four inoculations over 8 d, followed by two inoculations a week for 2 wk, then a second sequence (four inoculations over 8 d). For the remainder of the experimental period, mice were inoculated twice a week. Live *P. gingivalis* were recovered from the gingival tissue, as described previously (28).

From day 44, mice were administered daily doses of the compounds suspended in olive oil via oral gavage. The compounds were administered in olive oil due their lipophilic nature. This dose of olive oil was well below the levels reported to have an effect on bone (29). Day 44 was determined to be an appropriate time to commence treatment from preliminary experiments as disease had been adequately induced and this was also a clinically relevant time (24). Periodontitis was induced in the following three groups of mice by inoculation with *P.gingivalis* bacteria. Group 1: *P. gingivalis* inoculations and received 0.1 mL of olive oil only ($n = 5$) (known as oil treated); group 2: *P. gingivalis* inoculations and received 10 mg/kg/d dose of MS-275 (0.1 mL) ($n = 4$); and Group 3: *P. gingivalis* inoculations and received a 1 mg/kg/d dose of 1179.4b (0.1 mL) ($n = 4$). These doses were based on the *in vitro* results (20) and on the recommendations by the developers of the compounds. Group 4 were control mice (not inoculated with *P. gingivalis* or treated with HDACi) ($n = 5$). Control mice were used to give an indication of alveolar bone changes during normal mouse growth.

Three methods of analysis were used to determine the effects of HDACi: micro-CT scans of live mice to assess changes in bone volume; stereo imaging to assess the change in the area from the cemento–enamel junction (CEJ) to the alveolar bone crest (ABC); and histological analysis to determine effects on inflammation.

Bone volume analysis

Mice were scanned using the live animal micro-CT scanner [Sky Scan 1076 high-resolution *in vivo* scanner (SkyScan, Kontick, Belgium), Adelaide Microscopy Australia], as previously described (24). Mice were scanned on day 44 (before drug treatment) and at the completion of the study (day 81). The scanning specifications and machine details have been previously published (24). Mice were scanned at 74 kV/136 mA with a pixel size of 18 μM, 1 mm aluminum filter and frame averaging of 1. These parameters were used to minimize the radiation exposure to the animals and to significantly reduce the scanning time to around 12 min per animal. Before scanning, mice were anaesthetized via intraperitoneal injection [rat/mouse anaesthetic: 1 mL of xylazine, 2 mL of ketamine (100 mg/mL), 17 mL of water in injection, 0.3 mL for a 30-g mouse]. For each scan the mouse was positioned to ensure that the head was within the scanning area of interest. Following this, CT scans were then reconstructed using the SkyScan N Recon program for analysis (SkyScan). Bone volume analysis was then carried out using SkyScan's CTAn program. The three molars of the maxilla on both the left-hand and right-hand sides were used for the region of interest. For each area of interest, bone volume (mm^3) was calculated using CTAn software. The percentage change in bone volume over time was determined for each mouse.

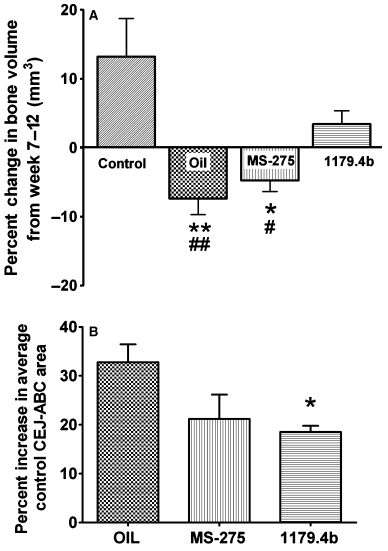

Fig. 2. (A) Percentage change in bone volume (mm^3) from week 7 (disease is evident) to week 12 (study completion). Control: mice with no disease or treatment ($n = 5$). Oil: mice ($n = 5$) treated with oil only. MS-275: mice ($n = 4$) treated orally with oil + 10 mg/kg/d of MS-275. 1179.4b: mice ($n = 4$) treated orally with oil + 1 mg/kg/d of 1179.4b. Bars represent mean ± standard error of the mean. Significance was determined using a one-way analysis of variance followed by Tukey's *post hoc* test. Significance was accepted when $p < 0.05$. $^{**}p < 0.01$, $^{*}p < 0.05$ compared to treatment with 1179.4b. $\#p < 0.01$, $\#p < 0.05$ compared with the control. (B) Average percentage increase in the control cemento–enamel junction (CEJ) to the alveolar bone crest (ABC) area. Oil: mice ($n = 5$) treated with oil only. MS-275: mice ($n = 4$) treated orally with oil + 10 mg/kg/d MS-275. 1179.4b: mice ($n = 4$) treated orally with oil + 1 mg/kg/d of 1179.4b. Significance was determined using a one-way analysis of variance followed by Tukey's *post hoc* test. Significance was accepted when $p < 0.05$. $^{*}p < 0.05$ compared with oil alone.

Bone area analysis

At the completion of the study, mice were killed, their heads were collected and defleshed, stained with Methylene Blue (0.003%) and the maxillae imaged using a stereo microscope (MZ16FA Leica Stereo microscope; Leica, Solms, Germany) (24). Right-hand and left-hand sides, and buccal and lingual surfaces were imaged using a 1.0 objective lens. The area from the CEJ to the alveolar bone crest (ABC) was measured for each molar (molars 1–3) using IMAGE J analysis software, as previously described (24). This was conducted by two blinded observers and the area from the CEJ to the ABC was calculated (in mm^2) for the three molars combined. This was then presented as the percentage increase in area relative to the average control area from the CEJ to the ABC.

Histological analysis

Standard hematoxylin and eosin staining was conducted on jaw tissue fixed in 4% formalin for 24 h, decalcified in 4% formic acid for 2 wk, then paraffin embedded. Three histological sections per mouse were scored based on previous methods (24). Scoring was carried out by two independent observers, blinded to the tissue type, using a four-point scale, and was based on the total numbers of inflammatory cells present within the tissues (lymphocytes, plasma cells, neutrophils or macrophages). Normal tissue (< 5% inflammatory cells) was scored as 0; mild inflammation (5–20% inflammatory cells) was scored as 1; moderate inflammation (20–50% inflammatory cells) was scored as 2; and severe inflammation, with massive infiltration of immune cells (> 50% of cells), was scored as 3. The number of multinucleated osteoclast cells (more than three nuclei) per square millimeter was also determined as described previously (30,31). An area encompassing 4×2 mm, and which included the alveolar bone between the first and third molars, was analysed.

Statistics

A one-way analysis of variance, followed by Tukey's *post hoc* test, was conducted to assess changes in bone volume between the groups (group 1, oil only; group 2, oil + MS-275; and group 3, oil + 1179.4b). Statistical significance was accepted when $p < 0.05$.

Results

Micro-CT scans of live mice confirmed that periodontitis had been induced with significant ($p < 0.001$) bone loss in mice inoculated with *P. gingivalis*. Control mice averaged a 13.2% increase in bone volume compared with a 7.4% decrease in mice inoculated with *P. gingivalis* (oil treated) (Fig. 2A and Table 1). Bone loss was confirmed by stereo imaging of the mouse heads (Fig. 3A,B). In mice inoculated with *P. gingivalis* (oil treated), an average 32% increase was observed in the area from the CEJ to the ABC compared with controls ($p < 0.001$) (see Fig. 2B). The presence of an inflammatory infiltrate in the gingiva of both drug-treated and oil-treated animals was noted (Fig. 4). The numbers of osteoclastic cells resorbing bone were also significantly elevated in mice inoculated with *P. gingivalis* (oil treated) compared with control animals (Fig. 5A).

No side effects or weight loss were noted in the treated mice. 1179.4b (1 mg/kg/d, orally) significantly reduced bone loss induced by *P. gingivalis* inoculation (Fig. 2A). There was an average 3.4% increase in bone volume in the 1179.4b-treated mice compared with a 7.4% decrease in bone volume in mice treated with oil only ($p < 0.01$) (Fig. 2A). Stereo imaging similarly showed a 20% increase in the CEJ to the ABC in 1179.4b-treated mice compared with an increase of 32% in mice treated with oil only ($p < 0.05$) (Fig. 2B). This observation was also confirmed by a significant reduction in osteoclast numbers in treated mice. Despite this suppression of bone loss, compound 1179.4b did not suppress inflammation, as demonstrated in Fig. 4E,F ($p > 0.05$). There was still a significant inflammatory cell infiltrate present in the periodontal tissues, as assessed by semiquantitative analysis (described in the Material and methods) (Fig. 5B).

In contrast to treatment with 1179.4b, treatment with MS-275 had little effect on bone loss. The change in bone volume in MS-275-treated mice was not significantly different from that of oil-treated animals ($p > 0.05$). There was a significant ($p < 0.05$) difference in bone volume in the mice treated with 1179.4b compared to the mice treated with MS-275 (Fig. 2A). Micro-CT scans of live mice revealed an average decrease in bone volume of 4.8% in the mice treated with MS-275, demonstrating that MS-275 did not

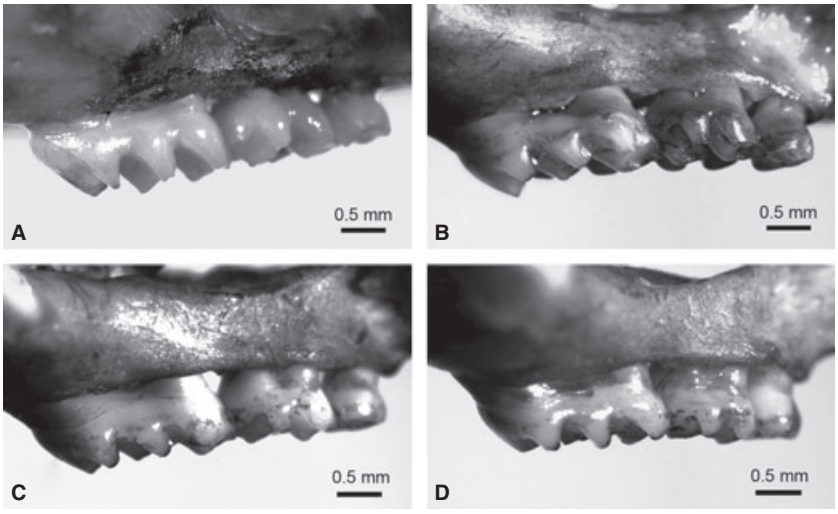

Fig. 3. Stereo images of the mouse heads at completion of the study (week 13) showing the 3 molars of the maxilla. Images were taken using a stereomicroscope, as described in the Material and methods, using a 1.0× objective lens. (A) Control mouse; (B) oil-treated mice (inoculated with *Porphyromonas gingivalis*, administered oil only, no drug treatment); (C) mouse treated with oil + 1 mg/kg/d of 1179.4b; (D) mouse treated with oil + 10 mg/kg/d of MS-275.

have a significant effect on *P. gingivalis*-induced bone loss ($p > 0.05$). However, inflammation was reduced in mice treated with MS-275, as demonstrated by semiquantitative analysis of the histological sections, but this was not significant ($p > 0.05$) (Fig. 5B).

Discussion

In this study, the bone-protective effects of two HDACi – 1179.4b and MS-275 – were investigated in a mouse model of periodontitis. In this model, periodontitis was induced by oral inoculations with *P. gingivalis* over a period of 44 d. A chronic inflammatory reaction was established with associated alveolar bone destruction (24), shown by micro-CT scans of live mice, histology and stereo imaging. Treatment with HDACi suspended in olive oil or treatment with olive oil alone was commenced once disease had been established as this is clinically relevant to the human situation.

The results of this study demonstrated that a novel HDACi, 1179.4b, administered at 1 mg/kg/d, significantly reduced alveolar bone loss compared with oil-treated mice. The other HDACi, MS-275, administered at 10 mg/kg/d, did not suppress bone loss in this model. Interestingly, inhibition of alveolar bone resorption by 1179.4b occurred even though there was no reduction in inflammation of the gingival tissues, as assessed histologically. This could suggest that the mechanism of action involves a direct effect on the bone rather than an indirect effect through suppression of inflammation.

The findings of the current study are consistent with our recent *in vitro* findings in which 1179.4b, an inhibitor of both Class I and Class II HDACs, suppressed osteoclast bone resorption.

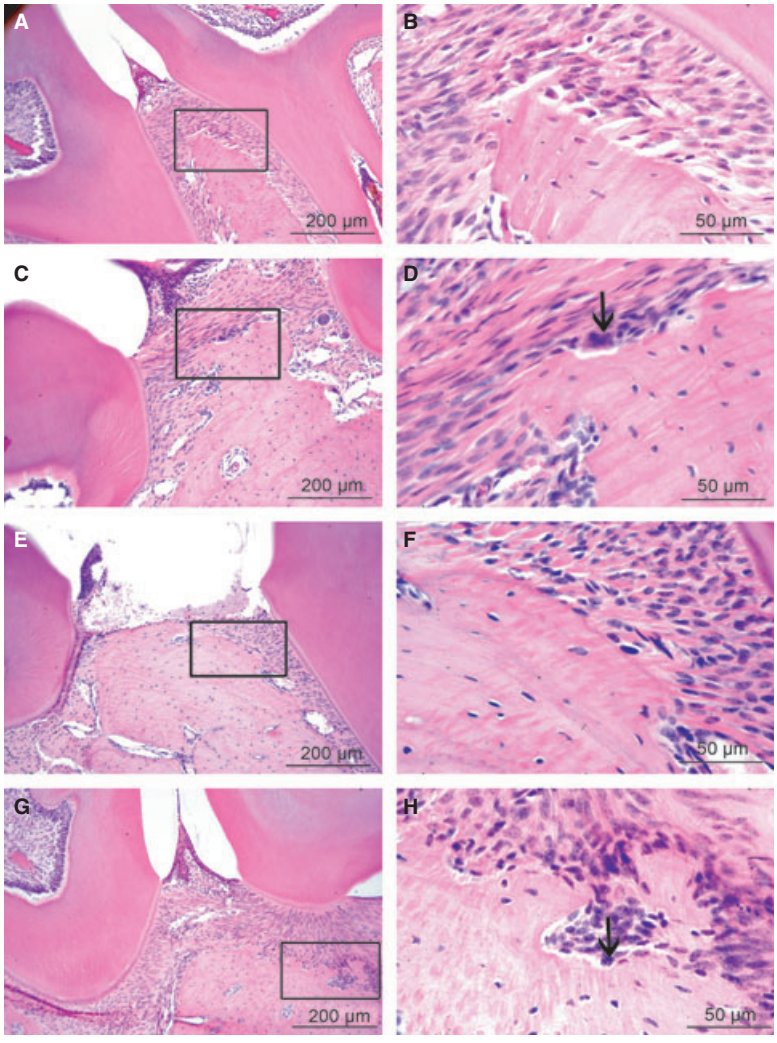

Fig. 4. Standard hematoxylin and eosin staining of the gingival tissue near the first and second molars in (A) control (10× magnification); (B) control (40× magnification of the boxed area in A); (C) oil-treated mice (inoculated with *Porphyromonas gingivalis*, administered oil only, no treatment) (10× magnification); (D) oil-treated mice (40× magnification of the boxed area in C); (E) 1179.4b (1 mg/kg/d) (10× magnification); (F) 1179.4b (40× magnification of the boxed area in E); (G) MS-275 (10 mg/kg/d) (10× magnification); (H) MS-275 (40× magnification of the boxed area in G). Arrows in (D) and (H) show multinucleated osteoclasts at resorption.

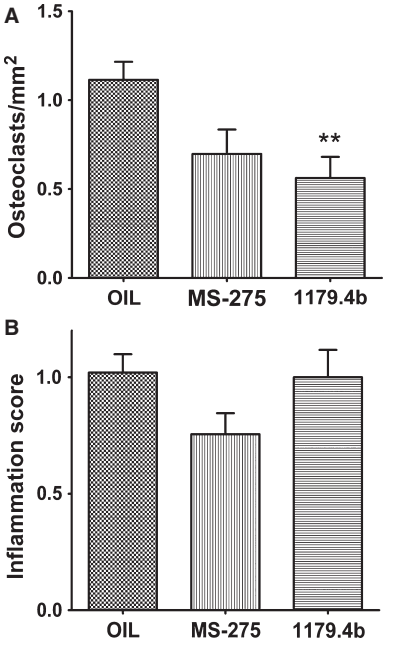

Fig. 5. (A) Average number of osteoclasts on the surface of alveolar bone supporting the 3 molars per section. Scoring details are described in the Material and methods. Three sections for each animal were quantified. Bars represent mean ± standard error of the mean. Significance was determined using a one-way analysis of variance followed by Tukey's *post hoc* test. $^{**}p < 0.01$ compared with oil. (B) Average histological scoring of the periodontal tissues for inflammation, as described in the Material and methods. Bars represent mean ± standard error of the mean. Significance was assessed using a one-way analysis of variance followed by Tukey's *post hoc* test. No significant differences were observed in inflammation.

Table 1. Average bone volume (BV) values for scan 1 (after inducing disease but before treatment) and scan 2 (after treatment), determined using micro-computed tomography of live mice

	Scan 1 BV (mm^3)	Scan 2 BV (mm^3)	Average change in BV during treatment (mm^3)
Control ($n = 5$)	1.320 ± 0.024	1.484 ± 0.030	+0.164 ± 0.037
Oil ($n = 5$)	1.208 ± 0.069	1.124 ± 0.049	−0.080 ± 0.038*
MS-275 ($n = 4$)	1.176 ± 0.026	1.119 ± 0.021	−0.057 ± 0.015*
1179.4b ($n = 4$)	1.076 ± 0.026	1.109 ± 0.020	+0.033 ± 0.027**·***

Results are given as mean ± standard error of the mean.
The final column shows the average change in bone volume for each group during the treatment period. Control: mice with no disease or treatment ($n = 5$). Oil: diseased mice (administered oil only, no drug treatment) ($n = 5$). MS-275: mice treated orally with oil + 10 mg/kg/d of MS-275 ($n = 4$). 1179.4b: mice treated orally with oil + 1 mg/kg/d of 1179.4b ($n = 4$). Significance was determined using a one-way analysis of variance followed by Tukey's *post hoc* test.
*$p < 0.05$ **$p < 0.01$ compared with control. ***$p < 0.001$ compared with oil.

Class II HDAC 5 and Class I HDAC 8 have also been shown to be up-regulated during the late stages of osteoclast development *in vitro* (20), suggesting that these HDACs may play a key role in osteoclast differentiation and activity. MS-275 has also been shown to have anti-inflammatory effects when administered at 10 mg/kg/d in a rat model of arthritis (22), with marked suppression of serum interleukin-6 in this model. In the present study, histological assessment indicated that MS-275 did appear to reduce inflammation in the gingival tissues of treated mice, although this was not statistically significant. MS-275 did not have any effect on bone destruction, confirmed by both CT analysis and histology. Although the exact activity of MS-275 is not known, it is reported to inhibit HDAC 1 at concentrations below 0.1 μM but does not affect HDAC 6 (32), indicating its selectivity towards Class I HDACs. It is also reported not to inhibit the Class I HDAC 8, suggesting its selectivity towards HDAC 1 and HDAC 3 (33). 1179.4b, on the other hand, effectively suppressed bone loss in this mouse model. This compound has been shown to inhibit HDAC 1 and HDAC 6 at concentrations of < 0.1 μM, indicating that it interacts with both Class I and Class II HDACs. This observation could explain differences in HDACs targeted by these compounds. As well as the selectivity of the compounds, there may also be differences in their potency. However, we noted significant bone-protective effects, even though 1179.4b was administered at a 10-fold lower concentration (1 mg/kg/d) than MS-275 (10 mg/kg/d). This is consistent with our previous *in vitro* findings, where 1179.4b was found to be more than 30-fold more active than MS-275 (IC_{50} of < 0.16 nM for 1179.4b vs. IC_{50} of 54.4 nM for MS-275) (20).

It has recently been demonstrated that HDAC 1 is highly expressed in synovial fluid from RA patients compared to patients with osteoarthritis (34). High levels of HDAC 1 have also been observed in RA tissues and these levels also correlate with higher TNF-α expression (34,35). Other HDACi compounds (TSA and SAHA) have demonstrated suppression of osteoclast-mediated bone resorption in various animal models of RA (14,22,23). Periodontitis shares many common features with RA (36) and there is a relationship between the two diseases (29,37) so it may not be surprising that similar results were seen in this current model of periodontitis. More extensive, detailed studies are needed to determine which specific HDACs of both classes are most important in various aspects of the periodontitis disease process (i.e. inflammation and bone resorption).

Although this class of compound is reported to be generally well tolerated as chemotherapeutic agents, they can have adverse side effects (38). While we did not observe any adverse effects at the doses used, the possibility of using these compounds in the clinic will have to be well monitored. For the management of periodontitis, side effects might be minimized by treating accessible sites in the mouth locally with these compounds. Another possibility is to specifically target only those HDACs required for osteoclast formation, with the advent of more HDAC-specific inhibitors on the immediate horizon. In addition, topical HDACi treatment may be a less toxic way of HDACi administration. Topical administration of HDACi – TSA and phenylbutyrate – was found to reduce paw swelling in an adjuvant arthritis model, and rats treated with HDACi exhibited no pannus formation or joint destruction (12). This method of application could potentially reduce any risk of systemic effects caused by the widespread expression of HDAC enzymes.

In conclusion, this study supports recent findings that HDACi prevent bone loss and demonstrates that the novel HDACi, 1179.4b, reduces alveolar bone loss in an *in vivo* model of periodontitis. The inhibition of bone loss was independent of the anti-inflammatory effects of the compounds and is likely to be a result of their ability to target osteoclast formation directly. While further studies are needed, the findings indicate that HDACi treatment could be used for the treatment of periodontitis in humans in the future.

Acknowledgements

This work was supported by National Health & Medical Research Council of Australia, an ARC Federation Fellowship (to D.P.F.) and by Australian Dental Research Foundation (ADRF) Grants. We acknowledge Mr Dale Caville for his photographic help.

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