論　文　内　容　要　旨

An *in vitro* study on the usefulness of LL37

as a pulp capping material

(覆髄剤としてのLL37の有用性に関する基礎的研究)

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Introduction

Pulp capping is worthwhile because it can preserve pulp vitality, which is crucial to the tooth's long-term survival. Moreover, it is easier to be performed, cheaper and less time-consuming than pulpectomy. However, with present materials available, the success rates of pulp capping of teeth with cariously exposed pulp are low, because the inflamed pulp responds poorly to these materials and has a diminished capacity for repair. Therefore, there is a need to develop a biological active material, which can regulate pulpal inflammation and promote pulp wound healing. Ideally, such a material should have the following properties: bacterial elimination, inflammatory regulation, pulp cell activation and angiogenesis stimulation.

LL37, the only human cathelicidin, possesses many other bio-functions apart from its antimicrobial activity. Interestingly, the previous studies have demonstrated that LL37 is effective against cariogenic bacteria and stimulates pulp cell migration. Hence, LL37 may be a potential pulp capping material. However, before it can be used for this purpose, LL37 still needs to meet some remaining criteria, such as angiogenesis and pulp tissue breakdown inhibition (inflammatory regulation).

This study aimed to investigate the possible application of LL37 as a pulp capping material by addressing the two remaining criteria, that is angiogenic effects on pulp cells and the inhibition of pulp tissue breakdown. In order to achieve this aim, firstly, I examined the effects of LL37 on the expression of vascular endothelial growth factor (VEGF), which is the most important angiogenic factor, in human pulp (HP) cells. Secondly, I examined the effects of LL37 on the expression of matrix metalloproteinases (MMPs) in peptidoglycan (PGN)-stimulated HP cells, since some members of MMPs are involved in pulp tissue destruction.

Materials and Methods

HP cells: Cells were obtained from the explant cultures of pulps taken from three healthy premolars extracted for orthodontic reasons from three donors.

Cell culture: HP cells at passage 6 were seeded at a density of 1×10^5 cells/well in six-well plates and cultured in DMEM supplemented with 10% FBS, with the media being changed every 3 days until cells became confluent.

mRNA expressions of VEGF and MMPs:

- VEGF mRNA: Under a serum free condition, confluent HP cells were treated with synthetic LL37 at 0.1-10 μg/ml for 0-24 hours. For the inhibition assay, cells were either pretreated or not for 30 minutes with 10 μM PDTC (an NF-κB inhibitor), 10 μM SB203580 (a p38 inhibitor), 50 μM PD98059 (an ERK kinase inhibitor) or 10 μM SP600125 (a JNK inhibitor) and stimulated with 10 μg/ml LL37 for another 3 hours. VEGF mRNA expressions were determined by real-time PCR.
- MMPs mRNA: HP cells were treated with 10 μg/ml PGN from *Staphylococcus aureus* for 0-24 hours. For the inhibition assay, cells were either pretreated or not for 30 minutes with the inhibitors mentioned above or with 10 μg/ml LL37 and stimulated with 10 μg/ml PGN for 24 hours. MMP-1, MMP-2 and MMP-3 mRNA expressions were determined by real-time PCR.

VEGF protein level: HP cells were treated with LL37 at 10 μg/ml for 0-24 hours. For the inhibition assay, cells were either pretreated or not for 30 minutes with 50 μM PD98059 and stimulated with 10 μg/ml LL37 for another 24 hours. VEGF protein production in the medium was measured by an ELISA kit.

Intracellular signal transduction:

- Involvement in VEFG expressions: HP cells were treated with 10 μg/ml LL37 for 0-60 minutes, or were either pretreated or not for 30 minutes with 50 μM PD98059 and exposed to 10 μg/ml LL37 for another 20 minutes. Phosphorylated ERK1/2, total ERK1/2 and β-actin levels were determined by immunoblot.
- Involvement in MMP expressions: HP cells were treated with 10 μg/ml PGN for 0-60 minutes, or were either pretreated or not for 30 minutes with 10 μg/ml LL37 and stimulated

with 10 μg/ml PGN for another 30 minutes. Phosphorylated p38 and total p38 levels were determined by immunoblot.

Results

1. LL37 dose-dependently increased VEGF mRNA expression, with a peak being observed at 3 hours, and time-dependently increased VEGF protein level.
2. The ERK kinase inhibitor suppressed LL37-induced VEGF expression, while the other inhibitors did not.
3. LL37 induced the phosphorylation of ERK1/2, with the maximal effect being observed 20 minutes after the LL37 treatment, but this was clearly blocked by the ERK kinase inhibitor.
4. PGN significantly increased the mRNA expressions of MMP-1, MMP-2 and MMP-3 in a time-dependent manner. However, the pretreatment with LL37 abolished these increases. Similarly, the ERK kinase, JNK and p38 inhibitors also suppressed PGN-induced MMP expressions, with the p38 inhibitor having the most suppressive effect.
5. PGN induced p38 phosphorylation, with the maximal effect being observed 30 minutes after the PGN treatment. However, PGN-induced phosphorylated p38 level was suppressed by LL37.

Discussion

LL37 is known to directly act on endothelial cells to induce angiogenesis. In this study, LL37 was demonstrated to activate ERK to boost VEGF secretion from HP cells. Since VEGF is a potent angiogenic factor, an increase in its level will promote angiogenesis, which indicates that LL37 also has an indirect angiogenic effect, making it an even more potent inducer of angiogenesis in the context of pulp tissue.

This study showed that LL37 suppressed PGN-induced MMP-1, MMP-2 and MMP-3 mRNA expressions in HP cells by suppressing the phosphorylation of p38 activated by PGN. Since these MMPs are involved in pulp tissue destruction, their suppression by LL37 means that LL37 can inhibit or at least reduce pulp tissue breakdown.

LL37 has been reported to be effective against cariogenic bacteria and induce pulp cell migration. Here, LL37 was revealed to increase VEGF expression and decrease PGN-induced MMP expression in HP cells, which in turn can promote angiogenesis and inhibit pulp tissue breakdown, respectively. Thus, with these additional activities, LL37 may contribute more significantly to the process of pulp wound healing.

Conclusion

LL37, which stimulates VEGF expression and suppresses PGN-induced MMP expressions in HP cells, is suggested to be a candidate for a pulp capping material.