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POSTER ABSTRACTS

321.COAGULATION AND FIBRINOLYSIS: BASIC AND TRANSLATIONAL

In Vitro Analysis of Bone Remodeling Alterations in Hemophilia: Influence of Coagulation Factors on Bone Cells

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Background. Today, one of the main comorbidities affecting the quality of life of the haemophilia patient is bone pathology. Hemophilia A (HA) is associated with reduced bone mass and mineral density (BMD). Due to the rarity of the disease and the heterogeneity among the studies, pathogenesis of bone loss is still under investigation and both processes of bone resorption and bone formation could be hypothesized to be altered. FVIII deficiency could alter BMD directly affecting bone cells or indirectly by decreasing thrombin generation. The reduction of BMD seems due to perturbations of the Receptor Activator of Nuclear factor-B RANK Ligand (RANKL) and osteoprotegerin (OPG) pathways. In haemophilia alterations of bone remodeling, osteoclasts seem to play a major role. Osteoclasts are multinucleated cells, which derive from the CD14+ monocyte/macrophage lineage. Until recently, the identity of osteoclast progenitors has not been well defined, but evidences report that CD16−CD14+ rather than CD16+CD14+ monocytes were prone to differentiate into osteoclasts (Komano *et al.* Arthritis Res Ther. 2006).
Objectives. To dissect the mechanism of bone loss in hemophilia, we studied the effects of coagulation factors, such as factor VIII, von Willebrand factor (VWF), activated factor X (FXa) and thrombin, on both osteoclasts and osteoblasts biology and we characterized the osteoclastogenic potential of HA patients' osteoclast precursors.
Patients/Methods: Peripheral Blood Mononuclear Cells (PBMC) isolated from healthy donors were induced to differentiate into osteoclasts and treated with plasma derived VWF/FVIII complex, human rVWF, human full length rFVIII, activated FX and thrombin. Osteoclastogenesis and expression analysis of RANK, TRAF6 (TNF receptor-associated factor 6), TCIRG1 (T Cell Immune Regulator 1) and osteoclasts protease CTSK (cathepsin K) expression were assessed. Moreover, in vitro assays assessed the osteoclastogenic potential of PBMC isolated from different hemophilic A patients. FACS analyses of osteoclast precursors isolated from patients were performed. Osteoblasts differentiation, mineralization and genes expression (Alkaline Phosphatase and COL1A2) were performed in the presence of aforementioned coagulation factors.
Results. We showed a significant reduction of mature osteoclasts after treatment of HD-PBMC with FVIII, VWF, FVIII/VWF, FXa and thrombin. VWF appears to play a major role to regulate osteoclast differentation from healthy donor-derived PBMC. Indeed, it inhibits by itself ˜45% the osteoclastogenesis comparable to OPG, and even more if is complexed with FVIII (53% inhibition). About 50% and 70% reduced levels of osteoclast differentiation were also revealed following treatment with FXa and thrombin, respectively. Interestingly, PBMC from HA patients showed increased ability to form mature osteoclasts compared to those obtained from healthy controls. Osteoclast precursors (CD16−CD14+CD11b+) are significantly higher in HA patients than age and sex matched controls. Moreover, transcriptional analysis revealed increased RANK, TRAF6, CTSK and TCIRG1 genes expression in adult hemophilia patient's osteoclasts compared to matched controls. FVIII and VWF treatments led also to a statistically significant reduction of ALP positivity in control osteoblasts; opposite effect was shown following thrombin treatment.
Conclusions. All these data support that bone loss observed in haemophilia patients could be related to increased osteoclast formation and activity and that coagulation factors directly impact on bone cells. These results may have important implications in the clinical management of hemophilic patients to prevent bleeding as well as to preserve bone health.

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