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Bisphosphonates as Chelating Agents in Uranium Poisoning

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Abstract

The study of uranium toxicity is very important for public health in general and especially for workers involved in the processes of uranium mining and milling because of the immediate and/or mediate risks of exposure. Most available studies show unsuccessful attempts to eliminate uranium from target organs once the poisoning has occurred. Our group has managed to avoid damage to target organs (short-term kidney and long-term bone damage) in a high percentage of animals treated with lethal doses of uranyl nitrate through the effective chelating action of a single dose of bisodic etidronate. In this context, the contributions of our team and other groups working on chelating therapies provide a starting point for progress in the search for agents for preventing and/or reducing the toxic effects of uranium.

Keywords: uranium, poisoning, bisphosphonates, bisodic etidronate, chelating agents

1. Introduction

As from the World War II, increasing interest in nuclear power brought about an increase in uranium exploration and the development of new plants for uranium processing and manufacture in many countries. These activities may involve accidental occupational poisoning for workers, and no protocol has yet been designed for rapid application in these cases to prevent rapid-onset life-threatening complications due to kidney failure. As the uranium-related industry increases, so does the potential for these accidental poisonings. In this chapter we present and discuss the papers published by our laboratory over almost 40 years of research in the field of the toxicology of uranium in animal models under the direction of Dr. Romulo Luis Cabrini, which replicated hypothetical situations of acute accidental exposure to uranium via different routes of entry. Our group has specifically studied toxicity on target organs (kidneys and bone) and developed a potentially effective protocol with bisodic etidronate used as a chelating agent that prevents kidney failure and bone alterations. We are currently processing histological and histomorphometrical kidney and bone samples from a long-term experiment with animals poisoned with lethal doses of uranyl nitrate and treated with a single subcutaneous dose of bisodic etidronate which survived for 1 year after treatment with good quality of life, similar to that of controls.

2. Biological impact of uranium: pharmacokinetics

Because uranium (U) is present in food, air, soil, and water, humans are constantly exposed to certain amounts of this element. Notwithstanding, the biological impact of such natural exposure on human physiology and pathophysiology is not yet fully known. However, it is known that overexposure to U may result in toxicity, which is derived from an excessive accumulation of the element in the organism. This accumulation depends on various factors, including route of entry, duration of exposure, dose, chemical compound of which it forms part, and absorption [1, 2].

Uranium can enter the body through different routes: oral, inhalation, percutaneous, or subcutaneous. Regardless of the route of entry, absorbed U enters systemic circulation, is distributed in the organism, and accumulates mainly in the bones (66%), kidneys (8%), and liver (16%) [3]. Approximately 1–5% of an oral dose is absorbed in the digestive tract [4], and nearly 60% of U is eliminated rapidly from the blood and slowly from organ depots with the urine by renal mechanisms in the first 24 h [5, 6]. In rats, most of the absorbed U is eliminated by the kidneys in a few days; half of it is excreted within 2–6 days [7] and the rest within 7 days. Ninety-five percent (95%) of U present in the kidneys of intoxicated rats is excreted in the urine within a week, and very small amounts remain in other organs [6, 8]. Uranium compounds can dissociate and form new compounds with various organic and inorganic anions. In body fluids, tetravalent uranium (+4) tends to oxidize to the hexavalent form (+6) followed by uranyl ion formation. Experimentally, using animal models, it was shown that uranyl ions are associated with ultrafiltered low molecular weight serum proteins, transferrin, and other plasma proteins [3]. In 2005, Vidaud *et al*. [9] were able to identify uranium-binding proteins in human serum fractions by means of an in vitro-sensitive procedure involving a combination of bidimensional chromatography with time-resolved fluorescence, coupled with proteomic analysis. These authors demonstrated that not all targets are metalloproteins, suggesting that uranyl ions can use a wide variety of binding sites, thus providing additional insights for a better understanding of uranium chemical toxicity. U also binds to phospholipids and membrane proteins of proximal contoured tubules [10].

On the other hand, when injected intravenously, almost 50% of the U is eliminated, while the other 50% is deposited in the skeleton (25%) and in soft tissues (25%), mainly kidneys. The U deposited in extrarenal soft tissues—mainly liver and spleen—is removed very slowly [11]. Orcutt *et al*. [12] reported that the percutaneous route constitutes an effective route of entry for soluble U compounds. De Rey *et al*. [13] demonstrated that uranyl nitrate (UN) can penetrate through the skin of adult Wistar rats in approximately 15 min and accumulate in the intercellular spaces between the granular and corneal layers. After 48 h, the U was not found in the skin, and the animals experienced signs of severe toxicity ranging from weight loss to death, clearly indicating its passage from this organ into the circulation. The retention of U particles after inhalation depends on the size of the particulate and the type of U compound. Harris *et al*. [14] reported that insoluble compounds (uranium dioxide and uranium trioxide) with average particle size below 2 µm have very long biological half-lives (120 days or more).

Uranium impact on human health may come from abandoned hard rock mines, which can contaminate the three natural resources—water, air, and soil—thus becoming a potential source of chronic exposure and toxicity for individuals living in the area.

It is worth noting that U toxicity depends on several factors, such as sex, age, body mass index [15], and species. Of all the mammals studied, humans seem to be the least sensitive to U [16]. Still, overexposure to U may cause pathological alterations in the different organs in both humans and animals.

2.1 Oral route

The oral route is important because of the possibility of the general population having to ingest U-contaminated water or food continuously, as well as the risk of workers ingesting toxic and/or lethal doses during accidents in some of the steps of the enrichment process. The literature contains interesting—though limited—data on the incorporation of U via the oral route, obtained from studies in both experimental animals and humans.

Harrison *et al*. [17] studied the gastrointestinal absorption of two U compounds administered orally by gastric intubation (gavage) in hamsters and demonstrated that soluble UN absorption was seven times greater than insoluble uranium dioxide (UD) absorption.

La Touche *et al*. [6] investigated the absorption and kinetics of UN administered orally in a model with fasting adult male Wistar rats that replicate the human intake of contaminated water after a night of fasting. It produced more absorption of the compound than if the rats had not been subjected to fasting.

Anke *et al*. [18] studied wild plants and cultivated plants from the immediate vicinity of uranium waste dumps and found that they stored up to eightfold higher uranium concentrations than controls.

In 2013, the ATSDR (US Agency for Toxic Substances and Disease Registry) [1] established a minimum risk level for the ingestion of U, known by the acronym MRL (minimum risk level), which is 0.002 μg of U per kilo of weight per day. The MRL is an estimate of the daily exposure of a human being to a dangerous substance that probably does not represent an appreciable risk of adverse effects (excluding cancer) beyond the time of exposure. MRL values for both oral and inhalation routes vary with different exposure times: acute (1–14 days), intermediate (15–365 days), and chronic (365 days or longer).

Zamora *et al*. [19] studied the effects of chronic ingestion of U in humans after drinking contaminated water. They suggested that intake of U doses such as those found in some underground water wells over prolonged periods of time altered the renal function. These observed effects may represent a manifestation of subclinical toxicity that does not necessarily lead to renal dysfunction or obvious injury. Instead, it may be the first stage in a spectrum in which chronic intake of elevated U levels can lead to irreversible renal injury [20].

In addition to being ingested via drinking water, U can be taken in orally through contaminated food, mainly beef and fish.

The concentrations of U in fish muscle (per gram of dry weight) extracted from a Canadian lake contaminated by tributaries of a U processing plant were 7–11 times higher than in those from uncontaminated lakes [21]. Lapham *et al*. [22] analyzed U concentrations in cattle muscle and found that although U levels in the muscle of the exposed cattle were almost imperceptible with respect to the controls, U concentration in the liver and renal tissues was 4 times higher in the exposed cattle than in the control. Moreover, U levels in the bone (femur samples) were found to be 13 times higher than in the controls.

Other than beef and fish, some underground vegetables such as potatoes, sweet potatoes, and turnips contribute approximately 38% of the U intake per diet according to the general food consumption rate [23].

Although there are no studies in humans regarding the lethal effects of U and its compounds when ingested orally, several animal studies have shown that a very high intake dose of U can be lethal for acute (1–14 days), subacute (14–365 days), and chronic (more than 365 days) exposures [24].

Maynard and Hodge [1] obtained an LD_{50} (lethal dose 50 population %) value for oral (UN) of 1579 mg of U/kg/day in rats of both sexes (without specifying

strain) in a 30-day study. Maynard *et al.* [25] found 16% mortality in rats that were intoxicated with 664 mg/kg/day of U for 30 days orally. In our laboratory, Martinez *et al.* [26] studied the effects of a lethal dose of UN administered by gavage in male mice: we found that 350 mg/kg was the LD_{99} (lethal dose 99 population %) on the third day of the experiment.

Studies in rats suggest that the primary pathway for gastrointestinal absorption of soluble U is through the small intestinal epithelium [27, 28] via the transcellular pathway [29]. In the event of ingestion, the digestive tract is the first biological system exposed to U intake via the intestinal lumen. However, little research has addressed the biological consequences of contamination with U on intestinal properties such as the barrier function and/or the immune status of this tissue. Dublineau *et al.* [28, 29] studied both acute contamination with Depleted Uranium (DU) at high doses and chronic contamination at low doses on inflammatory reactions in the intestine when orally delivered. They found that acute and chronic ingestion of DU modulated expression and/or production of cytokines in the intestine and had similar effects to those observed with lead on the nitric oxide pathway.

2.2 Inhalation route

Inhalation is a major route of human exposure to environmental particles. When inhaled, U particles, based on their size, may be deposited on the lung ciliated epithelial lining or may reach the lower respiratory tract. Small particles of U containing dust could be inhaled by U miners and people living close to the mines, penetrating deeply into their lungs. The particles can be phagocytosed by alveolar macrophages (AM) and/or cross the alveolar capillary barrier, thereby reaching the bloodstream.

Uranium toxicity depends, among other things, on the solubility, dose, and route of exposure. In general, the more insoluble U compounds (uranium trioxide, uranium dioxide, uranium peroxide, and triuranium octaoxide) have greater potential for long-term effects in the lung, probably due to the long-term retention of the compound [1]. On the contrary, soluble U compounds (uranyl fluoride, 1 uranium tetrachloride, and uranyl nitrate hexahydrate), due to their easier absorption in the lungs and passage into the bloodstream, exert their action mainly on the extrapulmonary organs [1]. Thus, lungs and extrapulmonary organs are susceptible to negative impact by U particles.

Even though U can enter the body through different routes, macrophages are always the first cells to respond to these or any other xenobiotic agents. Primary cultured AM is a suitable *in vitro* model for studying the effects of U at cell level and its cytotoxic mechanism. Tasat and de Rey [30] demonstrated the adverse effects of insoluble uranium dioxide using AM obtained from rat bronchoalveolar lavage. The study revealed the ability of macrophages to phagocyte U particles in a short time despite the high toxicity that metal exerts on cell membranes. The ultrastructural analysis detected the U particles confined within intracytoplasmic vacuoles or free in the macrophage cytoplasm, which in turn could lead to cell death. More recently, Orona *et al.* [31] demonstrated that exposure to the soluble U compound UN, also induced cell death in cultured rat AM. The cytotoxic mechanism studied in this *in vitro* model showed that at low doses, UN stimulated phagocytosis and generation of superoxide anion (O_2^-), while at high doses, it induced the secretion of TNFα. Therefore, Orona *et al.* suggested that cell death at low doses was principally mediated by reactive oxygen species (ROS), while the signaling pathway when exposed to higher UN doses was principally mediated by the release of pro-inflammatory mediators inhibiting the generation of ROS.

These authors hypothesized that an oxidant-antioxidant imbalance provoked by activated macrophages after U inhalation may lead to an alteration in macrophage

metabolism. This cell response may in turn modify the pulmonary tissue microenvironment, thereby participating in the development of lung pathologies associated with U exposure. Uranium exposure involves both an occupational risk for miners and workers who handle it constantly, and an environmental hazard to the health of the population at large. Katz *et al*. [32] updated the chemistry, pharmacokinetics, and toxicological effects of U on several systems in the mammalian body which were previously reviewed by Craft *et al*. [33] and Briner [34]. However, the adverse impact on human health is still controversial. In this context, we studied the effect of UN on macrophages differentiated from human THP-1 monocytes. As is clearly shown in **Figure 1**, increasing doses of soluble UN provoked a significant decrease in macrophage-like cell viability, similar to what was observed with murine macrophages. Extrapolated to the *in vivo* situation, these findings might help to explain, in part, how acute or chronic inflammatory states observed in U-exposed individuals with DNA lesions could be linked to the U compounds dose.

Despite all the research conducted by the scientific community to clarify and understand the effect of both insoluble and soluble U compounds, the data reported appear to have somehow been compromised by the political agendas of special interest groups at both national and international levels.
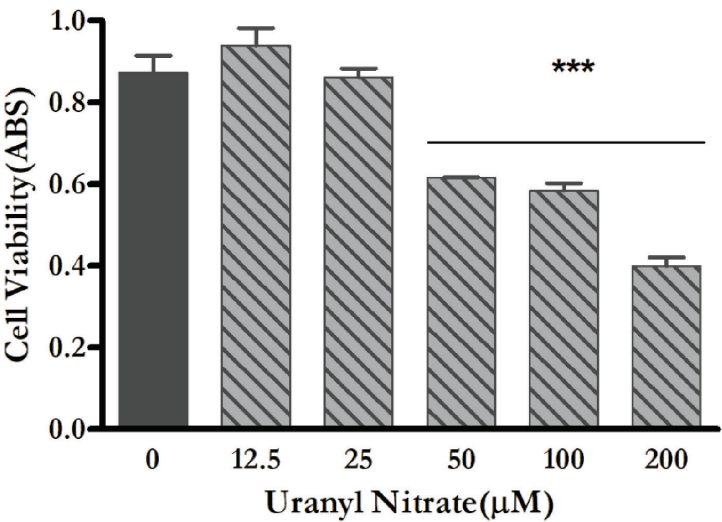

Figure 1.
Cell viability of macrophages differentiated from human THP-1 monocytes exposed to UN. Higher doses of UN caused a reduction in cell viability. Values are represented as mean ± SD. Results are compared employing one-way ANOVA followed by Dunnett's test P < 0.001.

2.3 Dermal contact

2.3.1 Percutaneous absorption

Chemically induced renal failure caused 100% mortality in male Wistar rats after five daily exposures to 237 or 1928 mg U/kg/day as UN hexahydrate or ammonium uranyl tricarbonate, respectively, applied in a water-Vaseline® emulsion. A 60% mortality rate was also reported for other male Wistar rats that received daily applications of 1965 mg U/kg as uranyl acetate dihydrate for 1–11 days. No death was reported for other Wistar rats similarly treated with 2103 mg U/kg/day as ammonium diuranate or to an unspecified dose of UD [13].

Decreased survival was observed in female Wistar rats following dermal application of 280 mg U as UN hexahydrate diluted in an oil–water emulsion; survival was inversely related to the duration of exposure and the application area [35]. A

24-h application to areas of 0.5, 1, 2, 4, 6, 8, or 16 cm^2 resulted in survival rates of 80, 83, 67, 29, 33, 0, and 0%, respectively; application to 8 cm^2 for 1 min, 7 min, 15 min, 30 min, 1 h, 8 h, or 24 h resulted in survival rates of 100, 100, 100, 67, 45, 43, 10, and 0%, respectively.

2.3.2 Subcutaneous absorption

Subcutaneous or intradermal U contamination takes place in the presence of a wound. This poses a real risk to workers handling U dust on a daily basis and to soldiers who fought in the modern wars (Balkan, Gulf, etc.). Penetration of DU shrapnel bullets into the skin has become the focus of increasing attention. In fact, the only documented cases of exposure to U are those of the Gulf War veterans who retained DU shrapnel fragments [36].

Subcutaneous implantation of insoluble UO_2 was investigated in our laboratory in an experimental animal model in rats by de Rey *et al*. [37]. This group showed that animals receiving doses higher than 0.01 g/kg died within the first 6 days due to acute renal failure. Histological analysis revealed the presence of deposits of uranium taken up by macrophages at 24 and 48 h postexposure. Deposits were found between the endothelial cells and the renal parenchyma, suggesting that the U insoluble compound implanted subcutaneously is transported and deposited.

3. Uranium toxicity and its main target organs

Regardless of the route of entry, U has two main target organs: the kidney and the bone. The magnitude of the adverse U impact in these two organs is dose- and time-dependent.

3.1 Renal toxicity

In 1991, the World Health Organization (WHO) identified U and other heavy metals such as lead, mercury, and cadmium as nephrotoxic elements. Early in 1949, Voegtlin and Hodge [38], in the framework of the Manhattan Project, studied the toxic effects of U in animal experimental models, finding characteristic histological features of kidney injury, regardless of the route of entry to the organism. Subsequently, many animal studies have shown that inhalation, oral exposure, or dermal exposure to uranium results in kidney damage [35, 39–41]. This damage was histologically manifested principally as glomerular and tubular wall degeneration. After being filtered by the renal glomerulus, the uranium-bicarbonate complex enters the glomerular urine. The bicarbonate is reabsorbed into the venous blood and loses the uranyl ion at the time of its passage through the proximal contoured tubule. The uranyl ion reacts with the membrane proteins of the cylindrical cells causing cell damage, and, when doses are high, cell death can occur, releasing the cell content into the urine [42]. Ultrastructural analysis showed damage to the endothelial cells in the glomerulus, such as loss of cell processes and reduction in the density of the endothelial fenestrae [43–46]. Although the most obvious effect of U exposure is damage to the proximal convoluted tubules, necrotic cells from the tubular epithelium have also been reported [19]. The histological alterations observed as a result of exposure to UN include partial degeneration, necrosis, and cast formation in proximal convoluted tubule although with damage to brush border, but glomeruli remain intact [47]. In our laboratory, we have observed that kidneys of exposed animals revealed the usual U-induced tubule necrosis lesions with abundant hyaline cylinders and extensive areas of necrosis after 48 h of 350 mg UN/kg b.w. exposure.

At glomerular level, although glomerulus structure appears to be intact, widening of the Bowman's capsule is evident [41] (**Figure 2**).

The analysis of autoradiographic, histological, and renal functioning studies showed that the site where the U produces greatest injury is the distal third of the proximal contoured tubule. If the tubular cells are not too damaged, they can repair the alterations and regenerate. Recovery is rapid despite the fact that the regenerated cells are atypical in some details, and within a few weeks or a month, both the biochemical parameters and renal histology are normalized. In 1982, Haley [44] studied different U compounds with special interest in their effects on the renal parenchyma. Of all the compounds studied, UN proved to be the most nephrotoxic compound, which explains why it is frequently used to produce experimental renal failure.
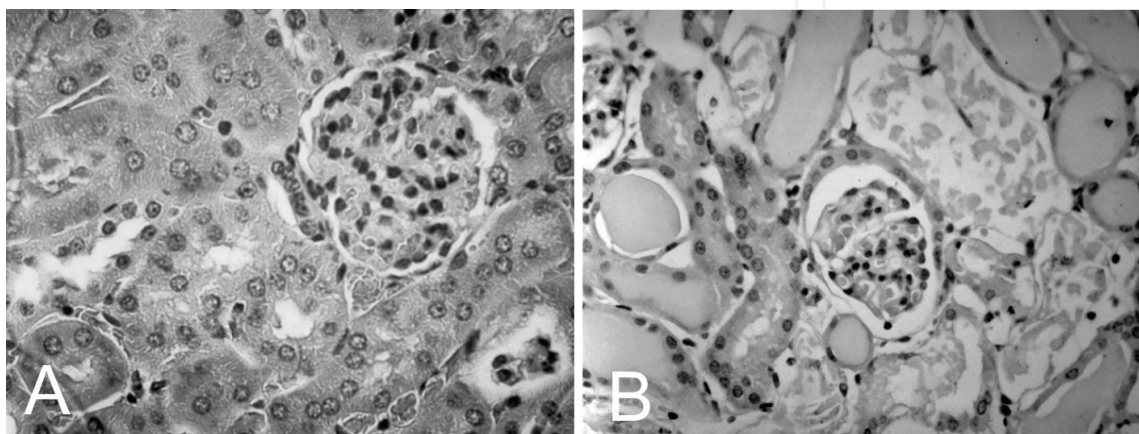

Figure 2.
Histopathology of kidney tissue from control and UN exposure groups. Cortical region of kidney: (A) control animal, intact tubular epithelium with no damage and (B) animal exposed to 350 mg UN/kg b.w., 48 h, tubular damage, loss of microvilli, extensive necrosis and cast formation in tubules, and necrosis of proximal tubular epithelium. Marked vacuolization of the tubules, abundant hyaline cylinders, and extensive areas of necrosis. Widening of the uriniferous tubule and of Bowman's capsule is also evident (HE 400X).

3.2 Effects on bone tissue

The incorporation of U compounds into bone tissue has been demonstrated by biochemical analysis, autoradiographic methods, neutron activation analysis, and X-ray microanalysis. Many U isotopes are considered more as a chemical risk than as a radiological risk. The radioactivity of UN can be considered negligible, since the radioactivity of ^{238}U, as we have seen, is very low [48–50]. This would explain that bone formation alterations could be due preferably to the chemical toxicity of U [51]. As mentioned above, Hursh *et al*. [11] reported that 25% of the systemically administered U is deposited in the skeleton and tends to bind to the newly formed bone. Since the bone is the only tissue in which U deposits can be found a long time after exposure, it is considered to be the critical organ in chronic exposures, displacing the kidney as a target. Autoradiographic studies demonstrated initial U deposits on the surfaces of the endosteum, the periosteum, and the haversian bone, particularly in areas where calcification occurs. Back in 1948, Neuman *et al*. showed that there was an association between U deposits and bone formation [52], but at that time, it was not known whether this process was affected. Three decades later, for the first time, it was found that U also affects bone metabolism in acute poisoning. Guglielmotti *et al*. [53, 54] were the first to demonstrate, by means of histological and histomorphometric methods, that the inhibition of bone formation was a result of acute intoxication with UN. The study was carried out with Wistar rats

(weighing 90 g/b.w.) intoxicated with a dose of 2 mg/kg of UN applied intraperitoneally, and observations were made at the level of the endochondral ossification of the tibia. After 14 days, inhibition of endochondral ossification of the tibia was observed in the intoxicated animals but not in the control animals. In a histomorphometric analysis of the bone of intoxicated animals, they found a decrease in bone surfaces covered by active osteoblasts and the consequent increase in inactive osteoblasts. Such results were attributed to U, which has been suggested to cause alterations in the osteoblast differentiation process or in their cell precursors. At the same time, the remaining osteoblasts may form the sealing trabeculae that were seen in the metaphyseal bone.

In 1987, Guglielmotti *et al.* [55] used the same animal model to study the effect of a low dose of UN (0.8 mg/kg) on tooth extraction socket healing over time (14, 30, and 60 days post-surgery). Results revealed a delay in socket healing with respect to controls. At low doses, U exerted its toxic effect on the recruitment and/or differentiation of osteoblasts, despite the cell damage, and dramatically inhibited bone formation observed after acute poisoning.

Ubios *et al.* [56] confirmed the results described above through ultrastructural studies in which they also detected the presence of small electron-dense deposits in the osteoblast cell membrane, inferring that they were U particles. The inhibitory UN effect on bone formation was evidenced as a reduction in bone growth of tibiae and mandibles [57, 58] and as a delay in tooth eruption [59]. In 2007, Tasat *et al.* [60] showed that UN modified osteoblast cell metabolism by increasing reactive oxygen species generation and reducing alkaline phosphatase activity, suggesting that ROS could play a more complex role in cell physiology than simply by causing oxidative damage.

The effects of U on bone formation are evident not only in situations of active ossification, but we have also observed them in bone modeling and remodeling, where, in addition, the reduction in bone formation activity was associated with an increase in bone resorption [61]. Nevertheless, while many studies have focused on the effect of U on bone formation and osteoblasts, the impact of U on bone resorption has been poorly explored. In this context, Ubios *et al.* [61] conducted a pioneering study demonstrating an increase in resorption of the alveolar bone of the jaws after intraperitoneal injection of UN in Wistar rats. Subsequently, in our laboratory, we have also observed a histomorphometrical increase in resorption surfaces in metaphyseal bone after oral administration of a lethal oral dose of UN [62]. Recent studies have revealed dose- and time-dependent U cytotoxicity on pre-osteoclast cell lines, impairing osteoclast formation and function [63].

4. Chelating agents in uranium poisoning

Uranium toxicity has been a concern for over 100 years. The toxicology of many forms of uranium, ranging from dust of several oxides to soluble uranyl ions, was thoroughly studied during the Manhattan Project in the United States in the 1940s [64]. Data available in the literature show that most studies have focused on finding a compound that accelerates U decorporation after it has reached the target organs: kidney (acute intoxication) and bone (chronic exposure).

From a pharmacological standpoint, different methods have been tested to counteract the toxic effect of U. Several chelating agents such as EDTA, Tiron, DTPA, or aminosalicylic acid have been experimentally assayed. However, even when these agents are able to reduce mortality, none of them achieve 100% survival. Bicarbonate can be administered to reduce U body burdens due to acute exposures. Bicarbonate ions form a complex with U and alkalize the blood, both

of which enhance the excretion from the kidneys by glomerular filtration [65], and such an application was described in a case of prophylactic treatment [66]. Experimental evidence in animals indicates that chelation therapy may reduce the body burden of U. Several compounds were found to enhance the urinary and fecal excretion of U if administered soon after U exposure. When administered immediately after exposure to U, Tiron® (sodium 4,5-dihydroxybenzene-1,3-disulfonate) resulted in the greatest reduction in renal and bone levels of U and acute lethal effects in animals [67, 68]. None of the chelating agents affected bone levels of U when administered ≥24 h after exposure to U [68]. Bicarbonate treatment is also limited to very near-term exposures. Another study that tested Tiron alone and in conjunction with either DTPA or ethylenediamine-N,N=-bis(2-hydroxyphenylacetic acid) (EDHPA) found that it reduced the U body burden by no more than about 35%, indicating that the administration of Tiron® is of limited practical value for the treatment of U exposures that do not greatly exceed the permitted intake level [69]. Our group began working with a bisphosphonate, ethane-1-hydroxy-1,1-bisphosphonate (EHBP) in 1986 based on the beneficial effect of bisphosphonates on bone when they are used in correct doses. Ubios *et al*. [70] showed the attenuation of the inhibitory effect of radiation on bone formation when the animals were treated with ethane-1-hydroxy-1,1-bisphosphonate (EHBP). In acute intoxication, U not only inhibits bone formation, but its excretion in urine also causes renal damage. The former effect is ameliorated by tetracycline (TC), probably due to its chelation property, which might also prevent U deposition in bone. Chemical determination of U incorporated in the bone and a histological study of the kidneys were performed by Guglielmotti *et al*. [71] on animals injected with U and then treated with TC. The results showed that TC was unable to prevent the binding of U to the bone, while it exacerbated U-induced renal damage. Ubios *et al*. [72] reported the beneficial effect of ethane 1-hydroxy-1,1-diphosphonate (EHDP) in restoring the inhibition of bone formation in cases of acute U intoxication in a post-extraction wound healing rat model. Ubios *et al*. [57] showed the use of a single subcutaneous injection of ethane-1-hydroxy-1,1-bisphosphonate to prevent mortality due to uranium poisoning in a rat model.

Of the chelation therapies that have been studied in animals, it appears that citric acid and citrate salts might be the most practical to employ in conflict areas where DU weapons have been used. Citric acid/citrate consumption should be recommended to anyone in areas where uranium aerosols might be found. Fructose and/or sucrose that may be present in some beverages that contain high levels of citrates should be avoided [64].

4.1 Bisphosphonates as chelating agents to avoid lethal poisoning by uranium

Bisphosphonates are used clinically to prevent osteoclastic bone resorption. Their use tends to achieve positive bone formation/resorption balance. These compounds are used to treat osteoporosis, a very common lesion in postmenopausal women in which the bone formation/resorption equation is altered (predominantly resorption), and the final matrix tends to decrease in volume, sometimes reaching mechanically dangerous situations due to fractures, among other problems.

The biological effects of bisphosphonates (BPs) as inhibitors of calcification and bone resorption were first described in the late 1960s. In the 50 years that have elapsed since then, BPs have become the leading drugs for the treatment of skeletal disorders characterized by increased bone resorption, including Paget's disease of the bone, bone metastases, multiple myeloma, osteoporosis, and childhood inherited bone disorders as osteogenesis imperfecta. The discovery and

development of BPs as a major class of drugs for the treatment of bone diseases is a paradigm for the successful journey from "bench to bedside and back again." Several of the leading BPs achieved "blockbuster" status as branded drugs. However, these BPs have now come to the end of their patent life, making them highly affordable. The opportunity for new clinical applications for BPs also exists in other areas of medicine such as aging, cardiovascular disease, and radiation protection.

In our laboratory, based in data published by Ubios *et al*. [57], we designed animal experiments to focus on the potential of EHBP to prevent death after the administration of lethal oral doses of UN. In 2000, Martinez *et al*. [26] demonstrated, for the first time, that a single administration of EHBP is effective in reducing the lethal effect of U, and it is at least as useful as subcutaneous administration for prompt therapy of oral U exposure, achieving a survival rate of almost 50%. Tubule necrosis lesions were present in kidneys of mice intoxicated with UN, whereas lesions were less severe in mice treated with EHBP.

Based on the aforementioned results, Martinez *et al*. [41] evaluated the efficacy of EHBP in preventing renal dysfunction induced by a lethal dose of UN, employing serum levels of urea and creatinine as endpoints. Two experiments were performed with different time periods: 48 h and 14 days in male Balb/c mice with 25 g average body weight. Three of these groups received 350 mg/kg body weight of UN by gavage (forced oral administration). Two of the three exposed groups were treated with EHBP either by gavage in a dose of 500 mg/kg body weight or with a subcutaneous injection of 50 mg/kg body weight. The fourth group served as control. Urea and creatinine serum levels were markedly lower at 48 h in exposed animals treated with EHBP than in untreated exposed animals. On day 14 these values in exposed and treated animals did not differ significantly from control values. The renal function of animals treated with oral or subcutaneous EHBP that survived UN exposure was markedly improved compared to the controls of untreated exposed animals at 48 h. At 14 days, treatment with EHBP averted renal damage and the histologic study of kidneys showed images of tissue recovery (**Figure 3**). These results suggest that the use of EHBP may be of great value in reducing renal damage.

Bozal *et al*. [62] showed that all growth cartilage and metaphyseal bone histomorphometric parameters were significantly lower in animals exposed to UN at 48 h than in controls. EHBP administration was found to prevent this condition at 48 h reaching similar values to those of controls. Although histomorphometric values did not reach control values at 14 days, they were higher than those of animals exposed to UN at 48 h not treated with EHBP. It is noteworthy that these values also decreased in animals
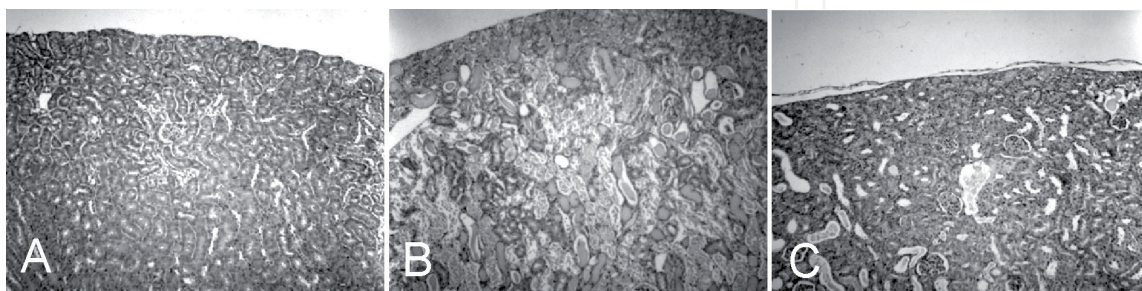

Figure 3.
(HE 100X) Histological sections of cortical zone of kidneys of a control animal (A), of an animal 48 h post-intoxication with uranyl nitrate (UN) (B), and of an animal intoxicated with UN and treated with oral EHBP, 14 days post-intoxication (C). (A) Note the integrity of tubule and glomerular structure. (B) Note the marked vacuolization of the tubules, abundant hyaline cylinders, and extensive areas of necrosis, all of them usual uranium-induced lesions. (C) Note that instead the presence of scattered hyaline cylinders, the tissue shows marked recovery of renal architecture.

only receiving EHBP at 14 days. Our results show that EHBP effectively ameliorates the adverse effects of a lethal dose of UN on endochondral ossification (**Figure 4**).

In our laboratory, we have also evaluated the effect of treatment with EHBP on the reduction in interradicular bone volume and the alteration of histomorphometric parameters of bone remodeling in animals intoxicated with a lethal oral dose of UN (unpublished data). These studies showed that 48 h after UN intoxication, EHBP treatment enables an interradicular bone volume to be maintained which is similar to the controls, and this condition is sustained 14 days post-treatment (**Figure 5**). Moreover, at 48 h, EHBP prevented the reduction in bone formation and increase in bone resorption caused by UN intoxication in the interradicular bone of intoxicated animals.
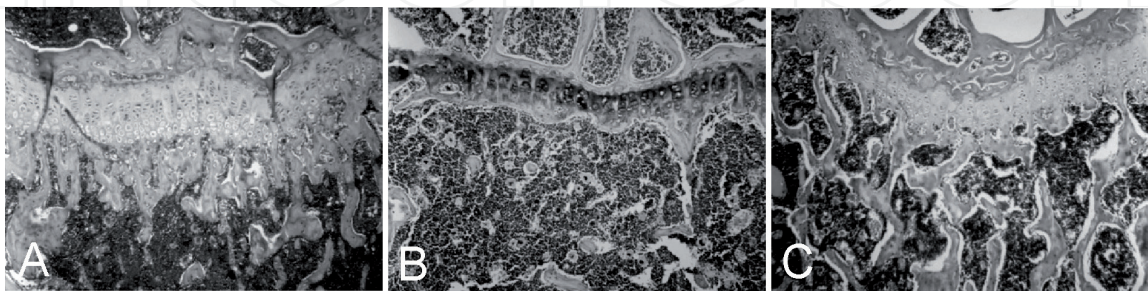

Figure 4.
(HE 100X) Histological sections of the metaphyseal bone of a control animal (A), of an animal 48 h post-intoxication with uranyl nitrate (UN) (B), and of an animal intoxicated with UN and treated with oral EHBP, 14 days post-intoxication (C). Note the marked reduction in cartilage width and the absence of proliferation cells in the growth cartilage as well as the absence of subchondral bone in UN-exposed animal (B) compared to the control (A). In animal intoxicated with UN and treated with EHBP (C), note that instead of the reduction in cartilage width compared to the control, proliferation cells in the growth cartilage and subchondral bone are evident.
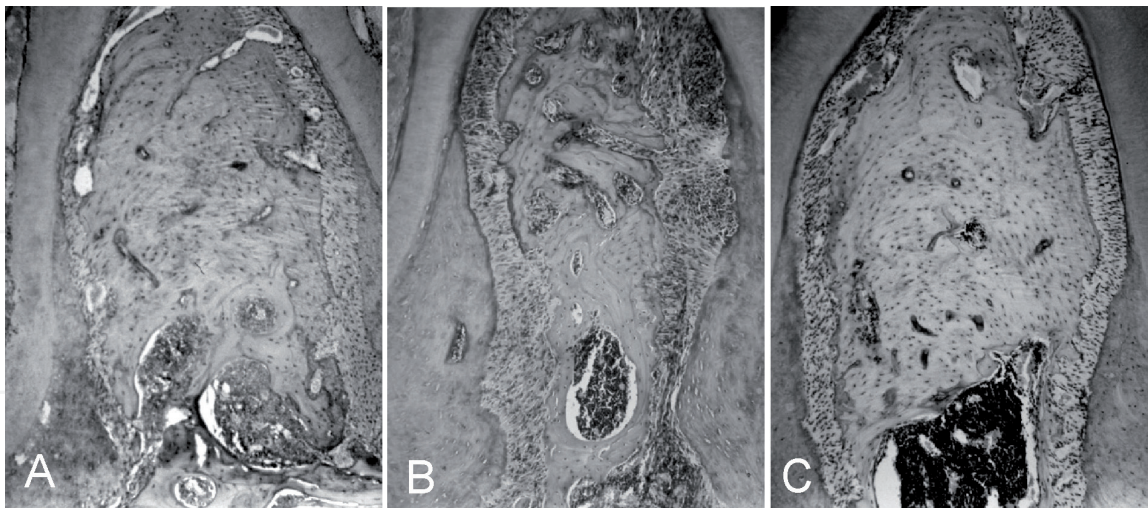

Figure 5.
(HE 100X) Histological sections of the interradicular alveolar bone of a control animal (A), of an animal 48 h post-intoxication with UN (B), and of an animal intoxicated with UN and treated with oral EHBP, 14 days post-intoxication (C). The images show the reduction in interradicular bone volume at 48 h in animals exposed to UN and how, in the exposed animals treated with EHBP, bone volume is similar to the control.

5. Discussion

Our research shows that in adult mice that had been exposed to a lethal dose of orally administered UN, a single dose of EHBP—either by mouth or subcutaneous—reduced mortality by about 50%. Surviving exposed animals

had adequate renal function and showed a reduction in the deleterious effects of uranium on endochondral ossification and alveolar bone [41, 62]. We decided to administer UN by mouth so that the experimental models would replicate, as closely as possible, the situation of workers exposed to potential accidents. Given the similarity in survival rates observed with both the EHBP administration routes tested at our laboratory, we suggest that its effectiveness as a chelator to reduce the lethal effects of uranium is independent of whether it is administered orally or subcutaneously. In contrast to other studies, it is important to highlight that in the experimental design tested at our laboratory, all animals had free access to food and drink throughout, in order to recreate a situation that would similar to what might happen in case of accidental U intoxication to humans. This information reinforces the potential use of EHBP as an antidote to U, highlighting its easy accessibility for use in accidental intoxications when it is impossible to know the content of the gastrointestinal tract of the individual and which may ultimately interfere with the pharmacokinetics or pharmacological efficacy of EHBP. It is worth noting that at that time, there was no report in the literature of exposure to uranium and administration of an antidote via the same route—in our case, by mouth. EHBP was selected as uranium chelating agent based on the findings of Ubios *et al*. [72], who postulated that since bisphosphonates have proven affinity for calcium [73], they may act as U chelating agents. That study demonstrated the efficacy of only one injection of EHBP to prevent renal damage and counteract mortality due to uranium poisoning with a success rate of 100% [72].

Several studies have focused on the efficacy of different chelating agents for removing uranium from tissue deposits. However, chelation of a heavy metal is more beneficial than its removal from tissue deposits because it prevents it from reaching target organs. This property has been demonstrated experimentally in biochemical, histological, and histomorphometric studies on the kidney [41] and bones [62] of animals exposed to lethal doses of uranyl nitrate.

One favorable factor was the brief time—20 min in our studies—elapsed between the administration of U and the administration of EHBP. The time that elapses between administration of U and the antidote is critical. Catsch *et al*. [74] demonstrated that there is no apparent benefit from administrating an antidote if the time elapsed is longer than 6 h. Ubios *et al*. [75] tested the application of single doses of two different bisphosphonates acting as chelating agents—EHBP and APD (pamidronate)—observing that the animals treated with EHBP or APD up to 24 h after the exposure achieved 100% survival until the 60th day. Only when it was given 48 h after the exposure to uranium, EHBP appeared unable to prevent death. The intervals proposed by other authors range from 10 min to 24 h [67, 76, 77], with better results having been achieved by those who administered the chelating agent 10 min to 3 h post-intoxication (Tiron, in this case). It is worth noting that none of the experimental cases reported achieved a higher survival rate than we did. Some authors highlighted the importance of administrating repeated doses of the chelating agent in order to achieve a higher survival rate in animals intoxicated with U compounds [67, 76, 77] without having achieved better results than with a single dose.

6. Conclusion

The effects of a lethal dose of uranyl nitrate can be counteracted by the chelating action of bisodic etidronate administered by mouth or subcutaneously. The therapeutic effect of EHBP has been demonstrated using an animal model of

uranium intoxication. Administration of EHBP provided a survival rate of 45–50%; returned serum renal biomarkers to values close to normal, which is consistent with reduction in hyalinization and necrotic areas; and reduced bone growth inhibition, reverting the damage typical of acute uranium intoxication. These results suggest that EHBP is a chelating agent capable of effectively neutralizing lethal uranium intoxication.

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