日本財団 助成事業
The Nippon Foundation

財団法人日中医学協会
2005年度共同研究等助成金－在留中国人研究者－報告書

2006年　3月　14日

財団法人　日中医学協会　御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書

中国人研究者名：陳 鋭

指導責任者名：五十嵐 薫　職名：教授

所属機関名：東北大学歯科研究科

〒 980-8575

所在地：宮城県仙台市青葉区星陵町4-1

電話：022-717-8375　内線：8375

1．助成金額：600,000 円

2．研究テーマ

歯周組織へ局所的OPG遺伝子導入による歯周病による骨破壊の阻止

3．成果の概要（100字程度）

７週齢Wistar系雄性ラットの上顎第一臼歯口蓋側歯周組織へLPS局所注入と局所的OPG遺伝子導入を２日毎に行った。歯周組織へのLPS局所注入は、破骨細胞数を増加させ、重篤な歯槽骨吸収を惹起した。また、同部位で歯根膜細胞や骨芽細胞にRANKLの発現増強が認められた。局所的なOPG遺伝子導入は、歯周組織におけるOPGタンパクの発現を増強し、LPSにより惹起された破骨細胞形成と歯槽骨吸収を有意に抑制した。

4．研究業績

(1) 学会における発表　　無　・　(有)（学会名・演題）

第64回日本矯正歯科学会大会（2005.10.）
歯周組織への局所的OPG遺伝子導入は、LPSによる歯槽骨吸収を抑制する

(2) 発表した論文　　無　・　(有)（雑誌名・題名）

Journal of dental research　(will be submitted)
Local OPG-gene transfer to Periodontal Tissue Inhibits Alveolar Bone Resorption

歯周組織への局所的 Osteoprotegerin 遺伝子導入は、LPS 誘導性歯槽骨吸収を抑制する-

研究者氏名　陳鋭
中国所属機関　中国華中科技大学同済医学院附属同済医院
日本研究機関　東北大学歯科研究科
指導責任者　教授　五十嵐　薫
共同研究者名　菅崎弘幸，千葉美麗

要旨

Previously, we discovered that local OPG-gene transfer to periodontal tissue inhibited RANKL-mediated osteoclastogenesis and inhibited experimental tooth movement. We hypothesized that local OPG-gene transfer to the periodontium would neutralized the RANKL activity induced by LPS injection, thereby inhibiting osteoclastogenesis and diminishing alveolar bone resorption in the experimental periodontal disease. Seven-week-old male Wistar rats were received LPS or PBS injection in palatal gingiva of the upper first molars on both sides. The inactivated HVJ envelop vector containing a mouse OPG expression plasmid [pcDNA3.1(+)-mOPG] or mock-vector was injected periodically into the palatal periodontal tissue of upper first molars. LPS injection induced severe periodontal bone resorption. Local OPG-gene transfer induced OPG production, and osteoclastogenesis was inhibited. Local OPG-gene transfer significantly diminished alveolar bone resorption. In this study, we report that OPG-gene transfer to periodontal tissue inhibited osteoclastogenesis in LPS-mediated experimental periodontal disease and inhibited experimental alveolar bone resorption.

Key Words　gene transfer, osteoprotegerin (OPG), alveolar bone, periodontal disease, osteoclastogenesis

緒言:

Recently, with the materials improving, adult orthodontic patients are increasing. In these patients, morbidity rate of periodontal tissues is high, and alveolar bone resorption can often be seen. Since orthodontic tooth movement under progressive periodontitis causes striking bone destruction, control of alveolar bone resorption induced by periodontal diseased becomes quite important for orthodontists (Cardaropoli et al., 2001; Frank and Long, 2002). Alveolar bone resorption in periodontitis is due to excess osteoclastic activity, leading to an imbalance in bone remodeling which favors resorption (Bezerra et al., 2000; Crotti et al., 2003; William et al., 2003). It has been reported that osteoclatstogenesis is primarily activated by the receptor activator of nuclear factor Kappa B ligand (RANKL) and inhibited by osteoprotegerin (OPG) (Simonet et al., 1997; Udagawa et al., 1999; Yasuda et al., 1999). And then, periodontal ligament (PDL) cells, which exist between teeth and alveolar bone, regulate osteoclastogenesis through RANKL stimulation and OPG inhibition, so as to affect processes such as periodontitis and orthodontic tooth movement (Kanzaki et al., 2001). Furthermore, we have reported that OPG-gene transfer to periodontal tissue inhibit RANKL mediated osteoclastogenesis and inhibited experimental tooth movement (Kanzaki et al., 2004). Combining this information, we hypothesized that local OPG induction in the periodontal tissue might neutralize RANKL-upregulation induced by periodontitis, and thereby inhibit osteoclastogenesis and diminish alveolar bone resorption.

To test this hypothesis, we used experimental periodontal disease induced by recurrent lipopolysaccharide (LPS) injection in rats with or without local OPG-gene transfer by means of a hemagglutinating virus of Japan (HVJ; Sendai virus) envelope vector gene delivery system.

対象と方法:

Foundation of experimental periodontal disease

Sixteen seven-week-old male Wistar rats with an average weight of 167 g were used in this study. Purified lipopolysaccharide from E. coli (Sigma chemical, St. Louis, MO, USA) was injected directly into the gingival adjacent to both of first molars (10 μg/site) every two days (Miyauchi, 2001; Ramamurthy, 2002).

In vivo Gene transfer

For *in vivo* transfection, we used an HVJ-envelope-vector Kit (GenomONE, Ishihara-sangyo kaisha Ltd., Osaka, Japan), according to the manufacturers instructions®. Administration of the HVJ-envelop-vector containing pcDNA-mOPG to the animals was started on the initial day of LPS injection. On the contralated side, 5 μl of mock vector was injected into the correspond area as the control.

Tissue preparation

After the scarifice and fixation by perfusion of the experimental animals, the upper jaws, including the molars, were dissected and further fixed overnight, then decalcified with 10% ethylenediamine-tetraacetic acid in 0.01M PBS (PH7.4) for 9 wks at 4℃, dehydrated, and embedded in paraffin. 8 μm serial frontal sections were prepared.

Observation of the periodontal tissues

Periodontal bone resorption on the palatal surface of the maxillary molars was observed with HE staining. TRAP staining and Immunohistochemical Analysis for RANKL and OPG were performed.

Statistics

A Student's paired *t* test was used for comparison of bone loss between the left and right side within each group. The other data were analyzed for statistical differences by Kruskal-Wallis analysis, followed by a Bonferroni-type multiple comparision (Tukey type). Differences with $P<0.05$ were considered significant. The values are expressed as the means ± standard deviation (SD).

結果:

Animal status

The LPS injection and local administration of the HVJ-envelop-vector containing pcDNA-mOPG did not affect the growth of the animals (Fig. 1). In addition, local gene transfer did not affect the bone mineral density (BMD) of tibiae (Fig. 2).

Periodontal status of experimental animals

LPS injection induced the RANKL expression in the periodontal tissue. Local OPG-gene transfer induced OPG upregulation in periodontal tissue, especially in the fibroblast cell of the periodontium.

Local OPG-gene transfer reduced osteoclastogenesis in periodontal tissue

The number of osteoclasts was increased by LPS injection (from 4±1 cells to 15±2 cells) ($P<0.01$), and local OPG-gene transfer inhibited the osteoclast induction stimulated by the LPS injection (from 15±2 cells to 7±2 cells) ($P<0.05$). When OPG-gene transfer was performed on the both two sides (group 3), there was no significant difference in the number of osteoclasts between side with LPS injection (7±2cells) and the side without LPS injection (3±1cells) ($P>0.01$) (Fig. 3).

Local OPG-gene transfer significantly diminished bone resorption

Length from cemento-enamel junction (CEJ) to the alveolar ridge (AR), longer value means the severer alveolar bone resorption, were measured. Compared to the control side (473.3 ± 38.7 μm), repetitive LPS injection showed larger value (588.3 ± 27.9 μm) ($P<0.01$) (Figure 4). Local OPG-gene transfer (522.9 ± 16.7 μm) significantly inhibited bone resorption compared to the LPS injected side (610.0 ± 98.4 μm) ($P<0.05$) (Figure 4).

考察:

Numerous reports have described the pharmacological control of alveolar bone resorption induced in periodontitis some of them focused on regulating of osteoclasts. (Bezerra et al., 2000; Di Paola et al., 2005; Lohinai et al., 2003) However, because

these drugs are rapidly flushed by blood circulation, daily systemic administration or daily injection is needed. Osteoprotegerin (OPG) is a potent inhibitor of osteoclast differentiation and activation (Doran et al., 2004; Simonet et al., 1997). OPG-gene transfer or OPG administration have been comfirmed to be a feasible and effective therapeutic candidate to treat or prevent bone loss in osteoclast-dependent skeletal disorders, such as osteolysis and arthritis (Doran et al., 2004; Lubberts et al., 2003; Yang et al., 2002). However, Effects of OPG-gene transfer on bone loss in periodontitis have been seldom reported. Inhibition of RANKL function with the decoy receptor OPG diminished alveolar bone destruction and reduced the number of periodontal osteoclasts after microbial challenge (Teng et al., 2000, Valverde et al., 2004). However, injections of OPG protein did not completely block alveolar bone destruction. The reason was considered to be related with some other proinflammatory cytokines (e.g., IL-1 and TNF-α). In this study, we used local OPG-gene transfer instead of OPG administration. Local gene transfer has two advantages (Blesing and Kerr, 1996): First, it can maintain a locally effective concentration and prolonged protein expression, regardless of blood circulation. Second, protein expression occurs at the local site, so that systemic effects are avoided. Since viral gene transfer vectors such as adenoviral and herpes viral vectors have been proven to have problems, especially in immunogenicity and cytotoxicity, hemagglutinating virus of Japan (HVJ)-liposome method was chosen for the OPG-gene transfer (Kaneda et al., 2002).

We were able to induce local OPG expression in the periodontal tissue without any systemic effects (Fig. 1d, 1f). Local OPG-gene transfer significantly reduced the numbers of osteoclasts at the periodontium caused by LPS injection (Fig. 3e). Thus, OPG-gene transfer almost completely inhibited the induction of osteoclastogenesis. It proved the high efficiency of transfection performed in this study. There was a little increasing of osteoclasts on the sides of LPS injection and OPG-gene transfer compared to the control sides. It must be considered some other cytokines regulating the RANKL/OPG balance, similar to the consideration in the study of OPG protein administration inhibiting the periodontal bone resorption, as described above (Valverde et al., 2004); alternatively, osteoclastogenesis inducted by LPS injection independent of RANKL/RANK/OPG regulatory axis, such as directly stimulating the osteoclasts to be activated (Daly et al., 1980; Tiranathanagul et al., 2004; Wang and Ohura, 2002).

Enforced OPG expression diminished the alveolar bone resorption induced by LPS injection almost completely (Figure 4). The result is consistent with the researches of controlling bone resorption in the other skeletal disorders by OPG-gene transfer (Doran et al., 2004; Yang et al., 2002).

Osteoclastogenesis in response to periodontal disease appears to be regulated primarily through RANKL signaling in the cells of periodontal tissues, such as fibroblasts, osteoblasts, activiated T cells. In this study, we demonstrated that OPG-gene transfer to the periodontal tissue inhibited RANKL-related osteroclastogenesis induced by LPS injection, and inhibited experimental alveolar bone resorption without eliciting any systemic effects. In conclusion, the present results suggest that OPG-gene transfer to periodontal tissue may be useful for the prevention of bone resorption in patients with periodontal diseases.

参考文献:

Bezerra MM, de Lima V, Alencar VB, Vieira IB, Brito GA, Ribeiro RA, et al. (2000). *J Periodontol* 71:1009-1014.

Blesing CH, Kerr DJ (1996). J Drug Target 3:341-347.

Cardaropoli D, Re S, Corrente G, Abundo R (2001). *Am J Orthod Dentofacial Orthop* 120:671-675.

Crotti T, Smith MD, Hirsch R, Soukoulis S, Weedon H, Capone M, et al. (2003). *J Periodontal Res* 38: 380-387.

Daly CG, Seymour GJ, Kieser JB (1980). *J Oral Pathol* 9:1-15.

Di Paola R, Mazzon E, Rotondo F, Dattola F, Britti D, De Majo M, et al. (2005) *Eur J Pharmacol* 516:151-157.

Doran PM, Turner RT, Chen D, Facteau SM, Ludvigson JM, Khosla S, et al. (2004). *Exp Hematol* 32:351-359.

Frank CA, Long M (2002). *Am J Orthod Dentofacial Orthop* 121:639-649.

Kaneda Y, Nakajima T, Nishikawa T, Yamamoto S, Ikegami H, Suzuki N, et al. (2002). *Mol Ther* 6: 219-226.

Kanzaki H, Chiba M, Shimizu Y, Mitani H (2001). *J Dent Res* 80: 887-891.

Kanzaki H, Chiba M, Takahashi I, Haruyama N, Nishimura M, Mitani H (2004). *J Dent Res* 83:920-925.

Kawai T, Eisen-lev R, Seki M, Eastcott JW, Wilson ME, Taubman MA (2000). *J Immunol* 164:2102-2109.

Kessler M (1976). *Am J Orthod* 70:154-162.

Lohinai Z, Mabley JG, Feher E, Marton A, Komjati K, Szabo C (2003). *J Dent Res* 82:987-992.

Lubberts E, van den Bersselaar L, Oppers-Walgreen B, Schwarzenberger P, Coenen-de Roo CJ, Kolls JK, et al. (2003). *J Immunol* 170:2655-2662.

Miyaushi M, Sato S, Kitagawa S, Hiraoka M, Kudo Y, Ogawa I, et al. (2001). *Histochem cell Biol* 116:57-62

Ramamurthy NS, Xu J-W, Bird J, Baxter A, Bhogal R, Wills R, et al. (2002). *J Periodont Res* 37:1-7.

Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. (1997). *cell* 89:309-319.

Teng YT, Nguyen H, Gao X, Kong YY, Gorczynski RM, Singh B, et al. (2000). *J Clin Invest* 106:R59-67.

Tiranathanagul S, Yongchaitrakul T, Pattamapun K, Pavasant P (2004). *Periodontol* 75:1647-1654.

Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K, et al. (1999). *Bone* 25:517-523.

Valverde P, Kawai T, Taubman MA (2004). *J Bone Miner Res* 19:155-164.

Wang PL, Ohura K (2002). *Crit Rev Oral Biol Med* 13: 132-142.

William J. Boyle, W. Scott Simonet, David L. Lacey (2003). *Nature* 423: 337-342.

Yang SY, Mayton L, Wu B, Goater JJ, Schwarz EM, Wooley PH (2002). *Arthritis Rheum* 46:2514-2523.

Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Goto M, et al. (1999). *Bone* 25:109-113.

Fig.1

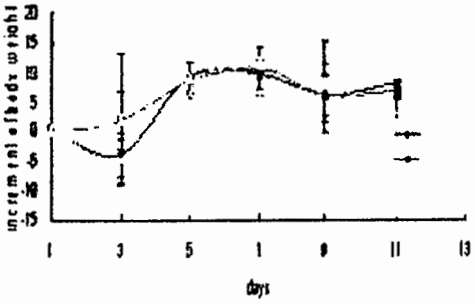


Fig.2

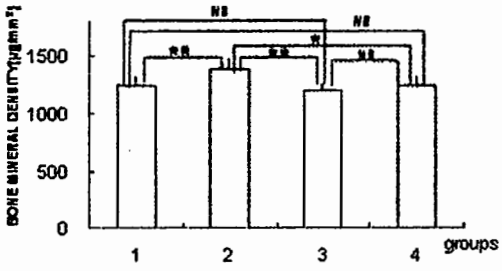


Fig.3

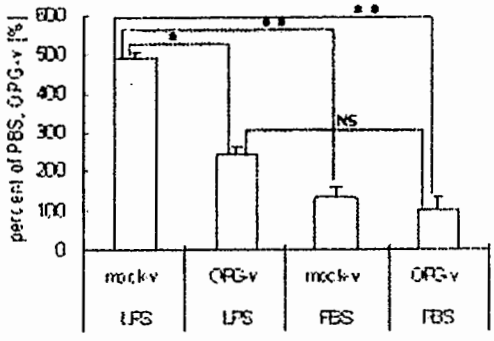
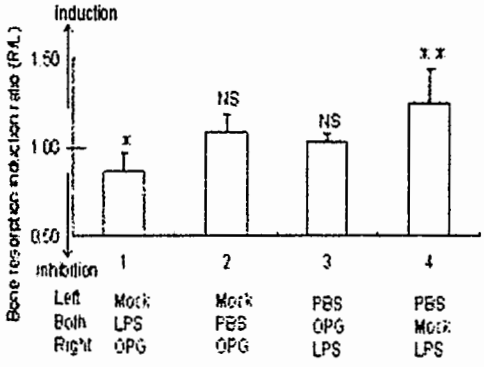


Fig.4



注:本研究は、2005 年 10 月　第 64 回日本矯正歯科学会大会にて POST 発表。

作成日:2006/03/01