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Lack of short-term adjunctive effect of systemic neridronate in non-surgical periodontal therapy of advanced generalized chronic periodontitis: an open label-randomized clinical trial

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Abstract

Aim: To determine if the adjunctive use of intra-muscular neridronate (NE) during non-surgical periodontal treatment (PT) provides, in patients with generalized chronic periodontitis (GCP), adjunctive benefits as compared with PT alone 3 months after the completion of a 3-month NE therapy.

Material and Methods: Sixty GCP healthy patients were randomly assigned to control (CG) or test group (TG). CG patients received PT only. Thirty subjects in TG also received adjunctive NE (12.5 mg in an i.m. injection/week for 3 months). Clinical parameters were evaluated at baseline, at the end of NE treatment (3 months after PT) and 3 months after the completion of NE treatment (6 months after the beginning of PT).

Results: Groups were balanced at baseline and all clinical parameters showed improvement between baseline and follow-ups. At 6 months improvements from baseline at sites with deep pocket depth ($\geqslant$7 mm) were 3.2 mm [95% confidence interval (CI): 2.7–3.9] in CG and 3.0 mm (95% CI: 2.3–3.8) in TG with a non-significant difference of 0.2 mm (95% CI: -1.0–0.5; ANCOVA; $p = 0.549$) between groups. Secondary outcomes did not show significant differences between groups. No major adverse events were reported.

Conclusions: The adjunctive use of NE during PT did not result in additional short-term improvements in periodontal conditions of GCP patients when compared with PT.

Key words: bisphosphonates; non-surgical periodontal therapy; chronic periodontitis

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Conflict of interest and sources of funding statement

The authors do not have any conflict of interest and they are not associated with AbiogenPharma.

The study was initiated by the company AbiogenPharma (Pisa, Italy), which only supplied the materials and supported the study financially. No other form of support was given. In addition, the study was not supported by any other party. The authors had full control of the data. Statistical analysis was conducted independently from the company.

Periodontitis is a multi-factorial chronic infectious disease characterized by a loss of the connective tissue attachment to the teeth and the resorption of the alveolar bone due to the inflammatory processes (Williams 1990). It is a common disease with a high prevalence of moderate forms which affect 40–50% of the adult population whereas severe

forms only affect a minority of adults (Papapanou 1996, Page et al. 1997).

The aetiology of periodontal disease is reducible to bacterial infection, which results in an inflammatory reaction (Pihlstrom et al. 2005). Indeed, bacteria proved to be essential but not sufficient to cause disease (Page & Kornman 1997). It is the local production of pro-inflammatory cytokines, such as interleukin 1 (IL-1) and tumour necrosis factor (TNF), that is responsible for the tissue destruction and osteoclast activation (Hou et al. 2003). Moreover, tissue damage is generated by collagenolytic enzymes such as matrix metalloproteinases (MMPs) which significantly contribute to periodontal tissue damage (Chen et al. 2000).

Treatment for periodontal disease aim at the mechanical elimination of subgingival plaque biofilm of the diseased dentition as the eradication of periodontopathogens has been proven to be successful in arresting disease progression (Haffajee et al. 2006). Nevertheless, in the last decade, due to the knowledge of the primary role of inflammatory response, anti-inflammatory and host–response modulators have been added to conventional treatment to further improve the effects of therapy (Salvi & Lang 2005).

Bisphosphonates (BPs) are unstable analogous of pyrophosphate that bind selectively to bone. BPs are supposed to act selectively on osteoclasts during high bone turnover, resulting in an anti-resorptive effect (Reszka & Rodan 2003). BPs are widely used in the management of systemic metabolic disorders such as osteoporosis and Paget's disease, moreover they are also indicated for the treatment of cancer-related bone disease (Fleisch 1997). Furthermore, BPs may down-regulate several MMPs involved in periodontal tissue destruction (Teronen et al. 1997, Kivela-Rajamaki et al. 2003). Thus, their action on bone metabolism and their ability to inhibit MMPs justify a possible use of BPs as adjunctive therapy in the management of periodontal disease (Giannobile 2008).

BPs have been used as inhibitors of bone resorption and MMPs in the treatment of periodontitis (Reddy 2003). Alendronate, an aminobisphosphonate, proved to be effective as adjunctive treatment in both patients with diabetes type 2 and post-menopausal women with little or no side-effects (Rocha et al. 2001, 2004). In humans daily administration of BPs improved periodontal condition also in systemically healthy patient (Jeffcoat et al. 2007). Within BPs neridronate (NE), a powerful nitrogen-containing aminobisphosphonate normally used in the management of osteoporosis (Braga et al. 2003) and bone metabolism dysfunction in cancer patient (Pittari et al. 2006) has never been applied to the treatment of periodontal disease.

The aim of this randomized controlled trial conducted in patients with advanced generalized chronic periodontal disease was to evaluate whether 3 months of aminobisphosphonate therapy with NE in association with conventional non-surgical periodontal treatment would provide additional clinical improvements as compared with the ones obtained with conventional treatment alone.

Material and Methods

Experimental design and patient selection

This study was an open label randomized, parallel design, single masked clinical trial with a 6-month follow up that involved periodontally affected subjects. Eligible patients were identified from the population referred to the Oral Surgery clinic of the University of Pisa, Italy. Ethical approval was obtained from the local Ethics Committee and the study was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects.

A complete periodontal examination was performed including patient history, an intra-oral examination and a full-mouth periodontal probing on six-sites per tooth. A radiographic examination was undertaken using either digital periapicals or a conventional ortopantomogram. Subjects who met the study inclusion criteria were invited to participate in the study.

The trial included subjects with (i) generalized advanced chronic periodontitis (Armitage 1999); (ii) at least 20 teeth present; (iii) good general health. Subjects were excluded from the study if they: (i) were pregnant or lactating females; (ii) required antibiotic pre-medication for the performance of periodontal examination and treatment or had received antibiotic treatment in the previous 3 months; (iii) suffered from bone pathologies or any other systemic diseases (cardiovascular, pulmonary, liver, cerebral, diseases or diabetes); (iv) were taking long-term anti-inflammatory drugs; (v) had received a course of periodontal treatment within the last 6 months; (vi) were allergic to BPs; (vii) required bone-metabolism altering drugs (estrogens, PTH, calcitonin, BPs, vitamin D) and (viii) were not able to consent to participate in the study. Informed consent was obtained from all the subjects to before starting the study.

Sample size calculation, randomization procedures and allocation concealment

Twenty-four subjects per treatment arm would be needed to provide 90% power to detect a true difference of 1.0 mm between test and control using probing pocket depth (PPD) reduction in pockets $\geqslant$7 mm as the primary outcome variable assuming that the common standard deviation is 1.0 mm. Thus, a sample of 60 subjects, 30 per arm were recruited to compensate for possible drop-out.

Subjects were randomly assigned by computer-generated table to receive one of the two treatments. The randomization table was saved by a research fellow not directly involved in the experimentation. Thirty plastic bags containing 12 ampoules of 12.5 mg/2 ml neridronic acid (Abiogen Pharma, Pisa, Italy) were matched with the treatment assignment number. Allocation to the treatment was concealed to the therapist and the examiner.

Examinations

Periodontal examinations were performed at baseline, at the end of the BPs treatment (i.e. 3 months after the first session of periodontal treatment) and 6 months after the first session of treatment (i.e. 3 months after the end of BPs therapy). A UNC-15 periodontal probe was used by a masked calibrated examiner at six sites/tooth excluding third molars. Full-mouth plaque score (FMPS) was recorded by assigning a binary score to each site and calculating the percentage of total tooth surfaces that revealed the presence of plaque detected by the use of a periodontal probe. Similarly, a full-mouth bleeding score (FMBS) was calculated after assessing dichotomously the presence of bleeding on probing from the bottom of the pocket when gently probing. Full-mouth PPD and recession of the gingival margin (REC) were recorded at

the same time, with measurements rounded to the nearest millimetre. Clinical attachment level (CAL) was calculated as PPD plus REC.

Treatment Procedures

A standard cycle of periodontal therapy consisting of oral hygiene instructions, supra and subgingival mechanical instrumentation of the root surface (scaling and root planing) was performed by a single experienced certified therapist (F. G.) using a piezoelectric instrument with fine tips (EMS, Nyon, Switzerland) and hand instruments as appropriate. Both groups received this treatment in four different appointments within a period of 2 weeks. Local anaesthesia was used as necessary. Patients then relied on standard oral hygiene methods as instructed at the commencement of the study and no oral rinses were prescribed.

Test subjects received an adjunctive course of systemic BPs consisting of 12.5 mg of NE i.m. once a week for 12 weeks, while control subjects did not receive any medication. NE was self-administered by the subjects during the course of treatment whereas the first dose was delivered by the research nurse at the end of the first session of treatment in an adjacent room. The research nurse provided proper guidance to subjects on self-administration of i.m. injections.

Follow-up

Patients were regularly seen to control and reinforce the oral hygiene habits, to monitor the early healing events, report on any adverse events or additional medications taken. Thus, patients were seen during the 2 weeks of treatment, at 12 weeks and 24 weeks after the first session of treatment. The number of not administered ampoules were documented based on each subject's self report.

Re-assessment visits occurred at 12 weeks (3 months) and 24 weeks (6 months) after the first session of treatment. During these appointments the examiner recorded any medical history changes and the clinical periodontal parameters recorded at the baseline visit were repeated. At the end of the final appointment a session of supragingival debridement was performed as necessary.

Outcome measures

The primary outcome measure of the study was PPD reduction in sites with initial PPD $\geqslant$ 7 mm. Secondary outcomes included differences between groups for the (i) changes in mean full-mouth PPD and the changes in PPD and CAL at different initial PPD categories; (ii) changes in FMPS and FMBS; (iii) percentage of sites with PPD reduction or CAL gain of $\geqslant$ 2 mm; (iv) percentage of sites that showed CAL loss $\geqslant$ 2 mm (disease progression); (v) percentage of sites with PPD changing from $\geqslant$ 5 to $\leqslant$ 4 mm and the percentage of sites with PPD changing from $\geqslant$ 4 to $\leqslant$ 3 mm (need for re-treatment); (vi) frequency distribution on the number of subjects who achieved different levels of treatment results (patient-centred outcome); (vii) description and frequency of adverse events and (viii) compliance with the systemic medication.

Data management and statistical analysis

Data were entered into an Excel (Microsoft office 2003) database and were proofed for entry errors. The database was subsequently locked, imported into SPSS for Windows (SPSS Inc. version 13.0) formatted and analysed. A subject-level analysis was performed by computing a subject-level variable (full-mouth or at different PPD categories) for each of the parameters. Numerical data were summarized as means and 95% confidence intervals (CI), categorical data were summarized as frequency distribution and the percentage-based measures (e.g. FMPS) were summarized as the median of the percentage and inter-quartile range. Significance of differences between test and control groups in terms of numerical data was evaluated via univariate analysis using the independent samples *t*-test. Likewise, significance of difference within each group before and after treatment was evaluated with the paired samples *t*-test. Categorical data were analysed with the χ^2 test, and the percentage data between the two groups were compared with the Mann–Whitney test while the within group percentage changes were evaluated with the Wilcoxon signed rank test. The significance of the treatment option (test or control) on the dependent variables PPD reduction and CAL gain at different initial PPD categories was estimated by analysis of covariance (ANCOVA). The models were adjusted for baseline values and controlled for smoking. Model estimates included adjusted means and 95% CIs. Data were also analysed as frequency distributions of treatment outcomes at a patient level using the Mann–Whitney test to compare the differences in the distribution of these outcomes at test and control groups. An intention-to-treat, last observation carried forward analysis was performed (Hollis & Campbell 1999). An ''on-drug'' analysis excluding data from subjects who incurred in a protocol violation (non-adherent to test medication) was also performed. In addition, the statistician was masked to the treatment group.

Results

Subject accountability

Sixty-eight subjects were assessed for their eligibility before entering the study. Of these, eight subjects were excluded; five because they did not meet the inclusion criteria, while the other three refused to participate (Fig. 1). Hence 60 subjects were randomly allocated to participate in the study. All participants received the allocated intervention. Five subjects from the test group were lost throughout the 6-month follow-up. Two of them stopped the NE injections during the first week due to adverse events while the other three were unable to attend clinical examinations. Four subjects from the control group were lost due to reasons unrelated to the study. All participants were included in the intent to treat analysis.

Study Schedule

Subject recruitment started in January 2003 and was completed by the end of June 2004. All the 6-month follow-up visits were completed by December 2004. Data entry of all information and statistical analysis were performed by the end of September 2005.

Subject characteristics at baseline

Baseline characteristics of the 60 participants who were treated non-surgically either with the adjunctive use of BPs or within the control group are displayed in Table 1. None of the demographic parameters showed a statistically significant difference between groups (*p*-values not

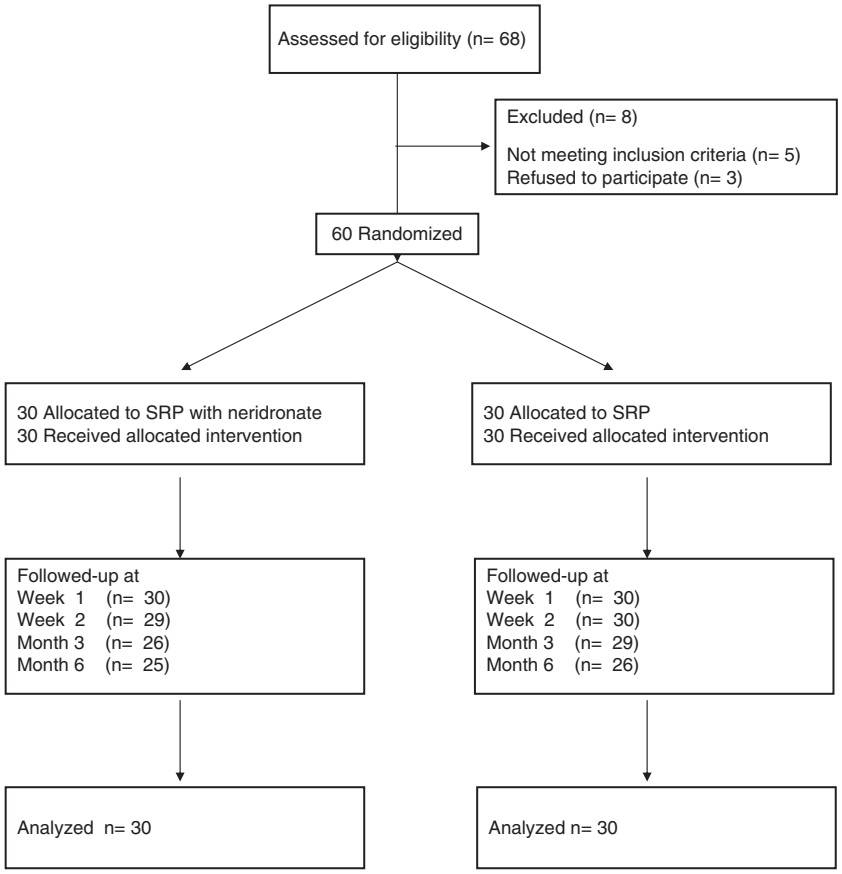

Fig. 1. Flow chart of patients during the study.

shown on tables). The two study groups displayed similar characteristics for number of teeth present, percentage of pockets $\geqslant 5$ mm, plaque and bleeding levels with no significant differences between the groups (Table 1). These data show that the subjects had retained most of their teeth, but had approximately 20% of sites exhibiting pockets requiring treatment and high levels of bleeding and plaque.

Mean values for clinical parameters

Mean full-mouth clinical outcomes and mean clinical outcomes at shallow ($\leqslant 3$ mm) moderate (4–6 mm) and deep ($\geqslant 7$ mm) pocket categories for baseline, and the differences between baseline and 3 months and baseline – 6 months are displayed in Table 2. At baseline there were no significant differences between test and control. All parameters, with the exception of mean CAL gain and mean PPD reduction at initial shallow pockets, showed a statistically significant difference between baseline and follow-up time points (*p*-values not shown on tables). On the contrary, no statistically significant differences were detected between test and control groups in any of the variables at any time point.

There were no significant treatment effects for any of the clinical variables included in the ANCOVA models (Table 3). Minor non-significant differences were detected for PPD reduction and CAL gain in $\geqslant 7$ mm pockets at 3 and 6 months, however, these differences were in favour of the control group.

In addition, smoking proved to be a significant factor on PPD reduction at 4–6 mm pockets at 6 months, and the difference between a non-smoker and a smoker on PPD reduction at moderate pockets was 0.4 mm (95% CI 0.0, 0.8). The corresponding value for the difference between non-smokers and smokers on full-mouth PPD reduction was 0.2 mm (-0.0, 0.5) demonstrating a borderline significance ($p = 0.053$) at 6 months. The effect of smoking was also significant on full-mouth CAL gain at 3 months ($p = 0.022$) and 6 months ($p = 0.039$), and CAL gain in 4–6 mm pockets at 3 ($p = 0.046$) and 6 months ($p = 0.008$).

Percentage of sites with pockets

The percentage of sites (median and inter-quartile range) with a PPD of a specific threshold at baseline and the reduction in the percentage of pockets within groups (difference between baseline 1 and 3 months and baseline – 6 months) are reported in Table 4. Analysis within groups (Wilcoxon Signed rank test) indicated that all PPD thresholds within each group showed a highly statistically significant difference between baseline and 3 months and between baseline and 6 months in both treatment groups (*p*-values not shown on the tables). However, the analysis of the differences between test and control treatments for the outcome of treatment did not show any statistically significant difference for the reduction in the percentage of pockets for any of the different PPD threshold used.

Oral hygiene and bleeding on probing

FMPS and FMBS at baseline, and the reduction within each group are displayed in Table 5. Plaque scores decreased in both treatments from baseline to 3 months and the difference was statistically significant for test and control groups. This was also true between baseline and 6 months (*p*-values not shown on tables). The effects of both treatments had a large impact on bleeding and these changes were statistically significant at 3 and 6 months ($p < 0.001$). However, there were no statistically significant differences between test and control groups for the improvement in the percentage of bleeding sites at 3 and 6 months.

Percentage of sites with clinically relevant changes

A subset analysis was carried out to test the changes of some clinically relevant parameters at 3 and 6 months (Table 6). All the median values (inter-quartile range) showed no statistically significant differences for the percentage of sites with CAL gain $\geqslant 2$ mm at 3 months ($p = 0.255$), at 6 months ($p = 0.882$), the percentage of sites with PPD reduction of $\geqslant 2$ mm at 3 months ($p = 0.620$) and at 6 months ($p = 0.988$), and the percentage of pockets that have converted from $\geqslant 5$ mm at baseline to $\leqslant 4$ mm at 3 months ($p = 0.407$) and at 6 months ($p = 0.780$). In addition, the percentage of sites with CAL loss $\geqslant 2$ mm were

very similar in the control group as compared with the test group at 3 ($p = 0.847$) and at 6 months ($p = 0.965$).

The percentage of pockets that converted from $\geqslant 4$ mm at baseline to $\leqslant 3$ mm also failed to show a statistically significant difference at 3 ($p = 0.438$) and at 6 months ($p = 0.953$).

Furthermore, Table 7 reports on the number of subjects who achieved different levels of reduction in the frequency of sites requiring further periodontal treatment for pocket depth reduction (using 5 mm as the discriminate depth). Five (16.6%) of the test subjects achieved a reduction of 100% of the sites that were $\geqslant 5$ mm at baseline and that converted to $\leqslant 4$ mm at 6 months as compared with two (6.6%) subjects in the control group. However, four (13.3%) subjects in the test group only achieved $<25\%$ of the sites converted from $\geqslant 5$ mm at baseline to $\leqslant 4$ mm at 6 months whereas this only occurred in one (3.3%) of the subjects from the control group. These differences did not show statistical significance between the groups ($p = 0.780$, Mann–Whitney test).

Adverse events, concomitant medication and compliance

During the first three weeks of treatment, eight subjects (26.7%) in the test group and two subjects (6.7%) in the control reported adverse events. Four subjects in the test group complained of musculo–skeletal pain. One subject experienced a large oedema at the injection sites. The types of adverse events at the 3 and 6 months follow-up visits are described in Table 8. Nine subjects (five in the test group and four in the control group) had a tooth extraction each between baseline visit and the 6 months visit. One subject from the control group lost one premolar due to a root fracture between the 3 months visit and the 6 month visit.

Concomitant medication during the study period was recorded. Two subjects in the test group and two in the control group took amoxicillin capsules for periodontal abscesses.

Compliance with the course of systemic medication and the number of ampoules not received were also documented. Two subjects (6%) did not complete the treatment as indicated. The reasons advocated by the two non-compliant subjects in the test group for not having all the injections were the following: one oedema at the injection site, one subject reported severe musculo–skeletal pain. These two subjects missed 91% and 66% of the whole number of ampoules respectively.

Secondary ''on-drug'' analysis did not report differences from the overall results (data not shown).

Table 1. Subject and clinical characteristics at baseline

Parameter	Test group ($N = 30$)	Control group ($N = 30$)
Age	44.7	42.2
Mean (95% CI)	(42.2, 47.3)	(38.7, 45.7)
Females (percentage)	19 (63.3%)	20 (66.7%)
Smokers (percentage)	10 (33.3%)	9 (30 %)
Body mass mean (95% CI)	23.9 (22.3, 25.4)	24.2 (23.1, 25.2)
Teeth at baseline mean (95% CI)	25.0 (23.7, 27.0)	25.0 (24.0, 28.0)
Percentage of pockets $\geqslant 5$ mm median (IQ)	21.9 (14.4, 33.5)	26.1 (11.0, 36.8)
Number of pockets $\geqslant 5$ mm	37.0	38.4
Mean (95% CI)	(28.3, 45.7)	(29.0, 47.8)
Percentage of pockets $\geqslant 7$ mm	3.3	4.2
Median (IQ)	(1.1, 8.4)	(1.2, 10.5)
Number of pockets $\geqslant 7$ mm	8.3	9.6
Mean (95% CI)	(4.7, 11.9)	(5.7, 13.5)
Full-mouth plaque score	76.5	66.0
Median (IQ)	(59.7, 82.0)	(45.7, 77.5)
Full-mouth bleeding score	31.5	29.7
Median (IQ)	(17.7, 42.0)	(20.0, 49.4)

CI, confidence interval; IQ, inter-quartile range.

Discussion

This was the first trial designed to assess the effect of NE as an adjunctive treatment of conventional non-surgical therapy of generalized advanced chronic

Table 2. Mean clinical outcome variables at baseline and differences between baseline – 3 months and baseline – 6 months

Clinical outcomes mean (95% CI)	Group	Baseline	Difference between baseline and 3 months	Difference between baseline and 6 months
Full-mouth mean PPD	Control	3.5 (3.2, 3.7)	0.7 (0.5, 0.9)	0.7 (0.5,0.9)
	Test	3.4 (3.2, 3.7)	0.8 (0.6, 0.1)	0.7 (0.8,0.9)
Mean PPD at pockets $\leqslant 3$ mm	Control	2.1 (2.0, 2.2)	0.0 (− 0.1, 0.1)	0.0 (− 0.2, 0.0)
	Test	2.1 (2.0, 2.2)	0.1 (− 0.1, 0.2)	0.0 (− 0.0, 0.1)
Mean PPD at pockets 4–6 mm	Control	5.1 (5.0, 5.3)	1.7 (1.4, 2.0)	1.8 (1.6, 2.1)
	Test	5.0 (4.8, 5.1)	1.7 (1.4, 2.0)	1.7 (1.4, 2.0)
Mean PPD at pockets $\geqslant 7$ mm	Control	7.6 (7.4, 7.8)	3.0 (2.4, 3.6)	3.2 (2.7, 3.9)
	Test	7.6 (7.3, 7.9)	2.7 (2.0, 3.4)	3.0 (2.3, 3.8)
Full-mouth mean CAL	Control	4.1 (3.6, 4.6)	0.5 (0.3, 0.8)	0.6 (0.3, 0.9)
	Test	4.2 (3.9, 4.6)	0.6 (0.3, 0.8)	0.5 (0.2, 0.8)
Mean CAL at sites with initial pockets	Control	2.7 (2.4, 3.0)	− 0.1 (− 0.3, 0.1)	− 0.1 (− 0.4, 0.1)
$\leqslant 3$ mm	Test	3.0 (2.7, 3.2)	− 0.0 (− 0.2, 0.1)	− 0.2 (− 0.5, 0.1)
Mean CAL at sites with initial pockets	Control	5.5 (5.1, 5.8)	1.2 (0.8, 1.5)	1.3 (0.1, 1.6)
4–6 mm	Test	5.4 (5.1, 5.6)	1.3 (1.0, 1.6)	1.1 (0.8, 1.5)
Mean CAL at sites with initial pockets	Control	8.3 (7.9, 8.8)	2.8 (2.2, 3.4)	3.1 (2.5, 3.7)
$\geqslant 7$ mm	Test	8.4 (7.8, 8.9)	2,2 (1.5, 2.9)	2.6 (1.8, 3.4)

CI, confidence interval; CAL, clinical attachment level; PPD, probing pocket depth.

Table 3. Analysis of covariance for PPD reduction and CAL gain at 3 and 6 months in different pockets categories

Multi-variate ''ANCOVA'' analysis models	Parameter	Difference baseline – 3 months		Difference baseline – 6 months	
		estimate (95% CI)	*p*- value	estimate (95% CI)	*p*-value
Full-mouth mean PPD reduction	Treatment group (test-control)	0.1 (– 0.1, 0.3)	0.440	0.0 (– 0.2, 0.2)	0.863
	Smoking (no-yes)	0.2 (– 0.0. 0.4)	0.129	0.2 (– 0.0. 0.5)	0.053
Mean PPD reduction in pockets 4–6 mm	Treatment group (test-control)	0.0 (– 0.2, 0.4)	0.646	– 0.1 (– 0.3, 0.3)	0.852
	Smoking (no-yes)	0.2 (– 0.6, 0.1)	0.160	0.4 (0.0, 0.8)	0.019
Mean PPD reduction in pockets ⩾7 mm	Treatment group (test-control)	– 0.3 (– 1.2, 0.5)	0.414	– 0.2 (– 1.0, 0.5)	0.549
	Smoking (no-yes)	0.1 (– 1.1, 0.8)	0.749	0.4 (– 0.4, 1.3)	0.317
Full-mouth mean CAL gain	Treatment Group (test-control)	0.0 (– 0.3, 0.3)	0.902	– 0.1 (– 0.5, 0.2)	0.428
	Smoking (no-yes)	0.4 (0.0, 0.8)	0.022	0.4 (0.0, 0.8)	0.039
Mean CAL gain in sites with initial PPD⩾7 mm	Treatment group (test-control)	– 0.6 (– 1.4, 0.3)	0.210	– 0.5 (– 1.4, 0.5)	0.342
	Smoking (no-yes)	0.0 (– 1.0, 1.0)	0.984	0.5 (– 0.6, 1.6)	0.084
Mean CAL gain in sites with initial PPD 4–6 mm	Treatment group (test-control)	0.1 (– 0.3, 0.5)	0.580	– 0.2 (– 0.6, 0.2)	0.435
	Smoking (no-yes)	0.5 (0.0, 0.9)	0.046	0.6 (0.2, 1.1)	0.008

CAL, clinical attachment level; PPD, probing pocket depth.

Table 4. Percentage of pockets at baseline and differences between baseline – 3 months and baseline – 6 months

Median of percentage (IQ)	Group	Baseline	Difference between baseline – 3 months	Difference between baseline – 6 months
Percentage of pockets ⩾4 mm	Control	41.0 (21.7, 52,4)	20 (6.1, 28.5)	21.2 (7.1, 28.3)
	Test	41.7 (31.0, 51.8)	22.2 (16.3, 29.1)	21.9 (17.3, 28.6)
Percentage of pockets ⩾5 mm	Control	26.1 (11.1, 36.9)	12.8 (3.7, 22.8)	12.6 (4.7, 27.4)
	Test	21.9 (14.5, 33.5)	13.1 (8.6, 19.9)	13.8 (7.4, 19.1)
Percentage of pockets ⩾6 mm	Control	12.0 (6.4, 25,0)	6.7 (2.6, 16.5)	9.0 (3.3, 17.8)
	Test	13.3 (7.3, 20.4)	6.8 (3.0, 13.5)	7.2 (3.3, 13.0)
Percentage of pockets ⩾7 mm	Control	4.2 (1.2, 10.5)	1.5 (0.0, 8.6)	2.6 (0.6, 9.3)
	Test	3.3 (1.1, 8.4)	1.4 (0.5, 4.2)	1.4 (0.6, 5.3)

No significant differences between groups in any of the variables.
IQ, inter-quartile range.

Table 5. Analysis on FMPS and FMBS at baseline and differences between baseline – 3 months and baseline – 6 months

Median (IQ)	Group	Baseline	Difference baseline – 3 months	Difference baseline – 6 months
Full-mouth plaque score (%)	Control	66.0 (45.7, 77.5)	43.5 (28.5, 60.2)	40.5 (26.0, 48.0)
	Test	76.5 (59.7, 82.0)	51.5 (29.0, 60.5)	50.0 (21.0, 63.2)
Full-mouth bleeding score (%)	Control	29.7 (20.0, 49.4)	18.3 (9.7, 26.5)	14.9 (5.9, 25.6)
	Test	31.5 (17.7, 42.0)	15.4 (5.1, 23.9)	11.4 (0.3, 21.3)

No Significant differences between groups in any of the variables.
FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; IQ, inter-quartile range.

periodontitis. NE was chosen for its effects on bone metabolism and its safety. Our data indicate that NE did not add any clinically significant benefit to scaling and root planning in otherwise systemically healthy patients.

Our results disagree with other two clinical trials on humans evaluating the effects of BPs therapy added to mechanical non-surgical treatment in patients with systemic diseases. In these studies BPs administration in association with non-surgical periodontal therapy had shown significant clinical improvement (Rocha et al. 2001, 2004). Rocha and colleagues assessed the effects of alendronate, an amminobisphosphonate, in diabetic patients and post-menopausal women six months after therapy. Their findings indicate that in subjects showing higher susceptibility to periodontal disease and impaired wound healing the added benefit of using BP were clinically small but significant.

One of the possible reasons for the lack of clinical effects 3 months after the end of drug intake could be related to the molecular action of the drug. The rationale of the usage of BPs for host-modulating periodontal treatment is based on their ability to decrease ostoblastic differentiation and inhibit osteoclast recruitment (Kantarci et al. 2006) and on their down-regulative action on MMPs enzymes (Nakaya et al. 2000). NE has not been tested on MMPs. Moreover, NE may either enhance (Frediani et al. 2004, Corrado et al. 2005) or decrease human osteoblasts biosynthetic activity according to the dosage and the metabolic stage of the cell (Corrado et al. 2005). Therefore, it could be also speculated that the dosage used in our study was not sufficient to expect an effective action on the osseous metabolism of the alveolar bone. Nevertheless, the total dosage used in this study (12.5 mg/week) is within the therapeutic effective range used for the intramuscular management of osteoporosis (Adami et al. 2008).

Table 6. Percentage of sites with clinically relevant changes in test and control groups at 3 and 6 months

Median of percentage (IQ)	Difference baseline – 3 months			Difference Baseline – 6 months		
	test group	control group	*p*-value Mann–Whitney test	test group	control group	*p*-value Mann–Whitney test
Percentage of sites with ⩾2 mm of CAL gain after treatment	24.8 (19.2, 34.0)	18.7 (8.3, 29.7)	0.255	21.7 (13.4, 34.6)	18.4 (13.3, 36.0)	0.882
Percentage of sites with ⩾2 mm of PPD reduction after treatment	24.8 (17.2, 34.0)	24.1 (9.5, 29.8)	0.620	25.2 (15.3, 30.7)	21.3 (12.0, 34.0)	0.988
Percentage of sites with CAL loss ⩾2 mm after treatment	7.3 (2.9, 11.3)	5.1 (3.2, 10.7)	0.847	5.1 (2.3, 16.0)	5.0 (1.8, 14.1)	0.965
Percentage of pockets converting from ⩾5 mm at baseline to ⩽4 mm after treatment	76.3 (63.0, 88.8)	70.4 (53.8, 85.7)	0.407	75.3 (65.9, 88.9)	74.4 (62.5, 92.0)	0.780
Percentage of pockets converting from ⩾4 mm at baseline to ⩽3 mm after treatment	66.6 (45.9, 76.1)	57.0 (48.2, 69.2)	0.438	66.4 (48.4, 80.0)	63.1(51.6, 75.3)	0.953

CAL, clinical attachment level; IQR, inter-quartile range; PPD, probing pocket depth.

Table 7. Number (percentage) of subjects who experienced different levels of reduction in the frequency of sites requiring further periodontal treatment for pocket reduction (using 5 mm as the discriminate value) at 6 months

Number of subjects (percentage)	<25%	25–49%	50–75%	75%	100%
Test ($N = 30$)	4 (13,3%)	1 (3,3%)	10 (33,3%)	10 (33,3%)	5 (16,6%)
Control ($N = 30$)	1 (3,3%)	4 (13,3%)	11 (36,6%)	12 (40,0%)	2 (6,6%)

$p = 0.780$, Mann–Whitney test.

Table 8. Type of adverse events at the 1- , 3-weeks, 3- and 6-months follow-up visit

Type of adverse event Number (percentage) of subjects	Time period	Test group ($N = 30$)	Control group ($N = 30$)
Stomach upset (nausea and vomiting)	1 week	0	0
	3 weeks	1	0
	3 months	0	0
	6 months	0	0
Gastrointestinal disorder (diarrhoea)	1 week	0	0
	3 weeks	0	0
	3 months	0	0
	6 months	0	0
Headache	1 week	1	0
	3 weeks	1	1
	3 months	1	0
	6 months	0	0
Periodontal abscess	1 week	0	0
	3 weeks	0	0
	3 months	0	0
	6 months	1	0
Musculo–skeletal pain	1 week	0	0
	3 weeks	2	0
	3 months	2	0
	6 months	0	0
Intra-oral tissue alteration	1 week	0	0
	3 weeks	0	0
	3 months	0	1
	6 months	0	0
General unwellness (irritability, flu, etc.)	1 week	0	0
	3 weeks	1	0
	3 months	2	0
	6 months	0	0
Pain at the injection site	1 week	2	NA
	3 weeks	0	NA
	3 months	0	NA
	6 months	0	NA

Furthermore, in this study NE was used in a cohort of systemically healthy patients showing no pathologies altering the susceptibility to periodontal disease. Interestingly, another trial conducted on systemically healthy patients showed that alendronate and risendronate did not present additional clinical benefit *versus* conventional treatment and placebo after 6 months (Lane et al. 2005). Apparently, a greater improvement of periodontal parameters appeared after 12 months. According to the authors the effect of scaling and root planning would be so evident to mask the adjunctive benefit of the drug during the first 6 months. Indeed, in our study periodontal treatment was successfully conducted in both groups. Mean PPD reduction and clinical attachment gain were higher than the expected standard of therapy as seen in Table 2 (Cobb 1996, Van der Weijden & Timmerman 2002). This was consistent with the observed reduction in bleeding scores and the percentage of pockets that needed further periodontal therapy. In this case, however, a longer follow-up could have helped us to gain further insight into a possible long-term added clinical benefit of NE in our patient sample. Thus, trials evaluating the possible effects of the BP during maintenance are advocated.

Smoking may alter the susceptibility of periodontal disease and also the response to treatment (Johnson & Guthmiller 2007). We did indeed find that smoking significantly affected some secondary outcomes such as PPD reduction and, in particular, CAL gain.

NE is an amminobisphoponate used mainly for the therapy of osteogenesi imperfecta (Gatti et al. 2005b), osteo-

porosis (Braga et al. 2003, Cascella et al. 2005), prevention of bone loss in cancer patients (Magno et al. 2005, Pittari et al. 2006) and Paget's disease (Adami et al. 2002, Merlotti et al. 2007). It appears to be a safe drug as it has been used also in paediatric patients affected by osteogenesis imperfecta (Gatti et al. 2005a). In our study the drug appeared to be generally safe. Some complications, such as flu and musculo–skeletal pain, occurred while no hypocalcaemia was detected as reported previously (Gatti et al. 2005a). However, as the subjects in the control group did not receive a placebo injection, no definitive conclusions could be drawn from the adverse events directly derived from the test medication.

Because of technical problems an open label study design with no placebo was chosen. Indeed, a placebo was not used for practical convenience and not to expose patients to multiple injections with no benefit. Nonetheless, the authors are aware that this may represent a limitation to the conclusion that can be drawn. However, in order to compensate the absence of a placebo (Koch & Paquette 1997), a masked examiner was chosen (Day & Altman 2000).

On the basis of our findings, NE did not appear to add significant benefits to conventional periodontal treatment 3 months after the completion of the adjunctive therapy. However, longer observation periods are needed in order to evaluate a possible long-term action of this adjunctive medication.

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Clinical Relevance

Scientific rationale for the study: BPs usage as adjunctive therapy in non-surgical periodontal treatment has been advocated on the basis of its host modulation activities. Indeed BPs down-regulates MMPs and modulates bone cell metabolism.

Principal findings: An adjunctive course of intra-muscular NE did not provide any additional clinical benefits as compared with scaling and root planing alone 6 months after periodontal treatment (3 months after the completion of BPs treatment).

Practical implication: On the basis of our findings there is no rationale for the usage of NE as an adjunctive medication in non-surgical periodontal treatment. Longer observation periods are needed in order to evaluate a possible long-term action of this adjunctive therapy.