Hepatocyte growth factor in saliva: a possible marker for periodontal disease status

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Abstract: Hepatocyte growth factor (HGF) acts as a mitogen, motogen, morphogen and anti-apoptotic factor for various kinds of epithelial cells. We previously showed that periodontal fibroblasts secreted an HGF-like chemoattractant for a gingival epithelial cell line and found that HGF content in gingival crevicular fluid was well correlated with probing depth, gingival index, and interleukin-1β concentration. To examine whether HGF in whole (mixed) saliva would be a useful marker for periodontal disease status, we investigated the relationship between salivary HGF levels and clinical parameters of 65 adults (50 men and 15 women). Unstimulated whole (mixed) saliva was collected from each subject and the HGF level was determined with an ELISA kit. After sample collection, probing depths and bleeding on probing were monitored. Significant correlations were found between salivary HGF levels and the number of probing depths exceeding 4 mm (r = 0.541), the number of probing depths exceeding 6 mm (r = 0.683), the deepest probing depth of each subject (r = 0.558) and the percentage of sites positive for bleeding on probing (r = 0.511). These results suggest that salivary HGF may be a novel marker for periodontal diagnosis in screening tests. (J. Oral Sci. 44, 35-39, 2002)

Key words: hepatocyte growth factor; saliva; marker; periodontal disease status.

Introduction

Hepatocyte growth factor (HGF), also known as scatter factor, is a heterodimeric protein secreted by cells of mesodermal origin (1-3). The factor induces a spectrum of biological activities in epithelial cells, including mitogenesis, stimulation of cell motility, and promotion of matrix invasion (1, 4-6). HGF is also a morphogen in vitro (7,8) and a potent angiogenic factor in vitro and in vivo (9,10). We previously showed that periodontal fibroblasts secreted an HGF-like chemoattractant for a gingival epithelial cell line (11,12); we hypothesized that HGF may be involved in epithelial invasion following the destruction of connective tissue attachment and that it had a role in the pathogenesis and progression of periodontitis. The presence of various markers of periodontal inflammation in gingival crevicular fluid (GCF) is well recognized (13,14). A variety of mediators or markers of the inflammatory response (e.g., lysosomal enzymes, prostaglandins, complement components, and cytokines, such as the interleukins and tumor necrosis factor) have been identified in GCF (13). Recently, we detected a much

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greater amount of HGF in GCF than in normal human serum, and found that HGF content was well correlated with probing depth, gingival index, and interleukin-1β concentration in GCF (15). For these reasons, we hypothesized that the HGF in whole (mixed) saliva would be predominantly derived from GCF. Therefore, we designed this preliminary study to evaluate the relationship between the levels of HGF in saliva and clinical measures of periodontal disease, and to assess whether the HGF levels in saliva would be a novel salivary marker for periodontal diagnosis.

Materials and Methods

Sixty-five sequentially selected subjects (50 men and 15 women; 40, 45, 50 and 55 years of age, mean age: 47.7 years) visiting the Health Care Unit of a company in Japan for medical and dental examinations were included in this study. Their periodontal status ranged from healthy to clinically diseased. All subjects gave their informed consent to the investigation. We excluded people with fewer than 19 teeth, people who had dental treatment or antibiotic therapy within the previous 6 months, and people with serious systemic diseases, such as diabetes.

All subjects were instructed to expectorate their unstimulated whole (mixed) saliva into a sterile 50-ml tube after a preliminary oral rinse with water. Samples (0.5-2.0 ml) were kept on ice for no longer than 2 h and then transferred to 1.5-ml microtubes and centrifuged at 10,000 × g for 20 min at 4 °C to remove particulate matter and bacteria. The supernatant was collected and stored at −20 °C until analysis.

After sample collection, probing depths at four sites (mesiobuccal, buccal, distobuccal, and lingual) around each tooth were measured, and bleeding on probing (16) was recorded to monitor the periodontal disease status of the subjects.

The HGF content of each sample was determined by using an ELISA kit (Quantikine human HGF immunoassay; R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions at room temperature. Briefly, 150 μl of Assay Diluent RD1W was added to each well of a HGF microplate (96 well polystyrene microplate coated with a murine monoclonal antibody against HGF). Then, 50 μl of standard or undiluted whole saliva sample was added to each well and incubated for 2 h. Fifty μl of Calibrator Diluent RD6X was used as a blank. After washing for 4 times, 200 μl of HGF Conjugate (polyclonal antibody against HGF conjugate to horseradish peroxidase) was added to each well, and incubated for 2 h. After washing 4 times, 200 μl of Substrate Solution (tetramethylbenzidine and hydrogen peroxide) was added to each well and incubated for 30 min. After the addition of 50 μl of Stop Solution (2 M sulfuric acid) to each well, absorbance was determined at 450 nm using a microplate reader (Multiscan Multisoft, Labsystems). The standard curve is shown in Fig. 1. The protein concentration in whole saliva was quantitated with the aid of Bio-Rad protein reagent by absorbance at 595 nm. Duplicate assays were performed and the data (means) were converted to pg/mg protein in whole saliva using bovine serum albumin (Fraction V, Bayer, Kankakee, IL, USA) as a standard.

Pearson’s correlation coefficients were used to analyze the relationship between HGF content and clinical parameters.

Results

HGF was detected in all saliva samples at between 154 and 9484 pg/mg protein, with a mean value of 1781 pg/mg protein. Significant correlations were observed between salivary HGF levels and the number of probing depths exceeding 4 mm (r = 0.541; P < 0.001) (Fig. 2), the number of probing depths exceeding 6 mm (r = 0.683; P < 0.001) (Fig. 3), the deepest probing depth (r = 0.558; P < 0.001) (Fig. 4), and the percentage of sites positive for bleeding on probing (r = 0.511; P < 0.001) (Fig. 5). There was no significant correlation between salivary HGF levels and...
Fig. 2 Correlation between salivary HGF levels and the number of probing depths exceeding 4 mm. Data were derived from 65 sequentially selected, untreated subjects. Following saliva collection for HGF quantification, subjects received a comprehensive periodontal examination. Data were significantly correlated ($r = 0.541, P < 0.001$).

Fig. 3 Correlation between salivary HGF levels and the number of probing depths exceeding 6 mm. Data were significantly correlated ($r = 0.683, P < 0.001$).

Fig. 4 Correlation between salivary HGF levels and the deepest probing depth. Data were significantly correlated ($r = 0.588, P < 0.001$).

Fig. 5 Correlation between salivary HGF levels and the percentage of sites positive for bleeding on probing. Data were significantly correlated ($r = 0.511, P < 0.001$).
subject age (data not shown).

**Discussion**

Our results show that HGF levels in whole (mixed) saliva (ranged from 0.154 ng/mg protein to 9.48 ng/mg protein, with a mean value of 1.78 ng/mg protein, and ranged from 0.127 ng/ml to 9.61 ng/ml, with a mean value of 1.80 ng/ml) were significantly correlated with periodontal disease extent; i.e., the salivary HGF was greater in subjects with increased periodontal probing depths and occurrence of bleeding on probing. Our recent study revealed that the HGF levels detected in GCF (ranged from 0.6 ng/ml to 729.6 ng/ml, with a mean value of 92.5 ng/ml) were much higher (mean: 308-fold) than those in normal human serum (0.3 ng/ml) (15). This result and the fact that GCF contributes continuously to whole (mixed) saliva, suggest that increased periodontal disease extent is associated with increased HGF in saliva. However, HGF levels in saliva are likely to be affected by factors such as salivary flow rate. Further, since whole (mixed) saliva represents a combination of fluids from major and minor salivary glands as well as GCF, these various sources could contribute differentially to the HGF; i.e., perhaps not all salivary HGF is of GCF origin. In fact, localization and expression of HGF in rabbit salivary glands and rat submandibular glands have been reported (17,18). However, the HGF content of human salivary gland-derived saliva remains unknown. Whereas our results are consistent with the hypothesis that GCF is one of the major source of HGF in saliva, the levels of correlation between salivary HGF and clinical measures (r = 0.511 to 0.683) suggest that other factors are also involved in periodontal inflammation. This is in agreement with current concepts of periodontal disease, where the combined interaction of the host and oral pathogens regulates the extent of tissue destruction and disease progression (19).

Possible salivary markers for periodontal diagnosis, such as enzymes, immunoglobulins, cytokines, and bacteria, have been reviewed and discussed (20,21). However, given the complex nature of periodontal disease, it is unlikely that a single marker will prove to be both sensitive and specific. Therefore, a combination of two or more markers may provide a more accurate periodontal assessment. A laboratory screening method based on whole (mixed) saliva would have the advantages of being non-invasive and of being quick and simple to collect in a wide variety of environments (20). Individuals who proved positive for any valid saliva-based marker of periodontal disease susceptibility or activity could then be called for a detailed clinical and laboratory work up, including site-specific examination of GCF and subgingival plaque. Although our results show that HGF in whole (mixed) saliva may be a novel marker for periodontal disease status, further study is needed to clarify whether HGF is a more reliable marker for periodontal diagnosis than other salivary markers for screening tests.

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