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Effect of Promethazine Hydrochloride on Bone Resorption in Tissue Culture[1] (41316)

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Abstract. The effect of promethazine hydrochloride, an established macrophage inhibitor, on bone resorption in tissue culture was examined. At nontoxic concentrations (5–25 μg/ml), promethazine hydrochloride inhibited, in a dose–response fashion, parathyroid extract-stimulated bone resorption and bone resorption in a bone remodeling system. Histological examination of sections prepared from cultured bones treated both with parathyroid extract and promethazine hydrochloride revealed an increase in number of macrophages in promethazine hydrochloride-treated cultures. At higher concentrations of this compound, but still within the same range of generally nontoxic concentrations (5–25 μg/ml), the macrophages appeared to be damaged. It is suggested that promethazine hydrochloride inhibits bone resorption in tissue culture by impairing macrophage function.

We have recently reported that sodium aurothiomalate, a gold salt that is used with beneficial effects in the treatment of rheumatoid arthritis, inhibits parathyroid extract-stimulated bone resorption in tissue culture. Histological examination of the cultured bones indicated that the gold salt was concentrated primarily in macrophages (1). Since it has been shown by others that gold salts impair the function of macrophages and monocytes (2–4), we postulated that the inhibition of bone resorption by sodium aurothiomalate might have a similar origin. In order to test this hypothesis, we studied the effect of promethazine hydrochloride on *in vitro* bone resorption. This compound, a phenothiazine derivative which is a depressor of central nervous system and H_1 receptor antagonist, has been reported to be a potent *in vitro* and *in vivo* macrophage inhibitor (5). Preliminary results of this study have been presented previously (6).

Material and Methods. For these studies two bone culture systems using 5-day-old mouse calvaria (7, 8) have been employed. The first, our standard "bone resorption system," consists of 60% heat-inactivated horse serum, 40% Gey's balanced salt solution, 100 u/ml penicillin, 100 μg/ml streptomycin, and 10 u/ml heparin. It uses parathyroid extract as the positive control and gives rise to various degrees of resorption depending on the potency and concentration of the bone resorption stimulating and/or inhibiting factor(s) present in the medium. During the 7-day maintenance period of the cultures, they were refed with fresh medium and regassed at two 2-day and one 3-day interval. Also, at these intervals, each bone culture was examined microscopically for extent of resorption present and scored essentially as described by Susi *et al.* (9). At the termination of all experiments, calvaria were fixed in formalin, sectioned serially, and stained with hematoxylin and eosin for microscopic examination. In addition, the final resorption scores observed in the living cultures were transformed into percentages as one index of the extent of bone resorption. Calcium released into the medium was used as a second index of the extent of resorption. Media collected at each interval and at the termination of the experiment were analyzed for calcium content using a Corning Calcium Analyzer Model 940. The individual values for calcium content that were obtained at 2- or 3-day intervals of the 7-day life span of the culture were summed to give the total

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calcium released. Appropriate corrections were made for calcium present initially in the various batches of freshly prepared media. Both sets of data were subjected to an analysis of variance.

The second, our "bone remodeling system," consists of 20% heat-inactivated horse serum, 20% chicken embryo extract, 60% Gey's balanced salt solution, 100 u/ml penicillin, 100 μg/ml streptomycin, and no heparin. It does not employ parathyroid extract, but over a 2-week period gives rise to both a moderate amount of bone resorption and excellent new osteoid formation due to the presence of unknown factors contained in the chicken embryo extract component of the medium. The remodeling system is utilized to determine whether a compound that inhibits resorption at a specific concentration in the bone resorption system also inhibits new osteoid formation or whether it has a differential effect on bone resorption versus new osteoid formation. Due to the formation of a calcium precipitate inherent in this system, the measurement of calcium in the medium cannot be used as an index of the extent of bone resorption. Therefore, in this system, the extent of bone resorption is determined only by microscopic observation of living cultures and scoring, as described above. In this series of experiments, the serial sections of all bones were examined histologically for the presence of new osteoid. The amount of new osteoid was scored as follows: 5—excellent new osteoid, 4—very good new osteoid, 3—good new osteoid, 2—fair new osteoid, 1—slight new osteoid, 0—no new osteoid, and T—toxic, where most of the cells are dead.

Results. As may be seen from Table I, there was a good correlation of results whether obtained by microscopic scoring of the living cultures or by measuring calcium

TABLE I. EFFECT OF PROMETHAZINE HYDROCHLORIDE ON PARATHYROID EXTRACT-STIMULATED BONE RESORPTION

Experiment No.	Treatment		Percentage bone resorption assessed microscopically (±SEM)	Calcium released into medium (mg/dl ± SEM)
	Parathyroid extract (u/ml)	Promethazine hydrochloride (μg/ml)		
1	—	—	8 ± 3.4	−0.4 ± 0.51
	0.1	—	74 ± 3.4	13.4 ± 0.51
	0.1	25	13 ± 3.4***	2.7 ± 0.51***
	0.1	10	63 ± 3.4*	10.3 ± 0.51***
	0.1	1	72 ± 3.4	12.5 ± 0.51
2	—	—	11 ± 4.4	2.2 ± 0.80
	0.1	—	88 ± 4.4	11.7 ± 0.80
	0.1	25	8 ± 4.4***	−0.6 ± 0.80***
	0.1	10	5 ± 4.4***	5.6 ± 0.80***
3	—	—	6 ± 3.0	0.2 ± 0.61
	0.1	—	91 ± 3.0	13.4 ± 0.61
	0.1	25	12 ± 3.0***	1.7 ± 0.61***
	0.1	20	51 ± 3.0***	4.8 ± 0.61***
	0.1	15	55 ± 3.0***	6.3 ± 0.61***
	0.1	10	66 ± 3.0***	8.7 ± 0.61***
4	—	—	13 ± 3.2	3.3 ± 0.80
	0.1	—	83 ± 3.2	14.0 ± 0.80
	0.1	20	5 ± 3.2***	2.0 ± 0.80***
	0.1	15	29 ± 3.2***	5.0 ± 0.80***
	0.1	10	57 ± 3.2***	9.6 ± 0.80***
	0.1	5	74 ± 3.2*	12.2 ± 0.80

* $P < 0.05$ compared to parathyroid extract-treated control.
*** $P < 0.001$ compared to parathyroid extract-treated control.

released into the medium. The nontreated control cultures had a low basal resorption, whereas the control containing PTE demonstrated extensive bone resorption. Promethazine hydrochloride, in concentrations ranging from 10 to 25 μg/ml, significantly inhibited PTE-stimulated bone resorption in all four experiments. Histological examination of sections prepared from bones cultured in the resorption system demonstrated that PTE treatment alone resulted in a typical *in vitro* picture of bone resorption accompanied by large numbers of osteoclasts. Of interest was the repeatedly observed finding that the heparin-containing control cultures developed large numbers of healthy macrophages over the 7-day culture period as compared to cultures that were not exposed to heparin (unpublished result). The presence of PTE in the culture medium together with heparin resulted in a significant decrease in the number of macrophages. However, the addition of promethazine hydrochloride to media containing both heparin and PTE seemed to reverse the decrease in macrophage number caused by PTE. At higher concentrations of promethazine hydrochloride but still within the same range of concentrations tested in the present study (5–25 μg/ml), the number of macrophages appeared to be increased beyond that found in heparin-treated controls. However, at these higher concentrations, the macrophages were frequently vacuolated and appeared to be damaged. Cultures treated with 50 μg/ml or more of promethazine hydrochloride were in most instances severely damaged or dead.

In the experiments conducted in the bone remodeling system, two parameters were assessed, namely bone resorption and new osteoid formation. As may be seen from Table II, promethazine hydrochloride, at concentrations ranging from 5 to 25 μg/ml, inhibits bone resorption significantly without preventing new osteoid formation. At 50 μg/ml, promethazine hydrochloride was toxic.

Discussion. The results of these experiments clearly indicate that promethazine hydrochloride, like sodium aurothiomalate, can significantly inhibit bone resorption in both our parathyroid extract-containing bone resorption system and in our bone remodeling system at concentrations that do not prevent new osteoid formation. The findings support the hypothesis that both compounds exert their effects by impairing macrophage function. As mentioned previously, promethazine hydrochloride has

TABLE II. EFFECT OF PROMETHAZINE HYDROCHLORIDE ON BONE RESORPTION AND BONE FORMATION IN THE BONE REMODELING SYSTEM

Experiment No.	Promethazine hydrochloride (μg/ml)	Percentage bone resorption assessed microscopically (±SEM)	New osteoid assessed microscopically
1	—	35 ± 2.7	4.5
	50	0***	T
	25	0***	1.0
	10	7 ± 2.7***	3.0
2	—	21 ± 1.8	5.0
	20	0***	4.0
	15	2 ± 1.8***	3.0
	10	4 ± 1.8***	4.0
	5	13 ± 1.8***	4.0
3	—	30 ± 2.5	5.0
	25	0***	2.5
	20	3 ± 2.5***	3.5
	15	2 ± 2.5***	4.5
	10	11 ± 2.5***	4.5
	5	14 ± 2.5***	4.5

*** $P < 0.001$ compared to nontreated control.

been reported to be a potent *in vitro* and *in vivo* macrophage inhibitor. It has been shown to inhibit glucose oxidation both in resting and phagocytizing rabbit alveolar macrophages, and *in vivo* to suppress the activation of alveolar macrophages by heat-killed Bacillus Calmette–Guerin (5). For several decades macrophages or monocytes have been implicated in bone resorption (10–14). In the past 10 years, additional evidence has accumulated to corroborate the involvement of these cells in the bone resorption process (1, 15–20). In the present study, it is of interest that PTE decreased the number of macrophages and increased the number of osteoclasts, whereas the presence of promethazine hydrochloride in the cultures that contained PTE apparently reversed this trend. This observation suggests that PTE acts on precursor cells and favors the differentiation of osteoclasts, whereas promethazine hydrochloride, in some unknown fashion, acts on the same precursor cells, but favors their differentation into macrophages. In addition, particularly at higher concentrations, promethazine hydrochloride damages these macrophages and apparently prevents them from participating directly or indirectly in the bone resorption process. The effects of promethazine hydrochloride resulting in its bone resorption inhibitory activity may be due in part to its lipophilic property, since the hydrophobic nature of phenothiazine drugs is a well-established fact (21). As a lipophilic agent, it may penetrate the lipid domains of cell membranes and thereby block the participation of membrane components in the cellular alteration required for the bone resorption process. Indeed, at approximately the same concentrations at which promethazine hydrochloride was used in this study, phenothiazine derivatives were shown to stabilize cell membranes in other systems (22), to suppress cell locomotion (23), and to affect several kinds of cell-to-cell interactions, such as cell fusion (24) and platelet adhesiveness (25). It should be noted at this point that both sodium aurothiomalate and promethazine were shown to be moderately potent inhibitors of prostaglandin biosynthesis (26, 27). In view of the relationship of collagenase and bone resorption (28, 29), it is of interest that prostaglandin biosynthesis appears to be essential for the elaboration of collagenase by macrophages (30). Since indomethacin does not inhibit parathyroid extract-stimulated bone resorption *in vitro* (31), we cannot explain the promethazine hydrochloride- or sodium aurothiomalate-inhibited bone resorption on the basis of prostaglandin biosynthesis inhibition.

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