II-5. Inhibitory effects of green tea polyphenol(-)-epigallocatechin(EGCG) on the MMP expression and the osteoclast survival

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Background

Alveolar bone resorption is a characteristic feature of periodontal diseases and involves the removal of both the mineral and organic constituents of the bone matrix. Osteoclasts are principally responsible for this process and osteoblasts play a role in the initiation of bone resorption by releasing matrix metalloproteinases(MMPs). MMPs are a family of zinc-dependent endopeptidases, which have the combined ability to degrade the organic components of connective-tissue matrices. Green tea is one of the most popular beverages in the world, and it has received considerable attention because of its many scientifically proven beneficial effects on human health. These effects have been largely attributed to the most prevalent polyphenol contained in green tea, polyphenol(-)-epigallocatechin gallate(EGCG). Recently EGCG has been shown to inhibit the activity and expression of MMPs. Furthermore, it has been reported that EGCG could induce the apoptotic cell death of osteoclasts. Apoptosis is a pathway of fundamental biochemical cell death. In many studies, the involvement of multiple caspases in the proteolytic cascade of apoptosis has been identified. Recently, EGCG has been shown to modulate caspase activation. However, the precise mechanism by which EGCG induces apoptosis remains to be elucidated.

Materials & Methods

In the present study, we investigated the inhibitory effects of EGCG on the gene expression of osteoblast-derived MMPs, and on the survival of osteoclasts. In addition,

we also investigated if EGCG mediates osteoclast apoptosis via caspase pathway.

The effect of EGCG on the gene expression of MMPs was examined by treating mouse calvarial primary osteoblastic cells with EGCG in the presence of sonicated *P.gingivalis*extracts (SPEs). The transcription levels of MMP-2, -9, and -13 were assessed by reverse transcription-polymerase chain reaction (RT-PCR). The effect of EGCG on osteoclast survival was examined by tartrate-resistant acid phosphatase (TRAP) staining in the co-culture system of mouse bone marrow cells and calvarial POB cells, and the system using RAW264.7 cells. In addition, we evaluated the apoptosis of osteoclasts by EGCG using DNA fragmentation analysis. The involvement of caspase in EGCG-mediated osteoclast apoptosis was evaluated by examining the effect of EGCG on the activity of caspase-3, which were assessed by western blotting and colorimetric assay.

Results

Treatment with the SPEs stimulated the expression of MMP-9 mRNA and this effect was significantly reduced by EGCG, whereas the transcription levels of MMP-2 and MMP-13 were not affected by either the SPEs or EGCG. In addition, EGCG significantly inhibited osteoclast survival in the co-culture and RAW264.7 cell systems. It was confirmed by DNA fragmentation pattern that this inhibitory effect of EGCG was mediated by apoptosis. Moreover, EGCG stimulated the activity of caspase-3 in the osteoclastic cells differentiated from RAW264.7 cells.

Conclusion

From these findings, we could suggest that EGCG might prevent the alveolar bone resorption which occurs in periodontal diseases by inhibiting the expression of MMP in osteoblasts and the survival of osteoclasts through the caspase-mediated apoptosis.

In conclusion, the inhibitory activity of EGCG could be usefully applied to the development of a therapeutic agent for the treatment of bone diseases such as periodontitis.

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