*LETTER TO THE EDITOR*

# COMBINATION THERAPY INCLUDING SERRATIOPEPTIDASE IMPROVES OUTCOMES OF MECHANICAL-ANTIBIOTIC TREATMENT OF PERIIMPLANTITIS

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**This study was designed as a retrospective analysis of clinical outcomes of cases of periimplantitis treated by mechanical debridement and the administration of antibiotics combined or not with the administration of either the proteolytic enzyme serratiopeptidase (SPEP) or non-steroidal anti-inflammatory drugs (NSAIDs). Clinical charts of 544 partially edentulous patients treated for periimplantitis between June 1996 and December 2010 were analyzed to obtain clinical data of the affected implants just before the beginning of treatment and 12 months later to evaluate the outcomes of combined mechanical antibiotic treatment alone or in combination with the co-administration of the anti-inflammatory SPEP or NSAIDs. The comparative analysis revealed that therapeutic outcomes were significantly different in the three groups. Failure rate in the group that received SPEP (6%) was significantly lower compared to the group that received NSAIDS (16.9%; $P<0.01$) and to the group that received no anti-inflammatory therapy (18.9%; $P<0.01$). Treatment including SPEP was associated with significantly better healing also when successful treatments alone were considered. The data reported in this paper strongly support the hypothesis that SPEP is a valid addition to protocols for the combined therapy of peri-implantitis. In fact, it allows to enhance success rates significantly and also favors better tissue repair around successfully treated implants as compared to other regimens.**

The discovery that biomaterials can be osteointegrated has been a striking revolution for modern dental practice (1, 2) and has allowed to produce increasingly reliable dental implants. In spite of the progression of knowledge that enabled to optimize their mechanical integration, dental implants are still subject to failure, mostly due to the incidence of early-onset (3) or late-onset infections (4, 5), sustained by bacteria that grow as strongly adherent biofilms at the surface of the implants (6). Thanks to low metabolic activity, biofilms give rise to infections that are rarely recognized early and which are quite difficult to treat with antimicrobials (7-9).

In the case of dental implants, easier access to the infected site makes valid therapeutic options available in a significant proportion of cases. These infections are commonly known as peri-implantitis (PI) and are caused by oral or other opportunistic bacteria (10, 11). Once clinically evident, they are

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often characterized by severe inflammation affecting peri-implant tissues and causing various degrees of loss of the implant-supporting bone (12).

Accessibility of dental implants for visual inspection and manipulation has favored development of periodic mechanical maintenance programs to prevent the onset of infections and has, in parallel, inspired treatment protocols that include mechanical debridement of the implant surfaces (12-15). Most researchers agree that these mechanical procedures are helped in their efficacy by the administration of anti-infective agents (5, 16).

In spite of a significant number of comparative studies being carried out, available data indicate that no single method of combined mechanical-antibacterial treatment of PI is superior (13). The key feature of a successful treatment protocol for PI is the complete removal of the bacterial biofilm adhering to the implant surface. This is confirmed by a recent report showing that resolution of PI with surgical treatment alone is possible (12).

Previous *in vitro* studies performed by Selan and co-workers (7), supported by *in vivo* trials (17) have shown that serratiopeptidase (SPEP), a bacterial protease commercially available as an oral anti-inflammatory drug, and favoring the penetration of antibiotics at infected sites, significantly enhances the susceptibility of bacterial biofilms to several antibiotics and enhances therapeutic outcomes of antibiotic treatment of different prosthetic infections. Basing on these observations and due to the fact that anti inflammatory therapy can be freely administered in the treatment of acute infections and following surgical manipulations and that SPEP is an anti-inflammatory drug with a very safe pharmacologic profile, some of us have used it as an adjunctive therapy in the combined mechanical-antibiotic treatment of PI from 1996 onwards.

This paper is the report of a retrospective study performed by analyzing clinical charts of patients treated for PI by combined mechanical-antibiotic therapy alone or including the administration of SPEP or non-steroidal anti-inflammatory drugs (NSAIDs).

## MATERIALS AND METHODS

*Study population*

Dental charts of 544 patients treated for PI in the period June 1996 to December 2010 were analyzed. Inclusion criteria were the following: i) diagnosis of PI on at least one dental implant supporting a fixed prosthetic restoration; ii) availability of endoral standardized radiographs of the implanted area obtained according to a long-cone paralleling technique at the moment of diagnosis and during follow-up at 12 months from the beginning of treatment; iii) treatment of PI with combined mechanical-antibiotic therapy, associated or not with the administration of SPEP (Danzen®) 2 x 5mg twice a day; iv) availability of recorded clinical data at 12 months following treatment. Charts of totally edentulous patients were not included in the study.

*Evaluated clinical parameters*

The clinical parameters on the evaluated implants obtained from charts were the following: (a) plaque accumulation recorded by the modified Plaque Index (mPII) (18); (b) gingival bleeding on probing recorded by the modified sulcus bleeding index (mSBI) (18); (c) periodontal probing depth (PPD); d) modifications of the depth of bone lesion (ΔDBL) calculated by comparing intra-oral standardized radiographs of sites of interest. Calculations of ΔDBL were confirmed by re-analyzing radiographies by one of the study investigators blinded to all clinical information contained in the charts.

*Statistical analysis*

Evaluation of significance of differences in clinical and radiographic parameters among groups receiving mechanical-antibiotic treatment alone or associated with the administration of either SPEP or NSAIDs, was carried out by Student's *t*-tests and by Fisher exact test or *chi*-square test, performed at a significance level of $P \leq 0.01$ for very significant differences and of $P$ in the range $>0.01-\leq 0.05$, using statistical analysis tools of Microsoft Excel software and the online resource available at http://www.quantpsy.org.

## RESULTS

Clinical charts of 544 patients treated for PI were evaluated for the outcome of combined mechanical antibiotic treatment in relation to the co-administration of no anti-inflammatory drug or of SPEP or NSAIDs. As shown in patient characteristics reported in Table I, the patients were equally distributed among sexes. Moreover, when patients

**Table I.** *Main characteristics of patients divided into categories according to anti-inflammatory therapy, antibiotic treatments and localization of perimplantitis-affected sites.*

| | | | Implanted site Nr (%) | | | | Antibiotic Nr (%) | |
|---|---|---|---|---|---|---|---|---|
| Patient category (Nr) | Mean age (±SD) | SEX (M/F) % males | UF | UP | LF | LP | β-lactam | Other |
| Overall (544) | 39.6 (±9) | (277/267) 50.9 | 142 (26.1) | 130 (23.9) | 125 (23) | 147 (27) | 435 (80) | 109 (20) |
| SPEP (182) | 40.4 (±8.9) | (95/87) 52.2 | 49 (26.9) | 43 (23.6) | 44 (24.2) | 46 (25.3) | 144 (79.1) | 38 (20.9) |
| NSAIDs (166) | 39.6 (±8.6) | (84/82) 50.6 | 42 (25.3) | 40 (24.1) | 36 (21.7) | 48 (28.9) | 133 (80.1) | 33 (19.9) |
| No AIF (196) | 38.9 (±9.5) | (98/98) 50 | 51 (26) | 47 (24) | 45 (23) | 53 (27) | 158 (80.6) | 38 (19.4) |

*LP (lower posterior): implants located in the lower premolar to molar area; LF (lower front): implants located in the lower canine to canine area; UP (upper posterior): implants located in the upper premolar to molar area; UF (upper front): implants located in the upper canine to canine area. SPEP: Serratiopeptidase; NSAIDs: non-steroidal anti-inflammatory drugs; No AIF: no anti-inflammatory drug.*

**Table II.** *Failure rates of the studied implants distinguished according to location and treatment group.*

| | Failures/total (Failure rate %) | | | | *P* | | |
|---|---|---|---|---|---|---|---|
| Implant Location | Overall | SPEP | NSAIDs | No AIF | SPEP *vs* NSAIDS | SPEP *vs* No-AIF | NSAIDs *vs* No-AIF |
| ALL | 76/544 (14) | 11/182 (6) | 28/166 (16.9) | 37/196 (18.9) | **≤0.01** | **≤0.01** | 0.62 |
| LP | 29/147 (19.7) | 3/46 (6.5) | 13/48 (27.1) | 13/53 (24.5) | **≤0.01** | <u>0.03</u> | 0.82 |
| LF | 8/125 (6.4) | 2/44 (4.5) | 1/36 (2.8) | 5/45 (11.1) | 1 | 0.43 | 0.22 |
| UP | 19/130 (14.6) | 3/43 (7) | 7/40 (17.5) | 9/47 (19.1) | 0.18 | 0.12 | 1 |
| UF | 20/142 (14.1) | 3/49 (6.1) | 7/42 (16.7) | 10/51 (19.6) | 0.18 | 0.07 | 0.79 |

*LP (lower posterior): implants located in the lower premolar to molar area; LF (lower front): implants located in the lower canine to canine area; UP (upper posterior): implants located in the upper premolar to molar area; UF (upper front): implants located in the upper canine to canine area. Values of P were calculated by the Fisher exact test for LP, LF, UP and UF and by the Chi Square test for ALL. SPEP: Serratiopeptidase; NSAIDs: non-steroidal anti-inflammatory drugs; No AIF: no anti-inflammatory drug. Values of P≤0.01 indicating very significant differences are evidenced in bold and values of P in the range >0.01-≤0.05 indicating significant differences are underlined.*

were stratified according to anti-inflammatory therapy into three groups, no statistically significant differences were evident between the 182 patients who had received SPEP and the remaining 362 patients (166 patients who had received NSAIDs and 196 patients who had received no anti-inflammatory therapy) with regard to age ($P$=0.15), sex ($P$=0.65), and implanted site ($P$=0.52, 0.64, 0.92 and 0.79 for LP,

**Table III.** *Clinical data obtained from patients charts, and aggregated according to therapy and therapeutic outcomes.*

| | | | | Antibiotic Nr (%) | | Mean value (±SD) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Patient category (Nr) | Mean age (±SD) | SEX M/F (% males) | β-lactam | Other | mPII | mSBI | PPD | Δ-DBL |
| Successes | Overall (468) | 39.5 (±9) | 234/234 (50) | 422 (90.2) | 46 (9.8) | 0.86 (±0.51) | 1.11 (±0.4) | 2.73 (±0.56) | 3.63 (±0.84) |
| | SPEP (171) | 40.6 (±8.9) | 88/83 (51.5) | 142 (83.0) | 29 (17) | 0.82 (±0.48) | 0.99 (±0.34) | 2.68 (±0.5) | 4.1 (±0.85) |
| | NSAIDs (138) | 39.7 (±8.6) | 70/68 (50.7) | 129 (93.5) | 9 (6.5) | 0.87 (±0.54) | 1.18 (±0.44) | 2.79 (±0.59) | 3.38 (±0.73) |
| | No AIF (159) | 38.2 (±9.4) | 76/83 (47.8) | 151 (95) | 8 (5) | 0.89 (±0.52) | 1.19 (±0.39) | 2.74 (±0.59) | 3.35 (±0.69) |
| *P* | SPEP vs Others | 0.05 | 0.63 | **≤0.01** | | 0.2 | **≤0.01** | 0.12 | **≤0.01** |
| | SPEP vs NSAIDs | 0.37 | 0.9 | **≤0.01** | | 0.38 | **≤0.01** | 0.07 | **≤0.01** |
| | SPEP vs No AIF | 0.02 | 0.51 | **≤0.01** | | 0.18 | **≤0.01** | 0.34 | **≤0.01** |
| | NSAIDs vs No AIF | 0.17 | 0.61 | 0.58 | | 0.7 | 0.88 | 0.43 | 0.77 |
| Failures | Overall (76) | 40.1 (±9) | 43/33 (56.6) | 13 (17.1) | 63 (82.9) | 2.04 (±0.26) | 2.86 (±0.35) | 5.91 (±0.64) | -1.55 (±0.72) |
| | SPEP (11) | 37.4 (±9.1) | 7/4 (63.7) | 2 (18.2) | 9 (81.8) | 1.91 (±0.30) | 2.91 (±0.30) | 5.82 (±0.75) | -1.27 (±0.79) |
| | NSAIDs (28) | 39.1 (±8.6) | 14/14 (50) | 4 (14.3) | 24 (85.7) | 2.07 (±0.26) | 2.71 (±0.46) | 5.82 (±0.77) | -1.32 (±0.72) |
| | No AIF (37) | 41.7 (±9.3) | 22/15 (59.5) | 7 (18.9) | 30 (81.1) | 2.05 (±0.23) | 2.95 (±0.23) | 6.00 (±0.47) | -1.81 (±0.62) |
| *P* | SPEP *vs* others | 0.29 | 0.75 | 1 | | 0.07 | 0.59 | 0.62 | 0.16 |
| | SPEP *vs* NSAIDs | 0.60 | 0.5 | 1 | | 0.10 | 0.20 | 0.99 | 0.85 |
| | SPEP *vs* No AIF | 0.19 | 1 | 1 | | 0.09 | 0.67 | 0.34 | <u>0.02</u> |
| | NSAIDs *vs* No AIF | 0.26 | 0.46 | 0.74 | | 0.78 | **≤0.01** | 0.25 | **≤0.01** |
| | Successes *vs* failures | 0.59 | 0.29 | **≤0.01** | | **≤0.01** | **≤0.01** | **≤0.01** | **≤0.01** |

*SPEP: Serratiopeptidase; NSAIDs: non-steroidal anti-inflammatory drugs; No AIF: no anti-inflammatory drug. Values of P≤0.01 indicating very significant differences are evidenced in bold and values of P in the range >0.01-≤0.05 indicating significant differences are underlined.*

LF, UP and UF respectively). β-lactamic antibiotics were prescribed in 80% of cases (Table I) and no statistically significant difference was observed in the prescription of antibiotics among the three groups ($P$=0.82 for serratiopeptidase *vs* NSAIDs, $P$=0.72 for serratiopeptidase *vs* no anti-inflammatory therapy, and $P$=0.91 for NSAIDs *vs* no anti-inflammatory therapy). The β-lactamic antibiotics which had been prescribed were amoxicillin-clavulanate in 338 cases (77.7%), amoxicillin alone in 88 cases (20.2%) and other penicillins in the remaining 9 cases (2.1%). The 109 cases that were not treated with β-lactamic antibiotics, were prescribed macrolides in 55 cases (50.4%), tetracycline in 42 cases (38.5%) and fluoroquinolones in 12 cases (1.1%). No significant differences were observed in the distribution of antibiotic prescription among the three groups which had received SPEP, or NSAIDs or no anti-inflammatory drug, respectively.

Comparative analysis of indices reported in the clinical charts and of standardized radiographic images of the implanted sites obtained just before treatment of PI and after 12 months revealed that therapeutic outcomes were significantly different in the three groups of patients. Therapy was considered as a failure if it resulted in an increase of PPD values and/or loss of bone around the treated implant, as evidenced by values of ΔDBL ≤0mm. Using these criteria, the overall failure rate of combined therapy was 14% (Table II). Failure rate in the group that received SPEP (6%) was significantly lower compared to the group that received NSAIDs (16.9%; $P$<0.01) and to the group that received no anti-inflammatory therapy (18.9%; $P$<0.01) (Table II). With the exception of implants located in LF sites, combined treatment including serratiopeptidase always yielded better outcomes, although the limited number of cases makes the differences significant for implants located in LP sites alone (Table III). Irrespective of anti-inflammatory therapy, failures were always characterized by higher values of mPII and mSBI, although some marginal but significant differences were evident in both mSBI and Δ-DBL (Table III). Moreover, for successful cases, treatment including SPEP was associated with significantly lower mSBI and more relevant reduction of bone defects as compared to the remaining treatments (Table III).

## DISCUSSION

Research in the field of biomaterials has greatly improved the performances of dental implants, but did not affect significantly the incidence of PI, sustained by biofilm growing at the surface of implants (9). Early diagnosis of PI is very difficult due to inconsistency of symptoms and lack of specific tests. Moreover, resistance of biofilms to antibiotics (7) makes pharmacologic treatment of PI inefficient. A combination of mechanic/chemical debridement of the implant surface, and administration of systemic antibiotics (5, 13, 14) is considered as the gold standard in the treatment of PI, although success rates with this approach are not outstanding. SPEP, a widely diffused anti-inflammatory drug, was shown to significantly enhance the activity of most antibiotics on bacterial biofilm (7) and therapeutic outcomes of prosthetic infections (17). Basing on these results, we added SPEP to protocols of combined treatment of PI. More recently, we also demonstrated that the combined systemic administration of antibiotics and SPEP associated to mechanical debridement of implant surfaces significantly enhances success rates in the treatment of PI (19). After 15 years of experience we decided to compare clinical outcomes of this potentially improved protocol with those of other colleagues adopting the standard combined protocol that does not include SPEP.

Of the 544 cases that were included in the study, 76 were judged as failures. The overall failure rate is comparable to that reported elsewhere for this therapeutic approach (5, 13, 14, 20). Comparison of therapeutic outcomes when cases were aggregated into three groups basing on the presence/absence of adjunctive anti-inflammatory therapy and on the prescribed anti-inflammatory drug (SPEP or NSAIDs), demonstrated that administration of SPEP significantly reduced failure rate. Analysis of clinical parameters highlighted that SPEP induced a better repair of bone lesions and reduced inflammation more than other regimens even in successful cases, indicating that SPEP influences not only the clinical healing but also its quality. When β-lactamic antibiotics were included in therapy, success rates were always higher than 95% , while when other antibiotics were prescribed, success rates were very low in the groups having received NSAIDs or no anti-

inflammatory drug (27.3 and 21.1%, respectively) as compared to the group treated with SPEP where the success rate was 76.3%. This confirms that β-lactamic antibiotics are the antibiotic of choice in the combined treatment of PI and that, whenever administration of a different antibiotic is mandatory, the co-administration of SPEP is fundamental to obtain a positive outcome. The data reported in this paper strongly support the hypothesis that SPEP is a valid addition to protocols for the combined therapy of PI.

The fact that the different therapeutic strategies were performed by different, although all well-skilled, specialists could constitute a bias to significance of the results presented in this paper, although the high number of cases that were analyzed should minimize the impact of it.

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