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INHIBITION OF PARATHORMONE-STIMULATED BONE RESORPTION BY TYPE I INTERFERON

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SUMMARY: The effect of Type I interferon on bone resorption was studied by measuring its effect on parathormone-stimulated calcium release from neonatal murine calvaria in vitro. A pure human recombinant leukocyte interferon hybrid of the A and D subtypes was used, which has high antiviral activity on mouse cells. Calcium release was inhibited in a dose dependent fashion with 50% inhibition at about 10^{-10} M or 600 U/ml, and the inhibition was reversible. The presence of interferon was required before or during the activation phase of the resorptive response, when the formation of osteoclasts from precursor cells would occur. When added to actively resorbing bone it had no effect. The data suggest that Type I interferon can inhibit the parathormone-regulated development of active osteoclasts, possibly by inhibiting osteoclast precursor differentiation.

The mechanism by which PTH causes resorption of bone is thought to involve the activation of resident osteoclasts and the stimulation of osteoclast formation from precursor cells (1-3), which are of monocytic origin (4,5). Since Type I interferon has been shown to inhibit the proliferation and/or differentiation of a number of cell types (6,7), including differentiation of monocytes to granulocytes (8), it was of interest to study the effects of this agent on PTH-stimulated bone resorption.

Since bone is responsive to a wide variety of hormones and growth factors, it was essential that the interferon used in this study be pure. For most of our studies we used pure leukocyte interferon prepared using recombinant DNA techniques and purified using anti-leukocyte interferon antibody affinity chromotography (9,10). This molecule, IFLrA/D, is a hybrid of two human leukocyte interferon subtypes and it has the unusual property of being active

Abbreviations: PTH, parathormone; IF, interferon; IFLrA/D, human leukocyte recombinant interferon hybrid of A and D subtypes, A(1-62/D(94-166); sCT, salmon calcitonin. A preliminary report of this work was presented at the 5th annual meeting of the American Society for Bone and Mineral Research (16).

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on mouse cells (9). We also used a commercial preparation of partially purified mouse interferon. Our results indicate that both IF preparations inhibit bone resorption.

MATERIALS AND METHODS

The in vitro bone resorption assay using ^{45}Ca labeled neonatal murine calvaria has been described (11). The extent of resorption was quantitated by calculating the percentage of the total calvarial label released from PTH-treated bone minus the percentage of the total label released from untreated control calvaria.

Mouse interferon (containing both alpha and beta forms) was purchased from Lee Biomolecular (San Diego, CA) and had a specific activity of 3×10^7 U/mg. Pure human IFLrA/D (specific activity 3×10^7 U/mg on mouse cells) was generously donated by Dr. Sidney Pestka, Roche Institute of Molecular Biology, Nutley, NJ. PTH (specific activity of 5000 U/mg) was prepared in our laboratory. sCT (specific activity of 4453/mg) was donated by J.W. Bastian, Armour Pharmaceutical Co., Kankakee, IL.

RESULTS

When calvaria were treated with IFLrA/D, inhibition of PTH-stimulated calcium release was observed throughout the subsequent assay period (Figure 1). This is in contrast to the kinetics of inhibition by sCT which is tran-

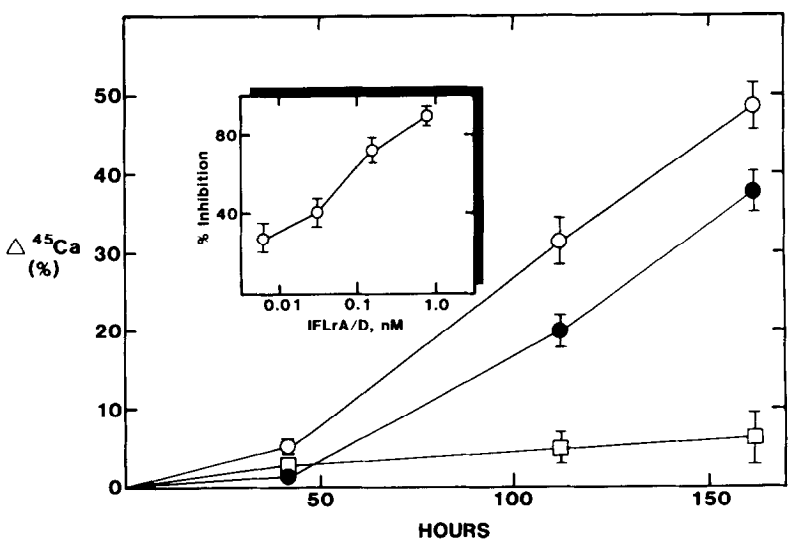

Figure 1. Effect of IF or sCT on PTH-stimulated calcium release. Calvaria were pretreated for 18 hours with control media (O—O), 30 nM sCT (●—●) or 0.3 nM IFLrA/D (□—□) and then transfered to medium containing 10 nM PTH at zero time. sCT and IFLrA/D were present throughout the incubation period. Calcium release from pretreated calvaria subsequently incubated without PTH was not significantly different from that of untreated control calvaria. Inset: calvaria were pretreated 18 hours with indicated concentrations of IFLrA/D. The percent inhibition of ^{45}Ca release was determined after 167 hours of culture in the presence of IFLrA/D, compared to PTH-treated controls.

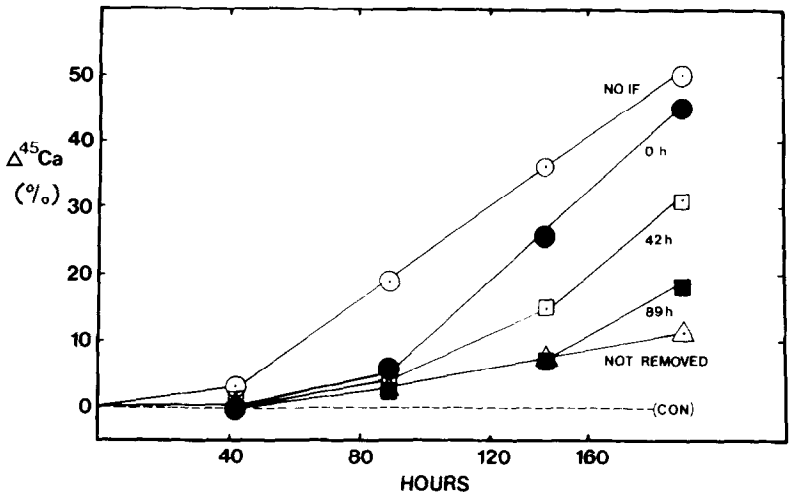

Figure 2. Reversibility of inhibitory effect of IF. Calvaria were preincubated with 0.16 nM IFLrA/D or without it (O—O) for 24 hours before the addition of 10 nM PTH at zero time. Appropriate calvaria were subsequently rinsed in resorption medium and transfered to IF-free medium at zero time just before addition of PTH (●—●), or 42 (□—□) or 89 (■—■) hours after addition of PTH or were not removed from IF medium (△—△). ^{45}Ca release from unstimulated calvaria incubated with or without IFLr/AD were not significantly different from each other (--CON).

sient (12). One-half maximal inhibition of resorption was achieved at approximately 10^{-10} M IFLrA/D. This corresponds to about 600 antiviral units/ml when assayed on mouse cells (9). Partially purified commercial preparations of Type I mouse interferon inhibits resorption by 43 ± 6% at 1000 U/ml. This result gives confidence in the use of IFLrA/D as an appropriate model for the action of Type I interferon on bone.

The inhibitory effect of IFLrA/D on PTH-stimulated resorption was reversible as shown by the data of Figure 2. Upon transfer of bone from medium containing IF to IF-free medium (containing PTH), enhanced calcium release was noted after a lag of 40-60 hours. Part of this lag period corresponds to the normal delay in calcium release seen upon addition of PTH to untreated calvaria. However, a significant amount of time seems to be required for recovery from the IFLrA/D treatment itself since there is a delay in the onset of calcium release in the case where interferon is removed at zero time, compared to PTH-stimulated calcium release from untreated bone. The rate of release of calcium from bone following removal from interferon was approximately the same as that of untreated bone. We also observe no effect on DNA,

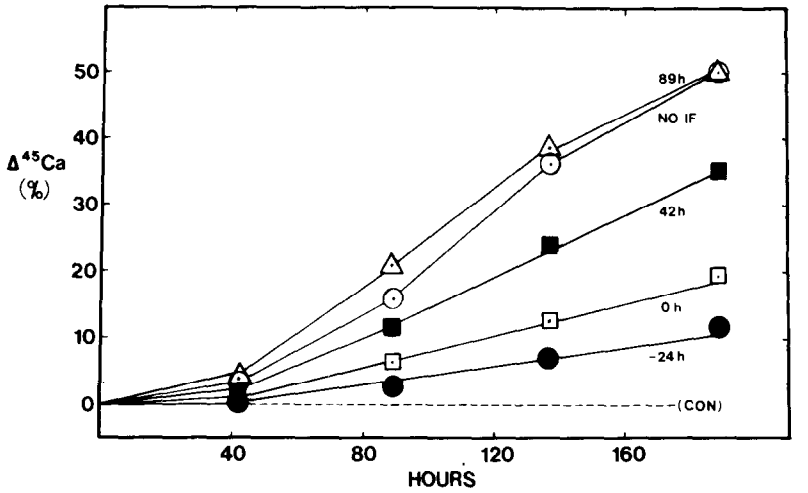

Figure 3. Effect of time of addition of IF on inhibition of PTH-stimulated calcium release. IFLrA/D (0.15 nM) was added to calvaria either 24 hours before addition of PTH (●—●), or at the same time as PTH (□—□) or 42 (■—■) or 89 (△—△) hours following addition of PTH at zero time. "NO IF" (○—○) calvaria were not treated with IFLrA/D. (--CON), same as Fig. 2.

RNA or protein synthesis in calvaria treated with IFLrA/D for 1-5 days (data not shown).

The period during which IFLrA/D must be present to inhibit PTH-stimulated calcium release was examined as shown in Figure 3. Significant inhibition was observed if IFLrA/D was added before or at the same time as PTH. When IFLrA/D was added 42 hours after PTH, the degree of inhibition was significantly less, even though calcium release had just begun at this time. When IFLrA/D was added 89 hours after addition of PTH, at which time calcium release had achieved a maximal rate, there was no effect on subsequent calcium release.

DISCUSSION

Our experiments document the ability of Type I interferon to inhibit PTH-stimulated resorption of bone in organ culture. The reversibility of the inhibitory effect indicates that IF is affecting specific PTH-dependent events in the bone rather than acting by a cytotoxic mechanism. The concentration of IFLrA/D, or mouse interferon, required to observe inhibition of calcium release is similar to that reported for inhibition of differentiation of monocytes to granulocytes in vitro (8). This is 2-3 orders of magnitude higher than that required for antiviral activity. Since both mouse interferon pre-

pared from mouse cells in culture, and IFLrA/D purified from E. coli containing the IFLrA/D gene could inhibit bone resorption in the same dose range, it is unlikely that a contaminant could be responsible.

It appears unlikely that IFLrA/D is capable of acting on active osteoclasts. Calcium release from bone already in the resorptive phase, 89 hours after stimulation by PTH, was unaffected by the addition of IFLrA/D. For inhibitory effects to be observed, IF needs to be present before or during the initial (i.e. first 40 hours) stages of the culture period, following addition of PTH. This suggests that IF may affect the mechanisms by which PTH either stimulates resident osteoclast activity or the formation of new osteoclasts. The former is thought to occur within a few hours after exposure to PTH (2), but this probably accounts for only a minor portion of the overall calcium release from neonatal calvarial bone. Rather, IF may block PTH-dependent formation of new osteoclasts from precursors resident in the bone. This process occurs 24-48 hours after exposure to PTH (3). Our data showing a lag of 40-60 hours before PTH-stimulated calcium release begins, following removal of bone from medium containing IFLrA/D, is most consistent with this later possibility, but does not rule out an effect of IF on the mechanism of activation of resident osteoclasts. Interferon has been reported to have both antidifferentiative and antiproliferative properties (6-8). However, recent data obtained using rat calvaria in vitro indicates that PTH-stimulated bone resorption does not depend on DNA synthesis (13). Thus, our observations are most consistent with the postulate that IF inhibits biochemical events which are required for differentiation of precursor cells to osteoclasts.

Other substances produced by cells of the immune system such as osteoclast activating factor (14) and interleukin-1 (15) stimulate bone resorption. Our data are the first to indicate that a leukocyte product, IF, could inhibit this process. This raises the possibility that interferon or an interferon-like molecule is produced in vivo to modulate bone resorption in a local fashion.

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