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Why not revisiting tinidazole as potential treatment of odontogenic infections?

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Tinidazole is a 5-nitroimidazole initially introduced into clinical medicine in 1969 for the treatment of unicellular parasites. Tinidazole offers selective bactericidal activity, not influenced by the inoculum size, against anaerobic bacteria, that make it of theoretical interest against periodontopathogen infections. This article reviews the required characteristics of an antibiotic directed to odontogenic anaerobic infections, as well as the pharmacodynamic pitfalls of common antibiotic treatments. In addition the *in vitro,* pharmacokinetic and pharmacodynamic properties of tinidazole are reviewed, assessing the degree of its adhesion to the required characteristics, as well as identifying the gaps to be fulfilled prior to its use in this medical field. Tinidazole offers interesting characteristics making worthy investigations as a candidate for the treatment of anaerobic odontogenic infections.

Key words:
Tinidazole. Odontogenic infections. Odontopathogens. Pharmacodynamia.

Rev Esp Quimioter 2008;21(3):198-202

¿Por qué no reevaluar el tinidazol como tratamiento potencial de infecciones odontogénicas?

El tinidazol es un 5-nitroimidazol que se introdujo en 1969 en la clínica para el tratamiento de infestaciones por parásitos unicelulares. El tinidazol ofrece una actividad bactericida selectiva, no influida por el tamaño del inóculo, frente a bacterias anaerobias, por lo que presenta un interés teórico en infecciones producidas por odontopatógenos. Este artículo revisa las características que requiere un antibiótico dirigido al tratamiento de infecciones odontogénicas por bacterias anaerobias, así como las carencias farmacodinámicas de los antibióticos habitualmente utilizados en este tipo de infecciones. Asimismo se revisan las propiedades *in vitro,* farmacocinéticas y farmacodinámicas de tinidazol, valorándose el grado de adhesión de este compuesto a las características requeridas para un antibiótico dirigido a este tipo de infecciones. También se identifican las lagunas de conocimiento sobre tinidazol que deben resolverse antes de su utilización en este campo. Tinidazol ofrece unas características interesantes que posibilitan realizar investigaciones como candidato al tratamiento de infecciones odontogénicas anaerobias.

Palabras clave:
Tinidazol. Infecciones odontogénicas. Odontopatógenos. Farmacodinamia.

INTRODUCTION

Periodontal disease is the most prevalent infectious disease in the community (50 % and 30 % of adult population present gingivitis and periodontitis, respectively)[1] and it can be considered as a subacute or chronic disease with acute reactivations along the individual life time[2]. The subgingival plaque is the basis of periodontal disease, and three hypotheses (that physiopatologically can be concomitant) have been postulated[3].

a) The specific plaque hypothesis: specific bacteria are the etiological agents of the disease.

b) The non-specific plaque hypothesis: the periodontal disease is an inflammatory disease, and the inflammatory process is a response to the bacterial biomass present in the plaque.

c) The environmental plaque hypothesis: the subgingival environment, when including in high amount some specific bacterial species, is responsible for the disease. This hypothesis may be considered a combination of the other two hypotheses.

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Regardless the responsibility of one of these three hypotheses on the disease, the therapeutic consequences are the same since therapeutic strategies (mechanical, pharmacological, or both) in periodontitis are directed to reduce the bacterial load, whether to preclude direct damage of periodontopathogens or to decrease the inflammatory stimuli[2].

While early odontogenic infections are usually produced by aerobic streptococci, subacute, chronic or late infections are produced by anaerobes[4]; thus the first treatment approach is to differentiate early from late infections[4-8]. Gingivitis and chronic periodontitis are subacute or chronic infections and, when antibiotic therapy is needed, amoxicillin/clavulanic acid or clindamycin are recommended[8] based on the high susceptibility rates of most anaerobic species, despite the non-susceptibility rates of some streptococci species[9].

An etiological based treatment should consider two types of periodontopathogens:

a) Odontopathogens as *Treponema denticola* (and other non-cultivable anaerobic spirochetes) whose prevalence increases as severity increases (greater gingival pocket depth), as basis of the consideration of periodontitis as an infectious disease.

b) Bacteria responsible for the non-specific bacterial growth and the subsequent inflammatory disease, being *Prevotella* spp. the most prevalent anaerobe isolate.

Anaerobic treatment should be directed against both types of bacteria. In early and acute infections, as periodontal abscess, coverage against facultative microorganisms (*Streptococcus viridans* species) should be added.

TARGET BACTERIA FOR ANTIBIOTIC TREATMENT

Different bacterial species are associated with health or disease in relation to odontogenic infections[2,10-14]. With respect to anaerobes, *Veillonella* spp. is the most common anaerobe in healthy individuals while *Prevotella intermedia* and *Fusobacterium nucleatum* are present in gingivitis. In addition to these three periodontopathogens, *Treponema denticola, Porphyromonas gingivalis* and *Tannerella forsythensis* are found in chronic periodontitis. In juvenile periodontitis, *Actinobacillus actinomycetemcomitans* is also present.

While the responsibility of streptococci has been clearly identified in focal systemic infections after odontological procedures in patients at risk (as endocarditis in patients with endocardic alterations) and in acute local infections as the periodontal abscess, their responsibility in subacute/chronic periodontal disease is not so evident since *Streptococcus* spp. (mainly *S. sanguis* and *S. mitis*) are found in healthy individuals as well as in patients with gingivitis and chronic periodontitis[2].

Analysis of the subgingival plaque has demonstrated that oral microflora can be defined as color-coded complexes (purple, yellow, green, orange and red)[3,15] since bacteria in different health/disease ecological multibacterial niches are present in different specific aggregations. This color sequence represents progression from health (purple complex) to disease, with orange (*Prevotella* spp., *Fusobacterium* spp.) and red (where *Prophyromonas* spp., *Tannerella* spp. and *Treponema* spp. are added) complexes associated with periodontitis. Thus, there is a progression of microflora from health (characterized by a predominantly gram-positive, aerobic, non-motile microflora) to disease (characterized by a gram-negative, anaerobic, motile microflora)[3].

In a study carried out in our country[9], PCR detection yielded positive results for *Tannerella forsythensis* in approximately 71 %, *T. denticola* in 63 %, *Porphyromonas gingivalis* in 58 %, and *Prevotella intermedia* in 36 % patients with periodontitis. Interestingly, detection of *T. denticola* ($p = 0.01$), *P. gingivalis* ($p = 0.05$) and *T. forsythensis* ($p = 0.1$) was associated (significantly higher detected, chi-square) with pocket depth > 4 mm[9]. Isolation rates (frequency per 100 patients) of the different species were approx. 60 % for *Prevotella buccae,* 40 % for *Prevotella denticola,* 38 % for *Fusobacterium nucleatum,* 35 % for *Prevotella intermedia,* and 23 % for *Veillonella* spp. and *Prevotella melaninogenica,* with respect to anaerobes. ß-lactamase production, rendering amoxicillin inactive, was positive in 54 % *Prevotella* spp., 39 % *F. nucleatum,* and 10 % *Veillonella* spp. Nearly 100 % anaerobes were susceptible to metronidazole and amoxicillin/clavulanic acid, while resistance rates for clindamycin ranged from 9 % to 21 % in *Prevotella* species[9].

With respect to aerobes and facultatives, isolation rate was approx. 71 % for *S. oralis* and *S. mitis* and 25 % for *Capnocytophaga* (30 % of them were ß-lactamase producers)[9]. Amoxicillin and clindamycin were the most active compounds against *S. mitis* and *S. oralis*[9], and this should be taken into account if aerobic/facultative coverage is needed.

PITFALLS OF APPROVED ANTIBIOTIC TREATMENTS/PHARMACODYNAMIA

Clinical trials in odontogenic infections are scarce and usually include a low number of patients; for this reason pharmacodynamic principles have been used to evaluate the degree of periodontopathogen coverage with different compounds[16]. Pharmacodynamia explores the relation between systemic antibiotic concentrations along the dosing interval and in vitro susceptibility, defining parameters that predict efficacy. The pharmacodynamic parameter predicting efficacy for antibiotics with time-dependent action (ß-lactams, macrolides, clindamycin) is the time (expressed as % dosing interval) that concentrations exceed the minimal inhibitory concentration (MIC) (T>MIC), while in the case of concentration-dependent antibiotics (azalides, metronida-

zole), the parameter predicting efficacy is the relation between the area under the serum concentration-time curve (AUC) and MIC (AUC/MIC). Classically it has been considered that T > MIC values > 40 % and AUC/MIC values >25 are associated to efficacy[16].

Given the prevalence of ß-lactamase producing anaerobes among periodontopathogens, amoxicillin given as monotherapy cannot be considered an adequate antibiotic in the treatment of infections caused by these bacteria, due to its low T > MIC values consequence of the enzymatic hydrolysis by the ß-lactamase produced.

Taking *Prevotella intermedia, Porphyromonas gingivalis, Fusobacterium spp.* and *Tannerella forsythensis* as anaerobic index bacteria, only three-times daily (tid) amoxicillin/clavulanic acid regimens and clindamycin, but not spiramycin, obtain adequate T > MIC values against them[16]. In the case of concentration-dependent antibiotics, twice daily (bid) regimens of metronidazole, but not of quinolones or azalides, obtain adequate AUC/MIC values[16].

The pharmacodynamic relationship of antibiotics and odontopathogens has also been described[9] using crevicular fluid concentrations (36.7 µg/ml for metronidazole, 14 µg/ml for amoxicillin —and 0.40 µg/ml for clavulanic acid—, 5 µg/ml for spiramycin, and 2 µg/ml for clindamycin, after standard doses)[17-19]. Mean gingival crevicular concentrations of amoxicillin/clavulanic acid and metronidazole cover MIC_{90} (MIC value inhibiting 90 % isolates) of anaerobic isolates, but this is not the case of clindamycin that pharmacodynamically does not cover resistant strains and several susceptible strains[9]. Spiramycin crevicular fluid concentrations are below MIC_{90} values for *Fusobacterium nucleatum*[16,17].

TINIDAZOLE AND ODONTOGENIC INFECTIONS

Tinidazole and metronidazole are the only nitroimidazoles available in the United States[20].

Tinidazole is a 5-nitroimidazole initially introduced into clinical medicine in 1969 for antiparasitic treatment of Trichomonas vaginalis infections and later on for the treatment of infections by *Entamoeba histolytica* and *Giardia lamblia*[21]. There is a common feature for these three unicellular parasites: they are anaerobic microorganisms. Tinidazole is intracellularly reduced (mediated by a ferredoxin system and a low oxidation-reduction potential) to active intermediates[21]. Most aerobic microorganisms do not generate low oxidation-reduction potential, explaining the selective activity of tinidazole against anaerobic microorganisms[21].

a) *In vitro* activity

Scarce (occasional reports) and old data are available regarding tinidazole activity against anaerobic bacteria of odontological interest. It has been reported that tinidazole is in vitro active against most anaerobic bacteria including *Tannerella* (previously *Bacteroides*) and *Fusobacterium* species[21], and that *Bacteroides* and *Fusobacteria* are regularly inhibited by 0.5 µg/ml nitroimidazoles[22]. Minimal bactericidal concentration (MBC) values for nitroimidazoles are equal to MIC values[23]. Tinidazole is rapidly bactericidal, its activity is not affected by the inoculum size, and emergence of resistance during treatment is rare[21].

b) Pharmacokinetics

Tinidazole in daily doses up to 2 g is free of toxicity, exhibiting a half-life of 12–14 h (doubling that of metronidazole) allowing once daily regimens[24]. Tinidazole is almost complete absorbed after oral administration and a single 2 g dose provides peak serum concentrations of 40 µg/ml declining to 10 µg/ml at 24 h and 2.5 µg/ml at 48 h[24]. Daily maintenance doses of 1g maintain drug concentrations above 8 µg/ml throughout the treatment period[24]. Six hours after administration of 1 g single dose, mean serum concentrations are 19.3 µg/ml[25]. AUC values range from approx. 600 µg × h/ml with the 1 g dose to 1,000 µg × h/ml with the 2 g dose[24].

Tinidazole is secreted in saliva with concentrations in mixed, parotid and submandibular saliva similar to those in serum at the same time[22,24]. Concentrations are detectable in saliva up to 7 days after 1 g single dose administration when serum concentrations are not found[25]. After 1g single dose administration, concentrations in mixed, parotid and submandibular saliva were approximately 10 µg/ml at 1 h, 20 µg/ml from 3 h to 6 h, and 10 µg/ml at 24 h[22]. Three hours after 1 g single dose administration, crevicular fluid concentrations are per-patient similar to those in serum (mean 18.5 µg/ml; range 14.6–24.6), and in alveolar bone, mean ± SD concentrations are 1.9 ± 0.5 µg/ml[22,26].

When measuring *in vivo* effects (quantitative counting of anaerobic bacteria in saliva) after 1 g single dose administration to 10 subjects, salivary anaerobic flora suffered a marked decrease in all subjects, with completely disappearance after 6 h of *Fusobacteria* in 8 subjects and *Veillonella spp.* in 4 subjects[25].

c) Theoretical pharmacodynamia and *in vivo* antibacterial activity

Resistance to metronidazole is 0 % in *Veillonella, Fusobacterium,* and most species of *Prevotella* (*melaninogenica, denticola, oralis...*), and <6 % in *Prevotella intermedia* and *buccae*[9]. MIC_{90} of metronidazole is 2–6 µg/ml for *Prevotella intermedia,* 2 µg/ml for *Porphyromonas gingivalis,* 1–8 µg/ml for *Fusobacterium nucleatum* and 0.25 µg/ml for *Tannerella forsythensis*[9,16]. Assuming the same intrinsic activity for both nitroimidazoles (tinidazole and metronidazole), MIC

values of tinidazole against most anaerobic periodontopathogens would be far below concentrations in serum, saliva and crevicular fluid.

When considering the value of the pharmacodynamic parameter predicting efficacy for nitroimidazoles (AUC/MIC >25[16]), this value is exceeded with tinidazole for MIC_{90} values against all *Prevotella* spp., *Veillonella* spp., *Fusobacterium nucleatum, Tannerella forsythensis* and *Porphyromonas gingivalis* with the 1 g dose if we assume that tinidazole MIC_{90} values are lower or equal to metronidazole MIC_{90} values.

GAPS IN THE KNOWLEDGE OF TINIDAZOLE FOR THE TREATMENT OF ODONTOGENIC INFECTIONS

Scarce data on tinidazole is available from investigations in the specific field of odontogenic infections, whether from the in vitro susceptibility, pharmacokinetics, pharmacodynamia, or clinical points of view, but previous data on tinidazole point it as a promising agent in this field. Compared with metronidazole, tinidazole has the potential of improving the pharmacodynamic coverage and/or the dosing regimen of the principal nitroimidazole.

From the in vitro perspective, susceptibility data obtained with panels of recent periodontopathogens isolates would be desirable. From the pharmacokinetic perspective, lower doses can be explored because the serum and crevicular fluid concentrations after 1 g dose administration are highly above the expected MIC. From the pharmacodynamic and clinical points of view it would be necessary to relate isolates (and the tinidazole MICs against them), crevicular fluid concentrations and outcome in patients with periodontitis.

On the other hand, treatments in Odontology are usually directed to both aerobic and anaerobic bacteria, and for this reason antibiotic combinations are usually prescribed[27,28]: *a)* spiramycin + metronidazole, despite the fact of high resistance rates to macrolides (frequently associated to resistance to tetracyclines, clindamycin and azithromycin) in *Streptococcus* viridans in our country[29-31], and *b)* amoxicillin + clavulanic acid to cover ß-lactamase producing isolates from *Prevotella, Fusobacterium and Veillonella* species. From this perspective, an association of tinidazole and amoxicillin seems an interesting possibility to be explored because it could improve the coverage of the whole bacterial spectrum in odontogenic infections. To this end, *in vitro* killing curves studies against multibacterial inocula (simulating a periodontopathogen multibacterial niche) with both aerobic/facultative and anaerobic microorganisms, comparing amoxicillin, tinidazole and amoxicillin + tinidazole would be the first step to explore the potential of this possible association: the most potent drug against the aerobic/facultative component (amoxicillin) and the anaerobicidal candidate tinidazole.

From the pharmacokinetic point of view this possible association needs to be synchronized because of the different half-life values of amoxicillin and tinidazole. Again, pharmacodynamic studies with lower doses of tinidazole are desirable. A possibility to be explored is bid regimens with high amoxicillin concentrations and tinidazole doses lower than those previously studied (1 and 2 g).

Tinidazole has been evaluated in the prevention of postoperative complications after surgical removal of the third molar[32-34] as well as in the treatment of adult periodontal disease with or without adjunctive scaling[35].

CONCLUSION

Tinidazole may offer interesting characteristics making worthy investigations as a candidate for the treatment of anaerobic odontogenic infections. Maybe it is time to revise a classic antibiotic considered mainly as an antiparasitic agent that may help to counter some pitfalls of previous antibiotic treatments in an area where 80 % of success of mechanical treatment is inexplicably accepted by clinicians[36], and where only in the 20 % cases of failure of mechanical treatment the antibiotic treatment is mandatory[37].

REFERENCES

1. Loesche WJ, Grossman NS. Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment. Clin Microbiol Rev 2001;14:727-52.
2. Aguilar L, Giménez MJ. La teoría unitaria en la etiopatogenia de la infección odontogénica. En: Bascones A, Noguerol B, Prieto J, editores. Infecciones odontogénicas en la comunidad y antibioterapia: dos factores a sincronizar. Madrid: Adalia Farma/GETECCU, 2006:25-36.
3. Preshaw PM, Seymour RA, Heasman PA. Current concepts in periodontal pathogenesis. Dent Update. 2004;31:570-2, 574-8.
4. Wynn RL, Bergman SA, Meiller TF, Crossley HL. Antibiotics in treating oral-facial infections of odontogenic origin: an update. Gen Dent 2001;49:238-40.
5. Flynn TR. The swollen face. Severe odontogenic infections. Emerg Med Clin North Am 2000;18:481-519.
6. Bascones A, Manso F. Infecciones odontógenas de la cavidad bucal y región máxilofacial. Av Odontoestomatol 1994;10:5-26
7. Bascones A, Manso F, Bascones J. Antibióticos. En: Bascones A, editor. Tratado de Odontología. Madrid: Ediciones Avances Medicodentales S.L., 2000;4317-30.
8. Bascones A, Maestre JR, Prieto J. Tratamiento y profilaxis antibiótica en odontología. En: Bascones A, Noguerol B, Prieto J, editores. Infecciones odontogénicas en la comunidad y antibioterapia: dos factores a sincronizar. Madrid: Adalia Farma/GETECCU, 2006:71-83.
9. Maestre JR, Bascones A, Sánchez P, Matesanz P, Aguilar L, Giménez MJ et al. Odontogenic bacteria in periodontal disease and resistance patterns to common antibiotics used as treatment and prophylaxis in odontology in Spain. Rev Esp Quimioter 2007;20:61-7.
10. Prescription des antibiotiques en odontologie et stomatologie. Recommandations et argumentaire. Agence Française de Sé-

curité Sanitaire des Produits de la Santé. 2001. Available in www.afssaps.sante.fr.
11. Chow AW. Infections of oral cavity, neck and head. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett principles and practice of infectious diseases, 6th ed. Philadelphia: Elsevier Churchill Livingstone, 2005:787-802.
12. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. Periodontol 2000 1997;14:12-32.
13. Tanner A, Stillman N. Oral and dental infections with anaerobic bacteria: clinical features, predominant pathogens, and treatment. Clin Infect Dis 1993;16 Suppl 4:S304-9.
14. Tatakis DN, Kumar PS. Etiology and pathogenesis of periodontal diseases. Dent Clin North Am 2005;49:491-516.
15. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134-44.
16. Canut A. Antimicrobianos en las infecciones odontogénicas: análisis farmacocinético/farmacodinámico (PK/PD). Seguridad y tolerancia. En: Bascones A, Noguerol B, Prieto J, editores. Infecciones odontogénicas en la comunidad y antibioterapia: dos factores a sincronizar. Madrid: Adalia Farma/GETECCU, 2006:51-69.
17. Poulet PP, Duffaut D, Barthet P, Brumpt I. Concentrations and in vivo antibacterial activity of spiramycin and metronidazole in patients with periodontitis treated with high-dose metronidazole and the spiramycin/metronidazole combination. J Antimicrob Chemother 2005;55:347-51.
18. Tenenbaum H, Jehl F, Gallion C, Dahan M. Amoxicillin and clavulanic acid concentrations in gingival crevicular fluid. J Clin Periodontol 1997;24:804-7.
19. Walker CB, Gordon JM, Cornwall HA, Murphy JC, Socransky SS. Gingival crevicular fluid levels of clindamycin compared with its minimal inhibitory concentrations for periodontal bacteria. Antimicrob Agents Chemother 1981;19:867-71.
20. Phillips MA, Stanley SL. Chemotherapy of protozoal infections. In: Bunton LL, Lazo JS, Parker K, editors. Goodman and Gilman's. The Pharmacological Basis of Therapeutics. 11 ed. New York: McGraw-Hill; 2005:1049-72.
21. Nord CE. Microbiological properties of tinidazole: spectrum, activity and ecological considerations. J Antimicrob Chemother 1982;10 Suppl A:35-42.
22. Von Konow L, Nord CE. Concentrations of tinidazole and metronidazole in serum, saliva and alveolar bone. J Antimicrob Chemother 1982;10 Suppl A:165-72.
23. Wust J. Susceptibility of anaerobic bacteria to metronidazole, ornidazole, and tinidazole and routine susceptibility testing by standardized methods. Antimicrob Agents Chemother 1977;11:631-7.
24. Wood BA, Faulkner JK, Monro AM. The pharmacokinetics, metabolism and tissue distribution of tinidazole. J Antimicrob Chemother 1982;10 Suppl A:43-57.
25. Heimdahl A, von Konow L, Nord CE. Effect of tinidazole on the human oral microflora: a comparison between high single and low repeated doses. J Antimicrob Chemother 1982;10 Suppl A:157-64.
26. Bascones A, Manso F. Aspectos diagnósticos y terapéuticos de las infecciones orofaciales. Av Odontoestomatol 1996;12 (Suppl A):9-22.
27. Granizo JJ. Impacto ecológico de los tratamientos antimicrobianos en odontología. En: Bascones A, Noguerol B, Prieto J, editores. Infecciones odontogénicas en la comunidad y antibioterapia: dos factores a sincronizar. Madrid: Adalia Farma/GETECCU, 2006:85-97.
28. Granizo JJ, Giménez MJ, Bascones A, Aguilar L. Impacto ecológico del tratamiento antibiótico de las infecciones odontológicas. Rev Esp Quimioter. 2006;19:14-20.
29. Rodriguez-Avial I, Rodriguez-Avial C, Culebras E, Picazo JJ. In vitro activity of telithromycin against viridans group streptococci and Streptococcus bovis isolated from blood: antimicrobial susceptibility patterns in different groups of species. Antimicrob Agents Chemother 2005;49:820-3.
30. Rodriguez-Avial I, Rodriguez-Avial C, Culebras E, Picazo JJ. Distribution of tetracycline resistance genes tet(M), tet(O), tet(L) and tet(K) in blood isolates of viridans group streptococci harbouring erm(B) and mef(A) genes. Susceptibility to quinupristin/dalfopristin and linezolid. Int J Antimicrob Agents 2003; 21:536-41.
31. Tomás I, Limeres J, Diz P. Antibiotic prophylaxis. Br Dent J 2005; 198:60-1.
32. Mitchell DA, Morris TA. Tinidazole or pivampicillin in third molar surgery. Int Oral Maxillofac Surg 1987;16:171-4.
33. Bystedt H, von Konow L, Nord CE. Effect of tinidazole on postoperative complications after surgical removal of impacted mandibular third molars. Scand J Infect Dis (Suppl.) 1981;26:135-9.
34. Mitchell DA. A controlled clinical trial of prophylactic tinidazole for chemoprophylaxis in third molar surgery. Dr Dent J 1986; 160:284-6.
35. Gallardo F, Huerta J, Cruz E et al. Efectos del tinidazol en el tratamiento de la enfermedad periodontal del adulto. Estudio clínico y microbiológico. Av Period Impl 1994;6:161-74.
36. Meador HL, Lane JJ, Suddick RP. The long-term effectiveness of periodontal therapy in a clinical practice. J Periodontol 1985; 56:253-8.
37. McFall WT Jr. Tooth loss in 100 treated patients with periodontal disease. A long-term study. J Periodontol 1982;53:539-49.