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Inflammatory response to chlorhexidine, minocycline HCl and doxycycline HCl in an in vivo mouse model

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Abstract

Aim: To examine the effect of locally delivered antimicrobial drugs on the inflammatory response in an in vivo mouse chamber model.

Material and Methods: Two weeks following chamber implantation, 24 BALB/c mice, in the experimental group, were given an intra-chamber challenge of heat-killed *Porphyromonas gingivalis*, followed immediately by injection of the specific antimicrobial drug: 2000 μg/ml chlorhexidine (CHX); 1500 μg/ml minocycline HCl;and 1500 μg/ml doxycycline HCl (concentrations achieved in the periodontal pocket with commercial controlled-release delivery systems). A second group of 24 animals received only the antimicrobial treatment without *P. gingivalis* challenge. Intra-chamber exudates were sampled at 2 and 24 h following the challenge, and leucocytes, TNFα, IFNγ and IL-10 were evaluated.

Results: At 2 h, minocycline HCl induced high levels of IL-10, TNFα and IFNγ, while CHX reduced the levels of TNFα and IFNγ. By 24 h, these responses were attenuated. Following bacterial challenge, the antibacterial agents attenuated the inflammatory process, each in its own fashion.

Conclusions: Antibacterial agents applied locally have the ability to induce an inflammatory response. They also modify the inflammatory response to *P. gingivalis* independent of their antimicrobial effect. CHX and doxycycline HCl appear to have the most marked anti-inflammatory effect.

Key words: antibacterial agents; chlorhexidine; cytokines; doxycycline HCl; inflammatory response; in vivo model; minocycline HCl

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The inflammatory periodontal diseases are widely accepted as being caused by bacteria associated with dental plaque (Socransky & Haffajee 1992). However, the nature of the periodontal disease resulting from dental plaque appears to depend on the interaction between the bacterial agent, the environment and the host defence mechanisms (Van Dyke et al. 1993). Traditionally, periodontal disease therapy has been directed at altering the periodontal environment to one that is less conducive to the retention of bacterial plaque. Classical therapeutic regimes to achieve this aim would include some or all of the following procedures: instruction in oral hygiene techniques, scaling, root planing, correction of inadequate restorative dentistry and the surgical elimination of pockets or other anatomical defects that aid bacterial retention and interfere with plaque removal. With the increasing awareness of the bacterial aetiology of periodontal disease (Socransky 1970, Slot 1979, Moor et al. 1983) and in particular the hypothesis that specific bacteria are involved (Loesche et al. 1985), a more direct approach using antibacterial agents has become an integral part of the therapeutic armamentarium (Mandel 1988, Van de Ouderaa 1991, Greenstein 1992).

The recent development of sophisticated, subgingivally placed delivery systems has provided the possibility of

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maintaining effective, intra-pocket, levels of antibacterial agents for extended periods. These systems have provided the profession with a new tool, which, in clinical trials, has been shown to alter the subgingival flora and significantly influence the healing of the marginal attachment apparatus (Goodson 1984, Soskolne 1997).

Several studies using tetracyclines (Chang et al. 1994, Ramamurthy et al. 2002) have demonstrated that these agents reduce the levels of pro-inflammatory cytokines induced by the injection of lipopolysaccharide (LPS) and increased the levels of the anti-inflammatory cytokine-IL-10. Tetracyclines as well as chlorhexidine (CHX) have been shown to reduce the activity of matrix metalloproteinases (MMPs) in experimental models (Azmak et al. 2002, Ramamurthy et al. 2002).

The goal in using an intra-pocket device for the delivery of an antibacterial agent is the achievement and maintenance of effective antibacterial levels of the drug for the required period. However, there does not appear to be any data available that report on the local effect of these agents on the tissues to which they are applied.

This study was designed to examine the effect of a selection of antimicrobial agents commonly used in controlled delivery devices on the inflammatory response in an in vivo local inflammation model.

Material and Methods

Antimicrobial agents

The antibacterial agents CHX digluconate, doxycycline HCl and minocycline HCl were kindly donated by Dexcel Pharma Technologies, Jerusalem, Israel. CHX was diluted to the desired concentrate of 2000 μg/ml (Soskolne et al. 1998) in phosphate-buffered saline (PBS) (pH 7.4). Doxycycline HCl and minocycline HCl were dissolved in double-distilled water to a concentration of 1500 μg/ml (Stoller et al. 1998, Paquette & Santucci 2000, Williams et al. 2001) (pH 3.5 and 4.4, respectively). The use of different solvents (PBS for CHX and double-distilled water for doxycycline and minocycline at room temperature) was in order to achieve stable solutions of the different materials. These dilutions approximate the maximum concentrations achieved in the gingival crevice when using controlled-release delivery systems available in the market [the PerioChip® (Dexcel Pharma, Jerusalem, Israel), Atridox™ (Tolmar Inc., Fort Collins, CO, USA) and Arestin® (Ora Pharma Inc., Warminster, PA, USA), respectively]. Two hundred microlitres from each agent was injected into the relevant chamber according to the study protocol. Two hundred microlitres of saline was used in the control group. At the time of injection, all the solutions were clear and showed no signs of precipitation of the solute.

The in vivo local inflammation model

The experimental protocol was approved by the Internal Review Board of the Hadassah–Hebrew University Medical Center. Female BALB/c mice, 5–6 weeks old (Jackson Laboratories, Bar Harbor, ME, USA), were used in this study. The in vivo local inflammation model described by Houri-Haddad et al. (2000) was used. Two titanium wire chambers were implanted into the subcutaneous dorso-lumbar region of each mouse. Two weeks following chamber implantation, 24 BALB/c mice were given an intra-chamber challenge of heat-killed *Porphyromonas gingivalis* [0.1 ml of 10^{10} colony-forming units (CFU)/ml], followed immediately by injection of the specific antimicrobial drug: 2000 mg/ml CHX, 1500 mg/ml minocycline HCl and 1500 mg/ml doxycycline HCl. Saline was used as the control (six animals per treatment group). A second group of 24 animals received only the antimicrobial treatment without *P. gingivalis* challenge. Chamber exudates were collected at 2 h (one of the two chambers in each animal) and 24 h post-challenge (the second chamber in each animal). The exudates were centrifuged for 5 min., and the supernatants were removed and stored at -20°C until analsed for cytokines. The pellets were immediately re-suspended in PBS, and the total leucocyte counts were evaluated after staining with trypan blue, using a hemocytometer.

Bacteria

P. gingivalis, strain ATCC 33277, was grown on blood agar plates in an anaerobic chamber with 85% N_2, 5%H_2 and 10% CO_2. After incubation at 37°C for 2–3 days, the bacterial cells were inoculated into a peptone yeast extract for 1-week incubation under the same conditions. The bacteria were washed three times with PBS and then heat killed at 80°C for 10 min. (Kesavalu et al. 1992). Using a spectrophotometer, the bacterial concentrations were standardized to an optical density of 0.1 at 650 nm, which corresponds to 10^{10} CFU/ml (Baker et al. 1994). The heat-killed bacteria were stored at 4°C. Immediately before use, the bacteria were resuspended in PBS by brief sonication.

Analysis of cytokines

The presence of cytokines in the chamber supernatants was determined by a two-site enzyme-linked immunosorbent assay (ELISA) (Frolov et al. 1998). The assays were based on commercially available antibody pairs (Pharmingen, San Diego, CA, USA; R&D Minneapolis, MN, USA). Ninety-six-well ELISA plates were coated with 1 μg/ml anti-mouse cytokine monoclonal antibodies and blocked by 3% bovine serum albumin. A secondary biotinylated antibody was used as the detecting antibody, followed by a streptavidin–horseradish peroxidase conjugate (Jackson Immunoresearch Laboratories, West Grove, PA, USA). The substrate used was *o*-phenylenediamine (Zymed, San Francisco, CA, USA). The reaction was stopped by the addition of 4 N sulphuric acid, and the optical density was read using a V_{max} microplate reader (Molecular Devices, Palo Alto, CA, USA) at 490–650 nm against a standard curve based on known concentrations of the recombinant cytokine.

Data analysis

Data analysis was performed using a statistical software package (SigmaStat, Jandel Scientific, San Rafael, CA, USA). One-way repeated measures analysis of variance (RM ANOVA) was used to test the significance of the differences between the treated groups. When significance was established, the inter-group differences were tested for significance using the Student *t*-test with the Bonferroni correction for multiple testing. The level of significance was determined at $p < 0.05$. All the results are presented as mean values $\pm$ the standard error of the mean.

Results

At baseline, before the chamber challenge, there were only a few isolated cells

present in the chamber exudates and the levels of the cytokines approached zero (data not shown). This corresponds with the results from our previous study (Houri-Haddad et al. 2000).

The local application of saline into the chambers induced a minimal response of the immune system at 2 h: a low level of leucocyte concentration and low levels of the cytokines TNFα, IFNγ and IL-10. The response remained similar at 24 h with no significant difference (Figs 1 and 2).

At 2 h, the *P. gingivalis* challenge induced an increase in cell migration (about sixfolds) and significant increases in the levels of TNFα, IFNγ and IL-10 compared with the saline group. After 24 h, the leucocyte concentration increased significantly (about fourfolds compared with 2 h). IFNγ levels remained similar to those at 2 h, while the levels of TNFα and IL-10 were significantly decreased (Fig. 4).

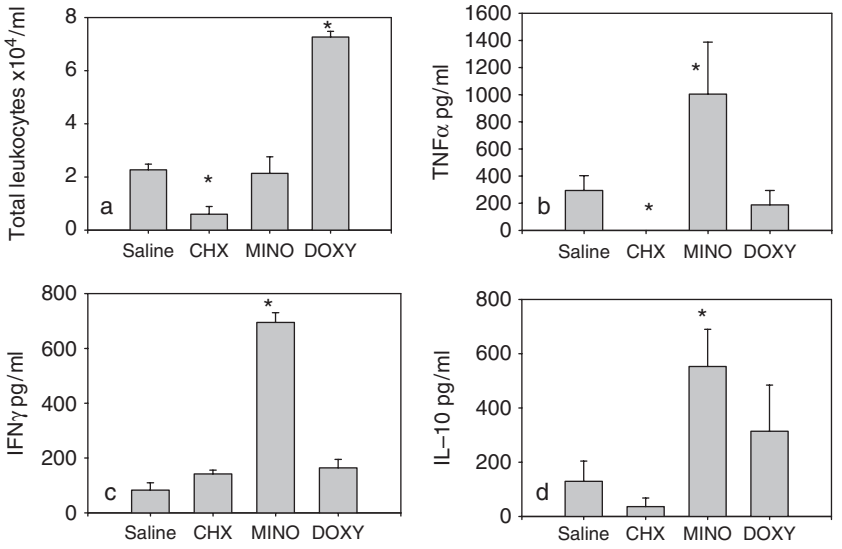

Fig. 1. Levels of leucocytes (a) TNFα (b), IFNγ (c) and IL-10(d) in the chamber 2 h following the challenge of the antibacterial agents. Antibacterial agents – chlorhexidine (CHX), minocycline HCl (Mino) and doxycycline HCl (Doxy) – were injected into the chambers of mice ($n = 6$, each group); saline served as control. Chamber exudates were harvested for analysis at 2 h post-challenge. Results are expressed as means ± standard error. *Significantly different from the respective control ($p < 0.05$).

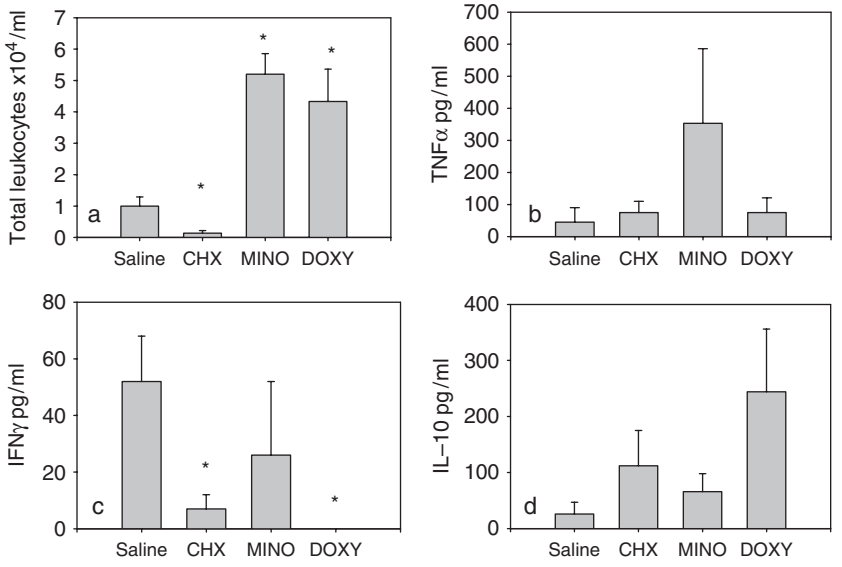

Fig. 2. Levels of leucocytes (a) TNFα (b), IFNγ (c) and IL-10(d) in the chamber 24 h following the challenge of the antibacterial agents. Antibacterial agents – chlorhexidine (CHX), minocycline HCl (Mino) and doxycycline HCl (Doxy) – were injected into the chambers of mice ($n = 6$, each group); saline served as control. Chamber exudates were harvested for analysis at 24 h post-challenge. Results are expressed as means ± standard error. *Significantly different from the respective control ($p < 0.05$).

Response to antibacterial agents alone (control = saline)

Recruitment of leucocytes into the chambers

The local application of the different antimicrobial agents into the chamber (with no bacterial challenge) resulted in alterations in cell migration into the chamber compared with the saline control. At 2 h, the CHX appeared to reduce the migration of the leucocytes to a minimum. Minocycline HCl did not affect the levels of the leucocytes, whereas the doxycycline HCl significantly increased their migration (Fig. 1a).

Twenty-four hours after drug application, the effect of CHX and doxycycline HCl showed no significant changes from the 2-h levels, while minocycline HCl induced a significant increase in leucocytes' migration (Fig. 2a).

Levels of cytokines in the chamber

TNFα. At 2 h, the introduction of antibacterial agents into the chambers altered the levels of TNFα in both directions. CHX reduced the levels below the detectable limit of the ELISA, while minocycline HCl enhanced the levels twofold (1005 ± 383). Doxycycline HCl did not alter the levels significantly compared with the saline control (Fig. 1b).

After 24 hours, the levels of TNFα were low in all the treatment groups and similar to the saline control, with no significant difference between them (Fig. 2b).

IFNγ. At 2 h, minocycline HCl increased the levels of IFNγ significantly (695 ± 35), while all the other treatments showed levels similar to the saline control (Fig. 1c).

After 24 h, the levels of IFNγ in all the groups treated with antibacterial agents were markedly reduced compared with their levels at 2 h (Fig. 2c). The levels of IFNγ were close to the lower detection limits of the ELISA.

IL-10. At 2 h, the levels of IL-10 in all the treatment groups were similar to the saline control, except the minocycline HCl group, which showed a significant increase (Fig. 1d).

After 24 h, the levels of IL-10 remained similar to those after 2 h, except the minocycline HCl group, which showed a significant reduction (Fig. 2d).

Response to the antibacterial agents following *P. gingivalis* challenge (control = *P. gingivalis* challenge)

Recruitment of leucocytes into the chambers (Fig. 3a)

At 2 h post-*P. gingivalis* challenge, CHX reduced the levels of the leucocytes compared with the *P. gingivalis* control group, and the minocycline HCl resulted in minimal changes, while the doxycycline HCl increased their levels (Fig. 3a).

Twenty-four hours post-challenge, the concentration of leucocytes in the chambers increased significantly from the 2-h levels in all the groups, except the doxycycline HCl group, where the increase did not reach significance (Fig. 4a). The 24-h leucocyte concentrations were not significantly different from each other.

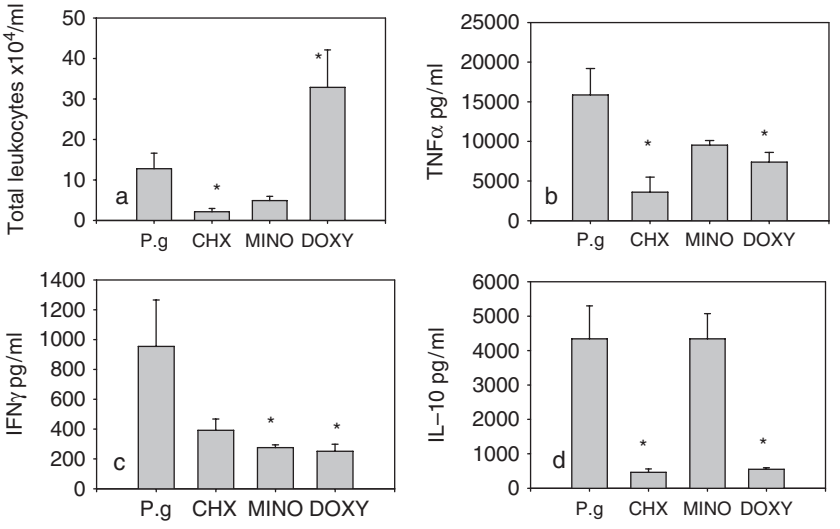

Fig. 3. Levels of leucocytes (a) TNFα (b), IFNγ (c) and IL-10 (d) in the chamber 2 h following heat-killed *Porphyromonas gingivalis* challenge. Antibacterial agents – chlorhexidine (CHX), minocycline HCl (Mino) and doxycycline HCl (Doxy) – were injected into the chambers of mice ($n = 6$, each group) following the bacterial challenge (saline served as control). Chamber exudates were harvested for analysis at 2 h post-challenge. Results are expressed as means ± standard error. *Significantly different from the respective control ($p < 0.05$).

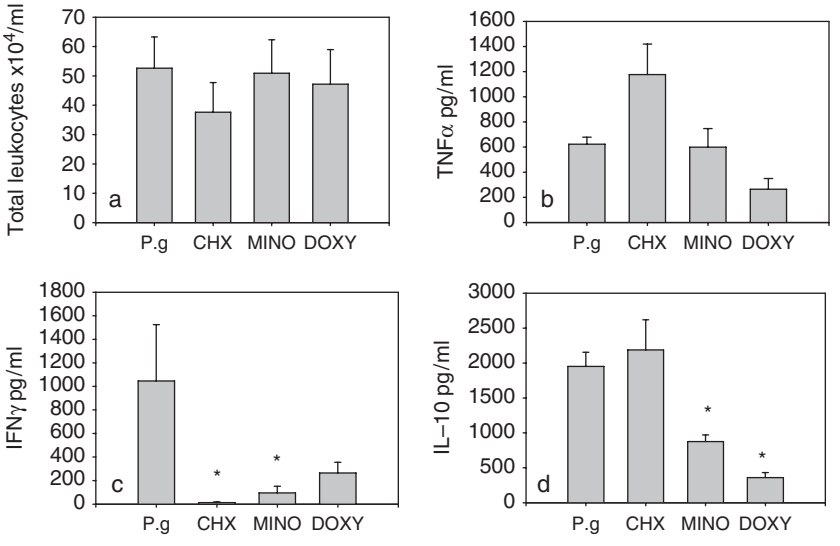

Fig. 4. Levels of leucocytes (a) TNFα (b), IFNγ (c) and IL-10(d) in the chamber 24 h following heat-killed *Porphyromonas gingivalis* challenge. Antibacterial agents – chlorhexidine (CHX), minocycline HCl (Mino) and doxycycline HCl (Doxy) – were injected into the chambers of mice ($n = 6$, each group) following the bacterial challenge (saline served as control). Chamber exudates were harvested for analysis at 24 h post-challenge. Results are expressed as means ± standard error. *Significantly different from the respective control ($p < 0.05$).

Levels of cytokines in the chamber

TNFα. At 2 h all the antibacterial agents reduced the *P. gingivalis* enhanced levels of TNFα secretion. This reduction was significant only in the CHX and doxycycline HCl-treated groups (Fig. 3b).

After 24 h, the levels of TNFα decreased in all treatment groups compared with 2 h, with the decrease being significant in the minocycline HCl and doxycycline HCl groups only. There were no significant differences between the groups and the *P. gingivalis* control (Fig. 4b).

IFNγ. At 2 h, doxycycline HCl and minocycline HCl reduced the IFNγ levels significantly compared with the *P. gingivalis* control group. The reduction in the CHX group was not statistically significant (Fig. 3C).

At 24 h, the levels of IFNγ in the CHX and minocycline HCl groups were significantly reduced compared with their 2-h levels. They were also significantly lower than the *P. gingivalis* control (Fig. 4C).

IL-10. At 2 h, the CHX and doxycycline HCl suppressed the IL-10 levels significantly compared with the *P. gingivalis* control. The IL-10 level in the minocycline HCl group remained similar to the control (Fig. 3d).

After 24 h, minocycline HCl and doxycycline HCl resulted in significant decreases in the levels of IL-10 compared with 2 h while CHX increased the levels of IL-10 significantly (Fig. 4d). The levels of IL-10 were significantly lower in the minocycline HCl and doxycycline HCl groups than in the *P. gingivalis* control.

Discussion

The changes in the chamber contents resulting from the introduction of the

antibacterial agents into the chambers represent the direct effect of these agents on the surrounding tissues. CHX had a strong anti-inflammatory effect by reducing the basal concentration of leucocytes migrating into the chamber compared with the control group. It also reduced the levels of the pro-inflammatory cytokines, resulting in a strong anti-inflammatory effect. TNFα had been reduced at 2 h and IFNγ at 24 h. Minocycline HCl had a minimal effect on leucocyte migration; however, the levels of the inflammatory cytokines (IFNγ IL-10 and TNFα) were increased at 2 h. This resulted in a pro-inflammatory effect that could be harmful to the surrounding tissues. This effect was short lived and had disappeared at 24 h. Doxycycline HCl induced an increase in leucocyte migration but the levels of cytokines remained similar to those seen in the saline group, except the reduction of IFNγ after 24 h. These results indicate that doxycycline HCl is the most inert of the three antibacterial agents tested. The differences in the activity of the three antibacterial agents might be explained, in part, by the differences in the pH of the different solutions at the time of injection (CHX = 7.4, doxycycline = 3.5 and minocycline = 4.4). Although the pH of both minocycline HCl and doxycycline HCl is similarly acidic, they resulted in different host responses, which in turn were different from the response to the pH neutral CHX. This suggests that the different responses seen in the chamber model are at least in part due to the differences in response to the drugs themselves. After 24 h, when the low pH of minocycline HCl and doxycycline HCl had probably been neutralized by the buffering capacity of tissue fluids, doxycycline HCl began to show an anti-inflammatory effect, while minocycline HCl lost its pro-inflammatory effect.

In an existing inflammatory response, CHX reduced the levels of all the cytokines induced by a *P. gingivalis* challenge in the short term with a tendency for the levels to return to those of the control after 24 h. Doxycycline HCl also resulted in an anti-inflammatory effect, reducing the levels of TNFα IL-10 and IFNγ, which persisted up to 24 h. Minocycline HCl also resulted in the reduction of the levels of IL-10 and IFNγ over the 24-h test period but, in contrast to doxycycline HCl, it did not reduce the levels of TNFα. In a previous study (Shapira et al. 1997), tetracycline was shown to reduce the secretion of TNFα by human monocytes challenged with *P. gingivalis* LPS. This reduction in secretion was shown to be due to the retention of the TNFα on the membrane fraction of the cells. This finding is similar to the results seen with doxycycline HCl in this study but different from the response to minocycline HCl. These findings would suggest that minocycline HCl would be a less desirable agent to use as a local anti-inflammatory agent than either doxycycline HCl or CHX. Doxycycline HCl would be the preferred anti-inflammatory agent for a single local application because of its neutral behaviour when introduced alone and its longer anti-inflammatory effect when used in conjunction with an inflammatory stimulus.

One of the ways that antibacterial agents may affect the inflammatory process independent of their antibacterial properties is through their known anti-collagenase activity (CHX – Gendron et al. 1999, Azmak et al. 2002 and the tetracyclines – Golub et al. 1984, 1985). It is known that the collagenases (MMPs) are important in the development of the inflammatory process (Chang et al. 2002), and it would therefore be important to examine the effect of the anti-collagenase activity of the different antibacterial agents on the levels of the MMPs in the chamber exudates and their relationship with cytokine secretion. This may well clarify the differing effects of the different antibacterial agents on the inflammatory response.

In summary, this model shows that each antibacterial agent modulates the cytokines in the inflammatory exudates in its own unique fashion. This modulation is time dependent and not necessarily purely pro- or anti-inflammatory in terms of cytokine secretion. These results represent the effect of a single dose. The clinical use of these agents in periodontology is usually via controlled-release delivery systems, where the drug concentration is maintained over an extended period. The high levels of the drugs over time would change the secretion profile of the cytokines, possibly extending the effects seen at 2 h.

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References

Azmak, N., Atilla, G., Luoto, H. & Sorsa, T. (2002) The effect of subgingival controlled-release delivery of chlorhexidine chip on clinical parameters and matrix metalloproteinase-8 levels in gingival crevicular fluid. *Journal of Periodontology* **73**, 608–615.

Baker, P. J., Evans, R. T. & Roopenian, D. C. (1994) Oral infection with *Porphyromonas gingivalis* induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. *Archives of Oral Biology* **39**, 1035–1040.

Chang, K. M., Ramamurthy, N. S., McNamara, T. F., Evans, R. T., Klausen, B., Murray, P. A. & Golub, L. M. (1994) Tetracyclines inhibit *Porphyromonas gingivalis*-induced alveolar bone loss in rats by a non-antimicrobial mechanism. *Journal of Periodontal Research* **29**, 242–249.

Chang, Y. C., Yang, S. F., Lai, C. C., Liu, J. Y. & Hsieh, Y. S. (2002) Regulation of matrix metalloproteinase production by cytokines, pharmacological agents and periodontal pathogens in human periodontal ligament fibroblast cultures. *Journal of Periodontal Research* **37**, 196–203.

Frolov, I., Houri-Haddad, Y., Soskolne, A. & Shapira, L. (1998) In vivo exposure to *Porphyromonas gingivalis* up-regulates nitric oxide but suppresses tumor necrosis factor alpha production by cultured macrophages. *Immunology* **93**, 323–328.

Gendron, R., Grenier, D., Sorsa, T. & Mayrand, D. (1999) Inhibition of the activities of matrix metalloproteinases 2,8 and 9 by chlorhexidine. *Clinical Diagnostic Laboratory Immunology* **6**, 437–439.

Golub, L. M., Ramamurthy, N., McNamara, T. F., Gomes, B., Wolf, M., Casino, A., Kapoor, A., Zambon, J., Ciancio, S. & Schneir, M. (1984) Tetracycline inhibit tissue collagenase activity. *Journal of Periodontal Research* **19**, 651–655.

Golub, L. M., Wolf, M., Lee, H. M., McNamara, T. F., Ramamurthy, N., Zambon, J. & Ciancio, S. (1985) Further evidence that tetracyclines inhibit collagenase activity in human crevicular fluid and from other mammalian sources. *Journal of Periodontal Research* **20**, 12–23.

Goodson, J. M. (1984) Dental applications. In: Langer, D. L. & Wise, D. L. (eds). *Medical Applications of Controlled Release*, Vol. II, pp. 115–138. Boca Raton, FL: CRC Press.

Greenstein, G. (1992) Periodontal response to mechanical non surgical therapy. A review. *Journal of Periodontology* **63**, 118–130.

Houri-Haddad, Y., Soskolne, W. A., Halabi, A., Barak, V. & Shapira, L. (2000) Repeat bacterial challenge in a subcutaneous chamber model results in augmented TNF-alpha and gamma-IFN response, and suppression of IL-10. *Immunology* **99**, 215–220.

Kesavalu, L., Ebersole, J. L., Machen, R. L. & Holt, S. C. (1992) *Porhyromonas gingivalis* virulence in mice: induction of immunity to

bacterial components. *Infection and Immunity* **60**, 1455–1464.

Loesche, W. J., Syed, S. A., Schmidt, E. & Morrison, E. C. (1985) Bacterial profiles of subgingival plaques in periodontitis. *Journal of Periodontology* **56**, 447–456.

Mandel, I. D. (1988) Chemotherapoitic agents for controlling plaque and gingivitis. *Journal of Clinical Periodontology* **15**, 488–498.

Moore, W. E. C., Holdeman, L. V., Cato, E. P., Smibert, R. M., Burmeister, J. A. & Ranney, R. R. (1983) Bacteriology of moderate (chronic) periodontitis in mature adult humans. *Infection and Immunity* **42**, 510–515.

Paquette, D. & Santucci, E. (2000) A pharmacokinetic study of a locally delivered minocycline therapeutic system (MPTS). *Journal of Clinical Periodontology* **27** (Suppl. 1), 12 (abstract.27).

Ramamurthy, N. S., Rifkin, B. R., Greenwald, R. A., Xu, J. W., Liu, Y., Turner, G., Golub, L. M. & Vernillo, A. T. (2002) Inhibition of matrix metalloproteinase-mediated periodontal bone loss in rats: a comparison of 6 chemically modified tetracyclines. *Journal of Periodontology* **73**, 726–734.

Shapira, L., Houri, Y., Barak, V., Soskolne, W. A., Halabi, A. & Stabholz, A. (1997) Tetracycline inhibits *Porphyromonas gingivalis* lipopolysaccharide-induced lesions *in vivo* and TNFα processing *in vitro*. *Journal of Periodontal Research* **32**, 183–188.

Slots, J. (1979) Subgingival microflora and periodontal disease. *Journal of Clinical Periodontology* **6**, 351–382.

Socransky, S. S. (1970) The relationship of bacteria to the etiology of periodontal disease. *Journal of Dental Research* **49**, 203–222.

Socransky, S. S. & Haffajee, A. D. (1992) The bacterial etiology of destructive periodontal disease: current concepts. *Journal of Periodontology* **63**, 322–331.

Soskolne, W. A. (1997) Subgingival delivery of therapeutic agents in the treatment of periodontal diseases. *Critical Review of Oral Biological Medicine* **8**, 164–174.

Soskolne, W. A., Chajek, T., Flashner, M., Landau, I., Stabholtz, A., Kolatch, B. & Lerner, E. I. (1998) An in vivo study of the chlorhexidine release profile of the PerioChip in the gingival crevicular fluid, plasma and urine. *Journal of Clinical Periodontology* **25**, 1017–1021.

Stoller, N. H., Johnson, L. R., Trapnell, S., Harrold, C. Q. & Garrett, S. (1998) The pharmacokinetic profile of a biodegradable controlled-release delivery system containing doxycycline HCL compared to systemically delivered doxycycline HCL in gingival crevicular fluid, saliva, and serum. *Journal of Periodontology* **69**, 1085–1091.

Van de Ouderaa, F. J. G. (1991) Anti plaque agents. Rational and prospects for prevention of gingivitis and periodontal disease. *Journal of Clinical Periodontology* **18**, 447–454.

Van Dyke, T. E., Lester, M. A. & Shapira, L. (1993) The role of host response in periodontal disease progression: implications for future treatment strategies. *Journal of Periodontology* **64**, 792–806.

Williams, R. C., Paquette, D. W., Offenbacher, S., Adams, D. F., Armitage, G. C., Bray, K., Caton, J., Cochran, D. L., Drisko, C. H., Fiorellini, J. P., Giannobile, W. V., Grossi, S., Guerrero, D. M., Johnson, G. K., Lamster, I. B., Magnusson, I., Oringer, R. J., Persson, G. R., Van Dyke, T. E., Wolff, L. F., Santucci, E. A., Rodda, B. E. & Lessem, J. (2001) Treatment of periodontitis by local administration of minocycline HCl microspheres: a controlled trial. *Journal of Periodontology* **72**, 1535–1544.

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Clinical Relevance

Scientific rationale for the study: The purpose of this study was to extend the minimal information available regarding the inflammatory response of tissues to local application of antibacterial agents.

Principal findings: This model shows that local release of antibacterial agents stimulates an inflammatory response in addition to modulating the cytokines in the inflammatory exudates.

Practical implications: The controlled release of antibacterial agents for the treatment of periodontal disease results in exposure of the tissues to antibacterial agents for extended periods. Although the results of this study represent the effect of a single dose of antibacterial agents for a short period, the resultant inflammatory response may be a significant factor in choosing the appropriate agent for therapeutic purposes.