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Influence of receptor activator of nuclear factor kappa B ligand, osteoprotegerin and interleukin-33 on bone metabolism in patients with long-standing ulcerative colitis

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Bone resorption;
C-terminal telopeptide (CTX)

Abstract

Background: Ulcerative colitis (UC) is a chronic disease with periods of remission and recurrences. Dysfunction of the local immune response leads to chronic inflammation within the large intestine which triggers morphological changes in the intestinal wall as well as induces the synthesis of numerous factors that have an adverse impact on the bone metabolism.
The aim of the study was to determine the expression of RANKL, OPG and IL-33 in mucosal biopsies of UC patients with long disease duration as well as serum level of these cytokines in the context of bone density and bone metabolism.
Materials and methods: The UC group consisted of 56 patients with average disease duration of 16 y. The control group comprised 37 healthy individuals. Local expression of cytokines was assessed in the biopsies of colonic mucosa by the real-time PCR and immunohistochemistry (IHC), and their serum concentration was measured by ELISA.
Results: The increased bone resorption observed in patients with UC was reflected by low bone density and high serum level of C-terminal telopeptide (CTX). Mucosal RANKL expression and serum concentration were similar in UC group and healthy subjects, however, UC patients had higher local expression of OPG and serum OPG concentration. Increased IL-33 gene expression was observed only in UC at the mRNA level. We propose that bone resorption in UC patients

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despite OPG up-regulation could be caused by IL-33-induced mucosal synthesis of a potent proinflammatory cytokine, such as TNF-α, known as a possible inducer of osteoclastogenesis in the way independent of RANKL.
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1. Introduction

The balance of pro- and anti-inflammatory cytokines in the colonic mucosa is not only fundamental for normal gut homeostasis but also for the proper bone metabolism. Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD) which are characterized by chronic inflammation of the colon (UC, CD) and/or small intestine (CD). Moreover, a significant reduction of bone mass during the course of disorder is observed.[17,19,20,27]

The receptor activator of nuclear factor κB ligand (RANKL), its receptor — RANK, and its soluble receptor — osteoprotegerin (OPG) play a key role in osteoclast differentiation and bone metabolism. OPG acts as a receptor for RANKL that prevents it from binding to and activating RANK. RANKL–RANK interaction is crucial for osteoclast maturation. RANKL and OPG are produced not only by osteoblasts and bone marrow stromal cells, but also by many other cell types including the cells involved in immune responses such as T lymphocytes.[7,26]

Interleukin-33 (IL-33) formerly known as nuclear factor from high endothelial venules (NF-HEV) is a new member of the IL-1 cytokine family, and is expected to be essential for induction of Th2-type immune response, which is a characteristic feature of UC.[13,14,23] IL-33 is a ligand for the IL-1 receptor-related protein (ST2), and is expressed in such cell types as endothelial cells, macrophages, and dendritic cells.[15,23] In vitro, human IL-33 (30 kDa) can be cleaved by caspase-1 to create a mature form (20–22 kDa) of the protein.[12]

The aim of our study was to assess local expression of RANKL, OPG and IL-33 in the mucosal biopsies of UC patients including serum levels of these cytokines in relation to the parameters of bone metabolism.

2. Materials and Methods

2.1. Patients

Histological and endoscopic classifications of specimens were based on endoscopic, clinical, and histopathological outcomes. UC patients (n = 65) were divided into 2 groups according to Modified Truelove and Witts Severity Index (MTWSI).[28] The first group consisted of 56 patients with moderate disease activity (30 patients with proctitis or left-sided colitis and 26 with pancolitis) and the second one comprised 9 UC patients in remission. The average duration of the disease was 16 y.

The control group included 37 healthy participants who underwent screening colonoscopy, showed normal colonic mucosa and had no history of immune-mediated diseases. All studies were confirmed by the local Ethics Committee and a voluntary written informed consent was obtained from all individuals involved into the study.

Patients with active UC were treated with aminosalicylates (sulfasalazine or mesalazine), corticosteroids or/and immunosuppressive drugs (azathioprine). Among UC patients, four of them underwent additional treatment with bisphosphonates (alendronate, ibandronate).

Dual-energy X-ray absorptiometry (DXA) was used to measure bone mineral density (BMD) of the femoral neck and lumbar vertebrae L1–L4. T and Z scores were determined for 32 patients with UC and 7 individuals with UC in remission according to the WHO definition of osteoporosis and osteopenia (T-score of −1 to −2.5 was classified as osteopenia and T-score ≤ −2.5 was classified as osteoporosis). The clinical characteristics of patients are shown in Table 1.

2.2. Tissue Harvest and Human Serum Collection

Biopsies from patient and control groups to determine mRNA and protein expression were immediately placed in liquid nitrogen, and stored at −80 °C until processed. Sera obtained from clotted blood after centrifugation at 2500 rpm for 15 min were stored at −80 °C until use.

2.3. Total RNA Extraction and Reverse Transcription

Biopsies were lysed in a tissue homogenizer (MagNA Lyser, Roche, Basel, Switzerland) and RNA was extracted using the commercially available Total RNA kit (A&A Biotechnology, Gdynia, Poland) based on the phenol–chloroform–isoamyl alcohol and silica membrane technique, according to the manufacturer's instructions. Isolated RNA was stored at −80 °C. Total RNA was reverse-transcribed to complementary DNA (cDNA) using M-MuLV Reverse Transcriptase (Fermentas, Inc., Hanover, MD, USA) and oligo-dT_{18} primer (Sigma-Aldrich, St. Louis, MO, USA). Samples were stored at −20 °C until processed.

2.4. Real-time PCR Analysis

The specific primers for RANKL, OPG, IL-33 and β-actin were designed using primer design tools: Primer3Plus and Vector NTI. For RANKL, forward primer was 5′-GCAGAGAAAGCGATGGTG GA-3′, reverse primer was 5′-GGGAACCAGATGGGATGTCG-3′. For OPG, forward primer was 5′-AAAGCACCCTGTAGAAAACA CA-3′, reverse primer was 5′-GTTGCCGTTTTATCCTCTCTA C-3′.[21] For IL-33, forward primer was 5′-GTGGAAGAACACAGC AAGCA-3′, reverse primer was 5′-AAGGCAAAGCACTCCACA GT-3′.[24] For β-actin, forward primer was 5′-TGTGCCCATCTAC GAGGGGTATGC-3′, reverse primer was 5′-GGTACATGGTGG TGCCGCCAGACA-3′.[16] The quantitative PCR analyses were performed in a fluorescent temperature cycler (iQ Cycler, Bio-Rad Laboratories, Inc., Hercules, CA, USA) using SYBR Green

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