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VITAMIN D_3 INVOLVEMENT IN NF-KB DEPENDENT CYTOKINE REGULATION OF BONE REMODELING UNDER EXPERIMENTAL OSTEOPOROSIS

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The underlying mechanism of osteoporosis is associated with the prevalence of osteoclast-dependent bone resorption over its osteoblast-mediated formation and mineralization leading to the loss of bone density, impairment of microarchitecture that ultimately increases the risk of fractures. A key role in controlling over the process of bone remodeling belongs to the cytokine systems. Specifically, osteosynthesis is provided by regulatory protein osteocalcin, which is a known marker of osteoblast-dependent bone remodeling, while the process of resorption is regulated by the system of osteotropic cytokines RANKL-RANK-OPG, which enhances differentiation and activation of osteoclasts. Reduced bioavailability of vitamin D_3 due to insufficient supplementation or deterioration of its metabolism is another major pathogenic factor of osteoporosis.

Based on the possible involvement of biologically active derivatives of vitamin D_3 in the regulation of cytokine activity, the study was aimed at investigating the relationship between vitamin D_3 bioavailability and such NF-κB dependent osteotropic system of cytokines as RANKL-RANK-OPG in experimental glucocorticoid-induced osteoporosis. Female Wistar rats received prednisolone (5 mg/kg of b.w.) with or without 100 IU of vitamin D_3 (for 30 days). The levels of RANK, RANKL, OPG, osteocalcin, phosphorylated nuclear factor κB (pNF-κB) p65 subunit, NF-κB inhibitor (IκB), CYP27A1, CYP2R1 and CYP27B1 were determined by western blot analysis. VDR mRNA level was measured by quantitative RT-PCR. The contents of $25OHD_3$, RANKL and OPG in blood serum were assayed by ELISA.

Osteoporosis induced by prolonged administration of synthetic glucocorticoid prednisolone was accompanied by a significant impairment of vitamin D_3 endocrine system. It was shown a marked reduction of $25OHD_3$ in blood serum, indicating the development of vitamin D_3 deficient state of experimental animals. Prednisolone-induced D-deficiency occured at the background of decreased activity of vitamin-D_3 25-hydroxylase in liver tissue and down-regulated protein expression of its key isoenzymes – CYP27A1 and CYP2R1. As a consequence, disrupted synthesis of the hormonally active form of vitamin D_3 – $1,25(OH)_2D_3$, due to altered 1α-hydroxylase (CYP27B1) expression, and the impairment of VDR-mediated cell signaling were observed. Expression of both VDR mRNA and protein was found to be significantly reduced in the hepatic and bone tissue under the influence of prednisolone.

The development of osteoporosis was assessed by the content of bone remodeling markers. Prednisolone administration led to hypocalcemia, hypophosphatemia and increased activity of bone isoform of alkaline phosphatase in blood serum that suggests glucocorticoid-evoked slowing of mineral metabolism. These changes were accompanied by increased RANKL and decreased OPG levels in blood serum and bone tissue. Along with the abnormal functioning of RANKL-OPG osteocytokine system, that contributes to bone resorption, a significant reduction of osteocalcin was also revealed, indicating the inhibition of osteosynthesis. Determination of the protein levels of phosphorylated form of nuclear factor κB and NF-κB inhibitor, key mediators of RANKL signaling pathway, showed tissue specific changes in bones and bone marrow. In bone tissue, prednisolone reduced the level of phosphorylated form of NF-κB, but increased IκB level, that indicates glucocorticoid-induced inhibition of transcriptional activity of this regulatory protein. Bone marrow, which contains only osteoblast and osteoclasts progenitors, unlike bone tissue, demonstrated activation of NF-κB signaling pathway, since the level of pNF-κB increased with decreasing IκB.

It was found VDR-mediated vitamin D_3 ability to efficiently normalize impaired bone remodeling associated with the abnormalities in NF-κB-dependent cytokine systems (RANK-RANKL-OPG, osteocalcin) induced by prolonged exposure to prednisolone.