

**POTENTIAL APPLICATIONS OF BIPHOSPHONATES  
IN DENTAL SURGICAL IMPLANTS**

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**For some time numerous studies have been centred on biphosphonates, drugs largely used for their unquestionable properties of inhibiting bone resorption by osteoclast in the treatment of various osteometabolic illnesses such as osteoporosis, multiple myeloma, tumors which metastasize to the bone and malignant hypercalcemia. In this literature review the physico-chemical properties, biologic activities and the action mechanisms of the biphosphonates are described. The use of these drugs is discussed, analyzing the quantity of results which have emerged through *in vitro* and *in vivo* experiments on animal models. In**

**this study the efficiency is demonstrated of these drugs in contrasting the osteolytic processes of the alveolar bone, in promoting the neoformation and in bettering the quality of bone implants. However, it is important to draw attention to a worrying correlation which has emerged during the last 3-4 years, between osteonecrosis of the jaw (ONJ) and the systemic administration of aminobiphosphonates. This collateral effect did not emerge following the use of non-aminobiphosphonates. The aim of this review is to identify the guidelines for the use of biphosphonates in oral implant surgery.**

Today, biphosphonates, for their unquestionable properties of inhibiting bone resorption by osteoclasts, are considered as being the elected therapy for osteometabolic pathologies such as osteoporosis (1-2), Paget's disease (3), tumors which metastasize to the bone, multiple myeloma and malignant hypercalcemia (4-6). Several data and recent revision literature, also recent, describe the chemical structure, the pharmacological and clinical applications of biphosphonates (1, 7-12), but few studies discuss the clinical applications of these drugs in implant surgery. According to the most recent data on the action mechanism and on the relative anti-resorption strength, it is important to distinguish two groups of biphosphonates: the non-amino and the aminobiphosphonates. The former are efficient in inhibiting lower bone resorption compared with the aminobiphosphonates (N-Bf) (11-13). In the last three years, however, a worrying correlation between osteonecrosis of the jaw and the systemic administration of biphosphonates has emerged (14-23) which, from our analysis of the relevant literature, has not emerged for non-aminobiphosphonates. The biological effects of biphosphonates are many and varied, based on the form of administration, the concentration and the type of molecule used. These drugs, in fact, act on skeletal homeostasis, inhibiting resorption mediated by osteoclasts by inhibiting the activity of the mature osteoclasts (24-25), the increase of the processes of apoptosis of the osteoclasts (26) and the inhibition of the formation and recruitment of new osteoclasts (27). Recently, it was noted that some of these drugs can even stimulate the differentiation and the activity of the osteoblasts (28-32) and have numerous and diverse biologic extraskelatal activities (33-40). The osteointegration of the implants represents the result of a series of events which involve all the constitutive elements of the bone. During this process diverse factors intervene which have been taken into consideration and valued singly in the attempt to increase the percentage of implant success, above all, in those cases where the quality and quantity of bone is lacking. We believe it is in this environment that the use of biphosphonates can give a significant contribution. In this regard it has still not emerged which type of biphosphonate is most efficient, the respective dose necessary and, above all, the system of administration. Therefore, the authors, making use of the concepts emerging from the analysis of

the numerous articles in literature, have the aim of identifying guidelines for the future clinical use of biphosphonates in implant surgery.

*Molecular structure, classification and molecular action mechanism of biphosphonates on osteoclasts*

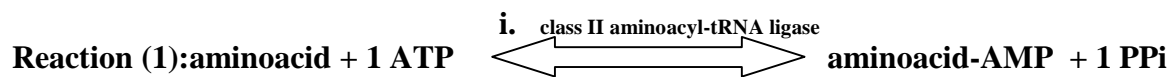
Biphosphonates were developed as non-hydrolysable parallels of the pyrophosphate since the use of the latter, as a strong inhibitor of the precipitation process of calcium phosphate crystals, results extremely limited from a clinical point of view because of the rapid enzymatic degradation regarding the gastrointestinal mucosa (41). The substitution in the molecule of the pyrophosphate of an oxygen atom with a carbon atom, or rather the presence of a bond P-C-P in place of a P-O-P, renders the biphosphonates resistant to rapid hydrolysis on the part of the pyrophosphatase of the organism, and therefore suitable for clinical use (Fig. 1). These drugs maintain the chemical-physical properties of pyrophosphates, such as the high affinity for bone mineralisation. They bind to the calcium phosphate and inhibit the growth, aggregation bonding and dissolution of the calcium phosphate crystals, *in vivo* and *in vitro* (41-42). As well as the anti-mineralising properties, from *in vivo* and *in vitro* studies (43), it emerges that they are also effective in inhibiting bone resorption by osteoclasts. Initially, it was thought that the action of inhibiting bone resorption could be correlated exclusively to the chemical-physical properties of these drugs. Successive studies have disproved this theory. Nowadays, it is clear that biphosphonates inhibit bone resorption by cellular effects on the osteoclasts rather than by a purely chemical-physical mechanism (see below). Because of their high affinity to calcium, biphosphonates adsorb to accessible bone surfaces. In fact, they localize mainly in sites covered by osteoclasts during the phase of resorption, where the calcium ions are more exposed (44). *In vivo* studies have demonstrated the presence of a radiolabelled biphosphonate inside the osteoclast in numerous intracellular vesicles and in other subcellular compartments like cytoplasm, nucleus and mitochondrions (45). These molecules, by a direct effect on these cells, block the bone resorption (46) [Fig. 2 (47)].

The lateral chains R1 and R2 of the basic structure of these drugs are of great importance as they permit being classified and they regulate the biological activity, and therefore the efficiency. Russell affirms that the two phosphate residues together with the lateral chain R1, if presenting a hydroxyl group, are responsible for the high affinity with bone mineral and act as a 'bone hook' (Fig. 3) (48). On the other hand, if a different group is present in R1, as in the clodronate, the bonding with the hydroxylapatite diminishes. The different structure and three-dimensional conformation of the lateral chain R2 determines the biological activity of the molecule, influencing the capacity of the drug to interact with specific cellular functions (48). In the lateral chain R2, the presence of a nitrogen group subdivides the compound into non-amino and aminobiphosphonates

(N-Bf). The latter, of a new generation, have a greater antiresorption potential compared to the former (11-13). To date, the mechanism by which these drugs carry out their action is still not completely clear, nor understood. Certainly, from the first studies carried out, these drugs have the osteoclast as a main cellular target, but they present two distinct molecular action mechanisms.

#### *Non-aminobiphosphonates*

Etidronate (*Didronel®*; Procter and Gamble Pharmaceuticals, Inc., Cincinnati, OH), clodronate (*Bonefos®*; Anthra Pharmaceuticals, Princeton, NJ) and tiludronate (*Skelid®*; Sanofi-Synthe Lab, Inc., New York, NY) present a structure very similar to pyrophosphate, and would seem to be metabolized inside the osteoclasts and macrophages in non-hydrolyzable parallels of the ATP (AppCp) by a reaction catalyzed by classe II aminoacyl-tRNA ligase cytoplasmatic enzymes. This was shown in diverse *in vitro* studies on amoebas such as *Dictyostelium discoideum* (49-50), or on cells of mammals like siml macrophagic murine cells J774 (51). The aminoacyl-tRNA ligase of class II catalyzes a reversable reaction in which an amino acid condenses with a molecule of ATP to form an aminoacyl adenylate (amino acid-AMP) with the accompanying release of pyrophosphate (PPi) (reaction 1). As this reaction is reversible, these biphosphonates substitute the PPi in the inverse reaction (inverse reaction 1). In such a reaction, the condensation of the biphosphonate (PcP) with the aminoacyl adenylate (amino acid-AMP), leads to the formation of the parallel of the ATP (AppCp) (47).



The AppCp, metabolically unusable, accumulates inside the cell, and would seem to alter numerous enzymes of the ATP-dependent cellular metabolism with damaging effects for normal functioning and for cellular survival. In particular, the active metabolite of the clodronate (AppCC<sub>12</sub>p: adenosine-5' [β,γ-dichloromethylene] triphosphate, would seem to inhibit the carrier ADP/ATP translocase (ANT) which is indispensable for the transport of the ATP from the mitochondrion to the cytoplasm (52). The effect of the clodronate regarding the osteoclasts can not be defined as a cytotoxic type because the action results reversible in the presence of ATP (53). A recent study on cellular lines, in fact, demonstrated that the clodronate determines an inhibition of the proliferation of the pre-osteoclastic cells. No effects on the cell vitality or cell necrosis have been highlighted, but the osteoclasts are found in various apoptotic stages (54).

#### *Aminobiphosphonates*

Last generation biphosphonates (N-Bf) include: pamidronate (*Aredia®*; Novartis Pharmaceuticals Corp., East Hanover, NJ) and alendronate (*Fosamax®*; Merck and Company, Inc., West Point, PA)

which contain a primary nitrogenated base; ibandronate (Bondronat®; Hoffmann-La Roche Inc., Nutley, NJ) a tertiary nitrogen; risedronate (Actonel®; Proctor and Gamble Pharmaceuticals, Inc., Cincinnati, OH) and zoledronate (Zometa®; Novartis Pharmaceuticals Corp.) a nitrogen inside a heterocyclic ring. These effective bisphosphonates are not metabolized by the enzyme aminoacyl tRNA ligase as are the non-aminobisphosphonates (49, 51) because of the steric conformation of the lateral chain R2 which is larger than the active site of the enzyme (47). Their action inhibits one or more phases of the metabolic chain of the mevalonate which leads to the synthesis of cholesterol. In particular, they inhibit the farnesyl diphosphate synthase (Fig. 4)(55). The inhibition of such enzymes determines the missing formation of some intermediate isoprenoid lipids such as isopentenylpyrophosphate (IPP), farnesyl-pyrophosphate (FPP) and geranylgeranyl-pyrophosphate (GGPP). Such lipids are essential for the post-translational modifications, also known as prenylation, of some small proteins, in particular of the GTP-binding proteins (GTPase), including Ras, Rho and Rac. These are important signalling proteins which regulate a great variety of fundamental cellular processes for the functioning of the osteoclasts. Their post translational modification with farnesyl or geranylgeranyl groups is essential for their locating in the cellular membranes and, ultimately, for their biological functioning (56). *In vitro* studies, (57-58) carried out firstly on macrophes J774 then on cellular organic bone cultures, have demonstrated that the inhibiting of the prenylation of proteins, including the GTP-binding protein, causes profound modifications on the cellular morphology and on the “ruffled border” of the membrane, alters the transduction of the intracellular signals and causes, finally, alterations of cell survival leading to apoptosis. These different molecular actions should lead to consideration of the amino and the non-aminobisphosphonate drugs which are of the same family but belong to different groups. This aspect is confirmed by the marked difference in the relative antiresorption strength which exists between the two groups (Table I) (11-13).

#### *Action on the osteoblasts and extracellular effects*

Recent data from *in vivo* and *in vitro* studies demonstrate that some bisphosphonates stimulate the differentiation and the activity of the osteoblasts (28-29). Alendronate seems to have a direct action on the osteoblasts, bettering the differentiation, the proliferation and the activity of forming bone tissue (30). In a recent study published in 2003, it emerged that clodronate is able to both stimulate bone mineralisation by osteoblasts, valued by the increase of the endocellular enzymatic activity of the alkaline phosphatase, and to stimulate the gene expression of the differentiation osteoblast markers (31). Lately, Dixon et al. (32) noted how the non-nitrogenated bisphosphonates, metabolized in parallel with the ATP, bonding to the purine receptors (P2X) observed in the osteoclasts diminish the activity, while bonding to the receptors (P2Y) observed in the osteoblasts increase the activity

and therefore increase bone formation. Regarding the extraskeletal effects, *in vitro* (33-34) and *in vivo* (35) studies have demonstrated that clodronate seems to have anti-inflammatory properties. In particular, reductions in the plasmatic levels of the mediators produced by synovial macrophages (IL-1, TNF- $\alpha$  and microglobulin  $\beta$ 2) in subjects with rheumatoid arthritis (36) and reductions of the release, *in vitro*, of cytokines and of NO on part of the macrophage-like cells (37), have already been documented. However, in the same studies (33-35), the pro-inflammatory effects of the aminobiphosphonates were highlighted. In fact, alendronate determined an increase in the secretion of cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) induced by the Lipopolissaccharide (LPS) on part of the macrophages (38). Such mechanism has been revealed to be cytotoxic and irreversible (38). The pro-inflammatory activity, following above all the parenteral administration of aminobiphosphonates, leads to the appearance of an acute phase reaction (39), with fever, arthralgia, arthritis (40).

#### *Systemic administration of biphosphonates in implantation*

Biphosphonates are the elected therapy for most patients affected by osteometabolic diseases. In these patients, such drugs are administered in a systemic way, intravenously or orally. The systemic administration of biphosphonates has also been studied in the field of orthopaedic and implant surgery. The aim of such studies, mostly on animal models, was to evaluate the effectiveness of the drugs in increasing and bettering osteointegration on which depends the stability and the survival of the implant or of the orthopaedic prosthesis over a long period of time (59-60). Eberhardt et al (61) tried to identify which dose of ibandronate, administered by subcutaneous injection, would be most effective to better the osteointegration of implants inserted by the press-fit technique into the marrow canal in rat femurs. In the histomorphometric analyses a statistically significant increase was noted of the osteointegrated implant surface (OIS) in the group with high doses of ibandronate (doses for tumours) compared with the group with low doses (doses for osteoporosis) and the control group. Another interesting study by Astrand and Aspenberg (62) agrees with the necessity of a high dose of alendronate and clodronate to reduce the bone resorption induced by the mechanical instability caused experimentally around prosthetic implants inserted into the tibias of rats. In the same year T. Hennigs et al. (63) demonstrated in humans, how high doses of alondronate are effective in preventing a decrease of periprosthetic bone mineral density (BMD). Lower doses did not have the same effect. This was also confirmed in a similar study but with a control after two years, previously carried out by B. Scammell et al (64). J.D Bobyn et al. also reported that a high dose of amino-biphosphonate could determine a positive modelling of the perimplant bone (65). Lastly, a study in 2004, carried out on healthy rats by B. Skoglund et al. (66), demonstrated by histological analysis and mechanical tests that the early bone remodelling around the implants have an important role in the stability of the implant. A systemic or local treatment of ibandronate may

be an efficacious pharmacological support for improving the stability of the implant in this critical stage. This is a very important orthopaedic task in that, on animal models two methods of drug administration are tested; systemic (daily subcutaneous injection) for steel screws spongy bone and topic (implants and wet sites) for screws set in more dense bone. In mechanical tests, both groups (treated with ibandronate, systemically and topically) gave better results than the control group (not treated). The histomorphometric analyses, were altered due to errors made by the authors, and did not give any statistically significant results. From this experimental protocol it is clear that to obtain a sensible increase in the osseointegration process a high dosage of the drug is necessary if administered systemically (Table II). Confirmation of this is also to be found in orthopedic literature. In fact, in experimental models, to inhibit the resorption of the bone around the implant, about 50 times the dosage was required in comparison to that used for treating osteoporosis (67).

*The main side effects of the systemic administration of aminobiphosphonates is osteonecrosis of the jaw bone*

The use of systemically administrated biphosphonates to counteract the osteolytic processes, promote the new formation of bone and improves the quality of the area around the implant, as well as the necessity of very high doses, must be critically considered from another important point of view, osteonecrosis of the jaw bone. Over the last three years a disturbing correlation has emerged in the literature between the use of strong aminobiphosphonates systemically administered and the appearance of a painful bone exposure in the jaw bone. In fact, an article by R.E. Marx (14), published in 2003, reported 36 cases of painful bone exposure, suggesting a causative effect of therapy with biphosphonates. Since then there have appeared numerous publications, mainly as case reports or letters to the editor, written by dentists, maxillofacial surgeons and oncologists (15-23). The term suggested for this form of painful bone exposure is osteonecrosis of the jaws (ONJ) because it is typically found in the jaw bone. From an analysis of these papers, it can be seen that the patients who presented osteonecrosis were all being treated with aminobiphosphonates. It is important to remember, however, that these patients took this drug because they had a serious osteometabolic or neoplastic illness. With regard to this, pharmacological therapies, together with chemotherapy or glucocorticoids, concur in promoting such lesions and must be considered the main risk factors. In the majority of the reported cases of OJN, the patients had previously had teeth extracted (14,15), therefore, invasive dental manipulations are considered the main trigger factor of the pathogenetic mechanism of such lesions. It is important to stress that, from our revision of the literature, no cases of OJN have been found in patients who were given, systemically or topically, a non-aminobiphosphonate such as etidronate and clodronate.

*Topical application of biphosphonates in implantology*



The close affinity of these drugs with bone mineral allows for taking into consideration a topical application. On this point the studies of A. Yaffe et al. towards the end of the '90s (68-69) demonstrated that the topical use gives a high concentration of the drug in the interested site, difficult to obtain with a systemic administration. M. Tanzer et al. (70) demonstrated that, in the dog ulna, a solution based on zoledronate directly applied with a pipette on the orthopedic implant coated with hydroxiapatite, gave an increase in the bone surrounding the implant.

Instead, S. J. Meraw et al. (71) were the first to study the efficacy of bisphosphonates applied directly onto dental implants. The bisphosphonate used was alendronate (Al) that was applied to the two surfaces of the implant with different techniques. The results obtained showed that the alendronate was efficacious in increasing the percentage of bone formation around the implant (71).

M. Yoshinari et al. in 2001 (72) proposed a new technique for fixing bisphosphonates to the titanium surface. According to the authors, the covering of the implant of thin layers of phosphate-calcium gave a higher stability of the drug. The strong affinity of bisphosphonates with calcium phosphate explains this phenomenon. In 2002, in an *in vivo* study (73), M. Yoshinari et al. again, evaluated the reaction of the surrounding bone to implants coated in amino bisphosphonates. These data suggest that the thin layer of calcium phosphate to which the amino bisphosphonate was fixed is effective in increasing the contact between the bone and the implant. The covering of calcium phosphate is effective because it contributes in regulating the local action of the bisphosphonate.

In another important *in vitro* study, published in 2003 by T. Goto et al. (74), the formation of mineralized tissue in osteoblastic cell cultures, on titanium plates, treated in different ways, was evaluated. In the experimental model the authors analysed pure titanium, titanium impregnated with calcium ions according to the Hanawa technique (75) and titanium in which was fixed amino bisphosphonate according to the Yoshinari technique (72). Pamidronate (Ciba-Geigy, Japan) and icadronate disodium (YM-175, Yamanouchi Pharmaceutical Co., Ltd., Japan) were the drugs used. In the groups where the drug was present there was an acceleration of the formation of bone as had already been hypothesized by other authors (28-29). In the group with pamidronate there was a percentage of bone-like nodules higher than in the group with sodium icadronate. It is important to stress that icadronate has a higher anti-reabsorption capacity than pamidronate. Therefore the analysis of the data obtained from this study suggest that the capacity of anti-reabsorption of a bisphosphonate does not result as proportional to the capacity to form new bone. What is stated above is also highlighted in an *in vivo* study carried out by H. Kajiwara et al. (76).

In the orthopedic field, P. Tengvall et al. (77), also demonstrated that an amino bisphosphonate fixed on pure steel screws gives an increase in the mechanical fixation of the metallic bio-material of the bone. In this study, the screws were first roughened and pre-treated with fibrinogen. Then



pamidronate was fixed followed by ibandronate. The “pullout force” as well as the “pullout energy” were higher in the group with amino-biphosphonates. Similar results to the mechanical tests were obtained in the already mentioned study by Skoglund et al. (66), where the implant site and the implant itself, were bathed in an ibadronate based solution.

The aspect that is common to the studies analysed so far is the role that amino biphosphonates, fixed on the surface of the implants, play in the process of neo-osteogenesis surrounding the implants. Data confirming this have also been obtained from a histological- histomorphometric (70,71,73,76) point of view as well as from a mechanical one (66,77). None of these authors had, though, done comparative evaluations on the different concentrations of the drug.

In a study published in 2005, B. Peter et al. (78) evaluated the response of the bone surrounding the implant to different concentrations of the drug. The authors aimed at identifying the concentration of drug to be used to obtain a bone density able to increase the stability of the implant. The authors identified a drug concentration range (0.2 – 2.1 µg/implant) that determines an increase in the mechanical stability of the implant. They conclude, therefore, that the bone surrounding the implant responds to the topical administration of Bf is concentration dependent, but there is no proportional dose-effect (Fig. 5) (78). It is also important to stress that the results of the mechanical tests of this study are higher than those reached in the abovementioned study by Tengvall et al. (77). This difference may depend on the type of drug administered and the fixing technique used. In the study by Peter et al., the drug used was zoledronate, a molecule that certainly has a higher anti-reabsorption strength than pamidronate and ibandronate which were used in the study by Tengvall. As regards the fixing technique used, the layer of fibrinogen used by Tengvall is less efficacious in respect to the coating of HA used by Peter et al. The importance of this aspect has been analysed by Tanzer et al. (70) in a study published in 2005. The authors found that in the implants in which the amino biphosphonates were fixed to a coating of HA, the release of the drug was gradual, constant and lasting. On the contrary, in the uncoated implants the release was immediate and had a duration limited to the first hour (Fig. 6) (70).

The studies mentioned so far though have all been carried out on animal models and with short periods of observation (Table III). No studies have so far been done on humans. All the same, the results obtained have been confirmed and lay a base for new protocol on animal models.

## CONCLUSIONS

From our analysis of the literature various important aspects emerge regarding the possibility of using biphosphonates in implant surgery. In first place, it is necessary to underline the worrying correlation between OJN and systemic therapy with azotized biphosphonates (N-Bf). This serious side-effect obliges us to exclude a systemic administration of amino biphosphonates for implants. It

is just as important to remember that in the literature no case of OJN in patients subjected to systemic treatment with non-amino biphosphonates is reported. Such correlation did not emerge even following a topical treatment with amino and non-amino biphosphonates and, therefore, this modality of administration may be taken into consideration for implant surgery. From the orthopedic literature it can also be seen that a topical administration of amino and non-amino biphosphonates allows us to obtain, in the interested site, a high concentration of the drug, difficult to reach with a systemic administration.

The response of the bone surrounding the implant seems to be influenced, as well as by the drug used, also by the time of release of it at the site of the implant. On this point various methodologies are discussed which all allow for the fixing of molecules on a coating of the implant. We find that it would be interesting to carry out studies to evaluate the efficacy of different concentrations of amino and non-amino biphosphonates, the latter not having been studied very much in this field of application, is fixed on the surface of the implant. It would be useful to compare the results obtained with those that had, instead, through the application of the drug, directly on the newly formed alveolus, a certainly simple method, but one that does not have much support in the literature. This comparative analysis would allow to determine the type of biphosphonate, the relative concentration and the method of the most efficient application for increasing the stability of the implant. Therefore, the use of this type of drug in human implant surgery seems to be a not too distant goal, but one certainly subordinate to the results of the studies on animals.

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