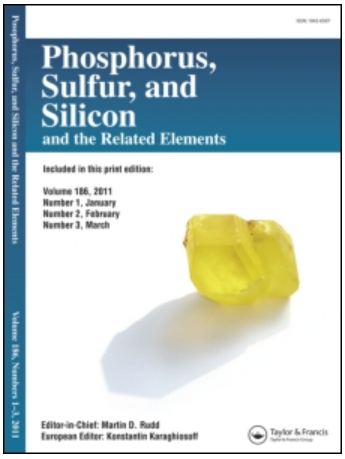


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

In Vitro and In Vivo Inhibition of Hydroxyapatite Formation and Dissolution by Bisacylphosphonates and Bishydroxyiminophosphonates

E. Breuer[a]; G. Golomb[a]; A. Hoffman[a]; A. Schlossman[a]; J. M. Van Gelder[a]; H. Saadeh[a]; M. Levi[a]; Y. Eitan[a]
[a] The Departments of Pharmaceutical Chemistry and of Pharmacy, School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel

To cite this Article Breuer, E. , Golomb, G. , Hoffman, A. , Schlossman, A. , Van Gelder, J. M. , Saadeh, H. , Levi, M. and Eitan, Y.(1993) 'In Vitro and In Vivo Inhibition of Hydroxyapatite Formation and Dissolution by Bisacylphosphonates and Bishydroxyiminophosphonates', Phosphorus, Sulfur, and Silicon and the Related Elements, 76: 1, 167 — 170
To link to this Article: DOI: 10.1080/10426509308032385
URL: http://dx.doi.org/10.1080/10426509308032385

Phosphorus, Sulfur, and Silicon, 1993, Vol. 76, pp. 167-170

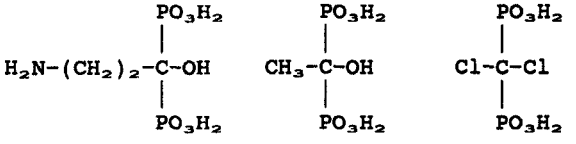
In Vitro and In Vivo Inhibition of Hydroxyapatite Formation and Dissolution by Bisacylphosphonates and Bishydroxyiminophosphonates

E. Breuer, G. Golomb, A. Hoffman, A. Schlossman, J. M. Van Gelder, H. Saadeh, M. Levi, and Y. Eitan,

The Departments of Pharmaceutical Chemistry and of Pharmacy, School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem 91120, Israel

Introduction

Certain irregularities in calcium metabolism such as Paget's disease, osteoporosis, as well as osteolysis in bone cancer, are characterized by excessive destruction of the bone by resorption. In contrast, a number of clinically important diseases, for example, atherosclerosis, kidney and renal calculus, arthritis, and bioprosthetic heart valve calcification are characterized by the deposition of calcium phosphate. Bisphosphonates are a relatively new class of drugs, that have been used in these diseases to inhibit both mineralization and bone resorption, following the recognition, that they are stable analogs of endogenous pyrophosphate, which is a physiological inhibitor of calcification and bone resorption.[1] A number of geminal bisphosphonates, namely, Pamidronate (ABP), Etidronate (HEBP), and Clodronate (ClMBP), have been approved for clinical use.



Pamidronate ABP | Etidronate HEBP | Clodronate ClMBP

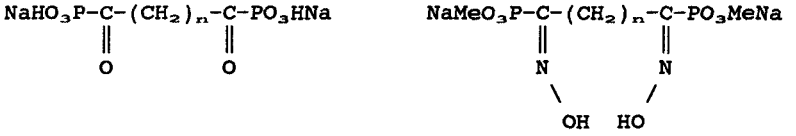
Since current research in this field has been confined solely to P-C-P type compounds, we considered it of interest to extend the synthetic effort to other types of structures which can reasonably be expected to interact with hydroxyapatite (HAP) and with calcium, and yield new biologically active compounds. In vitro and in vivo testing of new chemical classes of bisphosphonates would provide increased understanding of the relationship between chemical structure and biological ac-

tivity, and might stimulate the development of new generations of such drugs. In contrast to geminal bisphosphonates, monophosphonates, vicinal bisphosphonates (compounds characterized by the type of structure: P-C-C-P), and compounds in which the distance between the phosphoryl groups is greater (P-(C)n-P, n>2) have been reported to be inactive with respect to calcium-related disorders.[2] We hypothesized that long chain bisphosphonates might be made active in mineralization-related disorders, by introducing into them additional donor groups in positions adjacent to the phosphonic functions. In our approach to the problem of designing novel types of compounds to interact with calcium, we decided to concentrate on α,α'-diketobisphosphonates and their oximes as compounds having two calcium binding sites, each binding site consisting of one phosphonate function combined with an additional group capable to serve as a ligand. In this communication we wish to report on the synthesis and the study of anticalcification and antiresorptive properties of some bisacylphosphonates and bishydroxyiminophosphonates, studied in vitro and in vivo. We found that compounds belonging to these classes interact with HAP formation and dissolution in vitro, and inhibit in vivo both the pathological calcification of bioprosthetic heart valve tissue cusps and the release of tetracycline from bone, which is a means for measuring bone resorption.

Results and Discussion

Synthesis

Following previous reports from our laboratory on the synthesis and characterization of simple acylphosphonic acids[3] and oximes[4] we prepared the two bisacylphosphonate salts reported here, AdBP and SuBP, from the corresponding tetramethyl bisacylphosphonates, described earlier.[5] The disodium dimethyl bishydroxyiminophosphonates AdBPDOx and SuBPDOx were synthesized from the corresponding tetramethyl bisacylphosphonates by treatment with two equivalents of sodium iodide, which gave disodium dimethyl bisacylphosphonates, which were subsequently converted to the corresponding oximes by hydroxylamine.



n = 4, (AdBP); n = 6, (SuBP) n = 4, (AdBPDOx); n = 6, (SuBPDOx)

In vitro and in vivo characterization of bisphosphonates' activity

To characterize the anticalcification and antiresorptive properties of the new compounds, four experimental models were utilized: 1) inhibition of calcium phosphate (HAP) formation, 2) inhibition of HAP dissolution, 3) inhibition of the pathological calcification of bioprosthetic tissue implanted subdermally in rats, and 4) inhibition of the release of tetracycline absorbed in bone, as a means to monitor bone resorption. The inhibitory effect on the formation and dissolution of HAP of the new bisacylphosphonates and the new bishydroxyiminophosphonates was compared to a monoacylphosphonate a monohydroxyiminophosphonate, to 1,6-hexanebisphosphonic acid, to the clinically used bisphosphonates (ABP, ClMBP, and HEBP), and to the chelating agent EDTA.

1. Inhibition of hydroxyapatite (HAP) formation

The inhibitory effect of the various bisacylphosphonates and bishydroxyiminophosphonates on HAP formation was examined in supersaturated calcium phosphate solution. The results which will be presented show that all compounds significantly prevented calcium precipitation.

2. Inhibition of hydroxyapatite dissolution

The inhibition of HAP dissolution by the various compounds was examined by monitoring, over time at pH 5, the calcium and phosphate dissolution from the HAP-adsorbed bisphosphonate. The results from the HAP dissolution inhibition by the various bisacylphosphonates and bishydroxyiminophosphonates show that some of the bisacylphosphonates examined significantly inhibited HAP dissolution in vitro. In contrast, the bishydroxyiminophosphonates examined were found to be inactive.

3. Inhibition of ectopic calcification

The anticalcification effect of the novel bisphosphonates in vivo, was studied by examining the therapy of pathological calcification of bioprosthetic heart valve tissue implanted subdermally in rats, along with an ALZET osmotic pump delivering a solution of the drug tested. This is a representative model for cardiovascular calcific diseases.

The results show that both bisacylphosphonates and bishydroxyimino-phosphonates inhibited the calcification. The anticalcification effect of the various compounds was not associated with adverse effects on somatic growth, as could be seen from the weight gain observed in all experimental groups.

4. Inhibition of tetracycline (TC) release from bone

The influence of adipoylbisphosphonate (AdBP) on bone resorption in vivo was determined by monitoring the rate of release of tetracycline from the bone by measuring the amount of TC in the blood of rats which were pretreated by injections of tritiated TC. The results which will be presented show that this new bisphosphonate inhibits bone resorption in vivo, without having the adverse side effects of the currently used bisphosphonate ADP: namely impairment of bone mineralization, nephrotoxicity and inhibition on somatic growth. In addition, preliminary acute toxicological studies on bisacylphosphonates also show that the tolerated dose found (neither deaths nor weight loss) for AdBP was markedly higher than that for ADP. The apparent lower toxicity, and the better solubility properties of the newly reported bisacylphosphonates clearly indicate their potential for clinical applications.

Acknowledgment Supported in part by the German-Israeli Foundation for Scientific Research & Development.

References

1. H. Fleisch, Bone 8:S23 (1987); F. J. Schoen, H. Harasaki, K. M. Kim, H. C. Anderson, and R. J. Levy. J. Biomed. Mater. Res. 22:11-36 (1988).

2. M. D. Francis and R. R. Martodam, In R. L. Hilderbrand (ed.), The Role of Phosphonates in Living Systems, CRC Press, Boca Raton, 1983, pp. 55-96.

3. R. Karaman, A. Goldblum, E. Breuer, and H. Leader. J. Chem. Soc. Perkin Trans. 1 765-774 (1989).

4. E. Breuer, R. Karaman, A. Goldblum, D. Gibson, H. Leader, B.V.L. Potter, and J.H. Cummins, J. Chem. Soc. Perkin Trans. 1, 1988, 3047 - 3057.

5. R. D. Moss, US Patent No. 3,012,054, Chem Abstr. (1960), M. Kanaan and R. Burgada, Phosphorus & Sulfur, 37, 217 (1988).