Tartrate-Resistant Acid Phosphatase as a Marker of Bone Resorption

The application of cell and molecular biology techniques to study the action of osteoblasts and osteoclasts is providing valuable insight into the complexities of bone metabolism. It is also stimulating the search for new analytical methods (markers) to unlock what has, for many years, been a relatively closed world for clinical biochemists: bone metabolism and biochemical markers of bone disease. Although such markers as hydroxyproline and the phosphatases have been available for many years, the techniques have been limited. Today, however, there are immunoassays described for a range of precursor molecules, enzymes, and breakdown products derived from bone (1-3). The frequency of editorials in this journal bears witness to the expansion of work in this field and the clinical interest that it engenders (2, 4). The paper by Cheung et al. in this issue (5) describes the development of an immunoassay for a resorption marker, tartrate-resistant acid phosphatase (TRACP; EC 3.1.3.2). To gauge the potential utility of this marker, let us briefly review the claim that TRACP is a unique marker of bone resorption.

The process of resorption occurs after the attachment of osteoclasts to the bone surface and follows the secretion of acid and enzymes into a space created between the osteoclast and the bone. The acidic environment is produced by the action of carbonic anhydrase and an H⁺-ATPase proton pump (6). TRACP, one of the enzymes secreted into this space, has been located in the adjacent osteoclast membrane (known as the ruffled border) (7). The amount of ruffled border and the expression of enzymes, including TRACP, are increased when resorption is increased (8). Chambers et al., using osteoclasts dispersed on glass coverslips, had previously demonstrated that calcitonin (a potent inhibitor of bone resorption) was a powerful inhibitor of TRACP secretion (9). Furthermore, Miller (10) demonstrated that parathyroid hormone (parathyrin; a promoter of bone resorption) stimulated the secretion of TRACP by osteoclasts in the presence of osteoblasts. Zaidi et al. (11), using disaggregated osteoclasts on devitalized bone, showed a positive correlation between TRACP activity and the extent of resorption. Later, using similar techniques, they showed that adding molybdate, a powerful noncompetitive inhibitor of TRACP, to the medium led to reductions in the TRACP secreted and in the area of bone resorbed (12); furthermore, including a uteroferrin antibody, known to bind to TRACP, produced the same results (12).

Measuring TRACP activity in serum also indicates the involvement of the enzyme in bone turnover. Increases in activity have been demonstrated in patients with hyperparathyroidism, Paget disease, or bone malignancy, and in children (13). Increased serum TRACP activity has also been shown in postmenopausal women and in persons with osteoporosis, being positively correlated with concentrations of hydroxyproline and collagen cross-link molecules and with bone mineral density (14, 15). Increases are also seen with bone formation markers in these conditions, demonstrating the coupling between osteoblastic and osteoclastic activity. However, catalytic methods for measuring TRACP are problematical, given the relatively low activity and instability of the enzyme and the presence of inhibitors in the serum (16). The opportunity to monitor the status of a metabolic process, namely, bone resorption, by measuring with an immunoassay the amount of catalytic protein leaking into the blood is therefore very attractive. This notwithstanding, when there are several tissue sources of tartrate-resistant enzyme, the characterization of the immunogen, the calibrator, and the antibody are of critical importance.

TRACPs are found in osteoclasts, in alveolar and monocyte-derived macrophages, in hairy and Gaucher cells of the spleen, in the placenta, and in erythrocytes. Their pH optima for activity are reportedly between 4.9 and 6.0, and their molecular mass ranges between 30 and 40 kDa, except for erythrocytic TRACP, which is 17kDa. The 30-40-kDa TRACPs from different human sources have between 85% and 95% amino acid sequence homology, and variations between the TRACPs are thought to result from alternative splicing of the initial transcript, use of different translation initiation sites, or posttranslational modification of the protein, rather than from the existence of a multigene family. Only one TRACP gene and a single mRNA species have been reported (17).

A second acid phosphatase (ACP) found in the lysosomes of osteoclasts is sensitive to tartrate. While purifying this enzyme from chicken tibia, Lau et al. (18) demonstrated a time-dependent loss of tartrate sensitivity. Furthermore, this change was associated with a change in characteristics of ion-exchange chromatography, electrophoretic mobility, and reduction in molecular mass, to match the characteristics of the resistant enzyme. Lau et al., therefore, proposed that the tartrate-sensitive enzyme was the precursor of the resistant form. This same conclusion had also been reached by Fukushima et al. (19), who in histochemical studies of osteoclasts found the tartrate-sensitive enzyme to be primarily in the lysosomes and Golgi complexes, whereas the resistant enzyme was on the cell surface.
membrane. However, the idea that the lysosomal enzyme is transported to the ruffled border, being cleaved during the process, is challenged by the absence of amino acid sequence homology between the sensitive and resistant enzymes (17).

The main characteristics of the TRACPs include a high pi, reported in most instances to be between 8.5 and 9.0 (17); a lower pi has been reported for the placental enzyme (20), and Cheung et al. (5) refer to a lower value in an enzyme from rat bone. Many of the TRACPs are strongly inhibited by molybdate and phosphate and are activated by sulphhydril compounds, ferrous ions, and ascorbic acid (17, 21–24). However, as demonstrated by Hayman et al. (25), the TRACP from human osteoclastomas, although considerably homologous with spleen and uterus enzymes, was not activated by mercaptoethanol or ascorbic acid and was only moderately inhibited by molybdate. They also found that TRACP was inhibited by zinc and ferrous ions and EDTA.

The characteristics of the enzyme described by Cheung et al. (5)—a lower pi, weaker inhibition by molybdate and phosphate, and no significant effect of dithiothreitol—do not accord completely with the evidence in the literature on monocyte-derived TRACPs (17). This, therefore, poses the question as to the origin of the enzyme they purified. At present, there is only limited evidence of post translational modifications of these enzymes, and the existence of different isoforms unique to individual tissues or cell types has not been established; however, considerable variations in the characteristics, reflecting a degree of heterogeneity, are evident among the various TRACPs. Perhaps Cheung et al. have raised an antibody against placental TRACP that cross-reacts somewhat with the osteoclastic enzyme.

Few immunoassays of TRACP have been reported, although the porcine placental protein uteroferrin (which shows a high degree of homology with TRACP) (26) and the enzyme from hairy cells (27) have been used as immunogens; the fact that antibodies from the placental protein cross-react with the bone enzyme (26) and vice versa support the idea that the enzyme purified by Cheung et al. may be the placental type and may differ from the osteoclastic enzyme only in carbohydrate content. If true, then the specificity and the calibration of their assay require further consideration. Notwithstanding, the paper by Cheung et al. provides ample evidence of the potential utility of a method for measuring TRACP protein; indeed, among the several reports of immunoassays of TRACP, this paper (5) is the first to provide a substantial amount of clinical data. Furthermore, the data on the changes in the serum concentration of catalytic protein mirror the results found with activity assays, i.e., significant increases in children, postmenopausal women, and hyperparathyroidism compared with healthy young adults.

All of the evidence points to a role for TRACP in bone resorption. Its exact role is unclear (23), but it is likely to act in concert with other changes such as cell morphology, cell adhesion, and generation of free radicals (28). For clinical use of a TRACP immunoassay, it will be important to further characterize the enzyme, perhaps in relation to the extent of glycosylation in comparison with other TRACPs and thereby characterize the specificity of the antibodies. Similarly, the question of the existence of a unique osteoclastic TRACP will affect the production of an appropriate reference material and thus the "accuracy" of the immunoassays, as well as comparability and compatibility of results obtained with different sources of reagents.

The paper by Cheung et al. (5) therefore makes an exciting addition to the burgeoning range of biochemical markers of bone disease. Considered with immunoassays for bone alkaline phosphatase, the assay they describe heralds the prospect of assessing the balance of osteoblastic and osteoclastic function—which may be more important than the independent activity of either cell type in relation to uncoupling the functions of these important cells and the development of bone pathology. However, it will be a while before the assays are established and sufficient comparable clinical data exist for us to appreciate the complementarity of markers. Early signs are that, for detecting bone changes in premenopausal and osteoporotic women, the markers show comparable degrees of changes (3); also, as expected, markers of resorption decrease in concentration sooner than do markers of formation when resorption is inhibited (29).

We foresee an exciting future for bone markers in the diagnosis and management of disease as we increase our understanding of bone pathology and of the contribution that can be made by clinical chemistry.

References

Christopher P. Price
Aisling Kirwan
Christian Vader

London Hospital Medical College
Turner St.
London E1 2AD, UK
Fax 44-171-377-1544