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BASIC RESEARCH

Effect of vitamin D on osteogenic differentiation of hPDLSCs under inflammatory conditions

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Abstract

1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) enhances the osteogenic differentiation ability of human periodontal ligament stem cells (hPDLSCs).[1] The aim of this study was to investigate this effect under inflammatory conditions.

HPDLSCs were treated with different concentrations of $1,25(OH)_2D_3$ in the presence and absence of *Porphyromonas gingivalis* (*P.g.*) lipopolysaccharide (LPS) or Pam3CSK4. Gene expression levels of osteocalcin (OC) and osteopontin (OPN) were analyzed by qPCR.

$1,25(OH)_2D_3$ induced significant expression of OC and OPN in hPDLSCs in a concentration dependent manner. In the presence of Pam3CSK4, the $1,25(OH)_2D_3$-induced OC expression was significantly lower. Similarly, the enhancement of OPN gene expression by highest tested $1,25(OH)_2D_3$ was lower in the presence of Pam3CSK4, but this effect was not significant. The impact of $1,25(OH)_2D_3$ on expression of OC and OPN was not altered in the presence of *P.g.* LPS. According to our data, the effect of vitamin D on the expression of osteogenic markers is altered under inflammatory conditions, suggesting a compromised effectiveness of vitamin D on local bone metabolism during inflammatory diseases.

Background and Aim

HPDLSCs are similar to bone marrow mesenchymal stem cells and differentiate *in vitro* into osteoblasts, chondrocytes and adipocytes. $1,25(OH)_2D_3$ is known to promote osteogenic differentiation of hPDLSCs under physiological conditions.

The aim of this study was to investigate these effects under inflammatory conditions.

Methods and Materials

HPDLSCs of six donors were treated with different concentrations of $1,25(OH)_2D_3$ (0.1-10nM) in the presence and absence of *P.g.* LPS (1µg/ml) or TLR2 agonist Pam3CSK4 (1µg/ml) for 48 hours. Treatment of hPDLSCs with *P.g.* LPS or Pam3CSK4 alone was performed as control. Gene expression of OC and OPN was measured by qPCR.

Results

$1,25(OH)_2D_3$ induced significant expression of OC and OPN in hPDLSCs in a concentration dependent manner. In concentrations of 1nM and 10nM, $1,25(OH)_2D_3$ led to an increase of OC expression by about 6 and 14 times, respectively. In the presence of Pam3CSK4, same concentrations of vitamin D induced OC expression significantly lower by only about 1.5- and 2-fold, respectively. Similarly, the induction of OPN expression by $1,25(OH)_2D_3$ in concentrations of 1 and 10nM was significantly lower in the presence of Pam3CSK4, namely ~1.5 times vs. ~0.8 times and ~3 times vs. ~0.9 times. Treatment with *P.g.* LPS led to a significant decrease of vitamin D-induced OPN expression at concentration of 10nM (~3-fold vs. ~1,8-fold).

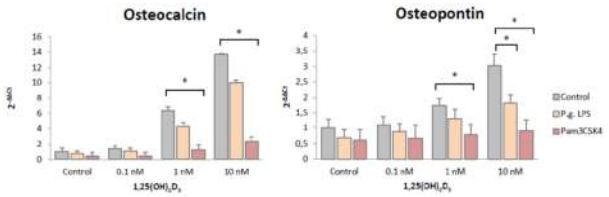

Figure 1. Gene expression levels of OC and OPN were measured by qPCR upon stimulation with $1,25(OH)_2D_3$ in different concentrations (0.1-10nM) and *P.g.* LPS (1µg/ml) or Pam3CSK4 (1µg/ml). Y-axes represent the n-fold expression levels of target genes in relation to non-stimulated cells. Data are presented as mean ± standard error of the mean of 6 independent experiments on 6 different donors. $1,25(OH)_2D_3$ significantly induced OC and OPN expression in a concentration dependent manner. The induction of OC and OPN expression by $1,25(OH)_2D_3$ in concentrations of 1 and 10nM was significantly lower in the presence of Pam3CSK4. Similarly, treatment with *P.g.* LPS led to significantly lower vitamin D-induced OPN expression at a concentration of 10nM.
* - means significantly different between physiological and inflammatory conditions, $p < 0.05$.

Conclusion

Our data show that the ability of vitamin D to stimulate osteogenic differentiation in hPDLSCs is strongly inhibited under inflammatory conditions. The effectiveness of vitamin D on local bone metabolism might be diminished in individuals with inflammatory disease.

References

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