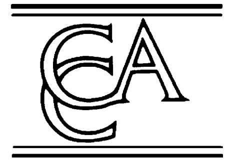
Clinica Chimica Acta 356 (2005) 154–163



www.elsevier.com/locate/clinchim

A bio-assay for effectors of osteoclast differentiation in serum from patients with bone disease

Marit-Naomi Dugard[a,*], C.A. Sharp[a], S.F. Evans[a], J.H.H. Williams[a,b], M.W.J. Davie[a], M.J. Marshall[a]

[a]*Charles Salt Centre, Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Trust, Oswestry, Shropshire, SY10 7AG, United Kingdom*
[b]*Chester Centre for Stress Research, Chester College, Chester, UK*

Received 12 November 2004; received in revised form 13 January 2005; accepted 13 January 2005

Abstract

Osteoclast differentiation and activity, and hence bone loss, depend on two opposing cytokines. Receptor activator of NF-κB ligand (RANKL) produced by osteoblasts and T-cells stimulates, while osteoprotegerin inhibits. Both of these cytokines are found in serum. Our aim was to develop a functional assay for any factors present in human serum that can affect osteoclast differentiation and to assess whether any such factors vary in diseases in which bone loss occurs.

Methods: Using a culture model of osteoclast differentiation in the presence of macrophage colony stimulating factor and soluble RANKL, we have measured the effects of different human sera on osteoclast differentiation. The production of a marker enzyme for the osteoclast, tartrate-resistant acid phosphatase (TRAP), was used to follow osteoclast differentiation.

Results: In general, human serum stimulates osteoclast differentiation as indicated by TRAP activity, but in patients with low bone density this stimulation was attenuated. Sera from 40 female subjects with low bone mineral density showed significantly lower TRAP cell differentiation activity than sera from the healthy female controls.

Conclusion: We describe a functional bio-assay for factors in human serum which can affect osteoclast differentiation. This assay may have application in monitoring the effects of therapy in bone disease.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Osteoclast; Differentiation; Serum; Bone disease; Bio-assay; RANKL

1. Introduction

The osteoclast and osteoblast are the two major types of bone cell that work in concert as part of a life-long programme of bone removal and renewal that maintains the mechanical integrity of the skeleton. The cell that removes bone, the osteoclast, is a multinucleate cell that differentiates from haemato-poietic cells of the monocyte lineage. Two key cytokines, macrophage colony stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL), are necessary and sufficient for the differ-

* Corresponding author. Tel.: +44 1691 404508; fax: +44 1691 404056.
E-mail address: naomi.rowlands@rjah.nhs.uk (M.-N. Dugard).

0009-8981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.cccn.2005.01.020

entiation of osteoclasts [1]. Osteoblasts, on the other hand, are derived from mesenchymal stem cells. In response to the influences of mechanical loading, hormones, and cytokines, osteoblastic cells orchestrate cycles of coupled bone remodelling that ensures bone architecture is exquisitely adapted to withstand the mechanical loads placed upon it. However, there are several commonly occurring conditions, including osteoporosis, rheumatoid arthritis, Paget's disease of bone, and hypercalcaemia of malignancy in which abnormal bone turnover results in excessive osteoclast activity, resorption of bone, and increased susceptibility to fractures. Understanding the mechanisms that control bone turnover is therefore an important objective that will lead to the effective prevention and treatment of bone loss.

The recent identification of two proteins produced by osteoblastic cells has offered a new insight into how osteoclastic bone resorption is regulated. RANKL is a tumour necrosis factor family member produced in response to a wide range of pro-resorptive hormones and cytokines [2,3] and is essential for the differentiation [1,4], activity [1] and survival [5,6] of osteoclasts. In this role it is counter-acted by a secreted decoy receptor, osteoprotegerin (OPG) [7], also produced by osteoblastic cells [8]. OPG acts by binding to RANKL and preventing its binding to its cognate receptor RANK on the osteoclast and its precursor [4]. Effectors that stimulate RANKL expression, for example PTH, prostaglandin E2 and 1,25 dihydroxyvitamin D3, inhibit OPG production [9]. On the other hand, factors that stimulate OPG production often inhibit RANKL production, for example oestrogen [9] and TGF-β [10]. Osteoclasts are now considered to be controlled by adjacent osteoblastic cells which can either activate or inhibit by the production of one or other of these two counter-balancing cytokines.

Both OPG and a soluble form of RANKL have been reported to be present in human serum [11,12] although the functionality of these two cytokines in serum has not been established. The commercially available assays for OPG do not distinguish between free and ligand bound OPG, and while free and total RANKL assays are available the values they give are anomalous. Hence, serum OPG is reported as about 2 pM and free RANKL is about 0.2 pM whereas total RANKL is 4 nM [12]. These values are not compatible with a simple model in which the molar concentration of total RANKL equals the sum of free RANKL and RANKL bound to OPG.

Our aim was to develop a functional assay for factors present in human serum that affect osteoclast differentiation and to assess whether any such factors vary in diseases in which bone loss occurs. Using an in vitro culture model of osteoclast differentiation in the presence of M-CSF and soluble RANKL, we have measured the effects of different human sera on osteoclast differentiation. The production of a marker enzyme of osteoclast differentiation, tartrate-resistant acid phosphatase (TRAP), was used to follow osteoclast differentiation. In general, human serum stimulates osteoclast differentiation as indicated by TRAP activity, but in patients with low bone density this stimulation was attenuated.

2. Methods

2.1. Subjects

Blood samples were collected from patients attending an outpatient metabolic bone disease clinic. In total, 168 subjects including 141 women and 27 men were included in the study. Of these, 40 patients (female) were diagnosed with low areal bone mineral density (BMD) at the proximal femur and lumbar spine (L2–L4) measured by the Hologic QDR4500 (Hologic, Waltham, MA, USA), defined by BMD values between 1 S.D. and 2.5 S.D. below the young normal mean. Also, there were 19 patients (10 male and 9 female) diagnosed with Paget's disease of bone, and 109 (17 male and 92 female) healthy volunteers recruited from the local population. The healthy volunteers were all ambulatory, free-living individuals who at the time of the study were not receiving any medication known to influence bone metabolism. Further subject group characteristics are shown in Table 1. The protocol was approved by the hospital research panel and the local research ethical committee.

2.2. Blood collection

Blood samples for the study were collected in serum clot-activator tubes. The samples were allowed

Download English Version:

https://daneshyari.com/en/article/9888130

Download Persian Version:

https://daneshyari.com/article/9888130

Daneshyari.com