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Gelatinase B is associated with peri-implant bone loss

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Abstract: The aim of this study was to clear whether gelatinase B is associated with peri-implant bone loss (PBL). Peri-implant sulcus fluid was collected from 46 implant sites in 12 patients. These sites were also characterized using modified Gingival Index (mGI). Activated and total gelatinase B levels, measured using a modified urokinase assay, showed correlation with PBL ($n = 46$, Spearman's rank correlation test). Activated and total gelatinase B values were significantly higher in PBL>3 mm group ($n = 6$) compared to PBL<1 mm ($n = 29$) and 1 <PBL<3 mm ($n = 11$) groups (rank sum test). Activated gelatinase B level in mGI>0.5 group ($n = 24$) was clearly higher compared to mGI = 0 ($n = 13$) and $\leqslant$0.5 ($n = 9$) groups (Rank sum test). We conclude that gelatinase B is associated with PBL. Activation of gelatinase B together with elevated mGI eventually reflect active phases of peri-implantitis and may prove to be diagnostically useful.

Dental implants are widely used in dentistry. Some complications, such as fracture of connecting screws, dislocation of dental implants or damage of the mandibular nerve, have become relatively rare (Goodacre et al. 1999). There are data to suggest that connecting screw problems and other issues remain. In addition, the incidence of mechanical failure of implant prosthesis is measurably greater than implant loss. However, according to a nationwide dental implant register of the National Agency of Medicines, the number of removed and loosened implants in Finland has remained small (1.9%) with only little variation. The most common reason for a failure is loosening of the implant without any clinically observable special event. The majority of failures, 60%, occurred during the first year after the operation. In most cases, no prosthetic restoration had been made on the implant. The second most common reason for an implant loss was infection (0.62%). However, very often the reason of implant failure remained unclear. Implant failures and losses occurred in the whole maxillofacial area, but the number of losses in the maxilla was slightly higher (Pihakari et al. 2001). Failure of dental implants in the long run is usually a result of prolonged infection-induced inflammation in the soft and hard tissues around the osseointegrated implant (Luterbacher et al. 2000). The incidence of mechanical failure is measurably greater than implant loss (Tonetti 1999). Peri-implant bone can be

destroyed to such an extent that their implants are finally lost and cannot be reimplanted.

Proteolytic enzymes are involved in the degradation of bone collagen matrix (Everts et al. 1999; Sorsa et al. 1999). Matrix metalloproteinases (MMPs) form a family of neutral endoproteinases with over 20 known members (MMP-1 to MMP-28). The common denominator is that they are able to function at the neutral body pH, under which circumstances they can degrade all components of the extracellular matrix (Brinckerhoff & Matrisian, 2002). In dentistry, they are best known for their ability to cleave across the interstitial collagen triple helix of type I and III collagen, which are the main components of the periodontal ligament. Periodontitis and peri-implantitis are often, probably erroneously, considered to have similar pathomechanisms. However, in contrast to natural teeth, dental implants are not fixed to alveolar bone with a periodontal ligament. Instead, they are directly osseointegrated to the alveolar bone. Therefore, peri-implantitis is an interesting model to study the eventual role of MMPs in bone destruction. Indeed, human osteoclasts express gelatinase B (Wucherpfennig et al. 1994), which seems to be a prerequisite for the recruitment of osteoclasts to bone resorption sites (Blavier & Delaisse 1995). Osteoclasts are increased in numbers in the bone resorption area during the progression of periodontitis (Makris & Saffar 1982). Gelatinase B contributes to the expansion of jaw cysts (Teronen et al. 1995). Gelatinase B degrades collagenase/cathepsin-cleaved bone collagen fragments (Birkedal–Hansen 1995; Kusano et al. 1998). It was hypothesized that increased activated and total gelatinase B levels in peri-implant sulcus fluid (PISF) are associated with peri-implant bone loss (PBL). Gelatinase B has not been analyzed in this clinical setting. We assessed the usefulness of modified Gingival Index (mGI) (Mombelli et al. 1987; Luterbacher et al. 2000) as a predictor for gelatinase activity.

Material and methods

Forty-six implant sites in 12 patients (6F, 6M; Mean age, 49.3 years, range 22–68 years) were studied. The mean loading time at the time of the study was 42 months. All implants had already been loaded. The detailed clinical data of the original 13 patients have been described in our previous article (Ma et al. 2000). However, one of these patients was not included in the present study, because all the PISF samples had been used up already. PISF samples, one sample per implant, were collected with a filter strip placed into the sulcus for 4 min. All PISF samples were collected from the mesial and/or distal locations. All PISF samples were collected from the site with maximum vertical bone loss as assessed from X-rays. These sites also mostly associate with the deepest peri-implant pockets. The absorbed PISF was diluted in 50 µl of 50 mM Tris-HCl, 0.2 M NaCl, 1 mM $CaCl_2$, pH 7.8, and centrifuged. The supernatants were stored at −20°C (Teronen et al. 1997). The protocol for collecting human samples was approved by the ethical committee of the Institute of Dentistry, University of Helsinki, Helsinki, Finland. All subjects gave their informed consent.

PBL and MGI

The distance between the fixture and alveolar crest was determined using the threads of the fixture of the inserted dental implant as an internal dimensional reference (Strid 1985). Accordingly, implants were grouped into three groups (Jeffcoat et al. 1995) (Table 1). mGI was determined according to Löe and Mombelli et al. (Löe 1967; Mombelli et al. 1987) (Table 2).

A modified pro-urokinase gelatinase B activity assay

To measure activated gelatinase B, 96-well plates (Costa) were coated with 100 µl (5 µg/ml) gelatinase B specific monoclonal TNO-S22.2 antibodies developed by Hanemaaijer (Hanemaaijer et al. 1998) for 16 h at +22°C and washed three times with PBS containing 0.05 percent Tween-20 (PBS-T). Purified gelatinase B as a positive control and 5 µl PISF samples were added and incubated for 16 h at +4°C. The wells were washed three times with PBS-T and incubated with assay buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 5 mM $CaCl_2$, 1 µM $ZnCl_2$, 0.01 percent Brij-35). 15 µl (50 µg/ml) modified pro-urokinase containing an MMP-specific activation site and 10 µl (6 mM stock) chromogenic substrate pyro-Glu-Gly-Arg-*p*-nitroanilide (Chromogenix, Mölndal, Sweden) were added. The color was measured in a Titertek Multiskan 8-channel photometer (Flow Laboratories, Irvine, Scotland). The results are expressed as U/ml (Verheijen et al. 1997; Hanemaaijer et al. 1998).

Table 1. **Comparisons (rank sum test) of activated and total gelatinase B levels (Medians [Q3−Q1]) in different categories of PBL**

	I	II	III	III vs. I	III vs. II	II vs. I
PBL	≤1 mm	<3 mm, >1 mm	≥3 mm			
n	29	11	6			
Activated gelatinase B (U/ml)	133 [225]	166 [200]	458 [979]	0.015	0.035	0.448
Total gelatinase B (U/ml)	183 [480]	250 [533]	1375 [2079]	0.013	0.027	0.797

Table 2. **Comparisons (rank sum test) of activated and total gelatinase B levels (Medians [Q3−Q1]) in different mGI groups**

	A	B	C	B vs. A	C vs. A	C vs. B
mGI	0	>0, ≤0.5	>0.5			
n	13	9	24			
Activated gelatinase B (U/ml)	50 [125]	216 [375]	225 [267]	0.17	0.012	0.52
Total gelatinase B (U/ml)	83 [217]	350 [1375]	416 [912]	0.02	0.043	0.65

To measure the total gelastinase B, PISF samples were first incubated with 0.5 mM p-aminophenylmercuric acetate (APMA) (Sigma, St. Louis, MO, USA) for 2 h at $+37^{\circ}C$ to activate progelatinase B. And then measured as activated gelatinase B. The assay detects as little as 3.75×10^{-15} mol gelatinase B in biological fluid samples (Verheijen et al. 1997).

Statistics methods

Statistical calculations were made using BMDP 7.2 for Windows software (Los Angeles, CA, USA). Shapiro and Wilk's W test were used to test for normality. Spearman's rank correlation test was used to calculate correlations between PBL and activated or total gelatinase B levels in all 46 implant sites. Kruskal–Wallis test was applied to compare activated and total gelatinase B levels in the three PBL or GI groups. When $P<0.05$, rank sum test was further used to check differences between two groups. The results were expressed as medians plus interquartile ranges [Q3–Q1].

Results

The degree of *in vivo* activated gelatinase B correlated ($r=0.942$; $P<0.0001$) with the total gelatinase B ($n=46$, Spearman's rank correlation test). Both total gelatinase B ($r=0.553$; $P=0.021$) and activated gelatinase B levels ($r=0.529$; $P=0.003$) in PISF correlated with PBL ($n=46$, Spearman's rank correlation test). The differences of activated ($P=0.044$) and total gelatinase B ($P=0.026$) levels were significant in the three PBL groups (Kruskal–Wallis test). Furthermore, gelatinase B levels were increased in PBL>3 mm group compared to PBL<1 mm and 1 <PBL <3 mm groups (rank sum test; Table 1).

There were significant differences of activated ($P=0.043$) and total gelatinase B ($P=0.031$) levels between the three mGI groups (Kruskal–Wallis test). Activated gelatinase B level in the mGI>0.5 group was increased compared to mGI = 0 and 0<mGI≤0.5 (rank sum test). Total gelatinase B levels in the 0<mGI≤0.5 and mGI>0.5 groups were clearly increased compared to the mGI = 0 group (Table 2).

Discussion

Recent evidence suggests that gelatinase B is involved in bone remodeling (Delaisse et al. 2000). Bone remodeling and destruction is considered to be mediated by bone morphogenetic units, which after initiation undergo sequential phases known as activation, resorption/reversal and formation, the so-called ARF cycle (Delaisse et al. 2000). Although MMPs, in particular MMP-9, have long been implicated in bone destruction, the exact phase of their involvement is not yet known. They may act at the activation or initiation stage (Chambers et al. 1985; Balvier & Delaisse 1995) or perhaps at the reversal phase when the bone resorption surface after osteoclast-mediated resorption is prepared for a subsequent osteoblast attachment followed by new bone formation. Gelatinase B is involved in the recruitment of osteoclasts to resorption sites (Blavier & Delaisse 1995). Gelatinase B, but not gelatinase A, is expressed by rabbit and human osteoclasts, the number of which is increased in periodontitis (Makris & Saffar 1982; Tezuka et al. 1994; Wucherpfennig et al. 1994). Gelatinase B also plays a role in jaw cyst expansion (Teronen et al. 1995). A specific synthetic gelatinase inhibitor can effectively prevent bone resorption *in vitro* (Hill et al. 1994). Increased gelatinase B in gingival crevicular fluid implies periodontal tissue destruction (Westerlund et al. 1996). Polymorphonuclear leukocytes, keratinocytes and macrophages contain gelatinase B (Salo et al. 1991; Westerlund et al. 1996). Osteoclasts also represent an important source of gelatinase B in periodontal tissues (Wucherpfennig et al. 1994). This may, in part, explain the correlation between gelatinase B in PISF and PBL. No clustering effect of the gelatinase B PISF levels was observed.

Two different proteolytic enzymes participate in the cleavage of bone collagen (Everts et al. 1999). Cathepsins, not collagenases, lead to resorption of long enchondral bones as in loosening of hip implants (Everts et al. 1999; Konttinen et al. 2001). However, both cathepsins and certain collagenases are required for osteoclastic resorption of calvarial/ membranous bone (Everts et al. 1999). Both have been detected in the subosteoclastic bone–resorbing compartment (Everts et al. 1992). Dental implants are implanted to the alveolar ridge, which is part of the membranous mandibular bone. Increased collagenase-2 and collagenase-3 contribute to PBL in loosening of dental implants (Teronen et al. 1997; Ma et al. 2000). After progelatinase B has been secreted, it can be activated in the extracellular space by low pH, bacterial proteinases or through host-derived proteolytic activation cascades (Okada et al. 1995; DeCarlo et al. 1997; Sorsa et al. 1997). Active gelatinase B degrades collagenase-cleaved denatured collagen type I 3/4 and 1/4 fragments and cathepsin-cleaved multiple fragments (Birkedal–Hansen 1995; Kusano et al. 1998). Therefore, gelatinase B is a cooperative enzyme, which participates in the activation–resorption and formation cycle involved in bone remodeling and in pathological bone destruction.

In vivo activated gelatinase B level was significantly increased when mGI was higher than 0.5. There is no gingiva around oral implants anymore. Therefore, it might have been better to use the modification of the GI utilizing the modified bleeding index described by Mombelli et al. (1987). This may help clinical dentists in the recognition of patients at risk for PBL. In such patients, monitoring of MMPs using a chair-side test (Sorsa et al. 1999), together with potent MMP inhibitors like tetracyclines and their chemically modified CMT derivatives (Mombelli et al. 2001; Ramamurthy et al. 2002) and CTTHWGFTLC-containing cyclic peptide gelatinase B inhibitors produced by phage display technology (Koivunen et al. 1999), may help in the prevention of progression of PBL.

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Résumé

Le but de cette étude a été de vérifier si la gélatinase B était associée à la perte osseuse paroïmplantaire (PBL). Du fluide créviculaire paroïmplantaire a été prélevé au niveau de 46 sites implantaires chez douze patients. L'état de ces sites était également estimé en utilisant l'index gingival modifié (mGI). Les taux de

gélatinase B activés et totaux, mesurés par un essai d'urokinase modifié, ont montré une relation avec PBL ($n = 46$, test de corrélation par rang de Spearman). Les valeurs de gélatinase activées et totales étaient significativement plus importantes dans le groupe avec PBL >3 mm (n = 6) comparées aux groupes avec PBL<1mm ($n = 29$) et 1 mm< PBL<3 mm (n = 11) (test par somme de Rank). Le niveau de gélatinase B activée dans le groupe mGI >0,5 (n = 24) était clairement plus élevé comparé aux groupes mGI = 0 (n = 13) et 0,5(n = 9). La gélatinase B est donc associée à PBL. L'activation de la gélatinase B avec un mGI élevé reflète finalement les phases actives de la paroïmplantite et semble un outil utile pour le diagnostic.

Zusammenfassung

Gelatinase B ist mit peri-implantärem Knochenverlust assoziiert

Es war das Ziel dieser Studie, zu klären, ob die Gelatinase B mit peri-implantärem Knochenverlust (PBL) assoziiert ist. Von 46 Implantatstellen bei 12 Patienten wurde peri-implantäre Sulkusflüssigkeit gesammelt. Diese Stellen wurden zusätzlich mit dem modifizierten Gingivalindex (mGI) charakterisiert. Die Konzentration der aktivierten und totalen Gelatinase B, gemessen mittels eines modifizierten Urokinasetests, zeigten Korrelationen mit PBL (n = 46, Spearman's rank correlation test). Die Werte der aktivierten und totalen Gelatinase B waren in der Gruppe mit PBL>3mm (n = 6) im Vergleich zur PBL<1mm (n = 29) und 1mm<PBL<3mm (n = 11) Gruppe signifikant höher (Rank sum test). Die Werte der aktivierten Gelatinase B in der Gruppe mit mGI>0.5 (N = 24) waren deutlich höher als in den mGI = 0 (n = 13) und 0.5 (n = 9) Gruppen (Rank sum test). Wir ziehen die Schlussfolgerung, dass Gelatinase B mit PBL assoziiert ist. Die Aktivierung der Gelatinase B zusammen mit erhöhten mGI reflektiert eventuell aktive Phasen der Peri-Implantitis. Dies könnte sich in der Diagnostik als nützlich erweisen.

Resumen

La intención de este estudio fue aclarar si la gelatinasa B esta asociada con la pérdida de hueso periimplantario (PBL). Se recogió el fluido del surco periimplantario de 46 lugares de implante en 12 pacientes. Estos lugares también se clasificaron usando el Indice Gingival modificado (mGI). Los niveles de gelatinasa B totales y activada, medidos usando una prueba modificada de uroquinasa, mostraron una correlación con PBL (n = 46, test de rango de correlación de Speakerman). Los valores de gelatinasa B totales y activada fueron significativamente mayores en el grupo PBL>3 mm (n = 6) comparados con los grupos PBL<1 mm (n = 29) y PBL<3 mm ($n = 11$) (test de suma de rangos). El nivel de gelatinasa B activada en el grupo mGI>0.05 (n = 24) fue claramente mayor comparado con los grupos mGI = 0 (n = 13) y 0.05 (n = 9) (test de suma de rangos). Concluimos que la gelatinasa B está asociada con la PBL. La activación de gelatinasa B junto con mGI elevada refleja eventualmente las fases activas de periimplantitis y pueden demostrar ser útiles para el diagnóstico.

要旨

本研究は、gelatinase Bがインプラント周囲の骨喪失（PBL）に関連しているかどうかを明らかにすることを目的に行った。インプラント周囲歯肉溝液を、患者１２名のインプラント部位４６箇所から採取した。これらの部位は、修正歯肉指数（mGI）を用いて特徴を調べた。gelatinase Bの活性化レベルと総レベルを、修正ウロキナーゼ・アッセイによって測定したところ、PBLと相関性を示した（ｎ＝４６、スピアマン順位相関検定）。PBLが３ｍｍ以上のグループ（ｎ＝６）は、PBLが１ｍｍ未満のグループ（ｎ＝２９）と１ｍｍから３ｍｍまでのグループ（ｎ＝１１）に比べて、有意に高いgelatinase Bの活性化レベルと総レベルを示した（順位和検定）。mGI＞０．５のグループ（ｎ＝２４）のgelatinase Bの活性化レベルは、mGI＝０（ｎ＝１２）及び≤０．５（ｎ＝９）のグループに比べて、明らかに高かった。我々はgelatinase BはPBLに相関していると結論した。gelatinase Bの活性化及びmGIの上昇は、インプラント周囲炎の活性期を反映しており、診断に有用であることを証明しうる。

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