The Effect of Paricalcitol on Osteoprotegerin Production by Human Peripheral Blood Mononuclear Cells

To the Editor:

It has been known for years that the ratio of receptor activator of nuclear factor- κ B ligand (RANKL) relative to its decoy receptor, osteoprotegerin (OPG), controls osteoclastogenesis. Under normal conditions the main source of RANKL and OPG is the osteoblast. Osteoblasts express on their surface RANKL that induces the formation of osteoclasts from their precursors as well as their survival and activation. OPG is also produced and then secreted by the osteoblasts, but it inhibits osteoclast formation by binding to RANKL and preventing binding to its receptor RANK on the surface of the osteoclasts' precursors¹. However, a recent study suggests that both B and T lymphocytes also play a significant role in basal bone turnover, since T cells help B cells to produce 65% of total OPG in bone marrow in mice². In inflammatory diseases the participation of activated lymphocytes in pathological bone erosion is established³.

Vitamin D promotes osteoclastogenesis through RANKL upregulation and OPG downregulation in the osteoblasts^{4,5}. *In vitro* experiments, in the absence of inflammatory stimuli, have shown that 1,25-dihydroxyvitamin D₃ initially downregulates OPG by accelerating the degradation of OPG mRNA and by transrepressing the OPG gene through its activating protein-1 (AP-1) binding site. But later the OPG gene becomes insensitive to 1,25-dihydroxyvitamin D₃ and reverts to its steady-state expression level over time⁴. Interestingly, under inflammatory conditions, inhibition of OPG production is more stable. Inflammatory cytokines such as interleukin 1 β (IL-1 β), IL-6, IL-11, IL-17, and tumor necrosis factor- α (TNF- α) stimulate osteoclast development by increasing the expression of RANKL in the presence of 1,25-dihydroxyvitamin D₃, whereas these cytokines further suppressed OPG production mediated by 1,25-dihydroxyvitamin D₃⁵. Importantly, experimental data and clinical observations confirmed that vitamin D has immunomodulatory/antiinflammatory properties⁶. Epidemiological studies have shown an inverse relation between serum levels of vitamin D and the occurrence or severity of various inflammatory bone erosive diseases. Rheumatoid arthritis is the best example^{7,8}.

Under inflammatory conditions vitamin D exerts osteolysis in part due to downregulation of OPG. Recently, we have shown that the vitamin D analog paricalcitol has antiinflammatory properties, since it reduces both basal and lipopolysaccharide (LPS)-induced TNF- α and IL-8 production by the peripheral blood mononuclear cells (PBMC)⁹. Additionally, paricalcitol is less calcemic than vitamin D¹⁰, which allows its administration in higher doses. In this context, its use for attenuating inflammation in chronic inflammatory diseases could be limited by its possible detrimental osteolytic effect on bone. Therefore, and considering the participation of activated lymphocytes in pathological bone erosion³, it is of interest to assess the effect of paricalcitol on OPG production by PBMC under inflammatory conditions.

In brief, 10 healthy volunteers (5 men and 5 women, mean age 40.3 $\pm$ 9.36 yrs) were enrolled into our study. Informed consent was obtained from each individual and the hospital ethics committee gave its approval to the study protocol. PBMC at a concentration of 10⁶ cells per well were cultured for 48 h in the presence or not of LPS as an inflammatory stimulus (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 100 ng/ml and in the presence or not of paricalcitol (Zemplar, Abbott Laboratories, North Chicago, IL, USA) at a concentration of 10⁻⁸ M. OPG was measured in the supernatants with ELISA (Bender MedSystems, Vienna, Austria). The normal distribution of the variables was confirmed using the 1-sample Kolmogorov-Smirnov test, and for comparison of means paired t-test was used.

Basal OPG concentration was decreased by paricalcitol (79.00 $\pm$ 25.87 pg/ml vs 52.40 $\pm$ 24.97 pg/ml; p < 0.001). LPS decreased OPG concentration (79.00 $\pm$ 25.87 pg/ml vs 56.80 $\pm$ 27.16 pg/ml; p < 0.001) as well. Paricalcitol failed to decrease further the OPG concentration in LPS-treated PBMC (56.80 $\pm$ 27.16 pg/ml vs 62.10 $\pm$ 26.38 pg/ml; p = 0.447).

In clinical practice, the main obstacle for vitamin D administration as an immunomodulatory/antiinflammatory agent is that at high doses it provokes hypercalcemia. Paricalcitol is a vitamin D analog that is safe, since it has been used for years in the treatment of secondary hyperparathyroidism in hemodialysis patients¹¹ and, importantly, it is less calcemic¹⁰. It has antiinflammatory properties as well⁹. In our study, paricalcitol did not decrease OPG production by the LPS-treated PBMC, indicating that under inflammatory conditions paricalcitol may not promote osteoclastogenesis. This feature of paricalcitol, along with its antiinflammatory properties and the less calcemic action, indicate that paricalcitol may have an advantage over vitamin D regarding its possible therapeutic role in inflammatory diseases characterized by pathological bone erosion.

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