Inhibition of Osteoclast-like Cell Formation by Bisphosphonates in Long-term Cultures of Human Bone Marrow

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Abstract

Bisphosphonates inhibit bone resorption in vivo and in vitro by unknown mechanisms. The effect of bisphosphonates on the formation of osteoclasts from their mononuclear hematopoietic precursors was investigated using human long-term marrow cultures in which multinucleated cells form that express most of the known features of the osteoclast phenotype (e.g., bone resorption, tartrate-resistant acid phosphatase, calcitonin responsiveness, and reactivity with specific MAbs). The five bisphosphonates that were tested strongly inhibited 1,25-dihydroxyvitamin D_3-stimulated formation of these cells with the same relative potencies as they inhibit bone resorption in vivo. Two representative compounds (3-amino-1-hydroxypropylidene-1,1-bisphosphonate and dichloromethylene bisphosphonate) failed to inhibit the proliferation of precursors of the osteoclast-like cells. However, these compounds decreased the proportion of mononuclear and multinucleated cells expressing an osteoclast antigen, thus suggesting a degree of specificity for cells of the osteoclast lineage. We conclude that bisphosphonates are potent inhibitors of osteoclast-like cell formation in long-term human marrow cultures, and that this may be related to their ability to inhibit bone resorption in vivo.

Introduction

The stable pyrophosphate analogues known as bisphosphonates (PCPs,[1] also designated diphosphonates) are potent inhibitors of bone resorption in vivo and in vitro (reviewed by Fleisch in reference 1) and are established therapeutic agents in Paget's disease of bone (1–7) and hypercalcemia of malignancy (8–10). The precise mechanism of action of these compounds is not understood. It was originally thought that PCPs might inhibit bone resorption through physicochemical effects on hydroxyapatite dissolution (11). More recently it has become clear that PCPs have a wide variety of effects on cells thought to be involved in bone turnover, such as osteoblasts (12, 13) and cells belonging to the mononuclear phagocyte system (14–16). However, the relative potencies with which different PCPs inhibit crystal dissolution or cause most of their cellular effects in vitro differ from the relative potencies of these compounds in experimental animals or as therapeutic agents in man. There is some evidence to suggest that PCPs may inhibit the recruitment or differentiation of the osteoclast precursor (17), which is thought to be a mononuclear cell of hematopoietic origin (18–20). We have investigated the effects of PCPs on the formation of multinucleated cells (MNC) in human long-term marrow cultures (described by MacDonald et al., reference 21). Many of the cells formed in these cultures display a number of phenotypic characteristics normally associated with osteoclasts. We have studied the effects of five PCPs on the formation of these cells stimulated by 1,25-dihydroxyvitamin D_3 (1,25 D) and colony-stimulating factors, and on the expression of an antigen expressed by osteoclasts but not by cells of the mononuclear phagocyte system.

Methods

Long-term human marrow culture. Human marrow was obtained from sections of rib removed during thoracotomy. The mononuclear fraction was separated by density gradient centrifugation through Ficoll-Hypaque (Sigma, Poole, Dorset, UK). These cells were washed and resuspended in MEM-alpha (Gibco, Paisley, Renfrewshire, UK) plus 20% heat-inactivated horse serum (Gibco). The cells were then added to 16-mm tissue culture wells (Becton Dickinson, Cowley, Oxford, UK) at 5×10^5 cells/well in 0.5 ml medium containing test substances, and were maintained for 3 wk at 37°C in a humidified atmosphere of 5% CO_2, 95% air. Media and test substances were partly replenished each week. At the end of this period the cells were fixed with 5% glutaraldehyde (Sigma) in 0.1 M phosphate buffer (pH 7.2) and stained with Wright's Geimsa stain (Sigma). MNC formation was stimulated either by 10 nM 1,25 D alone or by first stimulating the proliferation of precursors with giant cell tumor-conditioned medium (GCT-CM; Gibco), a source of colony-stimulating factors (22, 23). In the latter experiments, the cells were incubated with 10% GCT-CM, 10 nM 1,25 D, or no stimulus during the first week of culture, and 10 nM 1,25 D during the second and third weeks. PCPs were made up as 10-mM solutions in PBS (Gibco); the pH was adjusted to 7.4 where necessary and further dilutions were made in culture medium. Unless otherwise mentioned, the PCPs were present during the entire culture period. The following compounds were tested: 3-amino-1-hydroxypropylidene-1,1-bisphosphonate (AHPrBP, formerly APD), dichloromethylene bisphosphonate (Cl_2MBP, formerly Cl_2MDP), 1-hydroxethylidene-1,1-bisphosphonate (HEBP, formerly EHDP), 6-amino-1-hydroxyhexylidene-1,1-bisphosphonate (AHHexBP, formerly AHDP), and 3-pyridyl-1-hydroxyethylidene-1,1-bisphosphonate (3-PHEBP). The first four of these have been used clinically and the fifth represents a new group of highly active compounds. HEBP, Cl_2MBP, AHHexBP,

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1. *Abbreviations used in this paper:* AHHexBP, 6-amino-1-hydroxyhexylidene-1,1-bisphosphonate; AHPrBP, 3-amino-1-hydroxypropylidene-1,1-bisphosphonate; APAAP, alkaline phosphatase-anti-alkaline phosphatase; Cl_2MBP, dichloromethylene bisphosphonate; GCT-CM, giant cell tumor-conditioned medium; HEBP, 1-hydroxethylidene-1,1-bisphosphonate; MNC, multinucleated cells; PCP, bisphosphonate; 1,25 D, 1,25-dihydroxyvitamin D_3; 3-PHEBP, 3-pyridyl-1-hydroxyethylidene-1,1-bisphosphonate.

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and 3-PHEBP were obtained from Norwich Eaton Pharmaceuticals Inc., Norwich, New York; AHPrBP was obtained from the Instituto Gentili, Pisa, Italy. 1,25 D was obtained from Hoffmann-La Roche, Nutley, NJ. MNC formation was measured by counting the total number of cells per well containing three or more nuclei using an inverted stage phase microscope.

Cytotoxicity. The cytotoxicity of the PCPs was assessed in two ways: directly, by trypan blue exclusion after a 24-h incubation; and indirectly, by counting the total number of cells (both mononuclear and multinucleated) adherent after 3 wk in culture. Because of the large number of cells present in each well, a sample of eight serial fields magnified 50 times along the horizontal axis was taken. This sampling pattern was chosen because the distribution of the cells within each well was not random, the MNC in particular being more numerous towards the center of the well. The cell counting technique was chosen because of the necessity to distinguish between mononuclear and multinucleated cells. Such a distinction would not be possible using metabolic studies such as oxygen consumption.

Immunocytochemistry. For immunocytochemical purposes, mononuclear marrow cells were cultured in 6-mm tissue culture wells (Nunc, Roskilde, Denmark) at 10^5 cells/well in 0.2 ml medium. These cultures were maintained for 3 wk as described above, after which they were fixed with 4% paraformaldehyde, 2% sucrose in 0.1 M phosphate buffer (pH 7.2) at 4°C for 30 min. A murine MAb (13 C2) raised against giant-cell tumor osteoclast-like cells (kindly provided by Dr. M. A. Horton, Department of Hematology, St. Bartholomew's Hospital, London, UK) was used to detect the expression of an osteoclast antigen. This MAb does not react with monocytes or macrophages derived from a variety of tissues (24). 13 C2 was added as an undiluted hybridoma tissue culture supernatant for 18 h at 4°C. An irrelevant MAb (murine anti-rabbit IgG) was used as a control. Antibody binding was visualized using the alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique (25). Briefly, this technique consists of two stages: after incubation with the primary MAb (i.e., 13 C2 or control), rabbit anti-mouse IgG is added at a high concentration, followed by addition of soluble complexes of calf intestinal alkaline phosphatase (which is resistant to inhibition by levamisole) and murine monoclonal anti-calf intestinal alkaline phosphatase (APAAP complexes). The rabbit anti-mouse IgG binds to both the primary MAb and to the APAAP complexes, thus forming a link between the two. Naphthol AS-MX phosphate (0.2 mg/ml; Sigma) was used as a substrate and fast red TR salt (1 mg/ml; Sigma) was used to provide an insoluble red reaction product. Levamisole (1 M; Sigma) was added to the substrate mixture to block endogenous alkaline phosphatase. Hematoxylin was used as a counterstain. Total and 13 C2-positive mononuclear and multinucleated cells were counted using the sampling patterns described for the cytotoxicity and MNC counting experiments, respectively.

Statistics. In the MNC counting and cytotoxicity experiments, means were taken from four replicate wells and significance was assessed by analysis of variance. In the 13 C2 binding experiments, proportions (p)±SE [SE(p)] were calculated from pooled data from each treatment, according to the formulae

$$p = \frac{\text{positive cells}}{\text{total cells } (n)}, \quad \text{and} \quad \text{SE}(p) = \sqrt{\frac{p(1-p)}{n}}.$$

Values of p are expressed as percentages in the results. Significance was assessed by t test. All data shown are representative of at least three experiments.

Results

All of the five compounds tested inhibited MNC formation stimulated by 10 nM 1,25D in a dose-dependent manner at concentrations between 10^{-7} and 10^{-4} M (Fig. 1). Using an extended concentration range of 10^{-10}–10^{-4} M, IC_{50} values were estimated. The data shown in Table I show that although

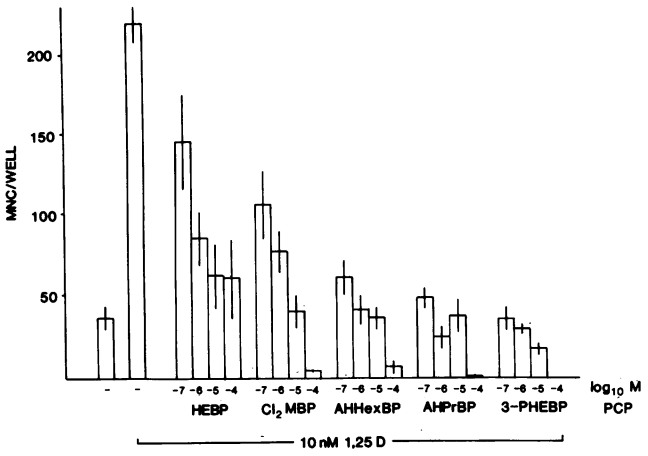

Figure 1. Effects of PCPs on MNC formation in long-term marrow cultures stimulated by 10 nM 1,25 D. Data shown are mean±SEM of four replicates. All concentrations of PCPs show significant inhibition ($P < 0.001$) except 10^{-7} M HEBP.

IC_{50} values obtained from three experiments varied within an order of magnitude, the relative potencies of the five compounds are the same in each case. The order of potency demonstrated here corresponds exactly to that observed in vivo (1, 26).

The possibility that these effects could be due to cytotoxicity of the drugs was investigated by trypan blue exclusion and by assessment of the total number of all cell types remaining in culture after 3 wk treatment with the PCPs. There was no evidence of toxicity at concentrations of 10^{-5} M or lower. 10^{-4} M AHPrBP, Cl_2MBP, AHHexBP, and 3-PHEBP caused decreases in viability as assessed by both methods (Table II). This suggests that at this concentration the reductions in MNC number may be partially or wholly due to toxic effects. Short-term cytotoxicity was also assessed using a biochemical technique that measures numbers of viable cells using a tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; reference 27). Using this technique, significant cytotoxicity was only detected in the presence of 10^{-4} M AHHexβP (data not shown). This technique, therefore, appeared to be less sensitive than trypan blue exclusion.

AHPrBP and Cl_2MBP were chosen as representative compounds for the studies on modulation of precursor prolifera-

Table I. Concentrations of PCPs Required To Half-maximally Inhibit the Effect of 10 nM 1,25 D on MNC Formation

Compound	IC_{50} Exp. 1	Exp. 2	Exp. 3	Mean±SEM	Order of potency
	nM				
HEBP	6,000	29,000	40,000	25,000±10,016	5
Cl_2MBP	130	140	650	307±172	4
AHHexBP	30	40	120	63±28	3
AHPrBP	22	29	80	44±18	2
3-PHEBP	4.5	1.6	11	5.7±2.8	1

The above values were estimated using a concentration range of each compound of 10^{-10}–10^{-4} M. Results from three separate experiments are shown.

Table II. Effects of PCPs on Cell Viability

Treatment		No. of adherent cells per well after 3 wk	% Viable cells after 24 h
1,25 D (10 nM)	PCP		
	M	*mean±SEM*	*mean±SEM*
−	−	263.3±33.2	98.7±0.7
+	−	682.0±51.7	99.3±0.4
+ HEBP	10^{-7}	494.8±98.3	99.7±0.3
+	10^{-6}	524.3±69.7	99.1±0.4
+	10^{-5}	500.5±76.0	99.5±0.5
+	10^{-4}	269.0±118.6‡	99.7±0.3
+ Cl_2MBP	10^{-7}	563.5±121.4	99.0±0.6
+	10^{-6}	620.5±76.4	99.4±0.5
+	10^{-5}	594.5±105.3	99.7±0.3
+	10^{-4}	72.0±16.5§	97.6±0.2§
+ AHHexBP	10^{-7}	509.5±23.1	99.0±0.56
+	10^{-6}	668.0±130.3	99.4±0.67
+	10^{-5}	847.5±149.8	99.3±0.38
+	10^{-4}	133.3±27.0§	96.7±0.76§
+ AHPrBP	10^{-7}	527.5±55.9	99.0±0.6
+	10^{-6}	668.8±100.8	99.1±0.9
+	10^{-5}	868.8±195.4	99.7±0.3
+	10^{-4}	85.0±11.9§	96.3±1.7§
+ 3-PHEBP	10^{-7}	618.5±73.9	99.7±0.3
+	10^{-6}	529.8±124.5	99.2±0.4
+	10^{-5}	815.0±199.5	98.2±1.0
+	10^{-4}	0.0±0.0§	88.0±3.6§

Viability was estimated by numbers of adherent cells present after 3 wk in culture and by trypan blue exclusion after 24 h. The number of adherent cells per well refers not to the total number of cells in each well, but to the number counted using the sampling technique described in Methods.
‡ $P < 0.01$; § $P < 0.001$.

tion and expression of the antigen recognized by 13 C2, as these compounds have been extensively studied in vivo and in vitro and have potent effects on MNC formation. Neither 10^{-5} M AHPrBP nor 10^{-5} M Cl_2MBP significantly inhibited the effect of GCT-CM when added during the first week of culture, although AHPrBP significantly inhibited the effect of 1,25 D when present only during this period (Table III). When the PCPs were present for the entire culture period, MNC formation was inhibited whatever the culture conditions during the first week. These results suggest that precursor proliferation is not the major site of the inhibitory action of PCPs. The data shown in Table III also demonstrate that in order to have their maximal inhibitory effect, the PCPs need to be present for the entire culture period.

The marrow cultures contain a heterogeneous population of both mononuclear and multinucleated cells. A change in the number of MNC may therefore not necessarily reflect a change in osteoclast number. The specificity of the inhibitory effects of PCPs for osteoclast-like rather than non-osteoclast-like MNC (presumably macrophage polykaryons) was assessed by counting the number of cells expressing the osteoclast antigen recognized by the MAb 13 C2. The proportion of 13 C2-positive mononuclear cells was also measured, as changes in the proportion of these cells may indicate an effect on the differentiation of the mononuclear osteoclast precursor. AHPrBP and Cl_2MBP decreased the numbers of 13 C2-positive MNC in a dose-dependent manner (Table IV). In the presence of 10 mM 1,25 D, 65.1% of the MNC and 33.0% of the mononuclear cells stained positively for 13 C2. Both AHPrBP and Cl_2MBP decreased the proportion of 13 C2-positive mononuclear and multinucleated cells at 10^{-5} M, and AHPrBP also had these effects at 10^{-6} M (Fig. 2).

Discussion

Some of the MNC that form in long-term cultures of human marrow appear to be osteoclasts by several criteria. These cells

Table III. Effects of AHPrBP and Cl_2MBP on GCT-CM-stimulated MNC Precursor Proliferation in Long-term Marrow Cultures

Treatment during week 1			Treatment during weeks 2 and 3		
GCT-CM (10%)	1,25 D (10 nM)	PCP (10^{-5} M)	1,25 D (10 nM)	PCP (10^{-5} M)	MNC/well
					mean±SEM
			+		68.8±6.5
		AHPrBP	+		66.0±6.4
		AHPrBP	+	AHPrBP	24.0±3.5‡
		Cl_2MBP	+		59.8±7.6
		Cl_2MBP	+	Cl_2MBP	36.3±6.8*
+			+		339.3±48.5
+		AHPrBP	+		284.3±12.9
+		AHPrBP	+	AHPrBP	43.8±11.0§
+		Cl_2MBP	+		304.8±39.3
+		Cl_2MBP	+	Cl_2MBP	53.0±14.3§
	+		+		197.0±14.9
	+	AHPrBP	+		114.8±18.9*
	+	AHPrBP	+	AHPrBP	48.8±5.4§
	+	Cl_2MBP	+		148.3±18.9
	+	Cl_2MBP	+	Cl_2MBP	69.4±13.9§

Data shown are mean±SEM of four replicates. * Significantly different from appropriate control ($P < 0.05$). ‡ Significantly different from appropriate control ($P < 0.01$). § Significantly different from appropriate control ($P < 0.001$).

Table IV. Effects of AHPrBP and Cl_2MBP on the Expression of the Osteoclast Antigen Recognized by the MAb 13 C2

Treatment		No. of 13 C2-positive cells	Total cells
1,25 D (10 nM)	PCP		
	M	*Mean±SEM*	*Mean±SEM*
Mononuclear cells			
−	−	17.0±1.4	81.8±12.4
+	−	21.8±1.8	64.8±8.4
+ AHPrBP	10^{-7}	28.7±5.1	78.5±11.4
+	10^{-6}	24.8±4.6	100.3±13.2
+	10^{-5}	21.6±4.6	102.5±7.2*
+ Cl_2MBP	10^{-7}	25.2±3.8	75.3±12.0
+	10^{-6}	24.0±6.1	78.2±21.9
	10^{-5}	23.5±5.4	93.2±9.4
MNC			
−	−	5.0±0.0	11.0±1.0
+	−	85.0±10.0	130.5±18.5
+ AHPrBP	10^{-7}	46.5±4.5‡	85.0±9.0
+	10^{-6}	32.0±3.0‡	61.0±12.0‡
+	10^{-5}	22.5±10.5§	48.5±8.5‡
+ Cl_2MBP	10^{-7}	56.0±1.0*	91.5±10.5
+	10^{-6}	38.0±9.0‡	68.5±22.5*
+	10^{-5}	24.0±5.0§	47.5±13.5‡

"No. 13 C2-positive cells" and "Total cells" refer to mean±SEM per field using the sampling pattern described in Methods for the cytotoxicity experiments for the mononuclear cells (eight fields each from duplicate wells) or mean±SEM per well for the MNC (using the same duplicate wells). The values obtained by calculating the percentages of mononuclear and multinucleated cells reacting with the 13 C2 MAb for each particular treatment in this experiment are shown in Fig. 2.

* Significantly different from appropriate 1,25 D-treated control, $P < 0.05$.

‡ Significantly different from appropriate 1,25 D-treated control, $P < 0.01$.

§ Significantly different from appropriate 1,25 D-treated control, $P < 0.001$.

have the biochemical characteristics of osteoclasts in that they express a number of osteoclast-specific antigens and the enzyme tartrate-resistant acid phosphatase which is generally considered to be osteoclast-specific in vivo. Secondly, they are morphologically similar to osteoclasts under transmission electron microscopy and have been shown in this and other laboratories to form resorption pits in devitalized bone (21, 28, 29). Finally, they respond to stimulators (21, 30) and inhibitors (21, 31) of bone resorption with appropriate increases or decreases in MNC numbers. Indeed, all stimulators of bone resorption so far tested in this system, including the cytokines tumor necrosis factor and IL-1 (unpublished results) have increased MNC numbers. This study provides further evidence for this by showing that PCPs, which are potent inhibitors of bone resorption, also inhibit MNC formation.

We have shown that five PCPs all inhibited the formation of MNC in long-term human marrow cultures stimulated by 1,25 D. 1,25 D was chosen because it consistently stimulates MNC formation in long-term marrow cultures and is a well-recognized stimulator of bone resorption. The potencies of these compounds in this system were greater than in any in vitro system reported to date. Furthermore, the relative potencies in vivo of the four of these compounds that have been used therapeutically (AHPrBP> AHHexBP> Cl_2MBP> HEBP, references 1 and 26) are the same as observed in our studies. 3-PHEBP has not been used in humans, but is more potent than the other compounds in animal models (32). Given that five compounds could have 120 different orders of potency, the probability of the same order occurring by chance in three experiments is 1 in 120^3 or 6×10^{-7}. The inhibition of MNC formation shown in these experiments may therefore be the major action of PCPs in vivo.

The inhibitory effect of AHPrBP and Cl_2MBP on the expression by MNC of the antigen recognized by 13 C2 suggests that PCPs inhibit the formation of osteoclast-like MNC more strongly than they inhibit the formation of non–osteoclast-like MNC. Although 13 C2 also reacts with renal proximal tubule cells and glomerular viscera, it can be considered as osteoclast-specific in the context of bone marrow cultures, as it does not react with other hematopoietic cells (24). This MAb also inhibits the resorption of devitalized bone by isolated osteoclasts (33), thus suggesting that it binds to an important functional site on the osteoclast. The identity of the mononuclear cells that react with 13 C2 is uncertain, but as neither monocytes nor bone marrow–derived macrophages react with this antibody, these cells are probably closely related to the osteoclast, possibly being precursors. Fuller and Chambers (34) have shown that when cultures of rabbit bone marrow are grown on devitalized bone, the presence of resorption pits detected by scanning electron microscopy correlates with the presence of mononuclear cells or cells of low multinuclearity reacting with another anti-osteoclast monoclonal (23 C6) in parallel cultures. This MAb like 13 C2, was raised by Dr. M. A. Horton against giant cell tumor osteoclast-like cells, and reacts with the same protein as 13 C2 (35). Furthermore, expression of tartrate-resistant acid phosphatase by mononuclear cells has

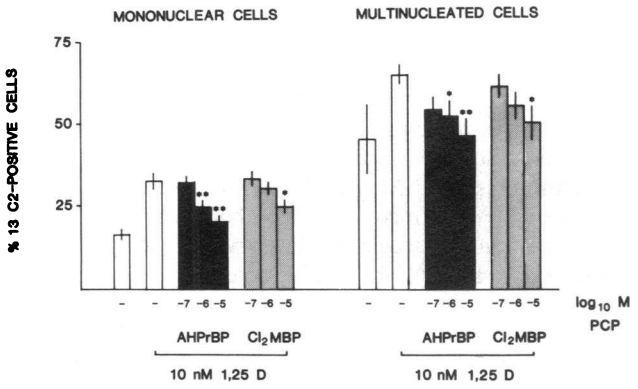

Figure 2. Effects of AHPrBP and Cl_2MBP on expression of the osteoclast antigen recognized by the MAb 13 C2. Data shown are percentage of cells staining positively for 13 C2±SEM from samples taken as described in Methods, calculated as total 13 C2-positive cells divided by total cells counted × 100%, from the data shown in Table II. *Significantly different from appropriate 1,25 D-treated control, $P < 0.05$. **Significantly different from appropriate 1,25 D-treated control, $P < 0.01$.

been demonstrated both in the marrow culture system described here (21) and on bone surfaces in vivo immediately before the appearance of osteoclasts in the bone remodeling sequence (36). These observations support the concept that the osteoclast precursor may express features of the osteoclast phenotype during the later stages of its differentiation, and that such cells may be present in long-term marrow cultures. Furthermore, recent studies suggest that the protein with which 13 C2 reacts is a receptor for a matrix protein (35). It is generally believed that mononuclear osteoclast precursors attach themselves to the bone surface before fusing (36) and it would therefore seem likely that these cells express such receptors. Addition of 1,25 D, which is a potent differentiating agent in other cell systems, increased the proportion of mononuclear and multinucleated cells expressing the 13 C2 antigen. However, this effect was abolished by simultaneous addition of PCPs. The decrease in the proportion of 13 C2–positive mononuclear cells observed in the presence of PCPs may therefore indicate that these compounds inhibit the differentiation of osteoclast precursors. This observation also suggests that PCPs do not simply inhibit fusion of osteoclast precursors, because if this were the case, increased numbers of 13 C2–positive mononuclear cells would be expected in PCP-treated cultures. A more precise interpretation of these results requires a better understanding of the identity of the various cell populations present in long-term marrow cultures.

Transforming growth factor-alpha and epidermal growth factor stimulate the formation of MNC in these cultures through enhanced precursor proliferation (30). Granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor, both of which are present in GCT-CM, have also been shown to have this effect in long-term cultures of baboon marrow (37). Neither AHPrBP nor Cl_2MBP significantly inhibited GCT-CM-stimulated precursor proliferation during the first week of culture. This might appear to contradict the findings of Cecchini et al. (38) who observed that three PCPs, including AHPrBP and Cl_2MBP, inhibited granulocyte-macrophage and macrophage colony-stimulating factor-stimulated colony formation in murine marrow cultures. However, these effects may be overcome in long-term cultures, and probably do not occur to a significant extent in vivo, as suppression of myelopoiesis has very rarely been reported in PCP-treated patients. Studies by Boonekamp et al. (17, 39) using organ culture systems have suggested that PCPs have two modes of action on osteoclastic bone resorption, inhibiting both the accession (migration to the bone surface and fusion) of osteoclast precursors and resorption by the mature osteoclast. Higher concentrations (10^{-5} M and above) were required to inhibit resorption by preformed osteoclasts than were required to inhibit accession of osteoclast precursors (10^{-6} M and above, in the case of AHPrBP). The relative potencies of the three compounds tested in these systems (AHPrBP, Cl_2MBP, and HEBP) also differed; Cl_2MBP inhibited resorption by mature osteoclasts most strongly, whereas the relative potencies of these three compounds in inhibiting osteoclast precursor accession were the same as those seen in our studies. It would therefore appear that although they are capable of acting directly on adult osteoclasts, PCPs inhibit bone resorption primarily by an effect on the formation of osteoclasts that appears to be at the level of the postmitotic precursor.

Long-term marrow culture may, therefore, prove to be a useful ex vivo technique for studying the efficacy of new therapeutic agents and the mechanism of action of existing ones. Bisphosphonates are potentially the most efficacious therapeutic agents for treating diseases of increased bone turnover such as Paget's disease and many cases of hypercalcemia of malignancy. Furthermore, PCPs may prove to have applications in other diseases of bone and mineral metabolism such as hyperparathyroidism (7) and certain forms of osteoporosis (40). It has been shown in a single case that bone marrow taken from a patient with hyperparathyroidism formed MNC in greater numbers than normal controls and that this effect was reversed after parathyroidectomy (21). The ability to study MNC formation in disease states illustrates the usefulness of this system for the study of the pathophysiological and pharmacological control of bone resorption. Unfortunately, the first PCP available for general use, etidronate (HEBP), causes mineralization defects during long-term administration (3, 41) and therefore should in time be replaced by more potent compounds (such as AHPrBP, Cl_2MBP, or AHHexBP) that lack this side effect at therapeutic doses. Other new, highly potent PCPs, for example 3-PHEBP, are currently being studied. Long-term marrow cultures may prove to be a valuable screening technique for these compounds.

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