S 22-2

IMPACT OF CHRONIC METABOLIC ACIDOSIS ON HUMAN BONE REMODELING PROCESS

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Early studies demonstrated that chronic metabolic acidosis resulted in marked elevation in urinary calcium excretion without accompanied by an increase in intestinal calcium absorption. In vitro and animal studies also demonstrated an increase in osteoclastic activity in acidosis condition. These findings indicate indirectly that metabolic acidosis induces bone resorption. Data in human, especially in chronic stage, is limited. Based on studies in distal renal tubular acidosis, a condition in which chronic metabolic acidosis resulted from impaired renal excretion of acid, a marked decrease in bone mineral density and impaired skeletal growth have been found, indicating the significant impact of chronic metabolic acidosis on bone remodeling process. However, histologic findings from transiliac crest bone biopsy in this group of patients demonstrated a suppression of bone formation rate and decrease in both osteoblastic and osteoclatic numbers without an increase in bone resortion. There was a modest increase osteoid formation. The histologic findings, in fact, are more compatible with adynamic bone disease rather than osteomalasia. The histologic abnormalities were corrected and an elevation of bone mineral density was demonstrated after an alkaline supplement. A suppression of serum parathyroid hormone, initially found during chronic metabolic acidosis, was also reversed toward normal level after correction of metabolic acidosis. The findings indicate the significant impact of metabolic acidosis on bone remodeling process in human. However, the causes of the discrepancy in the findings between studies in distal renal tubular acidosis and the in vitro or animal studies are still uncertain. The difference in the duration acidosis may result in a difference in body response. In chronic stage, the body may adapt to prevent further bone loss by a conversion into an adynamic condition. Further studies, investigating the mechanism of depleted bone mass and adaptive body response to chronic acidosis, should be performed.

S 22-3

METABOLIC ACIDOSIS INDUCES BONE RESORPTION VIA PROTON RECEPTOR-MEDIATED ACTIVATION OF INOSITOL PHOSPHATE-DEPENDENT CALCIUM SIGNALING

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Metabolic acidosis (Met) increases urine calcium (Ca) excretion without an increase in intestinal Ca absorption, resulting in net loss of bone mineral. In vitro, Met stimulates net Ca efflux from neonatal mouse calvariae by stimulation of a prostaglandin E_2-dependent increase in RANKL, leading to osteoclastic bone resorption. However, the pathway by which increased extracellular [H^+] transduces an intracellular signal is not clear. G protein-coupled proton sensing receptors (PSRs) provide a potential mechanism for transduction of extracellular acidosis into intracellular responses. Transcripts for the 4 known PSRs, OGR1, GPR4, TDAG8, and G2A, are detectable in total RNA isolated from cultured calvariae. To determine if OGR1, which is coupled to inositol phosphate (IP), modulates Met-stimulated bone Ca efflux, we utilized the OGR1 inhibitor $CuCl_2$ (100 μM) and found that it significantly inhibited Met-induced net Ca efflux from calvariae. Increased intracellular metabolites of IP lead to an increase in intracellular Ca (Ca_i). We measured Ca_i by fluorescent imaging of fura-loaded primary bone cells. The cells were analyzed in a closed chamber with entry and exit ports to facilitate rapid medium change at a fixed pH, Pco_2 and [HCO_3^-]. Infusion of physiologic Met medium (pH=7.11, Pco_2=45 mmHg, [HCO_3^-]=14 mM) induced a marked, rapid, flow-independent, transient increase in Cai in individual cells, which was inhibited by $CuCl_2$. We then tested the effect on bone resorption of several inhibitors that block different steps within the IP3 pathway: 2-aminoethoxydiphenyl borate (2-APB), which inhibits IP_3 receptors and the subsequent increase in Ca_i; thapsigargin (TG) an ER Ca-ATPase inhibitor which depletes Ca_i stores; and TMB-8, which blocks Ca release from ER. Neonatal mouse calvariae were incubated for 48h in Met (pH~7.11) or neutral (Ntl, pH~7.40) medium in the absence or presence of each of these inhibitors (inh). Medium was changed at 24 h. All three inhibitors significantly decreased the net Ca efflux which was induced by incubation in Met at 24~48h.

Inhibitor	Conc	Ntl	Met	Ntl+inh	Met + inh
APB	100 μM	131±55	670±101*	9±38†	264±138†
TG	100 nM	174±45	866±103*	122±32†	290±25*†§
TMB-8	100 μM	326±68	933±62*	1±26*†	319±80†§

Data are nmoles Ca released/bone/24 hr (mean±SE); n=6-8 for each group; *$p<0.05$ vs Ntl; $^{\dagger}p<0.05$ vs Met; $^{\S}p<0.05$ vs Ntl+inh

These results are consistent with Met activation of OGR1 to induce IP_3-dependent Cai transients, which may then modulate osteoblastic activity and lead to the subsequent increase in osteoclastic bone resorption.