Efficacy of Single Dose of Acyclovir in the Treatment of Chronic Periodontitis: A Pilot Study

Sepher Tabatabaee, Hengameh Khosropanah, Manoochehr Rasouli, K Vasegh, Nabil Khzam, Roy George

ABSTRACT
Background: Periodontitis is a common, chronic and progressive disease affecting majority of the population. Current evidence indicates that Porphyromonas gingivalis is one of the most common pathogens isolated in patients with periodontitis. Aims and Objectives: To compare the clinical parameters and presence of P. gingivalis following a single subgingival application of 2.5% acyclovir gel combined with Scaling and Root Planing (SRP) or SRP alone. Materials and Methods: Healthy patients (10 females; 10 males) diagnosed with moderate to severe chronic periodontitis received manual SRP and oral hygiene instructions before random assignment into intervention (2.5% acyclovir gel + SRP) and control (SRP) groups. Clinical parameters (periodontal pocket depth, clinical attachment level, and bleeding on probing) were recorded and subgingival plaque sampling performed before treatment and after one and three months. Results: There was a significant reduction in P. gingivalis count and the mean PPD between the baseline and at first and third months post-therapy for both groups, but no difference between the two groups. There was a significant decrease in CAL at each time point in the intervention group as compared with control. Conclusions: SRP with or without a single application of 2.5% acyclovir gel improves clinical parameters and reduce P. gingivalis count for patients with chronic periodontitis.

Keywords: Acyclovir; Chronic Periodontitis; Root Planing; Scaling

Introduction
Periodontal disease is a family of related diseases that have a common underlying chain of events or pathogenesis. It results from the complex interplay of various pathogenic events caused by the microbial challenge of dental plaque and modifiers, and/or factors that modify host response mechanisms or alter innate susceptibility. In periodontal disease, the components of host response that normally provide protection are the same ones that accomplish destruction.1

Recent studies have demonstrated an association between chronic periodontitis and the herpes group of viruses, the most common DNA viruses in oral pathology.2 Opportunistic bacterial infection is a common secondary consequence of viral infection, and various patient responses are proposed to result from a herpes viral-bacterial interaction in chronic periodontitis, including viral cytopathic effects, increased levels of pro-inflammatory cytokines and enhanced periodontal pathogenicity.2

A periodontal herpes virus infection is typically associated with an increased occurrence of periodontopathogenic bacteria.3 A study of chronic periodontitis found statistically significant associations between periodontal Epstein–Barr virus type 1 or cytomegalovirus and pathogens such as, P. gingivalis, Tannerella forsythia, Prevotella intermedia, Prevotella nigrescens and Treponema denticola.4 Quantitative polymerase chain reaction (PCR) between genome copy-counts of Epstein-Barr virus or cytomegalovirus and P. gingivalis and T. forsythia.5 The uncertainty about the infectious and clinical events of periodontal breakdown has resulted in several hypotheses about the etiology of periodontitis.

This study sought to assess the effect of a single, subgingival application of a commonly used anti-viral agent, acyclovir gel (2.5%), in combination with scaling and root planing (SRP), as compared with SRP alone, for treatment against P. gingivalis in patients diagnosed with chronic severe periodontal disease.

Materials and Methods
Sample population: This pilot, case-controlled study was conducted at the Department of Periodontics, with the approval from the Human ethic committee of the University of Shiraz. All participants were drawn from those visiting the periodontal department dental clinic. Participants were provided with information booklet and were required to sign a consent sheet prior to taking part in the study. For this study only 20 participants (10 males and 10 females) who met the inclusion criteria were requested to continue to take part in the study. All participants who did not meet the inclusion criteria were referred to a resident periodontist for further treatment.

The inclusion criteria included patients with severe chronic periodontitis as classified by the American Academy of Periodontology. Participants were excluded if they currently smoked, were pregnant, nursing, using contraceptives, had a systemic condition that would influence the course of the periodontal disease or treatment, had a medical condition that would require antibiotic prophylaxis for routine dental procedures, a history of antibiotic, antiviral therapy and/or anti-inflammatory drugs within the previous six months, or any periodontal treatment within the last six months. For the participants to be included in the study they should have at least one-tooth on each side of the same arch with a periodontal probing depth of ≥ 5 mm.

Periodontal examination: A single examiner with over 10 years of clinical experience treating periodontal conditions determined periodontal status. A Williams’ periodontal probe was used to record clinical parameters at the baseline and at first and third month review appointments. The parameters recorded were periodontal probing depth (PPD), clinical attachment level (CAL) and gingival bleeding on probing (BoP); bleeding points index suggested by Lenox and Kopczyk6 (0 or 1) at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual for all present teeth) was used to obtain records.
Periodontal treatment and microbiological sampling: After initial clinical measurements male and female participants were equally and randomly assigned into intervention (SRP + 2.5% acyclovir gel) and control (SRP) groups. All patients then received non-surgical periodontal treatment, comprising oral hygiene instructions, and SRP under local anesthesia, split over two consecutive days using manual instruments.

After one month of the treatment, patients were recalled, and microbial samples for all teeth and groups were collected using sterile Gracey curettes. The whole procedure was carried out with proper isolation to avoid saliva contamination. Blood-contaminated samples were discarded. Immediately following this, all participants in the intervention group received 2.5% acyclovir gel administered subgingivally using a 2 ml syringe with a side venting 27 gauge needle (maxi-I probe). The gel was carefully injected into treatment sites till excess was observed at the gingival margin. The treatment site was covered with a periodontal dressing for a week to prevent gel wash out. The study design allowed for collection of 20 samples (two per participant) for each treatment group for each time period (baseline, first, and third months). Participants were further followed up at one and three-month period, with each session involving a careful removal of supragingival plaque, extraction of microbial samples and reinforcement of oral hygiene instructions.

**Bacterial Culture:** Specimens (400 µl) were plated into Brucella Blood Agar and Phenylethyl Alcohol Blood Agar medium supplemented with vitamin K1 and Hemin, supplemented with sheep blood, and incubated in anaerobic containers at 37°C. Plates were examined after 48 hours and at seven days for gram staining. Gram-negative cocccobacilli and black pigmented colonies were inoculated on Laked Blood Agar containing kanamycin and vancomycin. Bacteroides colony growth inhibition suggests the presence of P. gingivalis. Antibiotic testing, using 5 µg/ml vancomycin, 1 µg/ml kanamycin, and 10 µg/ml colistin, was conducted on inhibited colonies. Colonies of Porphyromonas species that were resistant to colistin and kanamycin but susceptible to vancomycin were confirmed using the positive Indole test. Species identification was based on UV autofluorescence, with the absence of red fluorescence considered indicative of Porphyromonas species.

**Polymerase Chain Reaction:** Following P. gingivalis culture, 0.5 ml of thioglycolate medium containing the clinical sample was heated to release the bacterial genome, and centrifuged for 5 min at 13,000 rpm. The supernatant, containing the DNA, was collected for PCR using primers specific to P. gingivalis: F 5’-CCTGCCAGCCCGGTAATACG-3’ and R 5’-TAGATAGAAGCGGGAAGGAAGC-3’. Standard PCR amplification was carried out in PCR thermal cycler in a reaction volume of 50 µl. PCR products were examined using a trans illuminator.

**Statistical analysis:** All pre-operative and post-operative data were entered into an excel sheet and the data then examined for normality and then analyzed using ANOVA and a Post hoc test.

**Results**

The baseline PCR data showed that P. gingivalis in the intervention group (15.39%) was not significantly greater than the controls (13.17%). After one month, these numbers dropped to 6.22% and 5.22%, respectively. After three months, the average number of P. gingivalis was 4.6% and 2.66% in the intervention and control groups, respectively. No significant differences between the groups at first and third months were determined. The differences between the number of P. gingivalis at baseline and after first and third months were significant in both groups (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>1st month</th>
<th>3rd month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>15.39</td>
<td>6.22</td>
<td>4.6</td>
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<tr>
<td>Control</td>
<td>13.17</td>
<td>5.22</td>
<td>2.66</td>
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<tr>
<td>P Value</td>
<td>0.585</td>
<td>0.671</td>
<td>0.533</td>
</tr>
</tbody>
</table>

Table 1. Average number of P. Gingivalis

<table>
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<th>Groups</th>
<th>Baseline</th>
<th>1st month</th>
<th>3rd month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>4.23</td>
<td>3.56</td>
<td>3.52</td>
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<td>Control</td>
<td>4.36</td>
<td>3.81</td>
<td>3.54</td>
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<td>P Value</td>
<td>0.941</td>
<td>0.781</td>
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Table 2. Average Periodontal Probing Depth

<table>
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<th>3rd month</th>
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<tr>
<td>Intervention</td>
<td>4.73</td>
<td>4.17</td>
<td>4.13</td>
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<td>Control</td>
<td>5.01</td>
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<td>4.31</td>
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<tr>
<td>Average difference</td>
<td>0.046</td>
<td>0.157</td>
<td>0.177</td>
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<tr>
<td>P Value</td>
<td>0.908</td>
<td>0.708</td>
<td>0.696</td>
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Table 3. Average Clinical Attachment Level

<table>
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<th>Baseline</th>
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<th>3rd month</th>
</tr>
</thead>
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<tr>
<td>Intervention</td>
<td>83.3%</td>
<td>66.7%</td>
<td>44.4%</td>
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<tr>
<td>Control</td>
<td>94.4%</td>
<td>61.1%</td>
<td>72.2%</td>
</tr>
<tr>
<td>P Value</td>
<td>0.296</td>
<td>0.732</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 4. Average Gingival Bleeding on Probing
vention group had dropped to 4.17 and 4.13, respectively, and 4.52 and 4.31 respectively in the control group, also without significance. However, significant differences were observed between the average CAL in the control group between baseline and 3 months, and in the intervention group between baseline, first and third months (Table 3).

The bleeding indices at baseline were 83.3% and 94.4% in intervention and control group, respectively, without significance. After one month, the bleeding indices were 66.7% and 61.1% in the intervention and control groups, respectively, without significance. The differences between the groups were statistically significant at three months after treatment (Table 4).

**Discussion**

While the role of bacteria in periodontal diseases is well-established, the plausible association between herpes virus and periodontal disease is unknown. Given that herpes virus may cause periodontal pathology as a direct result of viral infection and replication, or as a result of virally mediated damage to the host defenses, it is important that this link be determined. Herpes viruses may exert their pathogenicity by: 1. direct cytopathic effects; 2. impairing cells involved in host defense; 3. promoting subgingival attachment and colonization of periodontopathic bacteria; 4. contributing to an increased concentration of inflammatory mediators and cytokines; 5. producing tissue injury as a result of immunopathological responses to virally infected cells. Contrastingly, factors that disturb the host’s defense mechanisms can activate latent viruses and increase their pathogenicity.

There is evidence to suggest that periodontal tissues are the initial site of viral infection and the possible source of latent viruses. Herpes virus reactivation in periodontal tissue and its subsequent transient immunosuppression might in part explain the episodic and progressive nature of human periodontitis. Tissue tropism in herpes virus infection might also account for the localized pattern of destruction in periodontitis. Given the central role of bacteria in periodontal disease, bacterial elimination forms the basis of periodontal treatment. Non-surgical treatments (SRP, oral hygiene instructions and patient motivation) form the initial and integral aspects of periodontal treatment, particularly when surgical treatment is not an option. Thus, to augment mechanical debridement, topical antiseptics are employed to reduce and suppress periodontal pathogenic activity.

Numerous studies advocate for the use antiseptics as an adjunct to SRP, and have detailed the local effects of different antibiotics and antimicrobial drugs on the periodontium. In this study, we examined the effects of subgingival application of a common anti-viral agent, 2.5% acyclovir gel, in combination with SRP on the clinical parameters and the number of P. gingivalis in 20 healthy patients diagnosed with generalized moderate to severe chronic periodontitis. At baseline, P. gingivalis was found in 69.44% of samples in culture and 77.77% of samples in PCR. This finding confirms the hypothesis that culture can underestimate the actual number of bacteria, as reported by Rooney and colleagues in 2002.

The average number of P. gingivalis in the intervention and control sites at baseline and first and third months later were statistically significant. However, there were no significant differences between the two groups. Daneshmand et al. also identified no benefit of a chlorhexidine chip against subgingival microorganisms in areas that had received periodontal debridement. Likewise, Cosyn and colleagues failed to find a therapeutic benefit of Chlorhexidine. However, Perinetti et al. found a significant reduction in the number of periodontopathic microorganisms in cases treated with 1% chlorhexidine gel or 1% metronidazole gel as compared with placebo. The ineffectiveness of the 2.5% acyclovir gel used in this study might be attributed to its single application and the constant flow of gingival cervical fluid that may reduced the concentration of the drug.

The average PPD in both groups differed significantly over time, but not between the intervention and control groups. Perinetti et al. also found no significant difference in PPD between groups that received 1% metronidazole or 1% chlorhexidine gel when compared with placebo. Azmak et al. revealed that a chlorhexidine chip with SRP significantly reduced PPD in the intervention group after third and sixth months compared with baseline levels; however, the difference between the intervention and control (SRP only) was not significant.

We also observed no significant changes in CAL. Likewise, Azmak et al. found no improvement in CAL with a chlorhexidine chip and SRP as compared with SRP only group after third and sixth months. Bonito et al. conducted a meta-analysis to evaluate the effectiveness of antimicrobial adjuncts to SRP therapy, identifying that the best results were obtained following treatment with tetracycline, minocycline, metronidazole and chlorhexidine. Although these drugs reduced the PPD, their effectiveness when compared with SRP alone was modest. Likewise, the increases in CAL were similar, but the effects were smaller and statistical significance was less common.

In both groups, the reduction in BoP percentage was insignificant. Perinetti et al. found that BoP decreased significantly in patients receiving 1% chlorhexidine or 1% metronidazole gel. A systematic review by Cosyn found that subgingival chlorhexidine gel as a monotherapy reduced the incidence of BoP. In our study, BoP was recorded as the presence or absence of bleeding on probing, and differs from the Bleeding Index (BI) used in other studies, which might explain this difference. There are numerous microorganisms involved in periodontal disease that were not studied here. Many of them unaffected by acyclovir gel and thus continue to be active even following acyclovir application. This could have resulted in the absence of a significant clinical difference between intervention sites receiving acyclovir gel and control sites. This pilot’s study provides a basis for further research into the beneficial effects of single and multiple doses in the treatment of chronic periodontitis.
Conclusion

The results of this pilot study demonstrate that SRP is an effective therapy for patients with chronic periodontitis, with or without the single application of acyclovir. Further research with sufficiently larger statistical power will be needed to establish if multiple dose of Acyclovir Gel can be beneficial in the treatment of chronic periodontitis.

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How cite this article

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Source of Support: Nil
Conflict of Interest: None Declared