Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
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Different Effects of Oxidized Parathyroid Hormone (ox-PTH) and Non-oxidized Parathyroid Hormone (n-oxPTH) on Sclerostin (SOST): based on in vitro studies

Autor: Mei Li
Institut / Klinik: V Medizinische Klinik
Doktorvater: Prof. Dr. B. Hocher

Sclerostin (SOST) is a negative regulator of bone homeostasis. It is mainly produced by osteocytes. Parathyroid hormone (PTH) acts on bones, intestines, and kidneys to regulate calcium, phosphate and vitamin D metastasis. Previous preclinical and clinical studies have proved that SOST is inversely associated with PTH both in vitro and in vivo. Currently, the specific mechanisms of negative association between SOST and PTH continue to be incompletely understood.
Two of the amino acids in PTH (positions 8 and 18) are easily oxidized in vivo. Prior studies have demonstrated that the oxidation of the PTH leads to a partial or complete loss of bioactive action, which depends on the specific oxidation position of the PTH. As a consequence, PTH oxidized at Met18 (Met18 (ox)-PTH) remains bioactive, whereas PTH oxidized at Met8 (Met8 (ox)-PTH) or at Met8 and Met18 together (Met8, Met18 (di-ox)- PTH) shows little or no bioactivity. In organisms, four forms of PTH co-exist in the peripheral blood including non-oxidized PTH (n-oxPTH), Met8 (ox)-PTH, Met18 (ox)-PTH, and Met8, Met18 (di-ox)-PTH. Currently, it is unclear how n-oxPTH and different forms of oxPTH induce SOST inhibition, respectively. Moreover, how four types of PTH interact with each other, and how their interplay influences the inhibition of SOST have not been elucidated.
In this study, we evaluated different effects of four forms PTH peptides on the expression of the SOST gene in UMR106 osteoblast-like cells, which were separately activated by n-oxPTH, Met8 (ox)-PTH, Met18 (ox)-PTH, and Met8, Met18 (di-ox)-PTH for 24 hours. Our in vitro study found both n-oxPTH and Met18 (ox)-PTH at doses of 1 nmol/l, 3 nmol/l, 20 nmol/l, 30 nmol/l significantly inhibit SOST gene expression, while Met8 (ox)-PTH has the weakest inhibition of SOST production. Based on our prior findings above, we primarily hypothesized that n-oxPTH, Met8 (ox)-PTH, Met18 (ox)-PTH, and Met8, Met18 (di-ox)-PTH can interact with one another, and their interplay can influence the SOST expression. Thus, we stimulated the UMR106 cells using two kinds of combinations of PTH peptides. One combination is n-oxPTH with concentrations of 3 nmol/l, 10 nmol/l,100 nmol/l and Met8, Met18 (di-ox)-PTH with concentrations of 0 nmol/l, 3 nmol/l, 10 nmol/l, 30 nmol/l, 100 nmol/l, respectively. The
aim is to investigate the interaction between n-oxPTH and oxPTH. The other combination is Met18 (ox)-PTH with concentrations of 3 nmol/l, 10 nmol/l and Met8, Met18 (di-ox)-PTH with concentrations of 0 nmol/l, 3 nmol/l, 10 nmol/l, 30 nmol/l, 100 nmol/l, respectively. We aimed to explore the interplay among various forms of oxPTH. However, our primary results in vitro were not inspiring. Our current study did not find the direct interaction either between n-oxPTH and Met8, Met18 (di-ox)-PTH, or between Met18 (ox)-PTH and Met8, Met18 (di-ox)-PTH. To strengthen our conclusions, further research design should be more complete including following improvements: (1) using different types of cell lines, such as osteocytic MLO-A5 cells lines or primary bone-derived cells to enhance our findings; (2) measuring the dynamic SOST gene expression by testing at different time points (such as after 6 hours, 12 hours, and 18 hours) to evaluate the changes of PTH-induced SOST inhibition over time; (3) assessing the effect of different forms of PTH on SOST expression at protein levels, measuring by the enzyme-linked immunosorbent assay or Western blot; (4) building animal models to evaluate the inverse association of n-oxPTH, Met8 (ox)-PTH, Met18 (ox)-PTH, and Met8, Met18 (di-ox)-PTH with SOST in vivo.
To conclude, bioactive PTH, including n-oxPTH and Met18 (ox)-PTH, inhibits SOST production in vitro significantly. Moreover, the addition of Met8, Met18 (di-ox)-PTH peptides to either n-oxPTH or Met18 (ox)-PTH did not affect effects of the n-oxPTH or the Met18 (ox)-PTH on SOST suppression.