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Review

Acidosis, hypoxia and bone

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ABSTRACT

Bone homeostasis is profoundly affected by local pH and oxygen tension. It has long been recognised that the skeleton contains a large reserve of alkaline mineral (hydroxyapatite), which is ultimately available to neutralise metabolic H^+ if acid–base balance is not maintained within narrow limits. Bone cells are extremely sensitive to the direct effects of pH: acidosis inhibits mineral deposition by osteoblasts but it activates osteoclasts to resorb bone and other mineralised tissues. These reciprocal responses act to maximise the availability of OH^- ions from hydroxyapatite in solution, where they can buffer excess H^+. The mechanisms by which bone cells sense small pH changes are likely to be complex, involving ion channels and receptors in the cell membrane, as well as direct intracellular effects. The importance of oxygen tension in the skeleton has also long been known. Recent work shows that hypoxia blocks the growth and differentiation of osteoblasts (and thus bone formation), whilst strongly stimulating osteoclast formation (and thus bone resorption). Surprisingly, the resorptive function of osteoclasts is unimpaired in hypoxia. *In vivo*, tissue hypoxia is usually accompanied by acidosis due to reduced vascular perfusion and increased glycolytic metabolism. Thus, disruption of the blood supply can engender a multiple negative impact on bone via the direct actions of reduced pO_2 and pH on bone cells. These observations may contribute to our understanding of the bone disturbances that occur in numerous settings, including ageing, inflammation, fractures, tumours, anaemias, kidney disease, diabetes, respiratory disease and smoking.

Introduction

Oxygen tension (pO_2) and extracellular pH within tissues are influenced by the vascular supply and the local metabolic activity of cells. This review will focus on some of the potential causes of pO_2/pH disturbances in bone, and how these might affect bone cell function and bone homeostasis.

Acid–base balance and the skeleton

Causes of acidosis

Precise maintenance of pH in the blood and extracellular fluid is needed because the machinery of cells is generally very sensitive to changes in H^+ concentration. Blood pH is mainly buffered via the CO_2/HCO_3^- system, with an important additional contribution from the histidine residues of haemoglobin. If the lungs are unable to expel sufficient CO_2 produced by respiration, an increase in blood H^+ concentration (i.e., pH decrease) occurs, leaving HCO_3^- concentration relatively unaltered; this is termed a respiratory acidosis. Conversely, addition of H^+ to the system (*e.g.*, as a result of the metabolism of sulphur-, nitrogen-, and phosphorus-containing molecules) will decrease blood pH and reduce HCO_3^- levels without greatly altering the CO_2 concentration. Protons generated in this way, together with associated waste anions, must be excreted via the kidneys; if insufficient H^+ is eliminated, a metabolic acidosis results.

There are numerous potential causes of systemic acidosis in addition to renal and respiratory disease. Many are associated with bone loss, including anaerobic exercise [1,2], gastroenteritis [3], excessive consumption of protein [4,5] or other acidifying substances, including chloride [6] and phosphoric acid-containing cola drinks [7], diabetes [8], ageing [9,10], the menopause [11] or androgen deficiency [12]. Acidosis can arise locally as a result of ischaemia/hypoxia [13–15], inflammation [16], fractures [17] and tumours [14,15,18]. Extracellular acidification can also result from hormone, growth factor or cytokine stimulation of cell metabolism; for example, parathyroid hormone and IGF-1 cause rapid acid efflux from osteoblasts [19,20]. Although the pH of arterial blood is normally close to 7.40, and that of venous blood ~7.36, the pH of the film of interstitial fluid bathing cells in tissues will generally be lower and subject to complex gradients, depending on the metabolic activity of the cells, their distance from the nearest capillary, and the quality of the microvasculature. Data are not available for bone but in normal tissue (rabbit ear), interstitial pH is ~7.2 [18].

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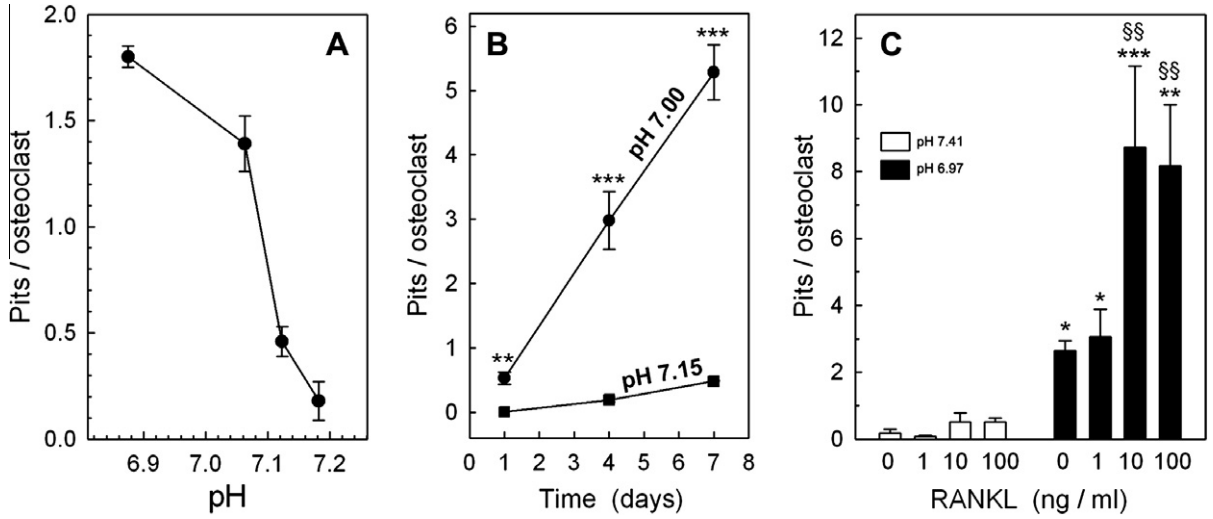

Fig. 1. *Activation of osteoclast function by acidosis*. Mature osteoclasts from neonatal rat long bones were cultured on ivory discs and resorption pit formation was assessed using reflected light microscopy after staining to demonstrate tartrate-resistant acid phosphatase (TRAP). A. In 26-h cultures, resorption is strongly activated when pH is ⩽6.9 and is almost 'switched off' when pH is ⩾7.2. B. Longer-term cultures emphasise the sensitivity of osteoclast function to small pH differences. Significantly different from pH 7.15 values: $^{**}p < 0.01$; $^{***}p < 0.001$. C. Activation of osteoclasts is a two-step process: RANKL exerts only a minimal effect on resorption at normal blood pH (7.41) in 26 h cultures but causes marked additional stimulation in acid-activated cultures (pH 6.97). Significantly different from respective pH 7.41 values: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; significantly different from zero RANKL control in acidified group: $^{§§}p < 0.01$. All data are means ± SEM ($n = 6$).

Acidosis and osteoclasts

The deleterious impact of systemic acidosis on the skeleton has long been known [21,22] but was thought to result from physicochemical dissolution of bone mineral: in other words, that the skeleton acted as an 'ion exchange column' to buffer systemic acidosis in a passive manner [23–25]. However, cell culture experiments showed that protons exerted a direct stimulatory effect on bone resorption by cultured rat osteoclasts [26]. Mature rat osteoclasts were observed to be almost inactive at pH 7.4, which corresponds to 'physiological' or blood pH, but resorption pit formation increased steeply as pH was reduced, reaching a plateau at about pH 6.8. Subsequent studies showed that avian [27] and human [28] osteoclasts also exhibit acid-activation responses. The sensitivity of OC to extracellular H^+ is such that pH reductions of <0.1 unit can cause a doubling of resorptive activity [29] (Fig. 1A). This effect is not subject to tachyphyllaxis (or 'escape') in longer-term cultures: acid-activated osteoclasts continue to form resorption pits over periods of 7 days or more, amplifying the effects of modest pH differences (Fig. 1B). Organ culture experiments showed that calcium release from calvarial bones was also extremely sensitive to acid stimulation. This H^+-activated calcium release is blocked by calcitonin, indicating that it is entirely osteoclast-mediated [30–31]. Acidosis is required for the initiation of resorption; once activated, osteoclasts can be further stimulated by factors such as receptor activator of NF-κB ligand (RANKL) (Fig. 1C), parathyroid hormone [26], 1,25$(OH)_2$ vitamin D [28] and ATP [32]. Thus, osteoclast stimulation is a two-step process, with acid-activation as the key initial requirement – and extracellular H^+ may be regarded as the long-sought 'OC activation factor' (OAF)[1].

Osteoclast activation involves upregulation of the key cellular machinery needed for resorption pit formation. Acidification rapidly increases expression of carbonic anydrase II [33] and the vacuolar-type H1-ATPase (required for the generation and pumping of the protons that solubilise bone mineral) in osteoclasts [34] and up-regulates cathepsin K (required for organic matrix degradation), tartrate-resistant acid phosphatase and TNF receptor-associated factor 6 [35,36].

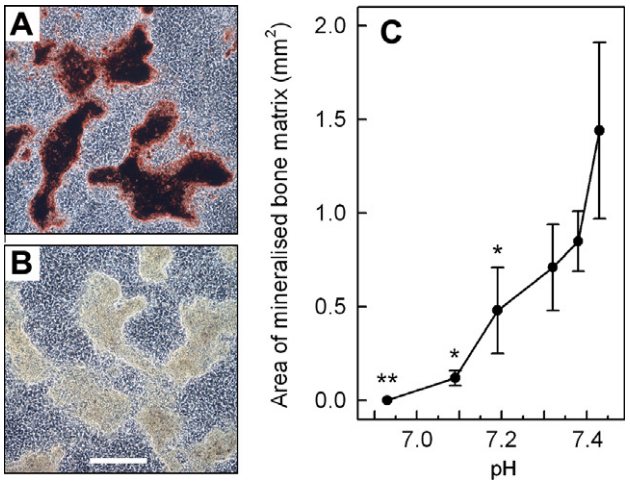

Fig. 2. *Inhibition of matrix mineralisation by acidosis*. Primary osteoblasts from neonatal rat calvariae were cultured for 16 days at pH 7.43 (A) or pH 6.93 (B), stained with alizarin red to demonstrate calcium deposition and viewed by phase contrast microscopy; scale bar = 0.5 mm. Organic matrix is deposited in the characteristic 'trabecular' manner in the acidic culture but mineralisation is completely prevented. Panel C shows the sensitivity of bone mineralisation, assessed by image analysis, to small pH changes. Values are means ± SEM ($n = 6$). Significantly different from the control (pH 7.43) value: $^{*}p < 0.05$; $^{**}p < 0.01$.

The effects of extracellular pH on osteoclastogenesis and lifespan are rather less clear-cut than the effects on activation. Acidosis is reported to inhibit the formation of mouse osteoclasts [37] but stimulate the formation of cat osteoclasts [35] and promote the survival of mature rat osteoclasts [38].

Acidosis and osteoblasts

Bushinsky and colleagues were among the first to study the direct effects of pH on osteoblast function. They reported that mild

[1] *Abbreviations used:* OAF, OC activation factor; HIFs, hypoxia-inducible transcription factors; HREs, hypoxia response elements; hPBMCs, human peripheral blood mononuclear cells.