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ScienceDirect
Biomedicine & Pharmacotherapy 61 (2007) 686–692
BIOMEDICINE & PHARMACOTHERAPY
www.elsevier.com/locate/biopha

Original article

Glycosaminoglycan, collagen, and glycosidase changes in human osteoblasts treated with interleukin 1, and osteodystrophy

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Received 30 March 2007; accepted 20 April 2007
Available online 24 May 2007

Abstract

Normal bone homeostasis involves a balance between osteoblast and osteoclast action, regulated by hormones and cytokine stimuli. Hemodialysis patients appear to have increased production of interleukin-1 (IL-1), interleukin-6 (IL-6) and glycosaminoglycans (GAG) in serum. IL-1 plays a role in the synthesis, degradation and degree of sulphatation of ECM components such as glycosaminoglycans. Also, continuous changes in the ECM involve enzymes such as β-*N*-acetyl-D-glucosaminidase (β-NAG) and β-D-glucuronidase (β-GLU) which act on different GAG classes and collagen fibers. We examined the effects of IL-1α on ECM synthesis and the related enzymes in human uremic osteoblast cultures. We also measured the levels of IL-1β, and IL-6 and alkaline phosphatase activity. In biopsies of uremic bone there was less ECM deposition than resorption associated with changes in osteoblast morphology. *In vitro* osteoblast proliferation was higher ($P \leq 0.01$), and extracellular GAG lower ($P \leq 0.01$) than in controls. The enzyme β-NAG was high ($P \leq 0.05$) but there were no noteworthy changes in β-GLU. ELISA of the medium indicated spontaneous production of IL-1β and IL-6, which significantly increased after IL-1α treatment compared to controls. IL-1α reduced alkaline phosphatase activity ($P \leq 0.01$) in uremic osteoblast cultures. IL-1 acts on osteoblasts with decreases in GAG synthesis and alkaline phosphatase activity, while β-NAG increases. This lead to a reduction in the organic component in ECM and its mineralization, and to changes in the regulation of cytokine activity by GAG. The enzymatic breakdown might be facilitated by metabolic acidosis and failed osteoblast differentiation; these factors could be correlated with different degrees of osteodystrophy.

Keywords: Interleukin; Osteodystrophy; Extracellular matrix

1. Introduction

Bone is a connective tissue composed of cells and mineralized extracellular matrix (ECM). Its normal remodeling and volume are maintained through the balance of bone formation by osteoblasts and resorption by osteoclasts. This interaction is a critical component of normal bone cell biology and is regulated by hormones and cytokine stimuli. In chronic renal failure caused by acid-base imbalances and calcium homeostasis abnormality resorption prevails, causing osteodystrophy [1–3]. Cytokine production is increased in hemodialysed patients [4]. Transforming growth factor β_1 (TGFβ_1) and interleukin-1 (IL-1) are two cytokines needed for the synthesis, degradation and degree of sulfatation of ECM components such as

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doi:10.1016/j.biopha.2007.04.003

hyaluronic acid (HA), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS) and heparan sulfate (HS). The glycosaminoglycans (GAG) control the availability and accessibility of cytokines in relation to cell functions [5–7]. What happens is that HA blocks the decreased proteoglycan concentration in osteoarthritic chondrocyte cultures [8], CS prevents nitric oxide damage [9] and is important for cartilage mineralization [10,11], while HS regulates gene expression in adult hepatocytes [12]. GAG are also involved in immunological processes and renal disease, and cytokines play a major role in acute graft rejection after renal transplantation [13]. This points to an important role for GAG, and suggests that their coordination with cytokines is essential for the differentiation and maintenance of adult tissue function. Continuous qualitative and quantitative changes are seen in GAG of the ECM in the processes by which they are produced and degraded. Lysosomal enzymes (exo- and endoglycosidases) are linked to these processes. Exoglycosidase, such as β-NAG, acts on HA, CS and DS and β-GLU acts on HA, CS, DS and HS.

The production of IL-1, IL-6 and GAG in serum is increased in hemodialysis patients [4,14]. IL-1 and IL-6 seem to induce osteoclast formation through a mechanism involving osteoblasts [15]. These latter synthesize GAG, which regulate cytokine activity and hence also osteoclastic activity.

We analyzed the *in vitro* effect of IL-1 on the synthesis of GAG and collagen, and the activity of β-GLU, β-NAG, enzymes involved in GAG turnover, and alkaline phosphatase which is related to bone mineralization in primary human osteoblasts. We measured IL-1α, IL-1β, IL-6 levels and did a histological analysis of human bone biopsies.

2. Materials and methods

Bone biopsies from 16 uremic patients (nine males and seven females) aged 49 ± 13.6 years who were on hemodialysis for 7.1 ± 3.6 years were examined under the optical microscope and cultured to obtain osteoblasts. All patients gave their consent to participation in the study. Biochemical and clinical data regarding their intact parathormone (PTH), parathormone 1-84 (PTH1-84), total parathormone (TPTH), bone alkaline phosphatase, total alkaline phosphatase, 1,25-dihydroxycholecalciferol, calcium and phosphate are reported in Table 1. Serum intact PTH was measured with a commercial immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) based on a double-antibody technique. PTH and total PTH were measured with an assay provided by Scantibodies Laboratory, Inc. (Santee, CA, USA). Serum 25-hydroxycholecalciferol was measured with a competitive protein-binding method, after purification on Sep-Pack C18 cartridges and extraction with acetonitrile; 1,25-dihydroxycholecalciferol was measured with a radioimmunoassay provided by Nichols Institute Diagnostics. Bone alkaline phosphatase (bAP) was measured with an immunoassay, using a monoclonal anti-bAP antibody, coated onto a microtiter strip to capture bAP in the sample, provided by Metra Biosystems (Mountain View, CA, USA). Serum total calcium was determined by a spectrophotometric assay using cresolphthalein as substrate. Serum phosphate and alkaline phosphatase were measured spectrophotometrically (DU-65 Beckman, Fullerton, CA, USA) using molybdate and *p*-nitrophenyl-phosphate as the respective substrates.

2.1. Optical microscopy

Bone biopsies from patients with renal failure were taken on the ilium, just below the anterior superior iliac spine. All specimens were placed immediately in fixative, then embedded in glycol methacrylate resin. Three-micrometer-thick sections from each specimen were cut with a Leica 3RM2155 microtome at intervals of ∼50 μm and stained with hematoxylin and eosin, Giemsa, methylene blue and modified Goldner trichrome.

Morphological analysis was done with an interactive image analyzer (Kontron-Zeiss) comprising a color camera (JVC-TK-C1381 EG) attached to a light microscope. Damaged microscopic fields were excluded from analysis.

2.2. Osteoblast culture

Bone specimens were cultured according to Maurizi et al. [16]. Primary cultures were grown in Falcon flasks containing medium 199 supplemented with 20% fetal calf serum (FCS) (GIBCO, Paisley, UK), 100 IU/mL penicillin, 100 μg/mL streptomycin and 25 μg/mL amphotericin B (Serva, Heidelberg, Germany). Subcultures were obtained 20–30 days later. Osteoblast cultures were used at the 4th subculture because the osteoblast MC3T3-E1 cell line presents molecular markers associated with replicative senescence in culture [17] and articular chondrocytes from young growing and mature old mice respond differently to IL-1 and TGFβ [18]. The nature of bone cells was assayed by labeling them with antiosteopontin antibodies (Fig. 1) (Santa Cruz Biotechnology Inc, Santa Cruz, CA). The confluent cultures were treated with 2 U/mL IL-1α (Boehringer Mannheim, Germany) for 24 h, then cell proliferation, GAG synthesis, glycosidase activity, IL-1β and IL-6 were determined.

Table 1
Patients' main biochemical data (mean ± SD and range)

iPTH (pg/L)	PTH Tot (pg/L)	1-25$(OH)_2D_3$ (pg/L)	AP (IU/L)	BAP (IU/L)	Ca (mg/dL)	P (mg/dL)
712.5 ± 628.7 (159–1203)	782.4 ± 655.3 (186–2100)	14.68 ± 17.11 (6.9–42.7)	394 ± 261 (44–1460)	85.4 ± 87.8 (36.4–299)	10.1 ± 1.21 (7.8–11.4)	5.41 ± 2.06 (4.7–6.8)

iPTH, intact parathyroid hormone; PTH Tot, total parathyroid hormone; AP, alkaline phosphatase; BAP, bone alkaline phosphatase; Ca, calcium; P, phosphorus.