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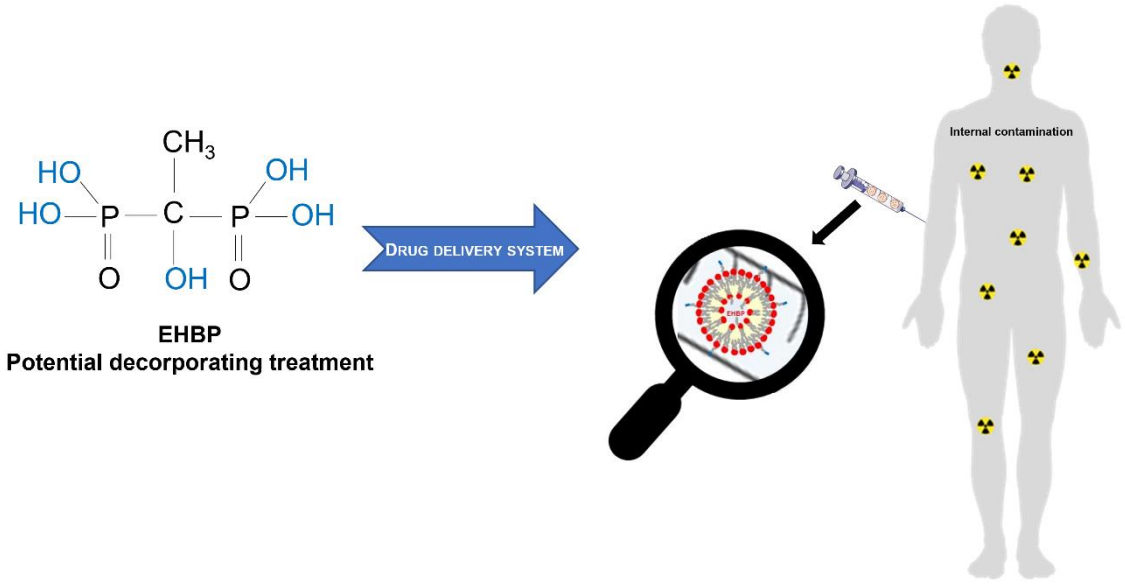


Abstract

In the event of internal contamination with radionuclides, decorporating treatment is crucial. It must be initiated as soon as possible to limit tissue retention of radiocontaminants and accelerate their excretion. However, the existing therapies for such contaminations often lack effectiveness. The conventional research strategy is based on selecting or synthesizing new chelating agents, a lengthy and costly process. Another approach is drug repurposing, such as molecules belonging to the biphosphonate series. This primary therapeutic class is mainly used to treat bone metabolism disorders. However, for decades, biphosphonates have offered a wide range of applications in research, including macrophage depletion or bone regeneration, but also in more unexpected areas such as skin decontamination and decorporation. In addition, controlled drug delivery and nanotechnologies have offered a promising approach for significantly improving the delivery and efficacy of these active substances. They increase their distribution to target organs and bypass issues related to their

toxicity or poor pharmacokinetic performance. After outlining the pharmaceutical properties of biphosphonates, the present review aims to present the central innovative systems for their delivery and targeting in the field of radiation protection.

Keywords

Bisphosphonate, internal contamination, radionuclide, nanotechnologies, controlled drug delivery, repurposing drug

1 Introduction

Bisphosphonates (BPs) have been initially used as corrosion inhibitors or complexing agents in the textile or petroleum industries. Etidronate (ethane-1-hydroxy-1,1-bisphosphonate, EHBP, or 1-Hydroxyethylidene-1,1-diphosphonate, HEDP) was the first clinically applied in the 1960s [1]. Other molecules (clodronate, pamidronate, risedronate, alendronate, tiludronate, zoledronate, and ibandronate) were then successively available on the market. As bone resorption inhibitors, they were employed as first-line treatment of metabolic bone diseases such as osteoporosis and Paget's disease. Prescription of these drugs has been growing extensively due to population aging, with the global prevalence of osteoporosis estimated at 18.3% [2]. BPs are also widely used in therapy to treat hypercalcemia and as an adjuvant in bone metastasis.

Besides their known therapeutic indications, BPs have been particularly interesting in radioprotection due to their mechanism of action and bone tropism. Since one of the target tissue after radiocontamination is the bone [3, 4], applications have included decontamination and decorporation [5, 6]. These studies demonstrated that BPs could be effective medical countermeasures for the population and workers exposed accidentally or intentionally to radiation.

The mechanism of action of BP molecules relies on their binding to hydroxyapatite (HA) crystals related to their strong affinity for calcium (Ca) [7]. A few conditional stability constants (log K) for Ca-BP complexes are available. For instance, for the CaL^{2-} complex, the values for clodronate, pamidronate, and EHBP are 5.77 ± 0.01 (0.1 M $(CH_3)_4NCl$, 25°C), 6.13 ± 0.1 (0.1 M KCl, 25°C), and 6.18 ± 0.03 (0.1 M $(CH_3)_4N(NO_3)$, 25°C), respectively [8-10]. Regarding the EHBP-Ca complex, the overall stability constant was determined as log β equal to 20.20 ± 0.06 [11]. Other elements, such as divalent cations like copper (Cu^{2+}), zinc (Zn^{2+}), or iron (Fe^{2+}), have

also been investigated for complexation and exhibit comparable affinities to BPs [12]. Recently, a detailed analysis of cobalt (Co^{2+}) complexation with EHBP was published and provided several overall stability constants up to 27.11 ± 0.1 [13]. Trivalent cations (Al^{3+}, Cr^{3+}, Eu^{3+}) were also investigated and displayed high values for the overall stability constants (log β > 25) [14, 15]. Moreover, a few studies have reported that BPs have a good affinity with hexavalent cations like uranium [16, 17]. Among all these elements, we can note that the recommended medical treatment for cobalt contamination has minimal effectiveness. The recent observations suggest that clinical indications of BPs could be extended to other radionuclides and increase the therapeutic arsenal in the event of internal contamination.

This review aims to update the knowledge of this significant class of drugs. After reviewing the pharmacological properties and mechanism of action of BPs, we will discuss the applications of BPs in the field of radiation protection.

2 Bisphosphonates: structure-activity relations

Also known as diphosphonates, BPs are a family of synthetic pharmaceutical molecules characterized by two phosphonate groups, $PO(OH)_2$, in geminal position, *i.e.* linked to the same carbon. BPs have a strong structural analogy with inorganic pyrophosphate, a degradation product of ATP that inhibits calcium phosphate precipitation by binding to HA crystals, which has a P-O-P bond (Fig. 1) [18-20]. However, replacing oxygen with a carbon atom in the center of the P-C-P bridge allows BPs to resist enzymatic degradation and are, therefore, non-hydrolyzable and, consequently, very stable molecules [21]. The differences between each BP are the two residues (R_1 and R_2), allowing the creation of multiple structures with different pharmacological properties.

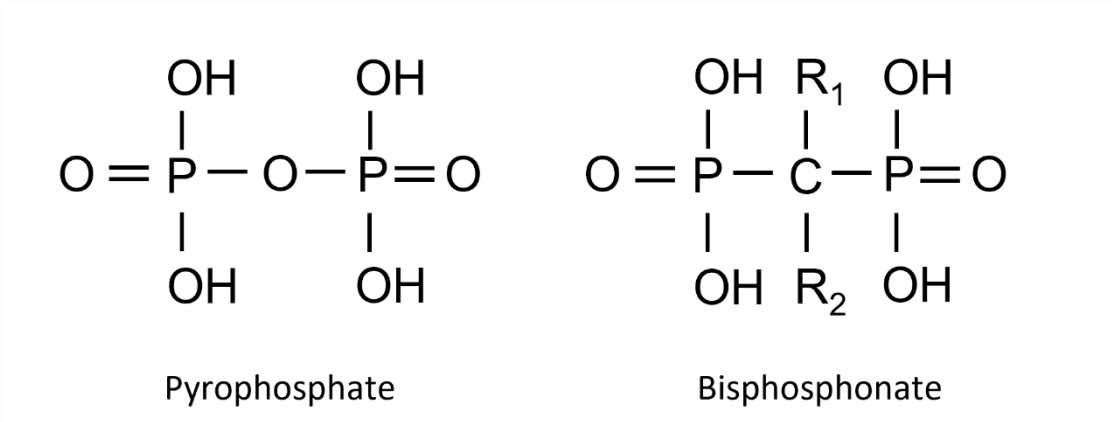


Fig. 1: General chemical structure of pyrophosphoric acid and bisphosphonic acid

BP molecules available on the market include clodronate, risedronate, ibandronate, pamidronate, alendronate, and zoledronate. EHBP is the reference BP. The chemical structure of these seven compounds is given in Fig. 2. These BPs share the P-C-P bridge and two phosphonate groups essential for bone resorption inhibition and targeting the bone mineral fraction [22]. Indeed, chemical modifications, such as adding carbon in the P-C-P bridge or replacing hydroxyl with methyl in the phosphonate groups, lead to a loss of bone affinity and resorption capacity [21, 23].

The short R_1 chain provides BP an affinity for bone tissue, notably *via* the presence of the hydroxyl group. In contrast, the function and the potential to inhibit bone resorption are essentially linked to the R_2 chain [24]. BPs can be classified into two categories according to the presence or absence of a nitrogen atom in the R_2 chain. The first category includes the first generation of non-amino BP (EHBP, clodronate, tiludronate) with mono-atomic or hydroxyl groups. The second category consists of a second generation of amino BP with a primary amine group in R_2, including pamidronate and alendronate, and a third generation of amino BP, which has a more complex structure and includes a tertiary nitrogen atom (ibandronate) or an aromatic N-heterocycle (risedronate, zoledronate).

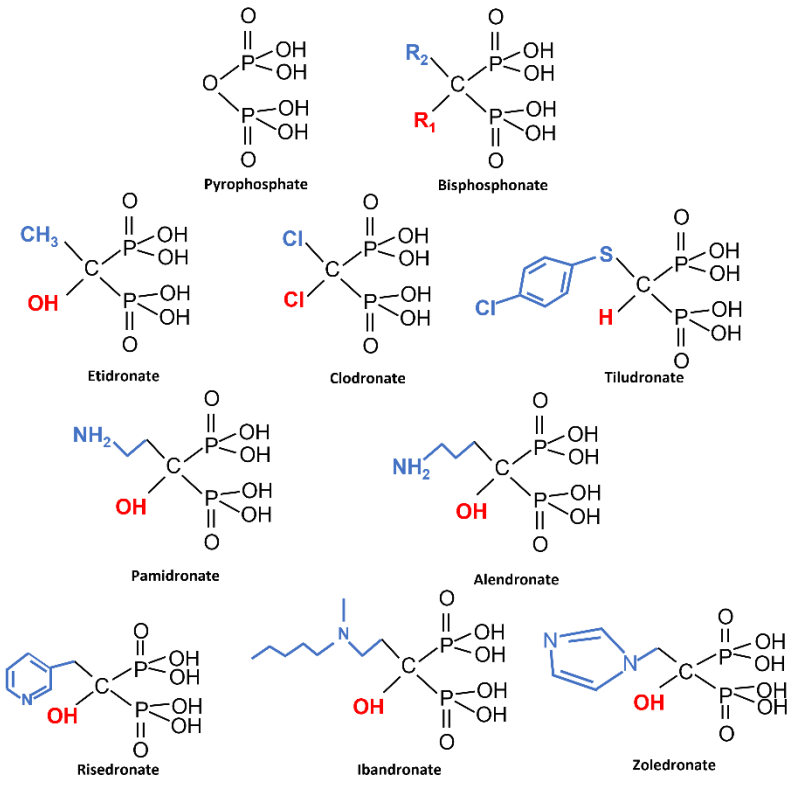
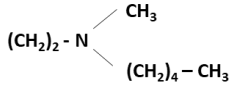


Fig. 2: Chemical structure of bisphosphonic acid family members

The presence of a pyridine-type heterocycle or the R_2 chain length dramatically influences the antiresorptive potency of the BPs molecules [18, 22]. The minimum dose of each BP required to induce osteopetrosis (a hereditary disease characterized by osteoclast dysfunction resulting in a defect in bone resorption) in young rats was determined to assess the potency of the BPs molecules. The doses were then compared to EHBP as a reference [25]. Table 1 shows the structural characteristics of BPs and their relative potency [26].

Table 1: Chemical structure and relative potency of bisphosphonates

Molecule	R_1 residue	R_2 residue	Generation	Potency
Etidronate	OH	CH_3	1st	1
Clodronate	Cl	Cl		10
Tiludronate	H	4-chlorothiophenyl		10
Pamidronate	OH	$(CH_2)_2NH_2$	2nd	100
Alendronate	OH	$(CH_2)_3NH_2$		1,000
Risedronate	OH	Pyridine	3rd	5,000
Ibandronate	OH			10,000
Zoledronate	OH	Imidazole		20,000

3 Pharmacokinetic properties of BPs

The administration of BPs usually occurs by the oral or intravenous route. Intramuscular and subcutaneous routes of administration are not considered because they induce irritation and tissue damage at the injection site [27, 28]. In humans, the oral absorption of BPs is minimal. The amount absorbed ranges between 2 and 2.5% for non-amino BPs and is about 0.7% for amino BPs [29]. Furthermore, in the presence of food and drink containing calcium and magnesium, the bioavailability of BPs collapses dramatically.

Once in the bloodstream, at physiological pH (7.4), BPs are partially ionized in the bloodstream, which explains their ability to bind to proteins present in plasma, mainly albumin. In humans, however, protein binding varies between BPs: for instance, on average, the binding fraction is 32% for zoledronate, 26% for ibandronate, 36% for clodronate [30, 31], and 78% for alendronate [32]. After a plasma half-life of around two hours, BPs disappear from the blood compartment and are retained in organs or undergo urinary excretion [33]. BPs are found throughout the human body in calcified (approximately 50-80%) and non-calcified tissues [34].

The distribution of BPs within calcified tissues is heterogeneous and complex [35]. Indeed, BPs will preferentially localize in trabecular bones where bone turnover is high. According to Lin's team, the concentrations of radiolabeled alendronate with carbon-14 (^{14}C) are two to three times higher in these areas [36, 37]. In humans, the available data support the above results. According to the work of Carnevale *et al.*, carried out on pre-and post-menopausal women using a technetium-99m-labeled methylene diphosphonate molecule, the distribution is predominantly in areas of high bone remodeling [38]. However, recent work by Roelofs' team suggests a more profound localization of BP. Indeed, 24 hours after administering fluorescent risedronate analogs to 3-month-old mice, the labeled molecule was found in osteocyte lacunae near vascular channels and in monocytes in the bone marrow [39].

The elimination half-life of BPs in skeletal tissue varies according to the molecule considered and depends on factors such as the rate of bone turnover and renal function [40]. In the case of alendronate, it is estimated to be more than ten years in healthy humans [41, 42].

The distribution of BPs in non-calcified tissues has not been investigated as much as in skeletal tissues. However, it appears to be relatively homogeneous with a preference for the liver, kidney, and spleen, but their retention decreases rapidly over time. After intravenous (IV) administration of 1 $mg.kg^{-1}$ of ^{14}C-alendronate in rats, the retention of the molecule in these tissues ranges from 63% of the dose at 5 min to 5% at 1 h. At the same time, retention in bone tissue increases to a peak at 1 hour after injection [37].

The characteristic of BPs structure based on a P-C-P bridge makes enzymatic hydrolysis difficult. However, in contrast to nitrogen containing bisphosphonate (N-BP), *in vitro* studies in mammalian cells (murine macrophage cells J774 and human osteosarcoma cells MG63) suggest that clodronate and EHBP are metabolized to non-hydrolyzable cytotoxic analogs of ATP identified by chromatographic analysis. No data on the percentage of the dose that would be metabolized is available. In contrast, for alendronate, no metabolites were detected [43], and N-BP was eliminated in an unchanged form [35].

The elimination route of BPs is almost exclusively urinary via glomerular filtration [35]. Some BPs are undergoing tubular secretion, as shown by Troehler's team on rodents [44]. The molecules used were EHBP and dichloromethylene diphosphonate labeled with ^{14}C. According to Ruggiero *et al.*, 50% of the absorbed BPs is excreted unmetabolized in the urine [24]. The removal of BPs is complex as it is dependent on bone turnover. Once integrated into the bone, BPs will only be mobilized during a bone remodeling phase. This slow removal kinetics explains

the continuous urinary and blood concentrations over long periods. A minor elimination route occurs via bile excretion (less than 0.5%) [37].

4 Mechanism of action of BPs

The principal therapeutic action of BPs is the inhibition of bone resorption. Bone tissue is, therefore, the main target of this class of drugs. Before detailing the mechanism of action, we will discuss the chelation process essential to this mechanism.

4.1 Chelation

The high affinity of BPs for calcium is necessary to inhibit bone resorption. Indeed, the binding intensity of BPs to calcium present in the mineral fraction of bone by adsorption forms the basis of their mechanism of action [7]. Chromatographic studies suggest that the phosphonate groups and the R_1 and R_2 residues of BPs significantly bind to HA crystals. These chemical differences explain the variability observed between BPs. The results suggest an increasing affinity in the following order: clodronate < EHBP < risedronate < ibandronate < alendronate < zoledronate [45]. More recently, DFT (Density Functional Theory*)* analyses based on quantum calculations of molecular structures have confirmed these results [46]. They support the stronger affinity to the mineral matrix of BPs with a nitrogen atom, attesting to a more consistent anti-resorption effect [47].

To better understand the chelation process, which is essential for the biological action of BPs, the Ca-BP bond has been studied extensively, particularly by Claessens and Van der Linden [8]. Among the ligands (L), EHBP and methylene diphosphonate were studied. According to the authors, the complexes would only exist at pH above 4. The predominant chemical complex at pH 5-7 would be a monoprotonated calcium one ($CaHL^-$) with a formation constant log K at 3.12 ± 0.06. Then, by increasing the pH, the unprotonated dicalcium species (Ca_2L) would be predominant with log K about 4.63 ± 0.06. The studies of Foti support these values [11]. Indeed, for the $CaHL^-$ complex, log K and log β (overall stability constant) are 2.87 and 14.31 ± 0.05, respectively. For the Ca_2L complex, log K and log β are 4.70 and 11.22 ± 0.06, respectively. Furthermore, it was shown that the bond between the BP and the calcium was bidentate or even tridentate when the R_1 residue contained a hydroxyl group [48].

In addition to calcium, the complexation between BP and especially EHBP (the head of BPs series) and other divalent elements has also been investigated, such as Cu^{2+}, Zn^{2+}, Fe^{2+}, nickel (Ni^{2+}), cadmium (Cd^{2+}), magnesium (Mg^{2+}), Co^{2+} and strontium (Sr^{2+}) [11-13, 49].

Complex formation with trivalent cations was also studied, especially with aluminium (Al^{3+}), chromium (Cr^{3+}) and europium (Eu^{3+}) [14, 15]. The stability constants of these cations with EHBP are listed in Table 2.

Table 2: Complexation constants for MH_2L complex

	log *K*	**log β**
	0.1 M KCl (20°C)[a]	
Co^{2+}	ND	8.52 ± 0.04
	0.1 M KNO_3 (25°C)[b,c,d]	
Sr^{2+}	1.52	ND
Co^{2+}	2.78	ND
Cu^{2+}	3.0 ± 0.4	20.1 ± 0.5
Zn^{2+}	3.8 ± 0.3	20.2 ± 0.5
Ni^{2+}	4.8 ± 0.1	20.3 ± 0.2
Cd^{2+}	4.5 ± 0.2	20.7 ± 0.3
Fe^{2+}	3.3 ± 0.3	21.0 ± 0.4
Cr^{3+}	4.0 ± 0.1	28.9 ± 0.1
Al^{3+}	1.8 ± 0.3	29.1 ± 0.3
	0.1 M NaCl (25°C)[e]	
Mg^{2+}	1.54	19.91 ± 0.2
Ca^{2+}	1.83	20.20 ± 0.06
	0 M[f]	
Eu^{3+}	5.6 ± 0.4	25.2 ± 0.4

M is metal and L is ligand (EHBP), ND means not determined.
[a][13], [b][49], [c][12], [d][15], [e][11], [f][14].

All the elements examined form stable complexes with EHBP molecules, and the results are pretty similar. Concerning Cu^{2+}, the complex M_2L_2 is predominant in a strongly alkaline solution. Log β was determined at 30.42 in 0.3 M NaOH solution at 25°c [50]. Differences can be observed and are function, among other things, of the ligand coordination sites, the charge of the complex, or the steric hindrance generated [49]. The use of BPs has also been considered for the decorporation of actinides such as uranium (U). A conditional constant for the complex UO_2^{2+}-HEDP (1:1 complex stoichiometry) was determined at

7.7 ± 0.3 by using a 0.1 M sodium perchlorate solution (at 20°c), and the stability constant was established at 19.1 [16].

In addition to their anti-bone resorption property, BPs are used for imaging through bone scintigraphy. They can be coupled to radiotracers, which are beta/gamma emitters such as ^{99m}Tc, ^{153}Sm, ^{186}Re, ^{188}Re, depending on the purpose for diagnostic purposes. The radiotracers can be coupled due to the chelation property of BPs towards these radionuclides [51, 52]. In summary, the powerful capacity of BPs, especially EHBP, to complex such cations is well documented and thus paving the way potentially for other elements.

4.2 Tissue level

At the tissue level, all BPs derivatives have a similar action. They bind preferentially to HA crystals, replacing inorganic pyrophosphate and inhibiting their degradation, thus preventing bone resorption. Employing a histomorphometric evaluation which allows a qualitative and quantitative analysis of the bone structure, it appears that in ovariectomized baboons treated with alendronate (0.05 or 0.25 mg.kg^{-1} by IV every 2 weeks for 2 years), there is a decrease in resorption gaps and then in a second phase (4 to 6 months after the start of treatment), an overall reduction in bone formation without any impact on osteoblast activity [53]. Consequently, a decrease in bone fragility and an increase in bone mass occur. Furthermore, as the newly formed bone is less prone to resorption, the mineralization phase is more complete, as it has been observed in women with post-menopausal osteoporosis treated with alendronate (5 to 10 mg.day^{-1} for 3 years or 20 mg.day^{-1} for 2 years followed by 5 mg for 1 year) [54]. From a densitometric point of view, the decrease in resorption is more significant than that of bone formation, resulting in a positive calcium balance.

4.3 Cellular level

Since the pharmacological action of BPs is an anti-bone resorption effect, their preferred target is the osteoclast cell. Different results with a direct or indirect action on the target have been reported [55]. Depending on the model used, experimental results regarding the effect of BPs on the osteoclasts provide additional information. Indeed, *in vitro* studies using osteoclast-like cells and mouse bone marrow have shown osteoclastogenesis inhibition after alendronate treatment [56]. On the other hand, *in vivo* studies in rats have shown an increase in non-active osteoclasts and then, after chronic administration of BP (EHBP), a decrease in their number [57]. Several studies, especially those carried out in mice, have demonstrated

the penetration of certain radiolabeled BPs, such as tiludronate, into osteoclasts and the dysfunctions caused, including modification of the actin structure [58]. The team of Hughes *et al*. revealed, through *in vitro* and *in vivo* studies in mice, that treatment with different BPs (risedronate, pamidronate, and clodronate) promoted the apoptotic induction of osteoclasts [59]. In the presence of BPs, mouse osteoblasts in marrow cultures would indirectly inhibit osteoclast recruitment (the last step in osteoclastogenesis) or their activity via inhibitory factors present in the culture medium [60]. Concentrations as low as 10^{-11} mol.L^{-1} and an exposure time of 5 min would be sufficient to cause this effect [22]. However, the exact mechanism of action has not been elucidated. Finally, at the cellular level, the mode of action of BPs will differ according to their chemical structure [61]. They can block osteoclastogenesis by inhibiting precursor osteoclast proliferation and recruitment, promoting osteoclast apoptosis, and inhibiting osteoclast activity [55].

4.4 Molecular level

The molecular mechanism of action of BPs has been elucidated in the late 1990s. During bone resorption, when the environment becomes acidic through the H^+/Cl^- transporter at the osteoclast membrane, the BPs will be protonated, making the binding with calcium less favorable, thus allowing their internalization by an endocytosis mechanism [62-64].

BPs will not have the same molecular target depending on their chemical structure. Indeed, once internalized within osteoclasts, non-nitrogenous first-generation BPs will be integrated into the ATP molecules pool. The newly formed non-hydrolyzable ATP cytotoxic compounds will accumulate intracellularly, inhibiting essential ATP-dependent functions of the osteoclasts (disruption of the mitochondrial machinery and cell death), leading to their apoptosis [65]. The principal mechanism of action of nitrogenous BPs (second and third generations) is quite different. After entering the cell, BPs will play a key role in inhibiting mevalonate/cholesterol cycle enzymes (Fig. 3, [66]) and particularly farnesyl pyrophosphate synthase (FPP-synthase) [67, 68].

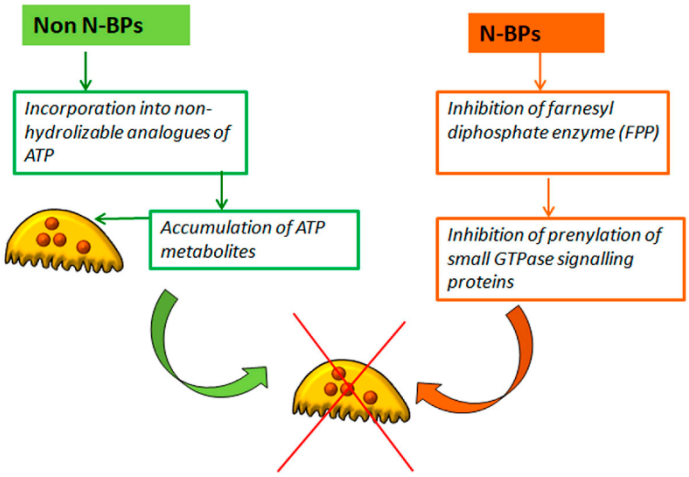


Fig. 3: Summary of the mechanism of action of BPs

By interfering with FPP-synthase, the prenylation (*i.e.,* the addition of an isoprenoid lipid farnesyl at C15 or geranylgeranyl at C20, via a thioether linkage to proteins at or near the C-terminal amino acid cysteine position) of numerous small proteins such as Ras, Rho, Rac will no longer take place. Rho/Rac proteins constitute a subgroup from the Ras superfamily of small GTPases. The biological functions of these proteins that regulate cellular processes essential to osteoclasts, such as the organization of the cytoskeleton, the modification of the cell membrane, or the transduction of the cellular signal, will no longer be ensured and will then lead to the loss of the anti-resorption activity of osteoclasts and their apoptosis.

The class of BPs could also have a beneficial role on osteoblastic cells by having an anti-apoptotic action. Indeed, all BPs activate a kinase enzyme called Extracellular Signal-Regulated Kinase (ERK). By increasing the phosphorylation of ERK enzymes, BPs would thus prevent the apoptosis of osteoblasts [69].

Due to their bone tropism and role as inhibitors of bone resorption, BP molecules are mainly used in treating bone pathologies, including osteoporosis and Paget's disease [24, 70]. In addition, they are prescribed for preventing bone complications in adult patients with advanced malignant disease with bone involvement and treating tumor-induced hypercalcemia [71, 72]. BPs are also used in nuclear medicine for diagnostic, metabolic imaging, or therapeutic purposes [73, 74]. Finally, BPs seem to have a potential antitumoral effect, especially by inhibiting tumor migration and angiogenesis, inducing tumor cell apoptosis, or impairing tumor cell adhesion to bone [64].

Generally, BPs are well tolerated if the recommendations for usage are respected (to take the treatment on an empty stomach, to remain in a sitting or standing position, and to respect a 30-minute delay before taking a meal). However, some patients experience potentially significant side effects that can cause great discomfort. Among the side effects noted are gastrointestinal symptoms (such as esophagitis, nausea, vomiting, diarrhea), kidney disorders, inflammation of certain membranes of the eye (such as uveitis, scleritis), metabolic troubles (hypocalcemia) [75-78]. Effects on the skeleton are also to be listed, such as atypical femoral fracture and osteonecrosis of the jaw [79-81]. Finally, minor cutaneous effects (eczema, urticaria, or Stevens-Johnson syndrome, which is more serious) and controversial effects (including atrial fibrillation and bone pain) have been described [82, 83].

Due to significant strengths (bone tropism, inhibitor of bone resorption, stable complex with various divalent cations), BPs, widely studied in research, have interested researchers in a more unexpected field: radiation protection. In this latter case, adverse effects seen in chronic treatments can be excluded since it becomes an emergency treatment.

5 Dosage forms and route of administration for the delivery of BPs

5.1 Nanotechnologies for targeted delivery

Among the formulations considered for the delivery of BPs are liposomes. They are small biodegradable vesicles composed of a phospholipid bilayer delimiting an aqueous compartment. This form allows the encapsulation of hydrophilic molecules in its core and lipophilic molecules within the bilayer. In oncology, clodronate liposomes are widely used to study the role of macrophages by inducing their apoptosis [84]. In addition to conventional liposomes, second-generation liposomes called stealth liposomes have also been applied to the delivery of BPs. They differ from the former by the addition, of hydrophilic polymers such as polyethylene glycol (PEG), allowing them to avoid opsonization (the process by which opsonins adsorb to the surface and promote phagocytosis) and thus to circulate longer in the body. These pegylated alendronate liposomes have been tested in mice to investigate the potential anti-tumor role of BPs [85]. In cancer-related research, pegylated nanoparticles of BP calcium salts were also tested in a model of mice with 4T1 tumors [86]. The results showed that M2 macrophages are depleted in large quantities and a drastic decrease in interleukin-10

secreted by M2 macrophages. This formulation has been responsible for a tumor microenvironment modification that allows for better radiotherapy efficacy.

BPs are also used as ligands to target bone in different diseases. One study describes micelles loaded with curcumin (potential anti-tumor effect) and functionalized with alendronate conjugated to a polymer (hyaluronan-octadecanoic acid) [87]. After injections every 2 days for 20 days in a mouse model with osteosarcoma, the results showed that the formulation of curcumin-loaded micelles in the presence of alendronate demonstrated a high affinity for HA and delayed tumor growth compared to the free molecule ($p<0.05$).

The IV route, one of the routes of BP administration in the clinic, allows for the distribution of various attractive targeting forms. Although it is invasive and requires a medical procedure, it will enable rapid delivery of the drug, in its totality, into the vascular compartment.

5.2 Dosage forms to improve BPs bioavailability.

5.2.1 Oral route

The oral route is one of the routes of administration of BPs in clinical practice. The main objective is thus to enhance the gastrointestinal absorption rate of the molecules. The use of ibandronate-based gastro-resistant reservoirs has been studied in rodents [88]. This formulation is composed of nanoparticles with citrus pectin as the raft former, calcium carbonate to enhance the raft strength, sodium bicarbonate as an effervescent mixture and PEG as the permeability enhancer. Pharmacokinetics studies showed a 2-fold increase in bioavailability of the gastro-resistant reservoir compared to the reference formulation (calculated area under the curve (AUC) of 6899 ± 3.5 ng/ml.h and 3708 ± 3.4 ng/ml.h). The resistance to stomach acidic pH was also explored in rabbits with gastro-resistant liposomes of alendronate [89]. Bioavailability was increased by 12 between the gastro-resistant formulation and the tablet form. Since 2012, alendronate is commercially available in Japan in a weekly gel form [90]. This interesting dosage form was shown to be more suitable for aging populations. It has the advantage of not requiring water and is easier to swallow. Although no difference was found with the tablet form, the results are significantly more favorable for oral-related adverse events (heartburn and epigastralgia). The effervescent solution of alendronate (taken weekly) has also been clinically assessed compared to the tablet form [91]. The results indicated a better follow-up of the treatment and a better

tolerance with the effervescent solution due to the dissolution of alendronate in a buffered appetent solution, limiting the risk of gastrointestinal irritation.

5.2.2 Pulmonary route

BP delivery through the pulmonary route was poorly studied. A dried powder formulation was proposed based on alendronate, leucine, and ammonium bicarbonate [92]. Administration of the formulation (with particle size less than 12 µm) was performed by the intra-tracheal route. The bioavailability of alendronate using this formulation (6.23 ± 0.83%) was increased by a factor of 3.5 compared to the oral route. Another study focused on risedronate sodium which was formulated into poly(lactide-co-glycolic acid) microspheres (with particle size mean of 3.68 ± 0.69 µm) to test the possibility for risedronate to be delivered through an alternative route instead of oral administration [93]. *In vivo* results showed high bone deposition compared to controls. The pulmonary route has many advantages: a large surface area of absorption, close vascularization (subject to a small aerodynamic diameter), no hepatic first-pass effect, and few notable adverse effects, unlike the oral route.

5.2.3 Transdermal route

A microemulsion (water/oil) formulation of alendronate has been tested in rat model [94]. At an equivalent dose (30 $mg.kg^{-1}$), statistically significant differences were reported between the oral and transdermal routes. The bioavailability of the microemulsion is twice greater than the solution. Another dosage form using the transdermal route was examined. It is a protransfersome gel loaded with risedronate [95]. Unlike liposomes, which have limited elastic properties and will primarily accumulate on the skin surface, protransfersomes are ultra-flexible lipid vesicles that can deform by absorbing water from the skin. Skin penetration is then increased without any sign of toxicity. Compared to the oral form, the results showed a statistically significant improvement in bone architecture and a strengthening of trabecular bone connectivity. Through these two examples, bioavailability was improved owing to the dosage form. The transdermal route displays real advantages such as no first hepatic passage, easy administration, the comfort of application, rapid withdrawal if necessary, and an absence of adverse effects related to oral administration.

6 BPs application in research in the field of radiation protection

As previously mentioned, BPs are a historical class of drugs and have been the subject of research for many years due to their attractive (tissue of interest and pharmacological property) and promising (affinity and different speciation with divalent cations) characteristics. The therapeutic molecules recommended in case of internal contamination by radionuclides are few and not entirely adequate. Using BPs as a decorporation treatment is a judicious strategy because, in the case of uranium, the retention organs include the kidneys and bone tissue [4]. Unlike other therapeutics, a decorporating agent must meet specific essential criteria [96]: (i) to be non-toxic; (ii) to have high affinity for the molecule of interest; (iii) to form stable M-L complexes; (iv) to form soluble and urinary excretable M-L complexes (v) to be orally administrable. Designing a decorporation treatment based on BP molecule could be hazardous due to the low gastrointestinal absorption. However, relying on new technologies and considering an alternative route of administration is a challenging but promising approach.

6.1 Skin decontamination

Research applications using BP molecules have included skin decontamination after exposure to actinides, particularly to uranium. Modes of exposure are either poorly soluble form (U(IV)) or soluble form (U(VI)), which is the most stable form in biological and environmental media [97, 98]. One of the first studies worth mentioning is the one from Houpert *et al*. dealing with early skin decontamination after exposure to industrial U compounds [6]. The treatment uses a paste or a dressing based on hydrocolloid and carboxymethylcellulose (with high absorbency) associated or not with potential chelating agents such as EHBP. The authors conducted *ex vivo* studies on bovine muscle and *in vivo* experiments carried out on two different models in rats. One with intermuscular U deposition to mimic a blunt object injury and the other with IM U deposition to mimic a sharp object injury. The results are reported in Table 3. Adding the BP molecules did not improve treatment efficacy in all these experiments. The reason is probably due to the insoluble character of the uranium oxides being unfavorable to chemical chelation.

In a second study, BP molecules incorporated into hydrogels were also considered for U decontamination [99]. Different types of hydrogels were formulated: pamidronate-based hydrogel (I), diethylene triamine pentaacetic acid (DTPA)-based hydrogel (II), pamidronate-

based polymeric hydrogel (III) and hydrogel in which BP molecule is linked to the motif of naphthalene phenylalanine-phenylalanine (NapFF) or NapFFP hydrogel (IV). For *in vivo* studies conducted on mice, a 1*1 cm^2 wound was created using a razor blade on the back of the animals. Different parameters were monitored, such as survival rate at 10 days, change in body weight over 10 days, and the amount of U in the organs, particularly the kidneys (target organ) (Table 3). All 4 treatments demonstrated significant efficacy compared to controls, but the pamidronate-based hydrogel (I) yielded better results.

The hydrogel-based treatment offers attractive results and combines interesting properties (absorptivity, stability, biocompatibility, biodegradability). However, the improvement of the effectiveness of these treatments by adding chelators to the formulas has not been demonstrated. Furthermore, the results obtained after using the dressing and paste forms are promising regarding reducing U in the kidneys. However, the results highlight the lack of efficacy of chelating agents within these dosage forms since they did not further retain the radionuclide. Nonetheless, in contrast to a simple EHBP solution, the dosage forms proposed (paste, dressing, hydrogel) are easier to use, ensure that the active molecule remains at the site of administration, and control the release of the drug by providing the carrier with a remanent effect. Effective emergency treatment after skin contamination appears essential to prevent the penetration of U in the body as much as possible and thus avoid long-term retention.

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Table 3: Treatments for the decontamination of uranium using BPs

Model	CONTAMINATION			TREATMENT						R_{Ligand} / RN	RESULTS	Bibliographical references
	U compound	Route	Single or repeated	Time before treatment	Molecule	Galenic form	Route	Single or repeated	Duration			
Bovine muscle	UO_2, UO_4 or U_3O_8	Deposition on incisions	Single	5 min	Carboxymethyl-cellulose-based hydrocolloids associated or not with EHBP	Paste	Application on incisions	Single	15 min (only for UO_4) or 1 h	90 (UO_2) 189 (UO_4) 602 (U_3O_8)	**U retention in the galenic form:** 15 min: 9% (6% with EHBP) 1h: 75-84% (no increase with EHBP)	[6]
						Dressing					**U retention in the galenic form:** 15 min: 48% (33% with EHBP) 1h: 89-97% (no increase with EHBP)	
Rat	UO_4	Inter muscular deposition	Single	2 min	Carboxymethyl-cellulose-based hydrocolloids associated (paste) or not with EHBP (paste/dressing)	Dressing or paste	Application on the inter or intra muscular pocket	Single	1 h	28	**Tissue retention of U at T0 + 1 h:** Dressing: ↘ 63% (wound site), ↘ 68% (kidneys) Paste: ↘ 39% (wound site), ↘ 65% (kidneys). Paste + EHBP: ↘35% (wound site), ↘ 70% (kidneys) **U retention in the galenic form at T0 + 1 h:** 63% (dressing), 40% (paste). No difference with EHBP with paste.	[6]
		IM deposition	Single		/	Dressing			15 min or 1 h	/	**U retention (regardless the treatment duration) at T0 + 1 day:** ↘ 41% (kidneys), ↘ 40% (femur), ↘ 38% (carcass) **U retention in the dressing at T0 + 1 day:** 28% (after 15 min), 30% (after 1 h).	
Mice	$UO_2(NO_3)_2$	3 drops on the wound	Single	15 min	Pamidronate-based hydrogel (**I**), DTPA-based hydrogel (**II**), Pamidronate-based polymeric hydrogel (**III**), NapFFP hydrogel (BP molecule linked to a naphthalene phenylalanine unit) (**IV**)	Hydrogel	Application on the wound site (0.125 cm^3)	Repeated (3 times)	3 min	NA	**Survival rate at T0 + 10 days:** 92% (I), 100% (II), 83% (III), and 58% (IV), 42% (control) **Body weight during 10 days**: Significantly gain for all treated groups (from day 5 to day 10 for I; at day 10 for II, III and IV) *vs* control group. **U quantity in kidneys**: ↘ 93% (II), ↘ 97% (III), ↘ 89% (IV) < DL for hydrogel (I)	[99]

U, uranium; RN, radionuclide; BP, bisphosphonate; NA, not available ; $R_{Ligand/RN}$ means molar ratio ligand to radionuclide.

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6.2 Decorporation

In the nuclear industry or following a nuclear incident/accident, the risk of internal contamination by inhalation, ingestion, or skin injury is real. It requires appropriate medical countermeasures using a decorporating agent. It removes radioactive elements from the body using a chelating agent or administering another pharmaceutical agent [100]. For some radionuclides (cesium-137 or iodine-131), treatments exist. However, for U, the proposed treatment is based on sodium bicarbonate, a non-specific molecule that lacks effectiveness. Until recently, various publications outlined the treatment strategies envisaged for the decorporation of this actinide [101-106]. Among the ligand families listed are polyaminocarboxylic acids, siderophores and bisphosphonates.

Some research groups are considering BP as a potential chelator of U. Wang *et al*. developed a conjugate of dopamine and BP that binds to iron oxide with a high partitioning constant at pH 7 [107]. Another team has synthesized different ligands formed by several BP compounds (EHBP) anchored on a calix[4]arene structure [98], including one with conditional stability constants with the uranyl ion UO_2^{2+} greater than 14 (at pH values ranging from 5.5 to 9.0 and hypothesizing a 1:1 ligand/uranyl complex formation), which is favorable to strong ligand-radionuclide complexations. Furthermore, the affinity of EHBP with U has also been demonstrated in rat serum using X-ray absorption spectroscopy (XAS) [108]. These *in vitro* data are promising clues for considering the application of this formulation to *in vivo* experiments.

Studies with EHBP have also been conducted in the rodent model. The results are listed in Table 4. Firstly, decorporation studies were considered after IM radiocontamination. Wistar rats received an IP injection with EHBP 1 h after being contaminated by IM injection of uranyl nitrate [109]. The treatment was pursued for 28 days, with one injection per day. The administration of EHBP resulted in a significant decrease in U concentrations between 40 and 50% in organs (kidney, liver, and femur) compared to controls. These encouraging data concerning the reduction of U retention in the kidneys had also been observed by Henge-Napoli's team, who carried out a study in the rodent model [5]. The treatment used was EHBP. Henge-Napoli *et al*. considered several protocols depending on the time elapsed between contamination and treatment (immediate, 5, or 30 min), the route (IM, IP), or the type of administration (single or repeated). The significant results were similar regardless of the ratio

493 ligand/RN or route of administration. Indeed, during early administration of EHBP, the
494 retention of U in the kidneys and the whole body declined by around 80% and 30% at
495 T0 + 24 h, respectively, compared to controls. After a delayed treatment (30 min), U retention
496 decreased by 45% in kidneys compared to controls. After repeated treatment, at T0 + 4 days,
497 EHBP reduced the U retention in the kidneys by 72% and
498

499 Table 4: Treatments studied for the decorporation of uranium using BPs

Model	CONTAMINATION			TREATMENT						$R_{Ligand/RN}$	Results	Biblio-graphical references
	U compound	Route	Single or repeated	Time before treatment	Molecule	Galenic form	Route	Single or repeated	Duration			
Rat	$UO_2(NO_3)_2$	IM	Single	1 h	EHBP	Solution	IP	Repeated (1/day)	28 days	8	**U retention at T 28 days** ↘ ~ 50% (kidneys), ↘ ~ 40% (liver), ↘ ~ 43% (femur) **Survival rate at T 28 days** 20% (control group), 50% (treated group)	[109]
Rat	$^{233}UO_2(NO_3)_2$	IM	Single	5 min	EHBP	Solution	IM	Single		5000	**After early administration, U retention (at T 24 h):** IM injection: ↘ 76% (kidneys), ↘ 30% (whole-body) IP injection: ↘ 83% (kidneys), ↘ 32% (whole-body)	[5]
				30 min								
				At the same time		Solution	IP	Single		2500	**After delayed administration, U retention (at T 24 h)**: IM injection: ↘ 45% (kidneys) IP injection: ↘ 45% (kidneys)	
				30 min								
				At the same time			IP	Repeated (1/day)	3 days		**U retention at T 4 days:** ↘ 72% (kidneys), ↘ 23% (whole-body)	
Rat	$^{238}UO_2(NO_3)_2$	IP	Single	At the same time	EHBP	Solution	IP	Single		7.5	For animals exposed and treated: bone healing did not differ from the non-exposed control group.	[110]
Rat	$^{238}UO_2(NO_3)_2$	IP	Single	At the same time	EHBP	Solution	IP	Single		8	**For animals exposed and treated**: - Survival rate: 100% over 60-day period - Body weight: no difference with control groups not exposed (untreated or treated with EHBP) at T 8, 30, and 60 days - Kidney tissue: no difference with control groups not exposed (untreated or treated with EHBP) on the 60th day	[111]
Rat	$^{238}UO_2(NO_3)_2.6H_2O$	IP	Single	At the same time, 24 h or 48 h	EHBP	Solution	IP or SC	Single		10	**Survival rate on the 60th day of the experiment**: 100% with EHBP (IP or SC) given immediately or 24 h after exposure (IP) **Biometric parameters of the mandibles 60 days after the beginning of the experiment**: better result with EHBP administered immediately by SC route	[112]
Rat	^{233}U	IP	Single	5 min	BP ligands or EHBP alone	Solution	IP	Single		100	**For 5 most promising ligands at T 5 days:** U retention: ↘ 53-58% (kidneys) for ligands n°3C, 5C and 7B, ↘ 18-30% (bone) for ligands n°5C, 6A and 7B U excretion: ↗ 31% for ligand n°3C only **For EHBP alone, at T 5 days**: ↘ 31% (kidneys), ↗ 23% urinary excretion	[17]
Mice	$UO_2(NO_3)_2.6H_2O$	Oral	Single	20 min	EHBP	Solution	Oral	Single		2 to 4	**For oral route ($R_{L/RN}$ = 3) and SC route ($R_{L/RN}$ = 0.3):** - Survival rate for at least 14 days: 50% - Urea and creatinine serum levels similar to control group at T 14 days - Kidney lesions less severe at T 48 h and signs of tissue recovery at T 14 days - Chelating effect of EHBP may prevent U from reaching acquiescent cells in the growth cartilage, allow normal formation of bone trabeculae.	[113 – 115]
							SC			0.3		

500 U, uranium ; RN, radionuclide ; BP, bisphosphonate; $R_{Ligand/RN}$ means molar ratio ligand to radionuclide.

in the whole body by 23%. Under favorable conditions, EHBP has been shown to be significantly more effective if administered earlier.

Decorporation studies have also been performed after radiocontamination with IP injection. Research was carried out on an alveolar bone healing model to study bone formation inhibition following uranium exposure [110]. After dental extraction, the rats were exposed to U and, at the same time, received a solution of EHBP at a ratio of 7.5 by the IP route. After an observation period of 14 days, unlike the exposed group in which bone healing did not occur, the exposed and treated group showed results similar to those of the control group. In another study, the researchers focused on renal function, another target of U [111]. A solution of EHBP was administered without delay to rats by IP at a molar ratio ($R_{L/RN}$) of 10. At the end of the 60-day experiment, the exposed and treated animals showed a 100% survival rate, body weight, and histological analysis of the kidneys similar to the control groups [111]. The Ubios team also confirmed a 100% survival rate after late treatment administration (24 hours after contamination) and by SC route without delay after exposure to U [112]. The results also highlighted that a single SC injection of EHBP immediately after contamination was more effective in preventing undesirable changes in mandibular growth (compared with IP). Other chemical entities have been proposed, dipodal or tripodal analogs bearing BP functions [17]. Almost all of these compounds showed high complexation constants (between 10^{15} and $10^{19.5}$) at pH 7.4 but also at pH 5.5 (pH of the kidney medium). An *in vivo* study was performed with the 5 most promising ligands (Table 4). At T0 + 5 days, for 3 ligands (n°3C, 5C, and 7B), U retention decreased by around 55% in kidneys, and for 3 ligands (n°5C, 6A, and 7B) 18-30% in bone, but only one ligand (dipod n°3C) showed a significant increase of 31% in U excretion compared to controls. For EHBP alone, at T0 + 5 days, urinary excretion of the radionuclide was increased by 10% compared to controls.

Finally, uranium decorporation has also been studied after oral exposure, a potential route of intake for the radionuclide. A solution of EHBP was administered 20 min after contamination by the oral route ($R_{L/RN}$ between 2 and 4) or by the SC route ($R_{L/RN}$ of 0.3) [113]. A survival rate of 50% for at least 14 days post-contamination was obtained after administration of BP by the oral route (at an $R_{L/RN}$ of 3) and by the SC route. The studies were pursued using the latter experimental conditions. In these groups of animals, the kidney lesions observed at 48 h were less severe than in the untreated exposed group and showed signs of healing at 14 days [114]. Finally, under these contamination/treatment conditions,

Bozal *et al* showed that EHBP allowed to improve the adverse effects regarding the endothelial ossification [115].

As mentioned above, the free form of EHBP has shown interesting efficacy in terms of clinical parameters (survival rate, body weight), histology, tissue retention of uranium, and excretion. However, using targeting systems such as colloidal nanoparticles could significantly improve the efficacy of these pharmaceutical molecules. Indeed, these dosage forms undoubtedly provide them with the following benefits: (i) to protect them from the environment by reducing the potential enzymatic degradation, (ii) to avoid the premature binding with endogenous compounds, (iii) to improve the ligand distribution and target tissues [116]. Combining free and encapsulated forms would ultimately act on circulating uranium and prevent U retention in the organs of interest.

7 Conclusion and perspectives

As discussed in this review, BPs represent a historical and significant class of drugs in the pharmaceutical field. Due to their macrophage inhibitory properties and their distribution in bone tissue, BPs have opened several avenues of research, and various future applications are proposed. The results of studies using BP molecules are attractive due to innovative dosage forms that improve their bioavailability or allow the targeting of the proper tissue. Drug delivery sciences appear essential in designing new treatments by allowing better delivery of BPs, limiting collateral effects, and ultimately allowing a benefit for the patient.

Among the applications mentioned, those related to skin decontamination and decorporation are promising, as evidenced by uranium studies. In addition, the global formation constants from the literature are attractive for both U and other divalent cations. For radionuclides such as radium, cobalt, or strontium, the efficacy of the therapy recommended by nuclear safety authorities worldwide is unsatisfactory, and the set-up of efficient medical countermeasures is essential for the population (civilian, responders, and nuclear workers). Thus, it appears worthwhile to continue the research with EHBP. Moving forward to nanotechnologies in the field of pharmacology must be considered for further investigations to maximize the efficiency of EHBP by focusing its action to decorporate the radiocontaminant.

Through this review, potential cases of drug repurposing are numerous. Even though drug repositioning remains a minority path compared to traditional research, this is a valuable

strategy that gives us early access to strong knowledge, particularly regarding toxicological and pharmacokinetic aspects. In addition to this precious time saving, there is a financial gain by avoiding lengthy and costly screening processes. Although there are many challenges to overcome, because of the abundant literature on the subject, drug repurposing of BPs is becoming feasible.

8 Declaration of interest

The authors have no conflicts of interest to declare.

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10 References

[1] Bassett, C.A., A. Donath, F. Macagno, R. Preisig, H. Fleisch, and M.D. Francis, Diphosphonates in the treatment of myositis ossificans, The Lancet 2(7625) (1969) 845, https://doi.org/10.1016/s0140-6736(69)92293-4.

[2] Salari, N., N. Darvishi, Y. Bartina, M. Larti, A. Kiaei, M. Hemmati, S. Shohaimi, and M. Mohammadi, Global prevalence of osteoporosis among the world older adults: a comprehensive systematic review and meta-analysis, Journal of Orthopaedic Surgery and Research 16(1) (2021), https://doi.org/10.1186/s13018-021-02821-8.

[3] Pors Nielsen, S., The biological role of strontium, Bone 35(3) (2004) 583-588, https://doi.org/10.1016/j.bone.2004.04.026.

[4] UNSCEAR, Sources, Effects and Risks of Ionizing Radiation, United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) 2016 Report, Annex D - Biological Effects of Selected Internal Emitters - Uranium

2017: New York. p. 147 pages.

[5] Henge-Napoli, M.H., E. Ansoborlo, V. Chazel, P. Houpert, F. Paquet, and P. Gourmelon, Efficacy of ethane-1-hydroxy-1,1-bisphosphonate (EHBP) for the decorporation of uranium after intramuscular contamination in rats, International Journal of Radiation Biology 75(11) (1999) 1473-1477, https://doi.org/10.1080/095530099139331.

[6] Houpert, P., V. Chazel, and F. Paquet, A local approach to reduce industrial uranium wound contamination in rats, Canadian Journal of Physiology and Pharmacology 82(2) (2004) 73-78, https://doi.org/10.1139/y03-113.

[7] Gałęzowska, J., Interactions between Clinically Used Bisphosphonates and Bone Mineral: from Coordination Chemistry to Biomedical Applications and Beyond, ChemMedChem 13(4) (2018) 289-302, https://doi.org/10.1002/cmdc.201700769.

[8] Claessens, R.A.M.J. and J.G.M. van der Linden, Stability constants of tin(II) and calcium diphosphonate complexes, Journal of Inorganic Biochemistry 21(1) (1984) 73-82, https://doi.org/10.1016/0162-0134(84)85040-0.

[9] Kaila, L., L.H.J. Lajunen, E.N. Rizkalla, and J. Eloranta, Thermodynamics of complexation of magnesium and calcium ions with dichloromethylenediphosphonate, Talanta 40(7) (1993) 999-1003, https://doi.org/https://doi.org/10.1016/0039-9140(93)80157-M.

[10] Le Cong, H., A.P. Boichenko, I.V. Levin, A.G. Matveeva, and L.P. Loginova, Complexation of Ca2+ and Mg2+ with aminopropylidenebisphosphonic acids in aqueous and micellar solutions of cetylpyridinium chloride, Journal of Molecular Liquids 154(2) (2010) 76-81, https://doi.org/https://doi.org/10.1016/j.molliq.2010.03.014.

[11] Foti, C., O. Giuffrè, and S. Sammartano, Thermodynamics of HEDPA protonation in different media and complex formation with Mg2+ and Ca2+, Journal of Chemical Thermodynamics 66 (2013) 151-160, https://doi.org/10.1016/j.jct.2013.07.005.

[12] Deluchat, V., J.C. Bollinger, B. Serpaud, and C. Caullet, Divalent cations speciation with three phosphonate ligands in the pH-range of natural waters, Talanta 44(5) (1997) 897-907, https://doi.org/10.1016/s0039-9140(96)02136-4.

[13] Scheglova, N.V., T.V. Popova, A.V. Druzhinina, and T.V. Smotrina, Spectrophotometric study of complexation of cobalt (II) with HEDP in aqueous solutions, Journal of Molecular Liquids 286 (2019), https://doi.org/10.1016/j.molliq.2019.110909.

[14] Heller, A., C. Senwitz, H. Foerstendorf, S. Tsushima, L. Holtmann, B. Drobot, and J. Kretzschmar, Europium(III) Meets Etidronic Acid (HEDP): A Coordination Study Combining Spectroscopic, Spectrometric, and Quantum Chemical Methods, Molecules 28(11) (2023), https://doi.org/10.3390/molecules28114469.

[15] Lacour, S., V. Deluchat, and J.C. Bollinger, Complexation of trivalent cations (Al(III), Cr(III), Fe(III)) with two phosphonic acids in the pH range of fresh waters, Talanta 46(5) (1998) 999-1009, https://doi.org/10.1016/s0039-9140(97)00369-x.

[16] Jacopin, C., M. Sawicki, G. Plancque, D. Doizi, F. Taran, E. Ansoborlo, B. Amekraz, and C. Moulin, Investigation of the interaction between 1-hydroxyethane-1,1'-diphosphonic acid (HEDP) and uranium(VI), Inorganic Chemistry 42(16) (2003) 5015-5022, https://doi.org/10.1021/ic0343509.

[17] Sawicki, M., D. Lecerclé, G. Grillon, B. Le Gall, A.L. Sérandour, J.L. Poncy, T. Bailly, R. Burgada, M. Lecouvey, V. Challeix, A. Leydier, S. Pellet-Rostaing, E. Ansoborlo, and F. Taran, Bisphosphonate sequestering agents. Synthesis and preliminary evaluation for in vitro and in vivo uranium(VI) chelation, European Journal of Medicinal Chemistry 43(12) (2008) 2768-2777, https://doi.org/10.1016/j.ejmech.2008.01.018.

[18] Fleisch, H., Development of bisphosphonates, Breast Cancer Research 4(1) (2002) 30-34, https://doi.org/10.1186/bcr414.

[19] Jung, A., S. Bisaz, and H. Fleisch, The binding of pyrophosphate and two diphosphonates by hydroxyapatite crystals, Calcified Tissue Research 11(4) (1973) 269-280, https://doi.org/10.1007/bf02547227.

[20] Russell, R.G.G., Z. Xia, J.E. Dunford, U. Oppermann, A. Kwaasi, P.A. Hulley, K.L. Kavanagh, J.T. Triffitt, M.W. Lundy, R.J. Phipps, B.L. Barnett, F.P. Coxon, M.J. Rogers, N.B. Watts, and F.H. Ebetino, Bisphosphonates: An update on mechanisms of action and how these relate to clinical efficacy, in Annals of the New York Academy of Sciences2007, Blackwell Publishing Inc. p. 209-257.

[21] Rogers, M.J., D.J. Watts, and R.G.G. Russell, Overview of bisphosphonates, Cancer 80(8 SUPPL.) (1997) 1652-1660,

[22] Rogers, M.J., S. Gordon, H.L. Benford, F.P. Coxon, S.P. Luckman, J. Monkkonen, and J.C. Frith, Cellular and molecular mechanisms of action of bisphosphonates, Cancer 88(12 SUPPL.) (2000) 2961-2978,

[23] Shinoda, H., G. Adamek, R. Felix, H. Fleisch, R. Schenk, and P. Hagan, Structure-activity relationships of various bisphosphonates, Calcified Tissue International 35(1) (1983) 87-99, https://doi.org/10.1007/bf02405012.

[24] Ruggiero, A., S. Triarico, A. Romano, P. Maurizi, G. Attina, and S. Mastrangelo, Bisphosphonates: From Pharmacology to Treatment, Biomedical and Pharmacology Journal 16(1) (2023) 221-229, https://doi.org/10.13005/bpj/2603.

[25] Carrel J.P., Abi Najm S., Lysitsa S., Lesclous P., Lombardi T., and Samson J., Phosphorus and bisphosphonates: or the forgotten lessons of the past!, Médecine buccale chirurgie buccale 12(1) (2006) 7-14,

[26] Yohann Bala., Etude multi-échelles des effets osseux des bisphosphonates au cours du traitement de l'ostéoporose ménopausique., 2011, Université Claude Bernard - Lyon I: ECOLE DOCTORALE INTERDISCIPLINAIRE SCIENCES - SANTE. p. 292.

[27] Ostovic, D. and G.S. Brenner, Development of subcutaneous and intramuscular formulations of calcium alendronate salts, Drug Development and Industrial Pharmacy 21(10) (1995) 1157-1169, https://doi.org/10.3109/03639049509026665.

[28] Rossini, M., V. Braga, D. Gatti, D. Gerardi, N. Zamberlan, and S. Adami, Intramuscular clodronate therapy in postmenopausal osteoporosis, Bone 24(2) (1999) 125-129, https://doi.org/10.1016/s8756-3282(98)00154-9.

[29] Cremers, S.C.L.M., G. Pillai, and S.E. Papapoulos, Pharmacokinetics/pharmacodynamics of bisphosphonates: Use for optimisation of intermittent therapy for osteoporosis, Clinical Pharmacokinetics 44(6) (2005) 551-570, https://doi.org/10.2165/00003088-200544060-00001.

[30] Weiss, H.M., U. Pfaar, A. Schweitzer, H. Wiegand, A. Skerjanec, and H. Schran, Biodistribution and plasma protein binding of zoledronic acid, Drug Metabolism and Disposition 36(10) (2008) 2043-2049, https://doi.org/10.1124/dmd.108.021071.

[31] Pentikainen, P.J., I. Elomaa, A.K. Nurmi, and S. Karkkainen, Pharmacokinetics of clodronate in patients with metastatic breast cancer, International Journal of Clinical Pharmacology Therapy and Toxicology 27(5) (1989) 223-228,

[32] Lin, J.H., I.W. Chen, and F.A. Deluna, Nonlinear kinetics of alendronate: Plasma protein binding and bone uptake, Drug Metabolism and Disposition 22(3) (1994) 400-405,

[33] Lin, J.H., Bisphosphonates: A review of their pharmacokinetic properties, Bone 18(2) (1996) 75-85, https://doi.org/10.1016/8756-3282(95)00445-9.

[34] Torregrosa, J.V. and A.M. Ramos, Use of bisphosphonates in chronic kidney disease, Nefrologia 30(3) (2010) 288-297, https://doi.org/10.3265/Nefrologia.pre2010.Mar.10320.

[35] Cremers, S. and S. Papapoulos, Pharmacology of bisphosphonates, Bone 49(1) (2011) 42-49, https://doi.org/10.1016/j.bone.2011.01.014.

688 [36] Lin, J.H., I.W. Chen, F.A. Deluna, and M. Hichens, Renal handling of alendronate in rats:
689 An uncharacterized renal transport system, Drug Metabolism and Disposition 20(4) (1992)
690 608-613,
691 [37] Lin, J.H., D.E. Duggan, I.W. Chen, and R.L. Ellsworth, Physiological disposition of
692 alendronate, a potent anti-osteolytic bisphosphonate, in laboratory animals, Drug
693 Metabolism and Disposition 19(5) (1991) 926-932,
694 [38] Carnevale, V., F. Dicembrino, V. Frusciante, I. Chiodini, S. Minisola, and A. Scillitani,
695 Different patterns of global and regional skeletal uptake of 99mTc-methylene
696 diphosphonate with age: Relevance to the pathogenesis of bone loss, Journal of Nuclear
697 Medicine 41(9) (2000) 1478-1483,
698 [39] Roelofs, A.J., F.P. Coxon, F.H. Ebetino, M.W. Lundy, Z.J. Henneman, G.H. Nancollas, S. Sun,
699 K.M. Blazewska, J.L.F. Bala, B.A. Kashemirov, A.B. Khalid, C.E. McKenna, and M.J. Rogers,
700 Fluorescent risedronate analogues reveal bisphosphonate uptake by bone marrow
701 monocytes and localization around osteocytes in vivo, Journal of Bone and Mineral
702 Research 25(3) (2010) 606-616, https://doi.org/10.1359/jbmr.091009.
703 [40] Bishop, N.J. and G. Russell, Bisphosphonates, in Osteogenesis Imperfecta: A Translational
704 Approach to Brittle Bone Disease2013, Elsevier Inc. p. 495-500.
705 [41] Gertz, B.J., S.D. Holland, W.F. Kline, B.K. Matuszewski, and A.G. Porras, Clinical
706 pharmacology of alendronate sodium, Osteoporosis International 3(3 Supplement) (1993)
707 13-16, https://doi.org/10.1007/bf01623002.
708 [42] Porras, A.G., S.D. Holland, and B.J. Gertz, Pharmacokinetics of alendronate, Clinical
709 Pharmacokinetics 36(5) (1999) 315-328, https://doi.org/10.2165/00003088-199936050-
710 00002.
711 [43] Frith, J.C., J. Mönkkönen, G.M. Blackburn, R.G.G. Russell, and M.J. Rogers, Clodronate and
712 liposome-encapsulated clodronate are metabolized to a toxic ATP analog, adenosine 5'-
713 (β,γ-dichloromethylene) triphosphate, by mammalian cells in vitro, Journal of Bone and
714 Mineral Research 12(9) (1997) 1358-1367, https://doi.org/10.1359/jbmr.1997.12.9.1358.
715 [44] Troehler, U., J.-P. Bonjour, and H. Fleisch, Renal secretion of diphosphonates in rats,
716 Kidney International 8(1) (1975) 6-13, https://doi.org/https://doi.org/10.1038/ki.1975.70.
717 [45] Lawson, M.A., Z. Xia, B.L. Barnett, J.T. Triffitt, R.J. Phipps, J.E. Dunford, R.M. Locklin, F.H.
718 Ebetino, and R.G.G. Russell, Differences between bisphosphonates in binding affinities for

719 hydroxyapatite, Journal of Biomedical Materials Research - Part B Applied Biomaterials
720 92(1) (2010) 149-155, https://doi.org/10.1002/jbm.b.31500.
721 [46] Ri, M.H., Y.M. Jang, U.S. Ri, C.J. Yu, K.I. Kim, and S.U. Kim, Ab initio Investigation of
722 Adsorption Characteristics of Bisphosphonates on Hydroxyapatite (001) Surface, Journal of
723 Materials Science 53(6) (2018) 4252-4261, https://doi.org/10.1007/s10853-017-1880-1.
724 [47] Russell, R.G.G. and M.J. Rogers, Introduction to bisphosphonates and the clinical
725 pharmacology of alendronate, British Journal of Rheumatology 36(SUPPL. 1) (1997) 10-14,
726 [48] Francis, M.D. and D.J. Valent, Historical perspectives on the clinical development of
727 bisphosphonates in the treatment of bone diseases, Journal of Musculoskeletal Neuronal
728 Interactions 7(1) (2007) 2-8,
729 [49] Rizkalla, E.N., M.T.M. Zaki, and M.I. Ismail, Metal chelates of phosphonate-containing
730 ligands—V Stability of some 1-hydroxyethane-1,1-diphosphonic acid metal chelates,
731 Talanta 27(9) (1980) 715-719, https://doi.org/10.1016/0039-9140(80)80164-0.
732 [50] Mukhametshina, R.M. Metal plating based on copper (II) complex compounds with
733 hydroxyethylidene diphosphonic acid. 2020. Institute of Physics Publishing.
734 [51] Bordoloi, J.K., D. Berry, I.U. Khan, K. Sunassee, R.T.M. De Rosales, C. Shanahan, and P.J.
735 Blower, Technetium-99m and rhenium-188 complexes with one and two pendant
736 bisphosphonate groups for imaging arterial calcification, Dalton Transactions 44(11) (2015)
737 4963-4975, https://doi.org/10.1039/c4dt02965h.
738 [52] Volkert, W.A., B. Edwards, J. Simon, D.A. Wilson, E.H. McKenzie, P. Oberle, and R.A.
739 Holmes, In vivo skeletal localization properties of 99mTc complexes of large phosphonate
740 ligands, International Journal of Radiation Applications and Instrumentation. 13(1) (1986)
741 31-35,37, https://doi.org/10.1016/0883-2897(86)90248-5.
742 [53] Balena, R., B.C. Toolan, M. Shea, A. Markatos, E.R. Myers, S.C. Lee, E.E. Opas, J.G. Seedor,
743 H. Klein, D. Frankenfield, H. Quartuccio, C. Fioravanti, J. Clair, E. Brown, W.C. Hayes, and
744 G.A. Rodan, The effects of 2-year treatment with the aminobisphosphonate alendronate
745 on bone metabolism, bone histomorphometry, and bone strength in ovariectomized
746 nonhuman primates, Journal of Clinical Investigation 92(6) (1993) 2577-2586,
747 https://doi.org/10.1172/jci116872.
748 [54] Liberman, U.A., S.R. Weiss, J. Bröll, H.W. Minne, H. Quan, N.H. Bell, J. Rodriguez-Portales,
749 R.W. Downs, Jr., J. Dequeker, M. Favus, E. Seeman, R.R. Recker, T. Capizzi, A.C. Santora, A.
750 Lombardi, R.V. Shah, L.J. Hirsch, and D.B. Karpf, Effect of oral alendronate on bone mineral

density and the incidence of fractures in postmenopausal osteoporosis, New England Journal of Medicine 333(22) (1995) 1437-1444, https://doi.org/10.1056/nejm199511303332201.

[55] Rodan, G.A. and H.A. Fleisch, Bisphosphonates: Mechanisms of action, Journal of Clinical Investigation 97(12) (1996) 2692-2696, https://doi.org/10.1172/jci118722.

[56] Schmidt, A., S.J. Rutledge, N. Endo, E.E. Opas, H. Tanaka, G. Wesolowski, C.T. Leu, Z. Huang, C. Ramachandaran, S.B. Rodan, and G.A. Rodan, Protein-tyrosine phosphatase activity regulates osteoclast formation and function: Inhibition by alendronate, Proceedings of the National Academy of Sciences of the United States of America 93(7) (1996) 3068-3073, https://doi.org/10.1073/pnas.93.7.3068.

[57] Schenk, R., W.A. Merz, R. Mühlbauer, R.G.G. Russell, and H. Fleisch, Effect of ethane-1-hydroxy-1,1-diphosphonate (EHDP) and dichloromethylene diphosphonate (Cl2MDP) on the calcification and resorption of cartilage and bone in the tibial epiphysis and metaphysis of rats, Calcified Tissue Research 11(3) (1973) 196-214, https://doi.org/10.1007/bf02547219.

[58] Murakami, H., N. Takahashi, T. Sasaki, N. Udagawa, S. Tanaka, I. Nakamura, D. Zhang, A. Barbier, and T. Suda, A possible mechanism of the specific action of bisphosphonates on osteoclasts: Tiludronate preferentially affects polarized osteoclasts having ruffled borders, Bone 17(2) (1995) 137-144, https://doi.org/10.1016/s8756-3282(95)00150-6.

[59] Hughes, D.E., K.R. Wright, H.L. Uy, A. Sasaki, T. Yoneda, D.G. Roodman, G.R. Mundy, and B.F. Boyce, Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo, Journal of Bone and Mineral Research 10(10) (1995) 1478-1487, https://doi.org/10.1002/jbmr.5650101008.

[60] Nishikawa, M., T. Akatsu, Y. Katayama, Y. Yasutomo, S. Kado, N. Kugai, M. Yamamoto, and N. Nagata, Bisphosphonates act on osteoblastic cells and inhibit osteoclast formation in mouse marrow cultures, Bone 18(1) (1996) 9-14, https://doi.org/10.1016/8756-3282(95)00426-2.

[61] Hampson, G. and I. Fogelman, Clinical role of bisphosphonate therapy, International Journal of Women's Health 4(1) (2012) 455-469,

[62] Coxon, F.P., K. Thompson, A.J. Roelofs, F.H. Ebetino, and M.J. Rogers, Visualizing mineral binding and uptake of bisphosphonate by osteoclasts and non-resorbing cells, Bone 42(5) (2008) 848-860, https://doi.org/https://doi.org/10.1016/j.bone.2007.12.225.

783 [63] Sato, M., W. Grasser, N. Endo, R. Akins, H. Simmons, D.D. Thompson, E. Golub, and G.A.
784 Rodan, Bisphosphonate action: Alendronate localization in rat bone and effects on
785 osteoclast ultrastructure, Journal of Clinical Investigation 88(6) (1991) 2095-2105,
786 https://doi.org/10.1172/jci115539.
787 [64] Teixeira, S., L. Branco, M.H. Fernandes, and J. Costa-Rodrigues, Bisphosphonates and
788 cancer: A relationship beyond the antiresorptive effects, Mini-Reviews in Medicinal
789 Chemistry 19(12) (2019) 988-998,
790 https://doi.org/10.2174/1389557519666190424163044.
791 [65] Drake, M.T., B.L. Clarke, and S. Khosla, Bisphosphonates: Mechanism of action and role in
792 clinical practice, Mayo Clinic Proceedings 83(9) (2008) 1032-1045,
793 https://doi.org/10.4065/83.9.1032.
794 [66] Bigi, A. and E. Boanini, Calcium phosphates as delivery systems for Bisphosphonates,
795 Journal of Functional Biomaterials 9(1) (2018), https://doi.org/10.3390/jfb9010006.
796 [67] Ebetino, F.H., A.M.L. Hogan, S. Sun, M.K. Tsoumpra, X. Duan, J.T. Triffitt, A.A. Kwaasi, J.E.
797 Dunford, B.L. Barnett, U. Oppermann, M.W. Lundy, A. Boyde, B.A. Kashemirov, C.E.
798 McKenna, and R.G.G. Russell, The relationship between the chemistry and biological
799 activity of the bisphosphonates, Bone 49(1) (2011) 20-33,
800 https://doi.org/10.1016/j.bone.2011.03.774.
801 [68] Luckman, S.P., D.E. Hughes, F.P. Coxon, R.G.G. Russell, and M.J. Rogers, Nitrogen-
802 containing bisphosphonates inhibit the mevalonate pathway and prevent post-
803 translational prenylation of GTP-binding proteins, including Ras, Journal of Bone and
804 Mineral Research 13(4) (1998) 581-589, https://doi.org/10.1359/jbmr.1998.13.4.581.
805 [69] Alanne, A.L., Novel Applications Related to Bisphosphorus Compounds, 2014, University
806 of Eastern Finland. p. 86.
807 [70] Cremers, S., M.T. Drake, F.H. Ebetino, J.P. Bilezikian, and R.G.G. Russell, Pharmacology of
808 bisphosphonates, British Journal of Clinical Pharmacology 85(6) (2019) 1052-1062,
809 https://doi.org/10.1111/bcp.13867.
810 [71] Macedo, F., K. Ladeira, F. Pinho, N. Saraiva, N. Bonito, L. Pinto, and F. Gonçalves, Bone
811 metastases: An overview, Oncology Reviews 11(1) (2017),
812 https://doi.org/10.4081/oncol.2017.321.
813 [72] Minisola, S., J. Pepe, S. Piemonte, and C. Cipriani, The diagnosis and management of
814 hypercalcaemia, BMJ (Online) 350 (2015), https://doi.org/10.1136/bmj.h2723.

[73] Bartel, T.B., M. Kuruva, G. Gnanasegaran, M. Beheshti, E.J. Cohen, A.F. Weissman, and T.L. Yarbrough, SNMMI procedure standard for bone scintigraphy 4.0, Journal of Nuclear Medicine Technology 46(4) (2018) 398-404,

[74] Finlay, I.G., M.D. Mason, and M. Shelley, Radioisotopes for the palliation of metastatic bone cancer: A systematic review, Lancet Oncology 6(6) (2005) 392-400, https://doi.org/10.1016/s1470-2045(05)70206-0.

[75] Bounds, L., F. McGrath, and M. Taubert, Hypercalcaemia to hypocalcaemia: tetany as a side effect of intravenous bisphosphonate treatment, BMJ Case Reports 15(4) (2022), https://doi.org/10.1136/bcr-2022-249141.

[76] Chartrand, N.A., C.K. Lau, M.T. Parsons, J.J. Handlon, Y.C. Ronquillo, P.C. Hoopes, and M. Moshirfar, Ocular Side Effects of Bisphosphonates: A Review of Literature, Journal of Ocular Pharmacology and Therapeutics 39(1) (2023) 3-16, https://doi.org/10.1089/jop.2022.0094.

[77] Cheng, L., M. Ge, Z. Lan, Z. Ma, W. Chi, W. Kuang, K. Sun, X. Zhao, Y. Liu, Y. Feng, Y. Huang, M. Luo, L. Li, B. Zhang, X. Hu, L. Xu, X. Liu, Y. Huo, H. Deng, J. Yang, Q. Xi, Y. Zhang, J.A. Siegenthaler, and L. Chen, Zoledronate dysregulates fatty acid metabolism in renal tubular epithelial cells to induce nephrotoxicity, Archives of Toxicology 92(1) (2018) 469-485, https://doi.org/10.1007/s00204-017-2048-0.

[78] Conte, P. and V. Guarneri, Safety of intravenous and oral bisphosphonates and compliance with dosing regimens, Oncologist 9(SUPPL. 4) (2004) 28-37, https://doi.org/10.1634/theoncologist.9-90004-28.

[79] Coropciuc, R., R. Coopman, M. Garip, E. Gielen, C. Politis, T. Van den Wyngaert, and B. Beuselinck, Risk of medication-related osteonecrosis of the jaw after dental extractions in patients receiving antiresorptive agents — A retrospective study of 240 patients, Bone 170 (2023), https://doi.org/10.1016/j.bone.2023.116722.

[80] Lu, L., J. Zhang, and J. Li, Potential risks of rare serious adverse effects related to long-term use of bisphosphonates: An overview of systematic reviews, Journal of Clinical Pharmacy and Therapeutics 45(1) (2020) 45-51, https://doi.org/10.1111/jcpt.13056.

[81] Silverman, S., E. Kupperman, and S. Bukata, Bisphosphonate-related atypical femoral fracture: Managing a rare but serious complication, Cleveland Clinic Journal of Medicine 85(11) (2018) 885-893, https://doi.org/10.3949/ccjm.85a.17119.

[82] Nassar, K. and S. Janani, Diffuse adverse cutaneous reactions induced by zoledronic acid administration: a case report: Eruptions cutanées diffuses induites par l'administration de l'acide zolédronique, Osteoporosis International 32(12) (2021) 2583-2586, https://doi.org/10.1007/s00198-021-06021-2.

[83] Rodríguez, A.J., N. Nerlekar, and P.R. Ebeling, Cardiac adverse events in bisphosphonate and teriparatide users: An international pharmacovigilance study, Bone 168 (2023), https://doi.org/10.1016/j.bone.2022.116647.

[84] Opperman, K.S., K. Vandyke, K.C. Clark, E.A. Coulter, D.R. Hewett, K.M. Mrozik, N. Schwarz, A. Evdokiou, P.I. Croucher, P.J. Psaltis, J.E. Noll, and A.C. Zannettino, Clodronate-Liposome Mediated Macrophage Depletion Abrogates Multiple Myeloma Tumor Establishment In Vivo, Neoplasia (United States) 21(8) (2019) 777-787, https://doi.org/10.1016/j.neo.2019.05.006.

[85] La-Beck, N.M., X. Liu, H. Shmeeda, C. Shudde, and A.A. Gabizon, Repurposing amino-bisphosphonates by liposome formulation for a new role in cancer treatment, Seminars in Cancer Biology 68 (2021) 175-185, https://doi.org/10.1016/j.semcancer.2019.12.001.

[86] Tian, L., X. Yi, Z. Dong, J. Xu, C. Liang, Y. Chao, Y. Wang, K. Yang, and Z. Liu, Calcium Bisphosphonate Nanoparticles with Chelator-Free Radiolabeling to Deplete Tumor-Associated Macrophages for Enhanced Cancer Radioisotope Therapy, ACS Nano 12(11) (2018) 11541-11551, https://doi.org/10.1021/acsnano.8b06699.

[87] Xi, Y., T. Jiang, Y. Yu, J. Yu, M. Xue, N. Xu, J. Wen, W. Wang, H. He, Y. Shen, D. Chen, X. Ye, and T.J. Webster, Dual targeting curcumin loaded alendronatehyaluronan- octadecanoic acid micelles for improving osteosarcoma therapy, International Journal of Nanomedicine 14 (2019) 6425-6437, https://doi.org/10.2147/ijn.s211981.

[88] Hanif, M., S. Shah, A. Rasul, G. Abbas, M. Zaman, M.W. Amjad, M.A.G. Raja, H.U. Khan, M. Ashfaq, and O. Iqbal, Enhancement of oral bioavailability of ibandronate through gastroretentive raft forming drug delivery system: In vitro and in vivo evaluation, International Journal of Nanomedicine 15 (2020) 4847-4858, https://doi.org/10.2147/ijn.s255278.

[89] Hosny, K.M., O.A.A. Ahmed, and R.T. Al-Abdali, Enteric-coated alendronate sodium nanoliposomes: A novel formula to overcome barriers for the treatment of osteoporosis, Expert Opinion on Drug Delivery 10(6) (2013) 741-746, https://doi.org/10.1517/17425247.2013.799136.

878 [90] Okimoto, N., Y. Uemura, T. Yoshioka, S. Arita, H. Tsurukami, H. Otomo, S. Nishida, T.
879 Ogawa, K. Hirao, S. Ikeda, H. Matsumoto, Y. Toten, Y. Katae, Y. Okazaki, T. Nakagawa, and
880 A. Sakai, Treatment with once-weekly alendronate oral jelly compared with once-weekly
881 alendronate oral tablet for Japanese patients with primary osteoporosis: An open-label,
882 prospective, observational study, Health Science Reports 2(1) (2019),
883 https://doi.org/10.1002/hsr2.107.
884 [91] Giusti, A., G. Bianchi, A. Barone, and D.M. Black, A novel effervescent formulation of oral
885 weekly alendronate (70 mg) improves persistence compared to alendronate tablets in
886 post-menopausal women with osteoporosis, Aging Clinical and Experimental Research
887 33(9) (2021) 2529-2537, https://doi.org/10.1007/s40520-020-01777-9.
888 [92] Cruz, L., E. Fattal, L. Tasso, G.C. Freitas, A.B. Carregaro, S.S. Guterres, A.R. Pohlmann, and
889 N. Tsapis, Formulation and in vivo evaluation of sodium alendronate spray-dried
890 microparticles intended for lung delivery, Journal of Controlled Release 152(3) (2011) 370-
891 375, https://doi.org/10.1016/j.jconrel.2011.02.030.
892 [93] Nasr, M., G.A.S. Awad, S. Mansour, I. Taha, A.A. Shamy, and N.D. Mortada, Different
893 modalities of NaCl osmogen in biodegradable microspheres for bone deposition of
894 risedronate sodium by alveolar targeting, European Journal of Pharmaceutics and
895 Biopharmaceutics 79(3) (2011) 601-611, https://doi.org/10.1016/j.ejpb.2011.07.010.
896 [94] Boche, M. and V. Pokharkar, Microemulsion assisted transdermal delivery of a hydrophilic
897 anti-osteoporotic drug: Formulation, in vivo pharmacokinetic studies, in vitro cell
898 osteogenic activity, Journal of Applied Pharmaceutical Science 10(8) (2020) 8-19,
899 https://doi.org/10.7324/japs.2020.10802.
900 [95] Gyanewali, S., P. Kesharwani, A. Sheikh, F.J. Ahmad, R. Trivedi, and S. Talegaonkar,
901 Formulation development and in vitro–in vivo assessment of protransfersomal gel of anti-
902 resorptive drug in osteoporosis treatment, International Journal of Pharmaceutics 608
903 (2021), https://doi.org/10.1016/j.ijpharm.2021.121060.
904 [96] Crisponi, G., A. Dean, V. Di Marco, J.I. Lachowicz, V.M. Nurchi, M. Remelli, and A. Tapparo,
905 Different approaches to the study of chelating agents for iron and aluminium overload
906 pathologies, Analytical and Bioanalytical Chemistry 405(2-3) (2013) 585-601,
907 https://doi.org/10.1007/s00216-012-6468-7.

908 [97] Chen, L., J. Liu, W. Zhang, J. Zhou, D. Luo, and Z. Li, Uranium (U) source, speciation, uptake,
909 toxicity and bioremediation strategies in soil-plant system: A review, Journal of Hazardous
910 Materials 413 (2021), https://doi.org/10.1016/j.jhazmat.2021.125319.
911 [98] Migianu-Griffoni, E., C. Mbemba, R. Burgada, D. Lecerclé, F. Taran, and M. Lecouvey,
912 Design and synthesis of new polyphosphorylated upper-rim modified calix[4]arenes as
913 potential and selective chelating agents of uranyl ion, Tetrahedron 65(7) (2009) 1517-1523,
914 https://doi.org/10.1016/j.tet.2008.11.076.
915 [99] Xu, K., W. Ge, G. Liang, L. Wang, Z. Yang, Q. Wang, I.M. Hsing, and B. Xu, Bisphosphonate-
916 containing supramolecular hydrogels for topical decorporation of uranium-contaminated
917 wounds in mice, International Journal of Radiation Biology 84(5) (2008) 353-362,
918 https://doi.org/10.1080/09553000802029902.
919 [100] Fisher, D.R., Decorporation: Officially a word, Health Physics 78(5) (2000) 563-565,
920 https://doi.org/10.1097/00004032-200005000-00015.
921 [101] Abergel, R.J., Chelation of Actinides, in RSC Metallobiology, R.J. Ward, et al., Editors.
922 2017, Royal Society of Chemistry. p. 183-212.
923 [102] Adriana Beatriz, M., B. Carola Bettina, O. Nadia Soledad, T. Deborah Ruth, and U. Angela
924 Matilde, Bisphosphonates as Chelating Agents in Uranium Poisoning, in Recent Techniques
925 and Applications in Ionizing Radiation Research, M.M. Ahmed and A. Basim, Editors. 2020,
926 IntechOpen: Rijeka. p. Ch. 8.
927 [103] Ansoborlo, E., B. Amekraz, C. Moulin, V. Moulin, F. Taran, T. Bailly, R. Burgada, M.H.
928 Hengé-Napoli, A. Jeanson, C. Den Auwer, L. Bonin, and P. Moisy, Review of actinide
929 decorporation with chelating agents, Comptes Rendus Chimie 10(10-11) (2007) 1010-1019,
930 https://doi.org/10.1016/j.crci.2007.01.015.
931 [104] Stradling, G.N., M.H. Hengé-Napoli, F. Paquet, J.L. Poncy, P. Fritsch, and D.M. Taylor,
932 Approaches for experimental evaluation of chelating agents, Radiation Protection
933 Dosimetry 87(1) (2000) 19-27, https://doi.org/10.1093/oxfordjournals.rpd.a032976.
934 [105] Wang, X., C. Shi, J. Guan, Y. Chen, Y. Xu, J. Diwu, and S. Wang, The development of
935 molecular and nano actinide decorporation agents, Chinese Chemical Letters 33(7) (2022)
936 3395-3404, https://doi.org/https://doi.org/10.1016/j.cclet.2022.04.017.
937 [106] Yue, Y.C., M.H. Li, H.B. Wang, B.L. Zhang, and W. He, The toxicological mechanisms and
938 detoxification of depleted uranium exposure, Environmental Health and Preventive
939 Medicine 23(1) (2018), https://doi.org/10.1186/s12199-018-0706-3.

[107] Wang, L., Z. Yang, J. Gao, K. Xu, H. Gu, B. Zhang, X. Zhang, and B. Xu, A biocompatible method of decorporation: Bisphosphonate-modified magnetite nanoparticles to remove uranyl ions from blood, Journal of the American Chemical Society 128(41) (2006) 13358-13359, https://doi.org/10.1021/ja0651355.

[108] Uehara, A., D. Matsumura, T. Tsuji, H. Yakumaru, I. Tanaka, A. Shiro, H. Saitoh, H. Ishihara, and S. Homma-Takeda, Uranium chelating ability of decorporation agents in serum evaluated by X-ray absorption spectroscopy, Analytical Methods 14(24) (2022) 2439-2445, https://doi.org/10.1039/d2ay00565d.

[109] Fukuda, S., H. Iida, M. Ikeda, X. Yan, and Y. Xie, Toxicity of uranium and the removal effects of CBMIDA and EHBP in simulated wounds of rats, Health Physics 89(1) (2005) 81-88, https://doi.org/10.1097/01.hp.0000156956.42935.28.

[110] Ubios, A.M., M.B. Guglielmotti, and R.L. Cabrini, Ethane 1-hydroxy-1, 1-diphosphonate (ehdp) counteracts the inhibitory effect of uranyl nitrate on bone formation, Archives of Environmental Health 45(6) (1990) 374-379, https://doi.org/10.1080/00039896.1990.10118758.

[111] Ubios, A.M., E.M. Braun, and R.L. Cabrini, Lethality due to uranium poisoning is prevented by ethane-1-hydroxy-1, l-biphosphonate (Ehbp), Health Physics 66(5) (1994) 540-544, https://doi.org/10.1097/00004032-199405000-00005.

[112] Ubios, A.M., E.M. Braun, and R.L. Cabrini, Effect of biphosphonates on abnormal mandibular growth of rats intoxicated with uranium, Health Physics 75(6) (1998) 610-613, https://doi.org/10.1097/00004032-199812000-00004.

[113] Martínez, A.B., R.L. Cabrini, and A.M. Ubios, Orally administered ethane-1-hydroxy-1,1-biphosphonate reduces the lethal effect of oral uranium poisoning, Health Physics 78(6) (2000) 668-671, https://doi.org/10.1097/00004032-200006000-00009.

[114] Martinez, A.B., P.M. Mandalunis, C.B. Bozal, R.L. Cabrini, and A.M. Ubios, Renal function in mice poisoned with oral uranium and treated with ethane-1-hydroxy-1,1-bisphosphonate (EHBP), Health Physics 85(3) (2003) 343-347, https://doi.org/10.1097/00004032-200309000-00010.

[115] Bozal, C.B., A.B. Martinez, R.L. Cabrini, and A.M. Ubios, Effect of ethane-1-hydroxy-1,1-bisphosphonate (EHBP) on endochondral ossification lesions induced by a lethal oral dose of uranyl nitrate, Archives of Toxicology 79(8) (2005) 475-481, https://doi.org/10.1007/s00204-005-0649-5.

[116] Liu, P., G. Chen, and J. Zhang, A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives, Molecules 27(4) (2022), https://doi.org/10.3390/molecules27041372.