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Predominant Cultivable Microflora on Spent Minocycline Strips

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INTRODUCTION

Local delivery of antimicrobial agents into periodontal pockets offers further possibilities in periodontal therapy. A considerable number of agents using different vehicles have been developed. Some clinical studies showed the agents tested to be as effective as conventional mechanical therapy, however in most studies the agents were adjunctive to mechanical therapy. A clinical project was carried out to investigate if local delivery of minocycline strips could produce added clinical effects in residual periodontal pockets one month after a course of non-surgical periodontal therapy. The present investigation was carried out to study the short-term effects of the minocycline strips on subgingival microbiology.

MATERIALS & METHODS

Subjects:

- 14 adults patients (8 tests, 6 controls) randomly selected from a group of 32 periodontitis patients who participated in a double-blind randomized parallel clinical trial on a local delivery Minocycline Strip (Vehicle-polycaprolactone, Dong Kook Pharmaceutical Co., Seoul, Korea).
- Bleeding on probing (BOP) and probing depth (PD) were measured using Florida Probe (Gainesville, FL) one month after non-surgical periodontal therapy.
- Strips (minocycline or control) were inserted into all residual periodontal pockets (PD ≥ 5 mm) of the participants, 2 times for 3 days each, one month after a course of non-surgical therapy. Strips were also placed into sample sites for 60 sec and then removed (day 0) to study the microbiology of the site at baseline. Each subject contributed one site.
- Strips were retained by Coe-pak®. (GC American Inc.)
- Chlorhexidine mouth rinses twice daily were performed during the period of strip retention.

Laboratory investigations:

- Spent minocycline and control strips retrieved at days 0, 3 and 6 were subjected to:
  - i) anaerobic culture using Columbia blood agar supplemented with 5% defibrinated horse blood, 5 mg/l hemin and 500µg/l menadione
  - ii) culture for coliform bacteria using MacConkey agar
  - iii) yeast culture using Sabouraud's dextrose agar

RESULTS

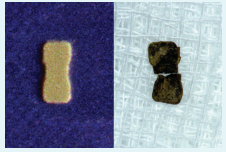

Figure 1. Minocycline strip used in the study. Left: new minocycline (test) strip; right: 3 day spent minocycline strip.

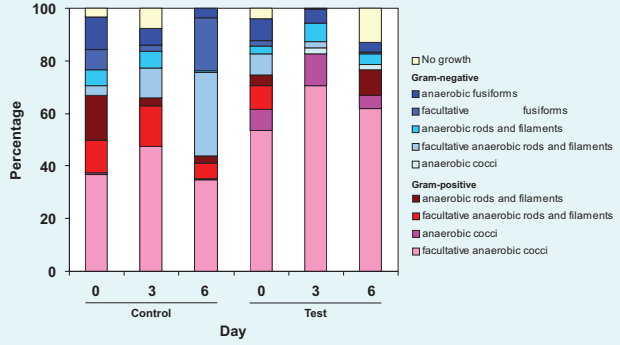

Figure 2. Relative mean proportion of predominant cultivable bacterial types from spent minocycline (test) and control strips. Significant reduced proportion of gram-negative rods and fusiforms was observed on day-6 test spent minocycline strips than on day-6 control strip ($P < 0.05$, Bonferroni multiple comparison).

Table 1. Demographic data and clinical parameters of subjects.

	Test	Control
n	8	6
Age (years)[a]	48.0 + 6.5	46.7 + 8.4
% Female	50.0	33.3
Oral condition post initial therapy		
%BOP[a]	45.7 + 20.7	43.0 + 22.0
No. of teeth with PD > 5mm[a]	3.1 + 1.6	4.7 + 2.5
No. of sites with PD > 5mm[a]	4.1 + 2.4	8.8 + 6.0
Sampling site PD (mm)[a]	7.3 + 2.0	7.5 + 1.0

[a] mean + SD

Table 2 Prevalence of isolation on spent minocycline (test) and control strips[a]

	Test			Control		
	day 0	day 3	day 6	day 0	day 3	day 6
Gram-positive						
Facultative anaerobic cocci						
Gemella haemolysans	37.5	0	37.5	33.3	33.3	16.7
Gemella morbillorum	62.5	62.5	37.5	50	33.3	50
Granulicatella adiacens	0	0	25	0	0	0
Micrococcus spp.	25	0	0	16.7	0	16.7
Staphylococcus auricularis	12.5	12.5	0	0	0	33.3
Staphylococcus lentus	0	25	0	0	0	16.7
Streptococcus constellatus	0	0	0	0	16.7	33.3
Streptococcus equinus	25	0	12.5	0	16.7	0
Streptococcus intermedius	25	12.5	25	16.7	0	33.3
Streptococcus mitis biovar 1[b]	50	37.5	37.5	16.7	0	0
Streptococcus oralis	50	62.5	37.5	33.3	66.7	16.7
Anaerobic cocci						
Anaerococcus prevotii	62.5	25	12.5	0	0	16.7
Facultative anaerobic rods						
Peptostreptococcus micros	37.5	12.5	12.5	0	0	0
Actinomyces naeslundii	12.5	0	0	50	0	33.3
Gram-negative						
Anaerobic cocci						
Veillonella spp.	0	25	25	0	0	0
Facultative anaerobic rods						
Kingella kingae	25	0	0	0	0	0
Anaerobic rods						
Campylobacter gracillis	25	12.5	0	33.3	16.7	0
Prevotella melaninogenica	25	12.5	0	0	0	0
Facultative anaerobic fusiforms						
Capnocytophaga gingivalis	25	0	12.5	66.7	33.3	16.7
Anaerobic fusiforms						
Fusobacterium necrogenes	25	0	12.5	16.7	0	0
Non-oral	25	37.5	25	33.3	50	16.7
Lost/unidentified spp	50	12.5	37.5	66.7	83.3	50

[a] Only species with frequency of isolation ≥ 20% in any sample are included.

[b] Significantly higher prevalence of isolation from test than control spent (minocylcine) strips, Fisher exact test, $P = 0.01$.

Table 3 List of minor microbes isolated[a]

Anaerobic culture

Gram positive

Facultative anaerobic cocci
Enterococcus faecalis
Kocuria varians
Lactococcus lactis subsp. *cremoris*
Lactococcus lactis subsp. *lactis*
Leuconostoc spp.
pneumoniae[c]
Staphylococcus aureus
Staphylococcus capitis[b]
Staphylococcus epidermidis
Staphylococcus hominis[b]
Staphylococcus lugdunensis
Staphylococcus saccharolyticus[b]
Staphylococcus xylosus
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus salivarius
Streptococcus sanguinis
Anaerobic cocci
Peptonipihilus asaccharolyticus
Facultative anaerobic rods
Actinomyces odontolyticus
Actinomyces pyogenes
Lactobacillus casei
Lactobacillus jensenii
Anaerobic rods
Actinomyces israelii
Actinomyces meyeri
Clostridium hastiforme[b]
Collinsella aerofaciens[b]
Propionibacterium acnes
Propionibacterium granulosum[b]

Gram negative

Facultative anaerobic rods
Enterobacter aerogenes[b]
Enterobacter cloacae[b,c]
Enterobacter gergoviae[b]
Haemophilus paraphrophilus
Klebsiella pneumoniae subsp.

Klebsiella oxytoca[b]
Pseudomonas aeruginosa[b,c]
Anaerobic rods
Bacteroides vulgatus[b]
Campylobacter rectus
Prevotella denticola
Prevotella disiens
Prevotella intermedia
Prevotella loescheii
Facultative anaerobic fusiforms
Capnocytophaga ochracea
Capnocytophaga sputigena
Anaerobic fusiforms
Fusobacterium nucleatum
Fusobacterium perfoetens[b]
Fusobacterium varium
Fusobacterium spp.

Coliform culture

Enterobacter sakazakii[b]
Raoultella terrigena[b]

Yeast culture

Candida albicans
Candida guilliermondii
Candida parapsilosis
Trichosporon mucoides[b]

[a] Species with frequency of isolation < 20% in any sample.
[b] Microbes that are not normally considered as members of the oral or oropharyngeal flora.
[c] Also isolated from coliform culture.

CONCLUSION

1) Minocycline (1.4 mg/strip) in polycaprolactone vehicle suppressed some bacteria but did not eliminate the subgingival colonization of the strip by microbial plaque species,
2) Predominant cultivable microbes from spent test and control strips were gram-positive cocci. Small amounts of gram-negative rods were also found colonizing spent strips,
3) *Enterobacteriaceae* colonization of spent test strips in the subgingival pocket environment was observed, indicating that it might be in the best interest of the patients to remove the minocycline strips after a shorter period of time,
4) Minimal amounts of yeast were recoverable from spent test and control strips within the study time period,
5) The microbiota colonizating the spent test strips from residual periodontal pockets of patients one month after non-surgical therapy was largely compatible with periodontal health.

ACKNOWLEDGEMENT

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