

BISPHOSPHONATES: STRUCTURE-ACTIVITY RELATIONS FROM A CLINICAL PERSPECTIVE

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Summary Bisphosphonates, synthetic compounds containing two phosphonate groups bound to a central (geminal) carbon (P-C-P) and two additional chains (R_1 and R_2 , respectively) bind strongly to calcium crystals, inhibit their growth and suppress osteoclast-mediated bone resorption. The availability of two side chains allows numerous substitutions and the development of a variety of analogs with different pharmacological properties. The R_1 structure together with the P-C-P are primarily responsible for binding to bone mineral and for the physicochemical actions of the bisphosphonates and a hydroxyl group at R_1 provides optimal conditions for these actions. The R_2 is believed to be responsible for the antiresorptive action of the bisphosphonates and small modifications or conformational restrictions of this part of the molecule may result in marked differences in antiresorptive potency. The presence of a nitrogen function in an alkyl chain or in a ring structure in R_2 greatly enhances the antiresorptive potency and specificity of bisphosphonates for bone resorption and most of the newer potent bisphosphonates contain a nitrogen in their structure. Recent evidence, however, suggests that the whole bisphosphonate molecule is essential for antiresorptive action. Thus, although the basic structural requirements for bisphosphonate actions have been defined, precise structure-activity relations have not been defined yet. These are essential for elucidating their molecular mechanism of action and for the rational design of compounds for various clinical indications.

Key words: bisphosphonate, chemical structure, antiresorptive action

Bisphosphonates bind strongly to calcium crystals, inhibit their growth and suppress osteoclast-mediated bone resorption. They have two phosphonate groups linked to a central (geminal) carbon, the so-called P-C-P bond, and two additional chains, R_1 and R_2 , respectively (Fig. 1). The availability of these two chains in the structure of geminal bisphosphonates allows the introduction of numerous substitutions which have resulted in the synthesis of a variety of analogs with different pharmacological properties. Although detailed

studies from various laboratories have greatly improved our current knowledge of the structure-activity relations of bisphosphonates, there are still many questions which remain unanswered. Defining such relations is necessary for exploring further their molecular mechanism of action and may lead to rational design of bisphosphonates rather than to empirical choices which has been the case up until recently. The purpose of this short review is to discuss certain structural features of the bisphosphonate molecules which are relevant for their *in vivo* actions. It is not intended as a detailed discussion of structure-activity relations. For that the interested reader is referred to some excellent reviews on the subject^{1, 2}.

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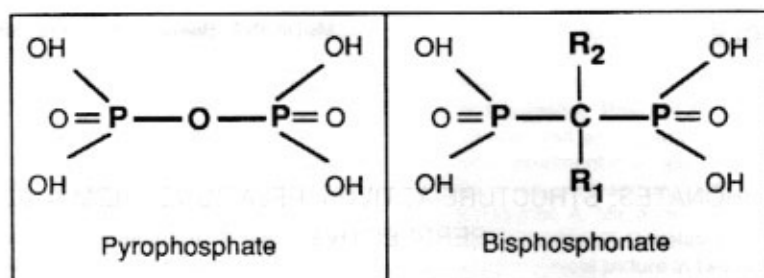


Fig. 1.— Structures of pyrophosphate and geminal bisphosphonates.

General considerations

It is generally believed that the R_1 chain together with the P-C-P part of the molecule are primarily responsible for the binding of bisphosphonates to mineralized matrices. This is why together they are usually referred to as the "bone hook". The R_2 chain is considered primarily responsible for the antiresorptive action of bisphosphonates and is thought to represent the "bioactive moiety". Small modifications or conformational restrictions of this side chain may result in marked differences in antiresorptive potency.

The "bone hook"

An important property of bisphosphonates is their strong affinity for calcium crystals and their selective uptake by mineralized tissues, an essential step in bisphosphonate pharmacology. This property is determined by the structure of the "bone hook". The structural requirements for binding of bisphosphonates to calcium crystals has been studied *in vitro* and it was shown that a hydroxyl or an amino group at R_1 increases binding affinity, whereas substitutions with H, Cl or no substitution decrease the binding of bisphosphonates to bone mineral³. This property is generally independent of the rest of the structure and bisphosphonates with variable R_2 chains but common R_1 bind to bone mineral with equal affinity. This is attributed to tridentate binding of hydroxybisphosphonates to active sites of calcium crystals^{4, 5}. Bidentate binding, as provided by the other substitutions, is less effective. The affinity of bisphosphonates for calcium crystals is mainly responsible for their physicochemical actions, the most important being the inhibition of crystal growth. In early structure-activity studies it was already shown that bisphosphonates can inhibit the growth of hydroxyapatite crystals dose-dependently^{4, 6}. This action is attributed to the structure of R_1 and compounds with a OH substitution are the most effective inhibitors. Changes in the alkyl chain of a series

of hydroxybisphosphonates had almost no effect in the degree of crystal growth while substitutions of R_1 in pentane bisphosphonates (same R_2) altered their ability to inhibit crystal growth in the same order of potency as with binding to the crystals. Similar results were obtained in studies of calcium incorporation to fetal bone explants in culture³. This physicochemical action of the bisphosphonates is probably responsible for their intrinsic property to inhibit the mineralization of bone *in vivo*. However, although all bisphosphonates can inhibit calcification of matrix *in vivo*, this action is clinically relevant only for etidronate which at effective therapeutic doses may induce rickets or osteomalacia.

Most of the bisphosphonates currently in clinical practice or development contain a hydroxyl group in R_1 (e.g. alendronate, etidronate, ibandronate, olpadronate, pamidronate, risedronate, zoledronate) while clodronate and tiludronate have different substitutions (Cl and H, respectively).

The "bioactive moiety"

From the early stages of bisphosphonate development it became apparent that their antiresorptive effect was not merely due to their physicochemical action but that it involved changes in cellular kinetics in the bone microenvironment which depended primarily on the structure of the R_2 chain. The breakthrough in the development of potent bisphosphonates was the recognition of the importance of a nitrogen function in R_2 ^{1, 7}. Consequently all potent bisphosphonates in clinical use contain a nitrogen in their structures. The less potent etidronate, clodronate and tiludronate do not have a nitrogen function. It was originally found that aminoalkyl hydroxybisphosphonates had the same binding affinity to crystals as alkyl hydroxybisphosphonates (e.g. etidronate) but they were much more potent inhibitors of bone resorption (Fig. 2). Although the importance of the nitrogen functionality in increasing the antiresorptive potency and the specificity of bisphosphonates for bone resorption has been unequivocally demonstrated, the mechanism

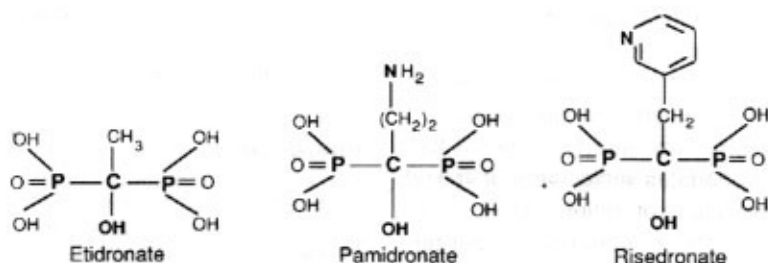


Fig. 2.— Structures of an alkyl, an aminoalkyl and a heterocyclic bisphosphonate (etidronate, pamidronate and risedronate, respectively). Bisphosphonic acid structures are shown; clinically used preparations as indicated by the names are usually the respective mono- or disodium salts.

underlying these actions is not yet known. Furthermore, it has been shown that the length of the R_2 carbon chain is important for antiresorptive potency. An R_2 with 3 carbons, as in alendronate, appears to be the optimal length for the antiresorptive potency of aminobisphosphonates. Reduction of the number of carbons as in pamidronate, or increase as in neridronate, result in decreased potency relative to alendronate. It was further shown that substitution of the hydrogen atoms linked to the nitrogen in pamidronate with either methyl groups or methyl/pentyl groups resulted in the design of compounds more potent than pamidronate. These are olpadronate and ibandronate, respectively, the latter being one of the most potent compounds available. Keeping the nitrogen as the most important function for antiresorptive action, research extended to the synthesis of heterocyclic, potent, nitrogen-containing compounds (e.g. risedronate, cimadronate, zolendronate). The heterocyclic-substituted 1-hydroxy alkane bisphosphonates are very potent but it should be noted that their potency does not always exceed that of aminoalkyl hydroxybisphosphonates^{1, 10}.

The main advantage of these potent compounds is that they have a very wide therapeutic window and concentrations which effectively suppress bone resorption do not impair the mineralization of newly formed matrix. Regarding optimal antiresorptive activity we have at present sufficient molecules for the management of the accepted clinical indications of bisphosphonates. Choices will be made on the basis of their toxicological profile and their ease administration which differ among the various compounds. Further theoretical considerations include possible differences in their mechanism of action and in their effects on the biomechanical properties of bone. However, identification of the precise structure-activity relations is essential for the study of interactions with potential molecular targets, elucidating their mechanism of action and for the design of compounds with optimal properties also for use in other potential indications of bisphosphonate therapy. From the designing point of view, chemists in various laboratories have been experimenting

with other modifications of the bisphosphonate molecule aiming at synthesizing compounds of low affinity for bone and antiresorptive action. This is a very interesting area of research which may eventually help in the identification of molecular targets of bisphosphonates but detailed description of these lies outside the scope of the present review.

The whole molecule

The studies described above provide a good basis for the interpretation of basic structure-function relations relevant to the clinical pharmacology of bisphosphonates. They do not, however, provide yet clear explanations for the great differences in antiresorptive potencies with minor structural modifications. In a series of recent studies in our laboratory we approached the subject from a different angle¹¹. Our starting point was the fact that bone targeting is an essential step in bisphosphonate pharmacology which, as already discussed, depends primarily on the "bone hook" of the molecule, with tridionate binding providing the optimal structure for binding to bone mineral. We, therefore, substituted the hydroxyl group at R_1 of 3 clinically useful bisphosphonates (etidronate, pamidronate, olpadronate) with an amino group which provides also a tridionate binding to calcium crystals. All three R_1 -aminosubstituted compounds and their hydroxy parent compounds bound with similar affinity to bone mineral and inhibited crystal growth to the same extent. Surprisingly, the antiresorptive effect of olpadronate was totally abolished by the aminosubstitution of the hydroxyl group while that of pamidronate was reduced by about 6-fold and that of etidronate did not change. These studies have

now been extended and the results were confirmed in vivo in mice as well as in a pure cellular system representative of bisphosphonate action (the slime mould *Dictyostelium discoideum*)¹². These studies provide now strong evidence that the entire bisphosphonates molecule is involved in the cellular mechanism of antiresorptive action and that there may be more than one cellular target. Moreover, the aminosubstituted analog of olpadronate which lacks any antiresorptive action but retains all other properties of olpadronate, provides an excellent tool to study further specific structure-activity relations at the cellular and molecular levels.

Resumen

Bisfosfonatos: relaciones estructura-actividad desde una perspectiva clínica

Los bisfosfonatos, compuestos sintéticos que contienen dos grupos fosfatos ligados a un carbono central (geminal) (P-C-P) y dos cadenas adicionales (R_1 y R_2 , respectivamente) se ligan fuertemente a los cristales de calcio, inhiben su crecimiento y suprimen la resorción ósea mediada por osteoclastos. La disponibilidad de dos cadenas laterales permite numerosas sustituciones y el desarrollo de una variedad de análogos con diferentes propiedades farmacológicas. La estructura R_1 junto con la P-C-P son las principales responsables de la unión al mineral óseo y de los efectos fisicoquímicos de los bisfosfonatos, en tanto que el grupo hidroxilo en R_1 optimiza las condiciones para estas acciones. Se supone que la R_2 es la responsable por la acción antiresortiva de los bisfosfonatos y pequeñas modificaciones o restricciones conformacionales de esta parte de la molécula podrían resultar en marcadas diferencias en la potencia antiresortiva. La presencia de una función nitrógeno en una cadena alquilo o en una estructura cíclica en R_2 mejora notablemente la potencia antiresortiva y la especificidad de los bisfosfonatos para la resorción ósea y la mayoría de los nuevos bisfosfonatos de gran potencia contienen un nitrógeno en su estructura. Evidencias recientes, sin embargo, sugieren que la molécula entera de bisfosfonato es esencial para la acción antiresortiva. Por lo tanto, aunque los requerimientos estructurales básicos para las acciones de los bisfosfonatos han sido definidos, no lo han sido todavía las relaciones más precisas entre estructura y actividad. Estas son esenciales para

la elucidación de su mecanismo molecular de acción y para el diseño racional de compuestos para distintas indicaciones clínicas.

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