J Periodont Res 2008; 43: 649–657
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JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2007.01053.x

Effects of cytomegalovirus infection on the mRNA expression of collagens and matrix metalloproteinases in gingival fibroblasts

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Botero JE, Contreras A, Parra B. Effects of cytomegalovirus infection on the mRNA expression of collagens and matrix metalloproteinases in gingival fibroblasts. J Periodont Res 2008; 43: 649–657. © 2008 The Authors. Journal compilation © 2008 Blackwell Munksgaard

Background and Objective: The purpose of this *in vitro* investigation was to study the effects of human cytomegalovirus infection on the mRNA expression for collagens I and III and for matrix metalloproteinases 1 and 2 in gingival fibroblasts.

Material and Methods: Gingival fibroblasts were experimentally infected with the Towne strain of human cytomegalovirus and the kinetics of expression of mRNA for collagens I and III and for matrix metalloproteinases 1 and 2 was studied at different time-points. Total RNA was isolated at the indicated time, and the reverse transcription–polymerase chain reaction was used to analyze the level of mRNA expression. In addition, gingival specimens were obtained from 14 periodontitis and from three non-periodontitis subjects and mRNA analysis for collagens and metalloproteinases was carried out. Nested polymerase chain reaction was used to determine the presence or absence of human cytomegalovirus in subgingival samples from each subject.

Results: The infection of gingival fibroblasts with human cytomegalovirus during a 0–72-h period resulted in progressive reduction in the expression of mRNA for collagens I and III ($p < 0.05$). A higher concentration of human cytomegalovirus resulted in varying degrees of mRNA reduction, suggesting a virally mediated effect. Biopsies from human cytomegalovirus-positive individuals with periodontitis had a higher expression of mRNA for collagens I and III than biopsies from human cytomegalovirus-negative individuals. An up-regulation in the mRNA expression of matrix metalloproteinases 1 and 2 over time was observed ($p < 0.05$). Analysis of mRNA expression in gingival biopsies demonstrated higher expression of matrix metalloproteinase-1 in human cytomegalovirus-positive periodontitis specimens compared with human cytomegalovirus-negative periodontitis specimens.

Conclusion: Altered expression of mRNA for collagens and metalloproteinases in human cytomegalovirus-infected gingival fibroblasts should be considered as possible modifying mechanisms in periodontitis-infected sites.

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Key words: collagen; gingival fibroblasts; human cytomegalovirus; metalloproteinases; periodontitis

Accepted for publication October 1, 2007

The structure of periodontal tissues is dependent on a matrix of protein components for support and homeostasis. Gingival connective tissues are rich in collagens, with type I and type III (1,2) being the major types. Collagens are regularly maintained and replaced throughout life and gingival fibroblasts mediate this process. For this reason, the appropriate functioning of gingival fibroblasts is crucial for periodontal tissue homeostasis. Virulence factors from periodontopathic bacteria, and cytokine production by immune cells, modify fibroblast functions, resulting in low cell growth and the release of collagenases and cytokines (3–6). This could halt periodontal healing or functioning and thus allow clinical attachment loss.

In pathologic processes, such as those associated with periodontal disease, the metabolism of connective tissue matrix components is altered such that more tissue is lost than replaced (7). This degradation of connective tissue matrix is mediated primarily by two mechanisms: phagocytosis and enzymatic degradation (8,9). The accumulation of bacterial plaque and subsequent inflammation induces the activation of phagocytic cells and the liberation of metalloproteinases (MMPs). Elevated levels of MMPs have been found in the gingival crevicular fluid and gingival tissues of periodontitis lesions (10–12). Both resident connective tissue cells and inflammatory cells produce MMPs in response to periodontal pathogens and inflammatory stimuli (13–15). This suggests that periodontal breakdown is dependent on the stimulation of host cells and the imbalance of inflammatory mechanisms.

With advancing research, some herpesviruses have been described as potential pathogens in periodontal disease and, of those indicated, human cytomegalovirus may be the most relevant (16). Considering that human cytomegalovirus replication is dependent on the transcription factors and protein synthesis machinery of the cell, this could impair some cellular functions and/or induce mechanisms that could be harmful to the host (17,18). This *in vitro* investigation studied the effects of human cytomegalovirus infection on the mRNA expression of collagens I and III, and of MMP-1 and MMP-2, in gingival fibroblasts.

Material and methods

The study protocol was reviewed and approved by the Institutional Review Board for Human Research of the Universidad del Valle (Cali, Colombia) in accordance with the Helsinki declaration of 1975, as revised in 2000. When human gingival biopsies were to be taken, previous authorization with a signed informed consent was required from periodontal patients referred to the dental clinics of the School of Dentistry at the Universidad del Valle between January 2006 and April 2007.

Gingival fibroblasts

Gingival fibroblasts were obtained from the connective tissue of a periodontally healthy donor during a crown-lengthening procedure. A 3-mm piece of connective tissue was cut into smaller pieces using a scalpel blade and immediately placed in a 25-cm^2 culture flask (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA), containing 3 mL of α-minimal essential medium supplemented with 10% fetal bovine serum, and incubated at 37°C and 5% CO_2. After 1 wk, the medium was replaced with 5 mL of fresh medium. The culture flask was examined daily for cell growth around the tissue. When fibroblasts had grown out of the connective tissue, the pieces of connective tissue were carefully removed from the flask and incubated until cell confluency. The medium was replaced every 3 d and, after cells reached 90% confluency, they were detached by trypsin treatment (0.25%), washed with α-minimal essential medium supplemented with 10% fetal bovine serum and split apart in 25-cm^2 culture flasks. Gingival fibroblasts were cultured by repeated passages and, every 2 wk, a stock of gingival fibroblasts was frozen in liquid nitrogen for future experiments. Cells between passages 10 and 13 were used in the experiments.

Virus

The human cytomegalovirus Towne strain was used in all experiments. To produce a stock of virus, gingival fibroblasts grown in a 25-cm^2 culture flask were infected with a high dilution of 10^{-6} of the original virus and incubated with α-minimal essential medium supplemented with 10% fetal bovine serum at 37°C and 5% CO_2. Supernatant was replaced with fresh medium once a week and the infected cells were maintained until a 100% cytopathic effect was observed (≈ 2 wk). At that time, supernatant was collected and stored at −70°C as the viral stock to be used in all the experiments. Cells were detached by trypsin treatment, washed with α-minimal essential medium supplemented with 10% fetal bovine serum and then stored in liquid nitrogen for future experiments.

To determine the concentration of virus in the stock, three aliquots of the supernatant were quantified by real-time polymerase chain reaction (PCR) using a commercial kit (LC CMV kit, Roche LightCycler ver. 2.0; Roche Diagnostics, Indianapolis IN, USA). The final viral stock used for the experiments contained 710,000 copies/μL (c/μL) of human cytomegalovirus.

In vitro infection of gingival fibroblasts

Gingival fibroblasts (100,000 cells/mL) were seeded in six-well plates (Falcon, Becton Dickinson Labware) and maintained in α-minimal essential medium supplemented with 10% fetal bovine serum, at 37°C and 5% CO_2, until 100% confluency was observed. At this point, the medium was replaced with α-minimal essential medium containing 2% fetal bovine serum, the cells were incubated for a further 24 h and then the medium was replaced with fresh α-minimal essential medium supplemented with 2% fetal bovine serum and 50 μg/mL of ascorbic acid (Sigma, St Louis, MO, USA), after which the cells were incubated for another 24 h (baseline).

For infection, 10-fold serial dilutions of the viral stock were prepared in α-minimal essential medium. Supernatants were removed from the cell

culture plates and 500 μL of each virus dilution containing 35.5×10^5 (71,000 copies/μL), 35.5×10^4 (7100 copies/μL), 35.5×10^3 (710 copies/μL) or 35.5×10^2 (71 copies/μL) viral copies were added to each well. The final density of gingival fibroblasts before infection consisted of 750,000 cells. Plates were incubated at 37°C in an atmosphere of 5% CO_2 for virus adsorption for 1 h and then 2.5 mL of α-minimal essential medium containing 2% fetal bovine serum was added to each well and incubation continued for 12, 24 and 72 h. As a non-infected control, gingival fibroblasts were detached and collected at baseline, and total RNA was extracted immediately.

An additional control included cells that were inoculated with ultraviolet light-inactivated human cytomegalovirus (71,000 copies/μL, 35.5×10^5). For ultraviolet light inactivation, 2 mL of the viral dilution was exposed to ultraviolet light (3 cm apart from the dilution), in a 10-mm sterile plate dish, for 20 min. The ultraviolet light-treated virus was then used to infect gingival fibroblasts, which were incubated for 72 h after infection. Exposure to ultraviolet light affects the infectivity of the virus. Viral infection was verified by immunofluorescence assay of infected cells (anti-human cytomegalovirus pp72) at the various experimental time-points (0, 12, 24 and 72 h postinfection). All experiments were performed three times.

Total RNA extraction

Gingival fibroblasts were detached at the indicated time-points (0, 12, 24 or 72 h) and total RNA was immediately isolated from the cell pellet. A commercially available kit for RNA purification was used according to the manufacturer's instructional protocol (Qiamp RNA blood mini kit; Qiagen, Valencia, CA, USA). To avoid DNA carryover, DNase treatment was included in the protocol following the instructions of the manufacturer (RNase-Free DNase set; Qiagen). Total RNA was quantified using a fluorometer (Qubit fluorometer; Invitrogen Corp., Carlsbad, CA, USA) and an RNA quantification kit (Quant-iT RNA Assay kit; Invitrogen Corp.). RNA was stored at −70°C for reverse transcription–PCR (RT-PCR).

RT-PCR

To determine the levels of mRNA expression for collagens I and III and for MMPs in human cytomegalovirus-infected fibroblasts, semiquantitative RT-PCR was performed. Primer sequence and amplification temperature are depicted in Table 1. The primer sequences used have been published previously (19,20), were based on GeneBank accession sequences spanning exon–exon junctions and were non-reactive to genomic DNA.

cDNA was synthesized using 3 ng of total RNA in a reverse transcription reaction. The first strand was synthesized using 1 μL of Oligo (dT) primer (Promega, Madison, WI, USA), 2.75 μL of diethylpyrocarbonate (DEPC) water and 3 ng of total RNA, incubated at 72°C for 5 min and immediately chilled on ice. A reverse transcription master mix was prepared using 4 μL of ×5 buffer (Promega), 1 μL of 10 mM dNTPs (Promega), 0.25 μL of RNasin (Promega) and 1 μL of Avian Myeloblastosis Virus (AMV) reverse transcriptase (Promega). After primer annealing, 6.25 μL of the master mix was added to each vial and programmed in a thermocycler (Peltier Thermal Cycler; MJ Research, Bio-Rad, Hercules, CA, USA) at 42°C for 1 h and at 95°C for 5 min. After reverse transcription, the volume of each cDNA was adjusted to 100 μL using DEPC-treated water and stored at −20°C for PCR analysis.

A 45-μL PCR reaction mix per tube was prepared as follows: 34.75 μL of PCR-grade water, 5 μL of buffer 10X (Promega), 2.5 μL of 2.5 mM dNTPs (Promega), 0.5 μL of primer mix, 2 μL of 25 mM $MgCl_2$ (1.0 mM, final concentration), 0.25 μL of 5 U/μL of *Taq* polymerase (Promega) and 5 μL of cDNA. The $MgCl_2$ concentration was optimized for each PCR product and varied as shown in Table 1. Standard PCR conditions were set to: 95°C for 1 min, 35 cycles corresponding to 94°C for 45 s, 58°C for 45 s (the annealing temperature varied according to the product, Table 1) and 72°C for 1 min; and a final reaction step at 72°C for 5 min.

Amplification products were electrophoresed in a 2% agarose gel, then observed and photographed on an ultraviolet transilluminator.

Gingival specimens

To measure the level of mRNA expression for collagen I (αI) and collagen III (αIII) and of MMPs in healthy and diseased periodontal tissues, RT-PCR was performed in gingival biopsies obtained from periodontitis patients and healthy donors. Determination of human cytomegalovirus in subgingival samples of each patient was performed according to Parra & Slots (21) and to Contreras & Slots (22).

In sum, gingival biopsies were obtained at baseline during the clinical examination of 14 periodontitis patients (human cytomegalovirus positive,

Table 1. Primer sequence and polymerase chain reaction amplification conditions for collagen I (aI), collagen III (aIII), matrix metalloproteinase (MMP)-1, MMP-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Product/cytokine	Primer sequence (5′–3′)	Amplicon size (bp)	Annealing T (°C)	$MgCl_2$ (mM)
Collagen I (aI)	F: AGGCCCTCAAGGTTTCCAAGG R: CCAGACCATTGTGTCCCCTAA	223	58	2
Collagen III (aIII)	F: TGGTGTTGGAGCCGCTGCCA R: CTCAGCACTAGAATCTGTCC	376	58	2
MMP-1	F: TGGACCTGGAGGAAATCTTGC R: AGAGTCCAAGAGAATGGCCGA	155	58	2
MMP-2	F: CTGATGGCACCCATTTACACCT R: GATCTGAGCGATGCCATCAAA	186	60	1
GAPDH	F: CGTCTTCACCACCATGGAGA R: CGGCCATCACGCCACAGTTT	220	58	2

F, forward; R, reverse; T, temperature.

$n = 10$; human cytomegalovirus negative, $n = 4$) and of three non-periodontitis patients (human cytomegalovirus negative, $n = 3$). Biopsies from periodontitis patients included a 3-mm bevelled incision in the palatal side of the maxillary teeth at a location that was intensively inflamed and had a pocket depth of > 5 mm. Specimens from non-periodontitis subjects were collected during mandatory periodontal surgery carried out for prosthetic reasons. All specimens were collected in microcentrifuge vials, immediately flash frozen in liquid nitrogen and then stored at −70°C for analysis.

Total RNA was extracted using a commercially available kit (RNeasy Fibrous Tissue Mini kit; Qiagen). To avoid DNA carryover, DNase treatment was included in the protocol according to the instructions of the manufacturer (RNase-Free DNase set; Qiagen). Total RNA was quantified, stored and analyzed as explained above.

Data analysis

The intensity of each PCR product band was analyzed using open source public software according to the manufacturer's instructions (IMAGEJ; NIH, Bethesda, MD, USA). Values were adjusted relative to the expression of glyceraldehyde-3-phosphate dehydrogenase in the same sample and expressed as the ratio of expression. The mean ratio of expression (± standard deviation) was calculated from three experiments and presented for each marker analyzed. Differences between infection times and human cytomegalovirus concentration were assessed using the unpaired *t*-test ($p < 0.05$). Data were analyzed using statistical software (STATA; Statistical Software for Windows StataCorp LP, College Station, TX, USA).

Results

In vitro human cytomegalovirus infection in gingival fibroblasts affects expression of mRNA for collagens I and III

The experimental infection of gingival fibroblasts (gingival fibroblasts) with 71,000 copies/μL of human cytomegalovirus for 0–72 h resulted in a progressive reduction in the expression of mRNA for collagens I and III (Fig. 1). Immunofluorescence staining of human cytomegalovirus pp72 antigen in the infected cells at the indicated timepoints showed that gingival fibroblasts were indeed infected with the virus (Fig. 1A). By contrast, infection was almost blocked in cells exposed to ultraviolet light-inactivated human cytomegalovirus, as observed at 72 h postinfection. The reduction in collagen mRNAs was readily appreciable at 12 h postinfection and was lower at 72 h postinfection when compared with baseline (0 h) or when compared with both non-infected and ultraviolet light-inactivated human cytomegalo-

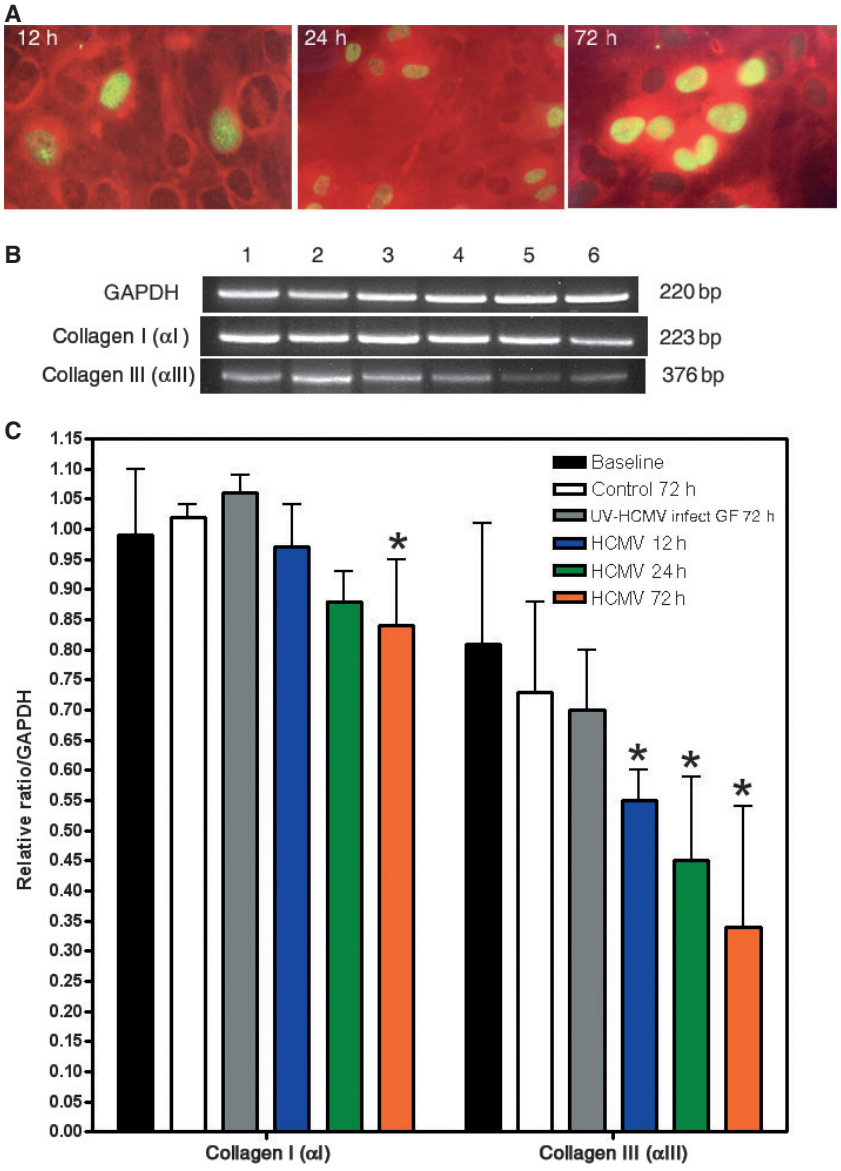

Fig. 1. mRNA expression of collagens I and III after HCMV infection (71,000 copies/μl). (A) Immunofluoresce assay showing staining for pp72 at 12, 24 and 72 h p.i. (B) PCR amplicons corresponding to GAPDH, Collagen I and Collagen III as follows: lane 1 Baseline, lane 2 Control non-infected GF 72 h, lane 3 UV-HCMV infected GF at 72 h, lane 4 HCMV infected GF at 12 h p.i, lane 5 HCMV infected GF at 24 h p.i. and lane 6 HCMV infected GF at 72 h p.i. (C) Relative ratio of expression of mRNA at the indicated times for collagens I and III. Data is presented as the mean of three different experiments ±SD. (*) Different as compared to baseline ($p < 0.05$).

virus-infected gingival fibroblast controls at 72 h. The level of mRNA for collagens I and III in gingival fibroblasts that were infected with ultraviolet light-inactivated virus were comparable to those of baseline or non-infected gingival fibroblasts. A higher reduction ($p < 0.05$) in the relative expression of mRNA for collagen III, as compared to baseline, at 12, 24 and 72 h (Fig. 1C) was noted. By contrast, reduction in the expession of mRNA for collagen I was not as marked as for collagen III (Fig. 1C). The reduction in mRNA expression for collagen I was lower than that determined at baseline ($p < 0.05$, Fig. 1C).

Infection of gingival fibroblasts with different doses of human cytomegalovirus resulted in an appreciable reduction over time of mRNA for collagen I only at 71,000 and 7100 copies/μL of virus. (Fig. 2A). Infection with a low viral dose consisting of 71 copies/μL had a negligible effect on the mRNA expression at 72 h. By contrast, reduction was more evident for collagen III (Fig. 2B). With low viral doses, a reduction of mRNA for collagen III was observed at 24 h followed by a slight increase at 72 h (Fig. 2B).

The analysis of mRNA for collagens I and III from gingival specimens is presented in Fig. 3. Subjects with periodontitis had a lower expression of mRNA for collagen I than subjects without periodontitis. By contrast, it was observed that biopsies from human cytomegalovirus-positive periodontitis individuals had a higher expression of mRNA for collagens I and III than did human cytomegalovirus-negative periodontitis specimens.

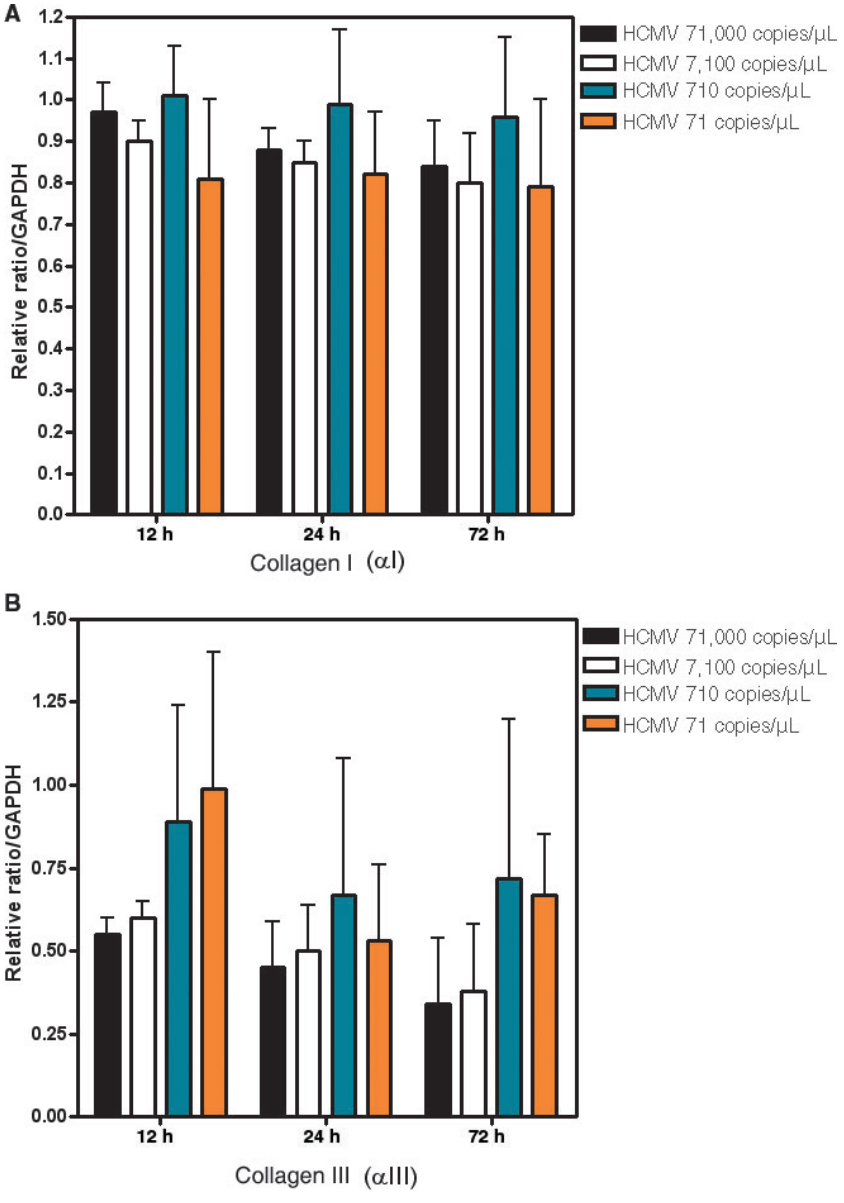

Fig. 2. Comparison of the effect of different HCMV infection doses on the mRNA expression of collagens I (A) and III (B) at the indicated time (X- axis, 12, 24 and 72 h). Data is presented as the mean of three experiments ± SD.

In vitro human cytomegalovirus infection in gingival fibroblasts resulted in up-regulation of mRNA expression of MMP-1 and MMP-2

The infection of gingival fibroblasts with 71,000 copies/μL of human cytomegalovirus induced an up-regulation of mRNA expression for MMP-1 and MMP-2 (Fig. 4A,B). Up-regulation was progressive over time for MMP-1 compared with baseline and control ($p < 0.05$). By contrast, mRNA up-regulation for MMP-2 peaked at 24 h when compared with baseline and control ($p < 0.05$). Irradiation of human cytomegalovirus with ultraviolet light partially reduced mRNA up-regulation at 72 h.

Different infectious doses of human cytomegalovirus had different effects over time (Fig. 5). Higher expression of mRNA for MMP-1 was observed with low doses of virus compared with higher doses of virus. By contrast, expression of mRNA for MMP-2 peaked at 12 and 24 h with 71,000 and 7100 copies/μL of human cytomegalovirus, respectively (Fig. 5).

The analysis of mRNA expression in gingival biopsies demonstrated a higher expression of MMP-1 in human cytomegalovirus-positive periodontitis in comparison to human cytomegalovirus-negative periodontitis specimens (Fig. 6). The levels of MMP-2 were similar in human cytomegalovirus-positive and human cytomegalovirus-negative periodontitis samples, but in both cases a higher level of expression was observed when compared with healthy specimens.

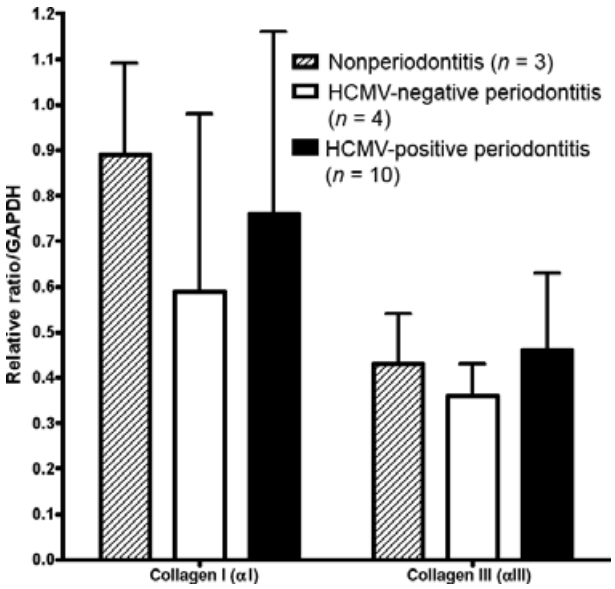

Fig. 3. Analysis of mRNA expression for collagen I and III in gingival specimens from non-periodontitis, HCMV(−) periodontitis and HCMV(+) patients. Data is presented as the mean ± SD.

Discussion

Collagens I and III are the major protein components found in connective periodontal tissues and are responsible for homeostatic and structural properties (23). Hence, it would be rational to think that any alteration in the production of these matrix components would directly affect the periodontal tissues, as observed in periodontal pocket formation (24,25).

As shown in the results presented above, human cytomegalovirus infection affects gingival biology. Collagen mRNA expression was found to be reduced *in vitro* but increased *in vivo*. It has been observed that infection of cells with human cytomegalovirus can result in chromosome alteration (26) and in multiple cellular function alterations (27–30) in different cell types. As a result of the *in vitro* evidence, human cytomegalovirus infection was considered to diminish the expression of mRNA for collagens I and III, resulting in lower or altered collagen synthesis. This biological alteration seems plausible and helps to explain the susceptibility of periodontally infected tissues to clinical attachment loss. Concomitant with the progression of inflammation in periodontal connective tissues, the volume of collagen becomes reduced to < 30% of the original volume. Nonetheless, results from mRNA analyses in gingival specimens showed that the expression of collagens was higher in human cytomegalovirus-positive periodontitis samples than in human cytomegalovirus-negative periodontitis samples, but lower than in healthy tissues. The above differences could be explained by the cellular and immunological interactions that occur within periodontal connective tissues. Another explanation for these differences might stem from the fact that the *in vitro* experiments were short term in nature, by contrast to gingival specimens in which a chronic infection is established. However, experiments in rats showed that murine cytomegalovirus infection enhances chronic renal allograft rejection (fibrosis) through the increased production of collagens I and III from myofibroblasts (31,32). One explanation for this might be a change in the phenotype of human cytomegalovirus-infected gingival fibroblasts to myofibroblasts, which occurs *in vivo*. However, this cannot be confirmed based upon the results of the present study because histopathology was not conducted. Myofibroblastic differentiation is an important event in gingival wound healing and chronic inflammation. This phenotypic change has been observed in cases of gingival overgrowth in either gingival hereditary fibromatosis or as a result of treatment with cyclosporine A (33,34). The clinical appearance of the gingival specimens analyzed here was inflamed gingival tissue, where a change in the normal architecture of the gingiva was evident. Furthermore, evidence from *in vitro* experiments showed that cytokines, such as transforming growth factor-β, regulate this myofibroblastic change in cultured gingival fibroblasts and that its production could be increased in periodontitis (35,36). Whether human cytomegalovirus infection was responsible for the results observed in gingival specimens should be studied in greater detail. Nonetheless, the results from *in vitro* experiments determined that, independent of other factors that may be acting *in vivo*, the infection with human cytomegalovirus *per se* is able to reduce the expression of mRNA for collagens in gingival fibroblasts. The evidence presented suggests that this biological event could be a modifier of periodontal healing or tissue integrity, resulting in a diminished ability to resist periodontal inflammation destruction. Nonetheless, *in vivo* conditions that mediate cellular function and human cytomegalovirus infection are far more complex than *in vitro* conditions and are beyond the scope of this study.

Periodontitis is a disease where connective tissue metabolism is increased through the production of collagenases (37). *In vitro* human cytomegalovirus infection of gingival fibroblasts demonstrated that the levels of mRNA for MMP-1 and MMP-2 increased over time after infection and were virally mediated. This biological event could have an important clinical impact in periodontal disease, favoring the loss of connective tissue attachment. MMPs can be classified, according to substrate, as: collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10) and membrane-type MMPs. While MMP-1 is responsible for the cleavage of native fibrillar collagens I and III, MMP-2 attacks denatured collagen fragments (38). Higher levels of MMP-1, MMP-2, MMP-3, MMP-8 and gelatinase-B (MMP-9) have been found in the gin-

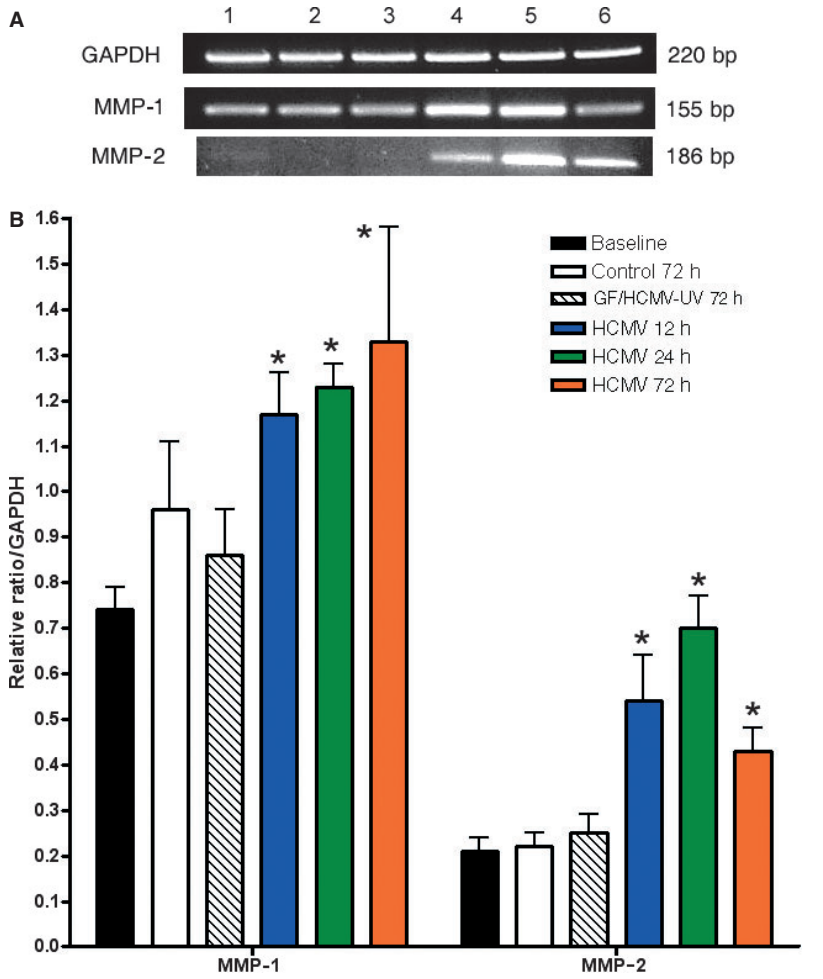

Fig. 4. mRNA expression of MMP1 and MMP2 after HCMV infection (71,000 copies/μl). (A) PCR amplicons corresponding to GAPDH, MMP-1 and MMP-2 as follows: lane 1 Baseline, lane 2 Control non-infected GF 72 h, lane 3 UV-HCMV infected GF at 72 h, lane 4 HCMV infected GF at 12 h p.i, lane 5 HCMV infected GF at 24 h p.i. and lane 6 HCMV infected GF at 72 h p.i. (B) Relative ratio of expression of mRNA at the indicated time for MMP-1 and MMP-2. Data is presented as the mean of three experiments ± SD. (*) Different as compared to baseline and control ($p < 0.05$).

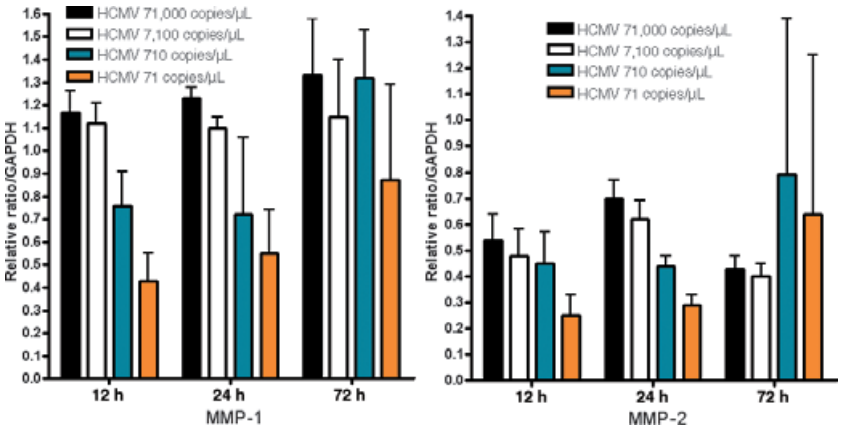

Fig. 5. Comparison of different HCMV infection doses on mRNA expression of MMP-1 and MMP-2 at the indicated time (X- axis, 12, 24 and 72 h). Data is presented as the mean of three experiments ± SD.

gival tissues and gingival crevicular fluid of patients with periodontitis (39,40). The mechanism proposed is that an imbalance between the production of MMPs and their inhibitors (tissue inhibitor of metalloproteinases) is responsible and helps to substantiate their relationship with tissue destruction (41). Human cytomegalovirus infection in periodontal connective tissues could lead to increased expression of mRNA for MMP-1 and MMP-2, resulting in higher levels of metalloproteinases. Therefore, the overproduction of MMPs as a result of bacterial challenge and viral infection could help to explain why periodontitis-infected sites suffer from increased periodontal attachment loss. This could be further supported by the fact that gingival biopsies from human cytomegalovirus-positive periodontitis subjects expressed more mRNA for both enzymes than did gingival biopsies from human cytomegalovirus-negative periodontitis subjects. However, more studies addressing the *in situ* production of MMPs in gingival connective tissues and gingival crevicular fluid, as related to human cytomegalovirus infection, are necessary to provide supportive evidence for the role of human cytomegalovirus in periodontal destruction.

In the present study it was demonstrated that the *in vitro* infection of gingival fibroblasts with human cytomegalovirus resulted in varying degrees of biological alterations. The expression of mRNA for collagens I and III was partially affected in human cytomegalovirus-infected fibroblasts. By contrast, human cytomegalovirus-positive periodontitis specimens showed the opposite effect. Although *in vitro* evidence demonstrated that mRNA alteration was a virally dependent event, differences between experimental and *in vivo* conditions should be considered carefully.

The expression of mRNA for MMP-1 and MMP-2 was consistent between experimental and gingival specimens. Human cytomegalovirus-positive infected fibroblasts and human cytomegalovirus-positive periodontitis samples showed an increase in the expression of mRNA for MMP-1 and

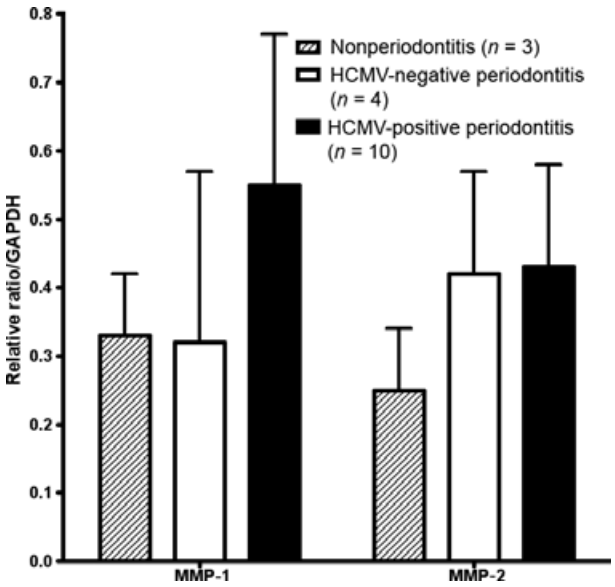

Fig. 6. Analysis of mRNA expression for MMP-1 and MMP-2 in gingival specimens from non-periodontitis, HCMV(−) periodontitis and HCMV(+) patients. Data is presented as the mean ± SD.

MMP-2. In this study, the results suggest that mRNA alterations in infected gingival fibroblasts were virally mediated because ultraviolet light irradiation of human cytomegalovirus partially reduced the effect observed. In conclusion, altered expression of mRNA for collagens and metalloproteinases in human cytomegalovirus-infected gingival fibroblasts should be considered as possible modifying mechanisms in periodontitis-infected sites.

Acknowledgements

This study was supported by a grant from COLCIENCIAS (Colombian Institute for Development of Science and Technology Francisco José de Caldas) and the Universidad del Valle (312-2004, 1106-05-16316). Javier E. Botero holds a scholarship from COLCIENCIAS. The authors are grateful to Dr Sandra Rich at the University of Southern California (USA) and Ronald Oliver at the University of Valle (Colombia) for their critical appraisal of the manuscript. Human cytomegalovirus Towne strain was kindly provided by Dr Mark Stinsky and Phil Lashmit from the University of Iowa (USA).

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