ANTI-INFLAMMATORY AND PRO-OSTEOGENIC EFFECTS OF PHYTOCYSTATIN CSINCPI-2

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Abstract

The phytocystatins are cystatins derived from plants and are natural inhibitors of cysteine proteinases(1). Among them, the CsinCPI-2 derived from orange have been investigated. The cysteine proteinases and the cytokines play an important role in the initiation and progression of immune-inflammatory processes (2). The inhibition of CTSK has been shown to augment bone formation and increase the concentration of BMP-2 and OC in osteoclast (3)

Background and Aim

The CsinCPI-2 was cloned and recombined by our group in E. Coli expression and showed inhibitory activity in human CTSK.

-The aims of the **present in vitro and in vivo studies** were:

- 1)To assess the inhibitory activity of CsinCPI-2 on gene expression of cathepsins and cytokines in murine macrophage cells (RAW 264.7) stimulated with heat-inactivated bacteria Porphyromonas gingivalis (P.g.) (10⁷ UFC/mL) and,
- 2)To assess the effects of CsinCPI-2 in the expression of BMP-2, OC and ALP in human dental pulp cells.
- 3)To assess in vivo the inhibitory activity of CsinCPI-2 in the production of TNF-α by rats blood cells stimulated by E.coli LPS.

Methods and Materials

IN VITRO STUDIES

✓ **RAW264.7 macrophage cells :**

1.Viability test.

2.Gene Expression (qPCR) – Cathepsins (B and K) and cytokines (TNF-α and IL-1β)

✓ **HUMAN DENTAL PULP CELLS:**

1. Gene Expression (qPCR)-BMP2, OC and ALP

IN VIVO STUDY

Results

IN VITRO STUDIES

Figure 1: Cellular viability of RAW 264.7 cells 24 h after addition of different concentration of CsinCPI-2.

Figure 2: Effects of the CsinCPI-2 on CTSK and CTSB gene expressions by RAW 264.7 cells stimulated by Pg.

Figure 3: Effects of the CsinCPI-2 on TNF-α and IL-1 gene expressions by RAW 264.7 cells stimulated by Pg.

*Significant difference in relation to C (control);
Significant difference in relation to Pg7. (p <0.05 ANOVA)

Figure 4: Effects of the CsinCPI-2 after 24hs in the gene expression of BMP-2, OC and ALP enzyme in human dental pulp cells.

Figure 5: Effects of the CsinCPI-2 after 72hs in the gene expression of BMP-2, OC and ALP enzyme in human dental pulp cells.

Results

IN VIVO STUDY

Figure 6: TNF-α production by rats blood cells after LPS-stimulus (IV)

Figure 7: TNF-α production by rats blood cells after LPS-stimulus (IV) and treated with CsinCPI-2 (0,8ug/gr) administered IV and IP

* Significant difference in relation to LPS group

Figure 8: TNF-α production by rats blood cells after LPS-stimulus (IV) and treated with CsinCPI-2 in different concentrations administered IP

* Significant difference in relation to LPS group

Conclusions

- ✓ It can be suggested that CsinCPI-2 has an anti-inflammatory effect decreasing the expression of cathepsins and cytokines by macrophages. Also, this phytocystatin has a pro-osteogenic effect, by inducing osteoblastic cell differentiation.
- ✓ The CsinCPI-2 may be evaluated in future studies as an adjunctive treatment option in controlling inflammatory diseases or diseases which affect bone tissue.

References

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