Regulation and Role of Heme Oxygenase-1 (HO-1) During Inflammation in Human Gingival Fibroblasts (HGF)

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ABSTRACT
Periodontitis is the most common cause of adult tooth loss in the U.S., with an estimated 1 in 3 adults suffering from some form and 10-15% of adults developing severe forms. In its advanced stage, periodontitis also contributes to the development of several other diseases, including cardiovascular disease, hemolytic anemia, and diabetes. Although the primary function of HO-1 is to reduce the levels of cellular oxygen and carbon monoxide, it has also been shown to play an important role in wound repair and the resolution of inflammation by mechanisms involving homoeostatic regulation of the redox state of cells. A series of experiments has been designed to determine whether and to what extent the levels of HO-1 mRNA and protein are regulated by inflammation cytokines in HGF isolated from individuals with and without periodontitis. Preliminary results show that HO-1 is downregulated in HGF cultures derived from patients with periodontitis and that HO-1 levels are inhibited over 60% by interleukin-1 (1 hour: 0.1 μg/mL IL-1β, p < 0.001). Interestingly, however, HO-1 protein levels as measured by ELISA are not decreased by IL-1β. Experiments are currently underway to address these apparent paradoxes, as well as the potential role of HO-1 in the regulation of inflammatory mediators.

INTRODUCTION
Periodontitis is the most common cause of adult tooth loss in the U.S., with an estimated 1 in 3 adults suffering from some form and 10-15% of adults developing severe forms. In its advanced stage, periodontitis also contributes to the development of several other diseases, including cardiovascular disease, hemolytic anemia, and diabetes. Although the primary function of HO-1 is to reduce the levels of cellular oxygen and carbon monoxide, it has also been shown to play an important role in wound repair and the resolution of inflammation by mechanisms involving homoeostatic regulation of the redox state of cells. A series of experiments has been designed to determine whether and to what extent the levels of HO-1 mRNA and protein are regulated by inflammation cytokines in HGF isolated from individuals with and without periodontitis. Preliminary results show that HO-1 is downregulated in HGF cultures derived from patients with periodontitis and that HO-1 levels are inhibited over 60% by interleukin-1 (1 hour: 0.1 μg/mL IL-1β, p < 0.001). Interestingly, however, HO-1 protein levels as measured by ELISA are not decreased by IL-1β. Experiments are currently underway to address these apparent paradoxes, as well as the potential role of HO-1 in the regulation of inflammatory mediators.

MATERIALS & METHODS

Cell Cultures
Human gingival tissues from periodontal surgical patients were obtained from Suresh S. Gurnani, D.M.D., Periodontist. Human gingival tissues from patients undergoing oral surgical removal of wisdom teeth, without inflammation, was obtained from Manosor Madani, M.D., D.M.D., Oral and Maxillofacial Surgeon. The tissues were transported in transport media primary culture, and were maintained in Eagle’s Minimal Essential Medium (EMEM) supplemented with 10% fetal bovine serum and antibiotics (penicillin, streptomycin, amphotericin B, 100 U/mL, 100 μg/mL, 2 μg/mL). Cells between passages 3 and 5 were used for experiments.

RNA extraction and Real-Time PCR
After gingival processing, cells were immersed, rinsed with IL-1 β 10 ng/mL for 12 hours and isolated with the indicated time points. RNA was isolated using the RNeasy Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions. Total RNA was treated with RQ1 RNase-free DNase (Promega, Madison, WI) according to the manufacturer’s instructions. The resulting RNA was reverse transcribed using the Omniscript First-Strand Kit (QIAGEN, Valencia, CA) as described in the manufacturer’s instructions. RNase-free DNase (QIAGEN) was added to the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Real-Time PCR was performed using the ABI 7500 Fast Real-Time PCR System according to the manufacturer’s instructions. Real-Time PCR reactions were performed using the Taq-Man Fast Advanced Master Mix (Applied Biosystems). Primer sequences for HO-1, β-Actin, and 18S ribosomal RNA (rRNA) are shown in Table 1. The qPCR efficiency was calculated by the formula 2^-1/ΔΔCt. The relative expression of each gene was calculated by the 2^-ΔΔCt method. The statistical significance of the differences between groups was determined using Student’s t-test. A P < 0.05 was considered significant.

RESULTS

Figure 1: Effects of IL-1 on expression of Heme Oxygenase-1 (HO-1) in HGF cells. A) HO-1 mRNA was quantified by real-time PCR and normalized to 18S rRNA in HGF cultures isolated from patients with periodontitis. B) HO-1 protein expression was quantified by Western blot analysis. C) The protein levels of HO-1 in HGF cells isolated from patients with periodontitis were compared with those in HGF isolated from patients undergoing an unrelated oral surgical procedure.

Figure 2: Effects of IL-1 on expression of Matrix Metalloproteinase-3 (MMP-3) in HGF cells. A) MMP-3 mRNA was quantified by real-time PCR and normalized to 18S rRNA in HGF cultures isolated from patients with periodontitis. B) MMP-3 protein expression was quantified by Western blot analysis.

Figure 3: Effects of HO-1 inhibition on basal and IL-1 induced expression of MMP-3 in HGF cells. A) MMP-3 mRNA was quantified by real-time PCR in HGF cultures isolated from patients with periodontitis. B) The protein levels of MMP-3 in HGF cells isolated from patients with periodontitis were compared with those in HGF isolated from patients undergoing an unrelated oral surgical procedure.

CONCLUSIONS

IL-1 inhibits expression of HO-1 mRNA in HGF isolated from patients with periodontitis.

These results suggest that increased expression of HO-1 in the context of chronic inflammation has the potential to attenuate expression of MMP-3, and may have an impact on disease expression and progression. Therefore, HO-1 may be a therapeutic target for anti-inflammatory therapies.

REFERENCES


