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Intracellular Metabolism of Uranium and the Effects of Bisphosphonates on Its Toxicity

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1. Introduction

Uranium is the heaviest naturally occurring element found in the Earth's crust. It is an alpha-emitter radioactive element that present both radiotoxicant and chemotoxicant properties. Uranium is present in environment as a result of natural deposits and releases by human applications (mill tailings, nuclear industry and military army). The release of uranium or its by-products into the environment (air, soil and water) presents a threat to human health and to the environment as a whole. Uranium can enter the body by ingestion, inhalation or dermal contact yet, the primary route of entry into the body is inhalation. Research on inhaled, ingested, percutaneous and subcutaneous industrial uranium compounds has shown that solubility influences the target organ, the toxic response, and the mode of uranium excretion. The overall clearance rate of uranium compounds from the lung reflects both mechanical and dissolution processes depending on the morphochemical characteristics of uranium particles. In this review we emphasize on one of the principal physical characteristics of uranium particles, its size. As is known, based on uranium chemical composition, three different kinds are defined: natural, enriched (EU) and depleted (DU) uranium. The radiological and chemical properties of natural uranium and DU are similar. In fact, natural uranium has the same chemotoxicity, but its radiotoxicity is 60% higher. DU, being a waste product of uranium enrichment, has several civilian and military applications. Lately, it was used in international military conflicts (Gulf and recently as the Balkan Wars) and was claimed to contribute to health problems. Herein, we reviewed the toxicological data in vivo and in vitro on both natural and depleted uranium and renewed efforts to understand the intracellular metabolism of this heavy toxic metal. The reader will find this chapter divided in three sections. The first section, describes the presence of the uranium in the environment, the routes of entrance to the body and its impact on health. The second section which is committed to uranium cytotoxicity and its mechanism of action stressed on the oxidative metabolism and a third section dedicated to the effect of different compounds, mainly bisphosphonates, as substances with the ability to restrain uranium toxicity.

2. Uranium in the environment, routes of entrance to the body and impact on health

Uranium is a natural and commonly occurring radioactive element to be found ubiquitous in rock, soil, and water. Uranium concentrations in natural ground water can be more than several hundreds μg/l without impact from mining, nuclear industry, and fertilizers. It is a reactive metal, so it is not found as free uranium in the environment. Besides natural uranium, antopogenic activities such as uranium mining and further uranium processing to nuclear fuel, emissions form burning coal and oil, and the application of uranium containing phosphate fertilizers may enrich the natural uranium concentrations in the soil, water and air by far. The wide dispersal of pollutants in the environment (heavy metals, pesticides, fuel particles, and radionuclides) created by various human activities are of increasing concern. In particular, the release of harmful constituents from uranium or its by-products into the environment presents a threat to human health and the environment in many parts of the world. For instance, the civilian and military use of uranium, as well as fuel in nuclear power reactors, counterweights in aircraft and penetrators in shrapnel, may lead to the release of this radionuclide into the environment. This was the case in Amsterdam after the aircraft crash in 1992 (Uijt de Haag et al., 2000), around uranium processing areas (Pinney et al., 2003) or following the drop of some 300 tons of depleted uranium (DU) during the Gulf War (Bem & Bou-Rabee, 2004). This uranium dispersion may cause pollution of the air and water wells and/or into the food chain (Di Lella et al., 2005), which may lead to a chronic contamination by inhaltion or ingestion of local populations.

Radioactive elements are those that undergo spontaneous decay, in which energy is emitted either in the form of particles or electromagnetic radiation with energies sufficient to cause ionization. This decay results in the formation of different elements, some of which may themselves be radioactive, in which case they will also decay. Uranium exists in several isotopic forms, all of which are radioactive. The most toxicologically important of the 22 currently recognized uranium isotopes are uranium-234 (^{234}U), uranium-235 (^{235}U), and uranium-238 (^{238}U). When an atom of any of these three isotopes decays, it emits an alpha particle and transforms into a radioactive isotope of another element. The process continues through a series of radionuclides until reaching a stable, non-radioactive isotope of lead. There are three kinds of mixtures (based on the percentage of the composition of the three isotopes): natural uranium, enriched uranium (EU), and depleted uranium(DU). Enriched uranium is quantified by its ^{235}U percentage. Uranium enrichment for a number of purposes, including nuclear weapons, can produce uranium that contains as much as 97.3% ^{235}U and has a specific activity of ~50 μCi/g .The residual uranium after the enrichment process is "depleted" uranium and possesses a specific activity of 0.36 μCi/g, even less radioactivity than natural uranium (Research Triangle Institute 1997).

There are three things that determine the toxicity of radioactive materials: its radiological effect, its chemical effect and its particle size. Regarding its radiological effect uranium releases alpha particles (1gr DU releases 13.000 alpha particles per second), chemically is a very toxic heavy metal, and regarding its size, uranium particles within the air fit in the nanometer range (aerodinamic diameter of 0.1 microns or less), being this third characteristic far more biologically toxic than the first two. It is because uranium is both a heavy metal and a radioactive element that it is considered among the elements an unusual

one. The hazards associated with this element are dependent upon uranium's chemical form (solubility, level of enrichment), physical form (morphology and size) and route of intake.

2.1 Chemical form

Uranium is a heavy metal that forms compounds and complexes of different varieties and solubilities. The chemical action of all isotopes and isotopic mixtures of uranium is identical, regardless of the specific activity (i.e., enrichment), because chemical action depends only on chemical properties. Thus, the chemical toxicity of a given amount or weight of natural, depleted, and enriched uranium will be identical. However, the chemical form of uranium determines its solubility and thus, transportability in body fluids as well as retention and deposit in various organs. On the basis of the toxicity of different uranium compounds in animals, it was concluded that the relatively more water-soluble compounds (uranyl nitrate, uranium hexafluoride, uranyl fluoride, uranium tetrachloride) were the most potent systemic toxicants. The poorly water-soluble compounds (uranium tetrafluoride, sodium diuranate, ammonium diuranate) were of moderate-to-low systemic toxicity, and the insoluble compounds (uranium trioxide, uranium dioxide, uranium peroxide, triuranium octaoxide) had a much lower potential to cause systemic toxicity. Harrison et al. (1981) studied the gastrointestinal absorption in animals of two uranium compounds with different solubilities. They showed that uranyl nitrate (soluble) was absorbed seven times more than uranium dioxide (insoluble). Generally, hexavalent uranium, which tends to form relatively soluble compounds, is more likely to be considered a systemic toxicant. However, particles with very low solubility could accumulate within biological systems and persist there for long durations.

Uranium is a reactive element that is able to combine with, and affect the metabolisms of: lactate, citrate, pyruvate, carbonate and phosphate. Uranyl cations bind tenaciously to protein, nucleotides, and as it can be absorbed by phosphate or carbonate compounds. In so, all different forms have singular biological activities and thus, different toxicities. As was already mentioned depleted uranium (DU) is a byproduct of the enrichment process of uranium, highly toxic to humans both radiologically as an alpha particle emitter and chemically as a heavy metal. Still, the major toxicological concern of U^{238} excess is biochemical rather than radiochemical. In fact uranium, in the form of uranyl nitrate hexahydrate, is considered the most potent toxicant (Stokinger et al., 1953; Tannenbaum et al., 1951). The variety of the molecular forms in which uranium can be presented extends by the ability of the uranium atom to form complex connections.

2.2 Physical form

It is very well known that for any kind of particles whatever their composition is (ordinary carbon, metallic-nonradioactive, etc), the smaller the particle the more harmful they are. This is exactly the case of micro or fine particles (aerodynamic diameter between 100 - 0.1 microns) and nano or ultrafine particles (aerodynamic diameter less than 0.1 micron). Reduction in size to the nanoscale level results in an enormous increase of surface to volume ratio, so relatively more molecules of the chemical are present on the surface, thus enhancing the intrinsic toxicity (Donaldson et al., 2004). Mankind has lived with low-level background radiation for as long as we have existed but, the uranium in a DU weapon

explodes on impact as it penetrates a target. It burns at extremely high temperatures (above 5,000 degrees centigrade) and in the process vaporizes into very small (micro and nano) particles. These particles become airborne like a gas, polluting the atmosphere and getting transported around the world being able to enter by inhalation to the population at large. Therefore, there are concerns regarding its potential health effects on the general population and due to internalization of DU during military operations, particularly on this subpopulation. The micro and nanometer size uranium particles released after impact are biologically dangerous and undoubtly a growing part of our world since 1991. It has been reported that inhaled nanoparticles reach the blood and may then be distribuited to target sites such as the liver, kidney, brain, lung, heart or blood cells (Oberdörster et al., 1994; MacNee et al., 2000; Kreyling et al., 2004). Still, the hazard from inhaled uranium aerosols or any noxious agent is determined by the likelihood that the agent will reach the site of its toxic action. The two main factors that influence the degree of hazard from toxic airborne particles are: the site of deposition in the respiratory tract and, the fate of the particles within the lungs. The deposition site within the lungs depends mainly on the particle size of the inhaled aerosol, while the subsequent fate of the particle depends on the physico-chemical properties of the inhaled particle as well as of the physiological status of the lung and target organs of the individual. For humans, inhalation is the most frequent route of access and therefore, the process of aggregation of the nanoparticles in the inhaled air has to be taken into account. Nanoparticles may translocate through membranes and there is little evidence for an intact cellular or sub-cellular protection mechanism. The typical path within the organ and/or cell which may be the result of either diffusion or active intracellular transportation is also of relevance. Very little information on these aspects is presently available and this implies that there is an urgent need for toxicokinetic data for nanoparticles.

2.3 Health effects by route of exposure

Uranium health effects studies derive largely from epidemiology and toxicological animal models. This contaminant can enter the body through inhalation, ingestion or by dermal contact and its toxicity has been demonstrated for different organs. Health effects associated with oral or dermal exposure to natural and depleted uranium (DU) appear to be solely chemical in nature and not radiological, while those from inhalation exposure may also include a slight radiological component, especially if the exposure is chronic. In general, ingested uranium is less toxic than inhaled uranium, which may be also partly attributable to the relatively low gastrointestinal absorption of uranium compounds. Because natural uranium and DU produce very little radioactivity per mass, the renal and respiratory effects from exposure of humans and animals to uranium is usually attributed to its chemical properties. Thus, the toxicity of uranium varies according to its chemical form as well as to the route of exposure.

2.3.1 Inhalation route

Inhalation represents one of the most important occupational risk of uranium exposure especially for workers at the uranium mines. Workers are exposed to both, natural uranium (moderately radioactive) as enriched uranium (highly radioactive). However, to a lesser extent, uranium dust can also enter percutaneously (direct contact or through contaminated clothes), subcutaneously (through wounds in the skin and mucous) and

orally (ingestion). Epidemiological studies indicate that routine exposure of humans to airborne uranium (in the workplace and the environment at large) is not associated with increased mortality. In fact, data of several mortality assessments of populations living near uranium mining and milling operations have not demonstrated significant associations between mortality and exposure to uranium (Boice et al., 2003, 2007, 2010). However, it has been reported in humans, that brief accidental exposures to very high concentrations of uranium hexafluoride have caused fatalities. In addition, laboratory studies in animals indicate that inhalation exposure to certain uranium compounds can be fatal (ATSDR). It has to be pointed out that these deaths are believed to result from renal failure caused by absorbed uranium.

The toxicity of uranium compounds to the lungs and distal organs varies when exposed by the inhalation route. The respiratory tract acts as a serial filter system and in each of its compartments (nose, larynx, airways, and alveoli). The mechanisms of particle deposition may change for each compartment as well as for the particle size that entered. Nanoparticles are primarily displaced by Brownian motion and therefore underlie diffusive transport and deposition mechanisms. It means that the smaller the particle, higher the probability of a particle to reach the epithelium of the lung. In general, by the inhalation route, the more soluble compounds (uranyl fluoride, uranium tetrachloride, uranyl nitrate hexahydrate) are less toxic to the lungs but more toxic systemically. Early studies with UF_6 demonstrated that this uranium type may present both chemical and radiological hazards. UF_6 is one of the most highly soluble industrial uranium compounds and when airborne, hydrolyzes rapidly on contact with water to form hydrofluoric acid (HF) and uranyl fluoride (UO_2F_2) as follows: $UF_6+2H_2O \longrightarrow UO_2F_2+ 4HF$. Thus, an inhalation exposure to UF_6 is actually an inhalation exposure to a mixture of fluorides. Chemical toxicity may involve pulmonary irritation, corrosion or edema from the HF component and/or renal injury from the uranium component (Fisher et al., 1991). The acute-duration LC50 (lethal concentration, 50% death) for uranium hexafluoride has been calculated for rats and guinea pigs (Leach et al., 1948). In these experiments, animals were exposed to uranium hexafluoride in a nose-only exposure for periods of up to 10 minutes and observed during 14 days. Lethality data suggested that rats are more resistant to UF_6 -induced lethality than are guinea pigs (total mortality of 34% and 46% respectively), proving that the biological response depends also on the host being species specific. It is worth to note that although animals were exposed to uranium via inhalation, histopathological examination indicated that renal injury, but not lung injury, was the primary cause of death (Leach et al., 1948, 1970). However, animals that died during or shortly after exposure had congestion, acute inflammation, and focal degeneration of the upper respiratory tract. The tracheas, bronchi, and lungs exhibited acute inflammation with epithelial degeneration, acute bronchial inflammation, and acute pulmonary edema and inflammation, respectively.

On the contrary, though inhalation exposure insoluble salts and oxides (uranium tetrafluoride, uranium dioxide, uranium trioxide, triuranium octaoxide) are more toxic to the lungs due to the longer retention time in the lung tissue, they are less toxic to distal organs. Harris et al. (1961) found prolonged half lives (120 days or more) for both dioxide and trioxide uranium insoluble compounds. Although insoluble uranium compounds are also lethal to animals by the inhalation route, it occurs at higher concentrations than soluble compounds.

Three different mechanisms are involved in the removal of particles from the respiratory tract. The first is mucociliary action in the upper respiratory tract (trachea, bronchi, bronchioles, and terminal bronchioles), which sweeps particles deposited there into the throat, where they are either swallowed into the gastrointestinal tract or spat out. The second mechanisms is the dissolution (which leads to absorption into the bloodstream) and the third one, the phagocytosis of the particles deposited in the deep respiratory tract (respiratory bronchioles, alveolar ducts, and alveolar sacs). After deposition of insoluble particles in the respiratory tract, translocation may potentially occur to the lung interstitium, the brain, liver, spleen and possibly to the foetus in pregnant females (MacNee et al., 2000; Oberdörster et al., 2002). It as to be emphasized that up to date there is extremely limited data available on these pathways. Several studies demonstrated that particles, whatever the element, triggered pro-inflammatory response characterized by upregulation of cytokine levels and/or immune cell density in lungs after inhalation of particulate matter. This inflammation was induced by particles of various sizes such as nanoparticles or ultra fine particles (Inoue et al., 2005; Stoeger et al., 2006), or by soluble transition metals (McNeilly et al., 2005). Induction of diverse inflammatory reactions was also reported following uranium contamination in different tissues. For instance, activation of cytokine expression and/or production was noted either in pulmonary tissues following uranium exposure by inhalation (Monleau et al., 2006) or in macrophages after in vitro contamination (Gazin et al., 2004; Wan et al., 2006)

2.3.2 Oral route (ingestion)

Experimental studies in humans consistently show that absorption of uranium by the oral route is <5%. Still, this is for the population at large, the main route of uranium entry to the body. UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation, 1993) has considered that limits for natural and depleted uranium in drinking water (the most important source of human exposure) should be based on the chemical toxicity rather than on a radiological toxicity, which has not been observed in either humans or animals. Evidence from several animal studies showed that the amount of uranium absorbed from the gastrointestinal tract was about 1% (La Touche et al., 1987), although other studies have reported even lower absorption efficencies. The most sensitive target of uranium toxicity to mammals, and perhaps humans, is the kidney. While acute, high-level exposure to uranium compounds can clearly cause nephrotoxicity in humans (Lu & Zhao, 1990; Pavlakis et al., 1996), the evidence for similar toxicity as the result of long-term, lower-level occupational exposures is equivocal. In 1987 ATSDR (Agency for Toxic Substances and Disease Registry U.S.) established a minimum level of risk (MRL) for uranium ingestion. Several epidemiology studies (Kurttio et al., 2002; Zamora et al., 1998, 2009) examined the possible association between chronic exposure to elevated levels of uranium in drinking water and alterations in kidney function. These effects may represent a subclinical manifestation of uranium toxicity not necessarily leading to renal dysfunction. By contrast, chronic ingestion of this toxicant could be the starting point of an irreversible renal injury (Wise Uranium Project 1999). Mao et al. (1995) found a significant association between cumulative uranium exposure (product of uranium concentration in drinking water) and urine albumin levels (expressed as mg/mmol creatinine) in adults living in households with elevated uranium levels in drinking water. In accordance, Zamora et al. (1998, 2009) found a significant

association between β2-microglobulin, and alkaline phosphatase levels observed in residents living in an area of high uranium levels in the drinking water.

Besides drinking water, uranium can entered the body through the ingestion of contaminated meat and/or fish. Smith & Black (1975) measured the uranium content in the tissue of cattle that graze near the uranium mines being slightly higher than the amount found in control non-contaminated animals. In humans, a study comparing uranium absorption between subjects primarily exposed to uranium in the diet and subjects exposed to elevated levels of uranium in the drinking water (Zamora et al., 2002) did not find significant differences in fractional absorption between these two subroutes.

Studies in rats suggest that the primary pathway for gastrointestinal absorption of soluble uranium is through the small intestinal epithelium (Dublineau et al., 2005, 2006) via the transcellular pathway (Dublineau et al., 2005). In the event of ingestion, the digestive tract is the first biological system exposed to uranium intake via the intestinal lumen. However, little research has addressed the biological consequences of a contamination with uranium on intestinal properties such as the barrier function and/or the immune status of this tissue. Dublineau et al. (2006, 2007) studied both acute contamination with DU at high doses and chronic contamination at low doses on inflammatory reactions in the intestine when orally delivered. The authors found that acute and chronic ingestion of DU modulated expression and/or production of cytokines in the intestine and had similar effects than those observed with lead on the nitric oxide pathway.

2.3.3 Dermal contact

2.3.3.1 Percutaneous entry route

For uranium workers, either in the mines or involved in the mining processes, the percutaneous route is after inhalation is the second main route of uranium contamination. The dust of uranium compounds can permeate clothes and, depending on its solubility, penetrate through the skin. Orcutt et al. (1949) reported that the percutaneous route is an effective mean of entry for soluble uranium compounds.

Our group demonstrated (de Rey et al., 1983), the existence of a differential percutaneous absorption for soluble and insoluble uranium compounds after topical application in rats. By transmission electron microscopy (TEM) we were able to localize these heavy compounds within the tissues. Almost immediately, dense deposits of soluble uranyl nitrate were observed at the epidermal barrier level, 24 h later these deposits were seen close to the endothelium and 72 h no traces of uranium was found neither in the epidermis nor in the dermis indicating that, the uranium had been absorbed into the blood. Mortality (due to renal failure) and body weight measurements indicated the high toxicity of uranyl nitrate and other soluble uranium compounds tested. On the contrary, no variations on these parameters were found when uranium dioxide was employed. Later, changes in skin thickness and permeability after percutaneously chronic exposure of uranium industrial products Peccorini et al. (1990), and of uranium trioxide (Ubios et al., 1997) were reported. We found that, in addition to the systemic effects, such as loss of body weight and nephropathy, the transepithelial penetration and the subcutaneous implantation of uranium induced structural alterations in the stratified squamous epithelium which lead to epidermic atrophy and increased permeability of the skin. In 2000, we demonstrated that there is an

inverse relation between the area of the surface exposed to uranium and the time of exposure with, the subsequent percutaneous toxicity (López et al., 2000). We concluded that the larger the area exposed to uranium or the longer the exposure time, the lower was the rate of survival.

2.3.3.2 Subcutaneous entry route

Subcutaneous or intradermal uranium contamination takes place in the presence of a wound. This possibility becomes a real risk to workers daily handling uranium dust and nowadays it also includes soldiers who fought in the modern wars (Balkan, Gulf, etc). Penetration of DU shrapnel bullets into the skin became an issue of increasing attention. In fact, the only cases in which there were documented exposures to uranium are those of the Gulf War veterans who retained depleted uranium shrapnel fragments (McDiarmid et al., 2000, 2004, 2007).

In an experimental model of subcutaneous implantation of uranium dioxide (insoluble) in rats de Rey et al. (1984) showed that animals receiving doses greater than 0.01 g / kg died within the first six days due to acute renal failure. Uranium contamination by this route of exposure showed no differences regarding the type of particle. Histological analysis revealed the presence of deposits of uranium taken up by macrophages at 24 and 48 h post exposure. Deposits were found between the endothelial cells and the renal parenchyma, suggesting that the transport and deposition of uranium insoluble compound implanted subcutaneously occurs.

2.4 Uranium biokinetics

As was mentioned before, uranium can enter the human body through inhalation, ingestion or through the skin. Measurement of the quantities of uranium and its biokinetics can be performed in vivo, ex vivo and in vitro. In vivo techniques measure the quantities of internally deposited uranium directly using a whole-body counter, ex vivo techniques permit estimation of internally deposited uranium by analysis of body fluids (urine, blood, feces), or (in rare instances) tissues obtained through biopsy or postmortem tissue sectioning and in vitro allows to study the mechanism by which uranium interferes with cellular organelles and molecules (USTUR 2011).

The large majority of uranium (>95%) that enters the body is not absorbed and is eliminated from the body via the feces. Excretion of absorbed uranium is mainly via the kidney. Absorption of inhaled uranium compounds takes place in the respiratory tract via transfer across cell membranes. The deposition of inhalable uranium dust particles in the lungs depends on the particle size, and its absorption depends on its solubility in biological fluids (ICRP 1996). Estimates of systemic absorption from inhaled uranium-containing dusts in occupational settings based on urinary excretion of uranium range from 0.76 to 5%.

Gastrointestinal absorption of uranium can vary from <0.1 to 6%, depending on the solubility of the uranium compound. Studies in volunteers indicate that approximately 2% of the uranium from drinking water and dietary sources is absorbed in humans (Leggett & Harrison, 1995), while a comprehensive review indicates that the absorption is 0.2% for insoluble compounds and 2% for soluble hexavalent compounds (ICRP 1996).

Data on dermal absorption of uranium is limited. In hairless rats, dermal exposure to uranyl nitrate resulted in 0.4% of the dose being absorbed (Petitot et al., 2007a, 2007b); damage to the skin resulted in higher absorption efficiencies.

Although no data are currently available regarding the metabolism or biotransformation of uranium in vivo, for either humans or animals it is known that following absorption, uranium forms soluble complexes with bicarbonates, citrates and proteins, all of which are present in high concentrations in the body (Cooper et al., 1982). Regardless of the route of entry, the absorbed uranium is distributed widely but preferably is deposited in bone, kidney and liver. Uranium, once in the bloodstream, has a very short plasma half-life. Approximately 60% is eliminated in the first 24h in the urine (Walinder et al., 1967). Laboratory animal data indicate that a fraction of the uranium in the plasma is associated with low molecular weight proteins ultrafilterable while the rest is bound to transferrin and other plasma proteins (ICRP 1995). In body fluids, tetravalent uranium tends to oxidize to the hexavalent form, followed by the formation of the uranyl ion. Wrenn et al. (1985) showed that 90% of the uranium is excreted in feces and the remaining 10% in urine while the uranium deposited in external soft tissues is removed very slowly (Hursh et al., 1969).

As the general population and workers involved in uranium mining and manufacture of uranium devices are exposed to this heavy metal toxicant, not only the study on health should be encourage but, , effective management of waste uranium compounds is necessary to prevent uranium exposure. In the next section, a more detailed description on the risks associated with uranium exposure is presented.

3. Toxicity of uranium: Cellular mechanisms

The primary purpose of this second section of the chapter is to provide an overall perspective on the toxicology of uranium. It contains descriptions and evaluations of toxicological studies in vivo and in vitro and provides conclusions, where possible, on the relevance of uranium toxicity and toxicokinetic data to public health.

As described in section I uranium, depending on the route of entry and the dose, produces structural and functional alterations in target organs mainly in bone, kidney, and lung, and may even compromise the individual's life.

3.1 Uranium in vivo toxicological effects: Uranium as a heavy metal particle

In general, when uranium enters the organism, it accumulates in a non soluble form in hepatocytes, kidney proximal tubule cells and macrophages or macrophages-like cells present in tissues throughout the body (lung, liver, spleen, skin and bone). In each of the cells mentioned above, uranium is specifically concentrated by lysosomes where the actinides are precipitated as insoluble phosphates. The mechanism of intralysosomal concentration may be explained by the high phosphatase activity of these organelles. Moreover, uranium and phosphate have a strong chemical affinity for each other thus, as the DNA and mitochondria are loaded with phosphate, uranium may be considered a DNA and mitochondria deep penetration bomb attacking on fundamental cellular levels. Limited data exists regarding in vivo genotoxicity in humans following exposure to uranium. The only cases in which there were documented exposures to uranium are those of the Gulf War

veterans who retained depleted uranium shrapnel fragments (McDiarmid et al., 2000, 2001, 2004, 2007, 2009)

It is well known that the biochemical reaction to heavy metals can alter cellular mechanisms, principally oxidative metabolism, leading to genetic mutations which in turn, can restrain cell growth and cause cancer. Heavy metals, that are also radioactive, amplify these effects. Several reports have shown that uranium, both toxic and radioactive, induces oxidative stress causing adverse biological effects which include as was seen for heavy metals DNA damage, cancer and other neurological defects (Miller et al., 2002; Abou-Donia, 2002; Barber et al., 2007). Among heavy metals lead, aluminum and mercury have been shown to dramatically increase cytogenotoxicity. Interestingly, lead is the final end product of the step by step radioactive decay of uranium. Therefore, it would not be farfetched to imagine that uranium and lead may have very similar chemical characteristics though, uranium is twice as dense. In fact, some results regarding the effect of depleted uranium (DU) on the nitric oxide (NO) pathway almost mimic the observed with lead (see below). The interaction of lead with sulfhydryl (SH) sites causes most of its toxic effects, which include impaired synthesis of RNA, DNA and protein, diminished antioxidants (glutathione), and interferes with the metabolism of vitamin D. Lead may also affect the body's ability to utilize the essential elements calcium, magnesium, and zinc.

3.1.1 Cancer

Generally reports examine lung cancer mortality among two subpopulations: smokers and non-smokers uranium miners and, soldiers who participated in the unfortunate modern armed conflicts during and after 1991. During the mining process, uranium particles and its decay products such as ^{222}Ra are released into the environment. Workers in uranium mines and the people living nearby are likely to inhale and ingest suspended air particles containing uranium and radon. Inhalation of uranium particle increases the frequency of chromosomal aberrations (WISE Uranium Project, 1999) and the risk of lung cancer (WISE Uranium Project, 1999). Therefore, uranium aerosolized nanoparticles, both as a heavy metal particle and due to its radioactivity when enter the respiratory system and deposited in the respiratory mucosa, are responsible for the induction of this pathology (lung cancer). Saccomano et al. (1996) in order to evaluate the incidence of tumors; their cell types; and the relationship of particulate size to their position in the bronchial tree conducted a retrospective and comparative study from 1947-1991. They studied a cohort of 467 uranium miners and 311 non-miners with lung cancer and concluded that inhaled uranium containing dust, radon, and cigarette smoke combine to form large particulates that deposit in the central bronchial tree. Furthermore, they show that the proportion of lung cancers in the central zone was significantly greater in miners than in non-miners presumably due to the deposition of radon decay products attached to the silica dust particles. More recently, two new reports show association between uranium miners and smokers. The first study took place in France revealing significant association between the relative risk of lung cancer and silicosis. Amabile et al. (2009) demonstrated that the relation between radon and lung cancer persisted even after adjusting the data for smoking and silicotic status but, these authors remind us of the complexity involved in assessing occupational risks in the case of multiple sources of exposure. The second investigation was done among German uranium miners (Schnelzer et al., 2010). Adversely in this study, the authors concluded that stability

of the uranium-related lung cancer risks with and without adjustment for smoking could suggest that smoking does not act as a major confounder at least in the cohort study. In brief, a number of studies reported death from lung cancers from occupational inhalation exposure of mine workers however; the available studies document no lung cancers solely from inhaled uranium- bearing dust. It is generally accepted that lung cancers developed subsequent to inhalation of uranium- containing dusts were principally due to radon daughters and long-term cigarette smoking, and not to uranium metallotoxicity or uranium radioactive emissions.

In the months and years following the Balkan (1999) and Gulf (1991) wars a large number of soldiers, UN peacekeepers, and civilians have exhibited unexpected and unexplained health problems, including excess leukemias and other cancers, neurological disorders, birth defects, and a constellation of symptoms loosely gathered under the rubric "Gulf War Illnesses", suggesting that the use of DU during these conflicts could be considered a possible cause. Thus, this is another subpopulation where the action of uranium and its possible link to cancer is important to be studied. In 2004, Tirmarch et al. (2004) reviewed the epidemiological knowledge of uranium, the means of exposure and the associated risk of cancer. These authors concluded that only studies with a precise reconstruction of doses and sufficient numbers of individuals could allow a better assessment of the risks associated with uranium exposure at levels encountered during conflicts using depleted uranium weapons. Nevertheless, it is well known that when uranium binds to DNA it can damage DNA directly, or indirectly by altering uranium related DNA signaling mechanism. Cell mutations can either result in cell death or may trigger a whole slew of protein replication errors, some of which can lead to different cancer types. In fact, the incidence of cancer has increased markedly in Iraq following the Gulf War. As was reported by Aitken et al. (1999) there are some areas in southern Iraq that have experienced a two- to five fold increase in reported cancers. In most of these cases the lung, bronchial tubes, bladder, and skin are damaged. In addition, increased incidence of stomach cancer in males and breast cancer in females has also been reported, as well as an overall increase in leukemia cases.

3.1.2 The respiratory system

Exposure by inhalation to uranium dust particles can lead, as function of its solubility, to uranium accumulation predominantly in the lungs and tracheobronchial lymph nodes, as well as the development of neoplasia and fibrosis at the pulmonary level (ATSDR, 1999). Particle genotoxicity can be caused by direct actions or by indirect mechanisms often mediated by reactive oxygen species (ROS) produced mainly by the inflammatory cells (Kirsch-Volders et al., 2003; Martin et al., 1997). In agreement with these observations, Monleau et al., (2006), demonstrated DNA strand breaks in lung rats after DU acute and chronic exposure by inhalation, was a consequence of oxidative stress and induction of pro inflammatory IL8 and TNFα gene expression. These effects seemed to be linked to the DU doses and independent of the solubility of uranium compound.

3.1.3 Excretory systems

As in the case of other heavy metals, a considerable body of evidence suggests that overexposure to uranium may cause pathological alterations to the kidneys in both humans

and animals. Studies have shown that the solubility of uranium compounds plays a significant role in the amount of damage occurred in the kidneys. Inhaled uranium compounds with slow- to medium-dissolution rates are relatively insoluble, and are therefore retained longer in the lungs, resulting in lower toxicity to the kidneys and other distal organs. In 1949, under the Manhattan Project, Voegtlin & Hodge observed, lesions in the renal tubules regardless the route of entry for uranium. In 1982 Haley showed that all uranium compounds, uranyl nitrate, proved to be the most nephrotoxic being the most obvious effect the damage of the proximal convoluted tubules (ATSDR, 1999). As detailed previously, uranium can link to carbonates. Human renal effects following acute exposure to DU includes proteinuria and abnormal phenol sulfonphthalein excretion. In addition, increased urinary catalase activity and diuresis have also been found (ATSDR, 1999). McDiarmid et al. (2000) reported an increase in a variety of renal function parameters such as serum creatinine, β2-microglobulin, retinol binding protein, serum uric acid, urine creatinine, and urine protein in patient veterans. Although β2-microglobulin concentrations were higher and urine protein concentrations were lower in patients exposed to DU, no significant relationships were found between these parameters in neither control and uranium exposed groups.

In agreement with McDiarmid et al., we found that the incorporation of uranium by oral exposure in mice (Martinez et al., 2000) provoke a markedly increase of urea and creatinine levels when compared to controls. These biochemical parameters corresponded to uranium-severe alterations such as widening of the urinary space, cell vacuolization in the convoluted tubules and a large amount of hyaline casts as was seen by the histopathological study of the kidneys (Martínez et al., 2003).

3.1.4 The bone skeletal system

The 25% of the systemically administered uranium deposits in the skeleton linked to the newly formed bone. It is possible to find uranium in bone formation fronts building a critical deposit organ in chronic intoxications. An initial deposit of uranium was demonstrated by autoradiography at the endosteal and periosteal surfaces and haversian bone, areas where particularly calcification took place (Neuman et al., 1948). Our group was the first to demonstrate that acute poisoning with uranyl nitrate, inhibits endochondral ossification with reduced bone surfaces covered by active osteoblasts and a consequent increase in inactive osteoblasts (Guglielmotti et al., 1984). In this case, we proposed that the toxic effect of uranium would be causing an alteration of the differentiation process of osteoblasts and/or their precursors, resulting in the formation of sealing trabeculae on metaphyseal bone. Guglielmotti et al. (1985) proposed alveolar wound healing as another useful model for the study of bone formation as it is considered a sensitive indicator of bone damage under various experimental conditions. In this model we observed after uranium acute intoxication, not only inhibition of bone formation but, inhibition of alveolar bone healing after extraction (Guglielmotti et al., 1987). Later, our group studied the toxic effect of uranyl nitrate on bone modeling and remodeling by performing histomorphometric measurements in the periodontal cortical bone in dental alveolus of mandibles of rats (Ubios et al., 1991). Our results revealed a decrease in bone formation in rats treated with uranium. On the remodeling side the decrease in bone formation was coupled to an increase in bone resorption where on the modeling side no bone resorption was observed and the decrease in

bone formation was linked to an increase in resting bone zones. Because osteoblasts play a significant role in bone formation, it is possible that uranyl nitrate can directly affect these cells and their precursors by binding to cell membranes. Based on these data, uranium toxicity may be viewed as a potential contributor to osteoporosis or other osteopenic diseases in exposed individuals (Ubios et al., 1991). Bone growth was found to be impaired in tibiae (Ubios et al 1995) and mandibles (Ubios et al 1998) after exposure to uranyl nitrate and to uranium dioxide (Diaz Sylvester et al 2002). In addition, we found a lower degree of eruption and tooth development in lactant rodents exposed orally to acute doses of uranyl nitrate (Pujadas Bigi et al., 2003). More recently, we demonstrated that uranyl nitrate induced severe ultrastructural alterations both in active and inactive osteoblast revealing fragmented and swollen RER, Golgi and puffy nuclei with fine granular content (Tasat et al., 2007).

It is well known that vitamin D (1,25(OH)(2)D(3)) is a hormone essential in mineral and bone homeostasis. The effect of acute and chronic DU exposure on the active vitamin D metabolism was investigated in an experimental animal model by Tissandie et al. (2006, 2007). In acute DU intoxicated-rats, cytochrome P450 (CYP27A1, CYP2R1, CYP27B1, CYP24A1), enzymes involved in vitamin D metabolism and, two vitamin D(3)-target genes (ECaC1, CaBP-D9K) were assessed by real time RT-PCR in liver and kidneys. It was seen that DU modulated both activity and expression of CYP enzymes involved in vitamin D metabolism and consequently affected vitamin D target genes levels. In DU chronic-intoxicated rats, through drinking water, active vitamin D (3) significantly decreased in plasma level. In kidney, a decreased gene expression was observed for cyp24a1, the principal regulators of CYP24A1. Similarly, mRNA levels of vitamin D target genes ecac1, cabp-d28k and ncx-1, involved in renal calcium transport were decreased in this organ. Then, it is clear that DU affected both the vitamin D active form, its receptor expression, and consequently modulated the expression of cyp24a1 and vitamin D target genes involved in calcium homeostasis.

3.1.5 The reproductive system

In vivo heavy metal studies have demonstrated that carcinogenic effects can occur in unexposed offspring. Similarly, preconception paternal irradiation has been implicated as a causal factor in childhood cancer and it has been suggested that this paternal exposure to radiation may play a role in the occurrence of leukemia and other cancers to offspring.

There are few studies that have examined the effects of DU on human reproductive tract and development.

Back in 1967, Muller et al. reported that male uranium miners were found to have more firstborn female children than expected. In early 2000, decreased fertility, embryo/fetal toxicity including teratogenicity and reduced growth of the offspring was observed following uranium exposure at different gestation periods by Domingo et al. (2001). In 2002, when monitoring veterans of Gulf War, McDiarmid et al. reported adverse health effects on the reproductive and central nervous systems of DU-exposed veterans. More recently, Miller et al. (2010) investigated the possibility that chronic preconception paternal DU exposure could lead to transgenerational transmission of genomic instability. They showed that implantation of DU pellets in male mice for seven months followed by

mating with untreated females resulted in transmission of genetic damage to somatic cells of offspring. Several issues remain unknown: the exact mechanism by which this occurred and if it is DU-radiation or DU-chemical effects theresponsible for transgenerational transmission of factor(s) leading to genomic instability in F1 progeny from DU-exposed fathers.

3.1.6 The nervous system

Despite evidence suggesting a link between neurological toxicity and DU exposure, as was reported by McDiarmid et al. (2002), there is no data clearly demonstrating that excess neurologic disease/mortality risk is associated with uranium exposure. Preliminary animal studies failed to demonstrate any performance deficiencies in locomotor activity, discrimination learning, and general functional observations (Pellmar et al., 1999). At that moment and because the methods used to examine those endpoints could have been insensitive to reveal subtle cognitive effects, the Gulf War Executive Summary recommended further studies of cognitive function, neurophysiological responses, and brain DU concentrations in Gulf War veterans (Fulco et al., 2000). Several follow-up studies on Gulf War veterans with DU fragments embedded in their soft tissues have led to suggest the possible role of DU as an inductor of neurotoxicity. At present, some experimental studies suggest a positive link between neurological toxicity and uranium. In 2005, studies in rodents indicated that DU readily traverses the blood-brain barrier, accumulates in specific brain regions, and results in increased oxidative stress, altered electrophysiological profiles, and sensorimotor deficits (Briner & Murray, 2005). In this case, after uranium crosses the blood-brain barrier behavioral changes and lipid oxidation were observed in rats, in as little as 2 weeks. In agreement Monleau et al. (2005) proved that after repeated exposure to uranium dioxide particles by inhalation, uranium bioaccumulates in the brain producing behavioral changes (Monleau et al., 2005). A possible mechanism of this neurotoxicity could be the oxidative stress induced by reactive oxygen species imbalance. After chronic ingeston of DU Lestaevel et al. (2009) analyzed the expression and /or the activity of the main antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in cerebral cortex and found that all of them increased significantly. These results illustrate that oxidative stress plays a key role in the mechanism of uranium neurotoxicity. On the contrary, the nitic oxide (NO) pathway was almost unperturbed.

3.1.7 Are there common metabolic pathways for in vivo uranium exposure?

From the above studies at least two common metabolic pathways for uranium exposure could be proposed.

3.1.7.1 The oxidative and antioxidative pathway

As was mentioned above, exposure to metallic environmental toxicants has been demonstrated to induce a variety of oxidative stress responses in mammalian cells. Environmental stressors such as chemical toxicants can create oxidizing imbalances in the cellular redox state resulting in a loss of reducing potential, a condition termed "oxidative stress". Heavy metals are environmental persistent toxicants that have been shown to exert oxidative stress on living systems through the production of reactive oxygen species (ROS),

which overwhelm the cell's capacity to maintain a reduced state thus, damaging various cellular components (Ercal et al., 2001; Stohs & Bagchi, 1995). The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (arsenic is a metalloid, but is usually classified as a heavy metal). These metals have been extensively studied and their effects on human health regularly reviewed by the international research community and organizations. Once again there are few studies exploring the effect of uranium on the oxidative metabolism. Nevertheless, as uranium and lead are both heavy metals, the mechanism/s by which they induce cell damage could be probably alike.

Studies on the pathogenetic effect of lead showed its action is multifactorial since it directly interrupts the activity of enzymes, competitively inhibits absorption of important trace minerals and deactivates antioxidant sulphydryl pools (Patrick et al., 2006). In 2001, Ercal et al. proposed two independent but related mechanisms for free radical-induced damage by lead (Ercal et al., 2001). The first involved the direct formation of reactive oxigen species (ROS) and the second, the depletion of the cellular antioxidant pool. Still, interrelations between these two mechanisms exist so that the increase in ROS on one side simultaneously leads to depletion of antioxidant pools on the other (Gurer & Ercal, 2000). The major effect of lead is on glutathione metabolism (Hunaiti & Soud, 2000) with glutathione representing more than 90% of the non-tissue sulphur pool of human body. The sulphydryl groups of glutathione bind effectively to toxic metals such as arsenic and mercury. Therefore, an organism exposed to lead has significantly lowered levels of glutathione, with respect to control groups, which may in turn induce an imbalance in the oxidative metabolism concomitantly increasing the production of ROS. Metal-induced ROS cause damage to cellular proteins, nucleic acids and lipids, resulting in a variety of cellular dysfunctions including cell death. Intracellularly, ROS can modulate gene expression by interfering with signal transduction cell proliferation pathway activating various transcription factors, thus controlling cell cycle progression and apoptosis (Evan & Vousden, 2001). The most important pathway involves the nuclear factors NF-kB, AP-1, etc., and the tumour suppressor protein p53. Furthermore, deregulation of cell growth and differentiation is a typical characteristic of the cancer phenotype, and cancer is a multifactorial disease, it could be hypothesized that uranium, as a heavy metal, could be one of the factors implicated. Mammalian cells have developed multiple homeostatic systems to counteract the effects of metal induced- oxidative stress by scavenging free radicals and repairing oxidant damage to biomolecules. There are two specific enzymes, glutathione reductase (GR) and deltaaminolevulinic acid dehydrogenase (ALAD) that are both inhibited by lead (Hoffman et al., 2000). In large part, in response to oxidative stress, the transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2) is activated and coordinates the expression of antioxidant gene products (Osburn & Kensler, 2008). The Nrf2 activation has been demonstrated in response to a variety of metals including lead (Korashy & El-Kadi, 2006). In this context, given that several metals are known to generate ROS, and that mammalian cells activate Nrf2-mediated transcription in response to ROS, it could be expected that uranium exposure could activate the Nrf2 pathway. Metal exposure has been shown to exert a number of effects on the Nrf2 pathway including reduction of sulfhydryl groups in Keap-1, MAPK activation, and inhibition of proteasomal pathways which stabilizes Nrf2. The cumulative impact of these events is stabilization and activation of Nrf2 and transcriptional upregulation of anti-oxidant genes. Effective chelators of lead like meso-2,3-dimercapto

succinic acid and calcium disodium ethylenediaminetetraacetic acid are used in treatment of lead toxicity (Gurer & Ercal , 2000). Similarly, several chelators have been assayed to inhibit uranium toxic effects in vivo on target organs. This aspect will be described in part 3 of this chapter.

3.1.7.2 The nitric oxide (NO) pathway

Although there are very few studies exploring into uranium on the nitric oxide (NO) pathway there is an extensive series of publications on the effects of lead on this signal pathway (Barbosa et al., 2006; Kong et al., 2000; Zhu et al., 2005). All these studies showed evidence that the NO pathway is a target for lead exposure. Lead-induced inhibition of NO production in central nervous system (Zhu et al., 2005), blood (Barbosa et al., 2006), kidneys (Dursun et al., 2005), inmmune cells (Bishayi & Sengupta, 2006) and in intestine (Kong et al., 2000), suggesting a common mechanism present for these tissues. Therefore, the NO pathway seemed to be a preferential target for uranium effects. DU-induced decreases gene expression, stimulation of enzyme activity of eNOS and slight decrease in iNOS activity, as well as diminution of NO metabolite content (Dublineau et al., 2007). Dublineau et al. (2007) postulated several hypotheses to explain these inhibitory effects of uranium on the NO pathway. The first one involved direct interference of uranium with calcium. An exchange of uranyl ion with calcium has been assumed at bone surface and inhibition of different calcium transporters by uranium was reported several years ago (Desmedt et al., 1993; Thompson & Nechay, 1981). Interactions between metals and calcium were already suggested for lead with transporters or calcium-binding proteins (Kern & Audesirk, 2000; Simons, 1993). Several authors demonstrated that Pb^{+2} could be substituted for Ca^{+2} in the activation of calmodulin, leading to negative effects on iNOS activity (Gribovskaja et al., 2005; Simons, 1993). It can be thus suggested that uranium inhibits the NO production via interaction with calmodulin, but further experiments have to be performed to validate this hypothesis. The second hypothesis for uranium-NO inhibition is the activation of inhibitors of iNOS, such as IL-4 and IL-10 (Simmons & Murphy, 1993). The third hypothesis is an inhibitory effect of uranium induced oxidative stress. In fact, oxidative stress has been observed following uranium contamination in different systems (Linares et al., 2006; Periyakaruppan et al., 2007; Tasat et al., 2007). Here again, similarity between uranium and lead may be noticed because lead-induced oxidative stress has been shown as causing NO inactivation (Vaziri & Ding, 2001).

3.2 In vitro uranium cytotoxicity

Although in vivo studies demonstrate that uranium toxicity removes the body's supply of antioxidant enzymes as glutathione, superoxide dismutase (SOD), catalase (CAT) allowing free radicals to run uncontrolled through tissues and organs, in vitro studies must be performed in order to understand the intracellular mechanisms of cell response to this toxicant (Periyakaruppan et al., 2007).

Unlike the abundant research on the effect of uranium in various organs in vivo, the cellular effects have only been studied in a limited number of cell culture models in vitro. After inhalation and deposition of uranium, particles principally reach two main target cells: macrophages and epithelial cells (Schins & Borm, 1999). Uranium uptake by alveolar macrophages has been shown to occur after inhalation of soluble and insoluble compounds

(Tasat & de Rey, 1987; Berry et al., 1997) and subsequent uranium accumulation was proved to activate macrophages which in turn, secrete different mediators like pro- and anti-inflammatory cytokines (Driscoll, 2000; Driscoll et al., 1997). Thus, herein we first describe uranium possible intracellular mechanism on these two cell types.

In rat lung epithelial cells Periyakaruppan et al. (2007) found that uranium induced a significant oxidative stress and a decrease in cell proliferation. These findings were attributed to a reduction in the antioxidant potential of the cells due specifically to loss of total glutathione and superoxide dismutase. This investigation pointed out the ineffectiveness of antioxidant system's response to induced -uranium oxidative stress in the cells. More recently, these same researchers (Periyakaruppan et al., 2009) showed that oxidative stress may lead to apoptotic signaling pathways. Epithelial lung cells treated with DU resulted in a dose and time-dependent increase in the activity of caspases-3 and -8, both enzymes involved in the apoptotic process. Xie et al. (2010) observed that DU human bronchial lung cells exposure can transform and induce significant chromosome instability. Most investigators agreed in that chronic DU contamination increases chemokine CCL-2, pro-inflammatory cytokine IL-1b and anti-inflammatory cytokine IL-10 (Wan et al., 2006).

Macrophages or macrophage-like cells are present in large numbers in tissues throughout the body including the lung, liver, bone, lymph nodes, brain, kidney, skin, and spleen. More often than not, alveolar macrophages are the cells in charge of cleaning organic and inorganic inhaled particles from the lungs (Fels & Cohn, 1986; Lohmann-Matthes et al., 1994). As we (Tasat & de Rey, 1987) previously showed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), after in vitro phagocytosis of uranium dioxide, macrophages are implicated in the retention of these particles resulting in what is known as "activated macrophages". Even more, we observed that uranium rat alveolar macrophage exposure resulted in time and concentration dependent uptake of uranium particles, citotoxicity, and induction of cell death. In vitro studies showed that these activated macrophages respond by secreting diverse intracellular mediators: reactive oxygen species (Tasat & de Rey, 1987), proinflammatory cytokines (TNFa, IL6) (Driscoll, 2000) and MAPK activation (Gazin et al., 2004) among others. In 2002, Kalinich et al. reported on cultured mouse macrophages that DU in the form of uranyl acetate induced cell death by apoptosis. In agreement with Kallinich we found that uranyl nitrate (12.5-200mM) on cultured rat alveolar macrophages impaired cell viability, induced secretion of superoxide anion (O_2^-), TNFα and apoptosis via caspase -3 and its clivated PARP substrate (Orona, 2009). Furthermore, we explored into the oxidative metabolism and the pro-inmflamatory cytokine profile and suggested that apoptosis could be reached by different intracellular signaling pathways depending on the uranium concentration. In our latest work (Orona et al., unpulished) we showed that when exposed to low doses of uranyl nitrate, high increases of superoxide anion generation may act as the principal mediator and directly damage alveolar macrophage DNA. On the other hand, when macrophages were treated with high DU doses, cell death seems to be encountered as the result of the TNFα receptor activation, also known as the cell death receptor. We suggested that the signaling pathway mediated by O_2^- may be blocked, prevailing damage to DNA by the TNFα route (figure 1).

In accordance Wan et al. (2006) showed in murine macrophages and CD4+ T lymphocytes, that DU cytotoxicity was concentration dependent. By microarray and real-time reverse-transcriptase polymerase chain reaction (RT-PCR) analyses these authors revealed that DU

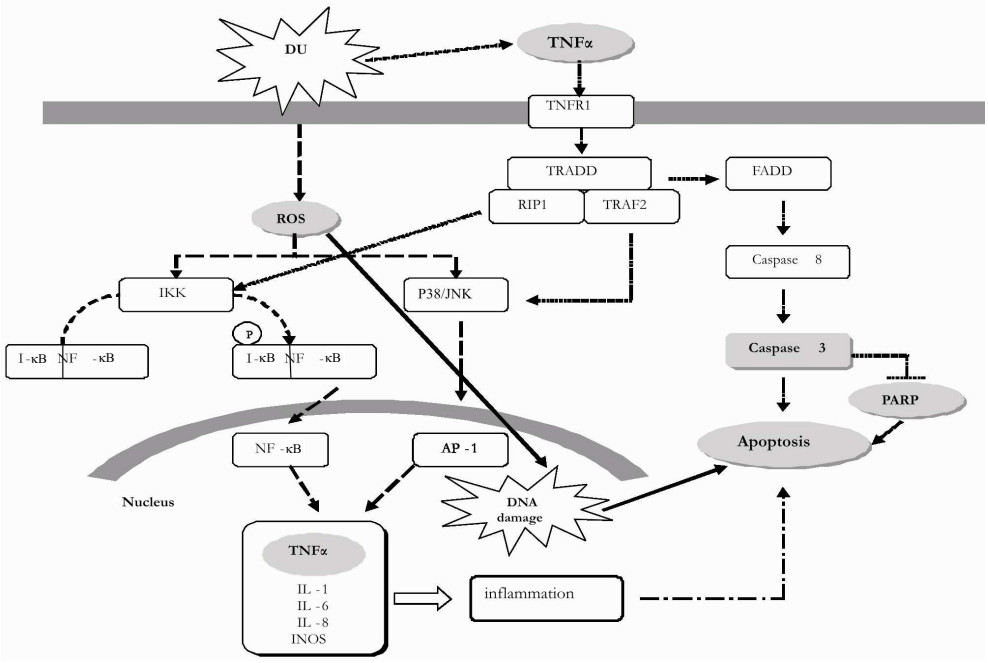

Fig. 1. Proposed model for the effect of low and high depleted uranium (DU) concentrations in cultured rat alveolar macrophages. Orona et al. (unpublished, 2011)

altered gene expression patterns in both cell types being the most differentially expressed genes the ones related to signal transduction, such as c-jun, NF- kappa Bp65, neurotrophic factors (e.g., Mdk), chemokine and chemokine receptors (e.g., TECK/CCL25), and interleukins such as IL-10 and IL-5, indicating a possible involvement of DU in cancer development and autoimmune diseases.

As previously mentioned, bone and kidney are main target organs for uranium deposition, so next we describe the effect of uranium on normal fetal and tumoral osteoblasts and renal cells in cultured. To evaluate the effects of DU in vitro, Miller et al. (2002) employed immortalized human osteoblast cells (HOS) demonstrating its genotoxic and neoplastic activity. Furthermore Miller et al. (2003) showed that DU exposure impacted genomic instability manifested as delayed lethality and micronuclei formation. Still, up to 2003 the cellular and molecular pathways of uranium toxicity in osteoblast cells were unknown. In 2007, we studied (Tasat et al., 2007) on human fetal osteoblasts cells (HFob) in culture, cell proliferation, generation of reactive oxygen species (ROS), apoptosis, and alkaline phosphatase (APh) activity. We found that 1-100 μM of urnayl nitrate in vitro failed to modify cell proliferation ratio, induced apoptosis, increased ROS generation in a dose-dependent manner and decreased APh activity. We then suggested that ROS could play a more complex role in osteoblast physiology than simply causing oxidative damage.

Concentration-dependent cytotoxicity was also observed in NRK-52E cells, an immortalized cell culture model representative of rat kidney proximal epithelium cells (Thiébault et al.,

2007). Researchers have also evaluated the transcriptomic and proteomic responses of HEK293 kidney cells, and renal tissue from rats exposed to DU and found that there were several oxidative-response–related transcripts that were up-regulated, and significantly increased peroxide levels that support the implication of oxidative stress (Prat et al., 2005). Recently, Prat et al. (2010) in a study based on DNA microarrays, analyzed gene expression after acute uranium exposure in several human cell lines taken from kidneys or lungs. They found no common gene between cells originating from lungs and kidneys but, higlighted a gene (SPP1) coding for osteopontin, a secreted protein linked to bone mineralization. They concluded that uranyl ions affected the excretion of osteopontin in a time- and dose-dependent manner and therefore proponed this protein as a potential biomarker of uranyl mineralization effects in vivo.

Other in vitro studies showed toxicity of uranium. On reproductive cells like Chinese Hamster Ovary (CHO), Lin et al. (1993) showed that DU decreased cell viability in a dose dependent manner, reduced cell cycle kinetics (CCK) and increased micronuclei (MN), chromosome aberrations (CA) and sister-chromatid exchanges (SCE) frequencies. This array of DU cytogenetic altered parametes could probably establish a biological basis for the potential teratogenic effect of uranium on developing fetal mice. In 2005, Stearns DM et al., showed in this same mammalian cell line that uranyl acetate could be chemically genotoxic and mutagenic through the formation of strand breaks and covalent U–DNA adducts. Once more health risks for uranium exposure could go beyond those for radiation exposure.

4. Inhibition of uranium toxicity

The general purpose of this third section is to review the studies on chelating agents employed as treatment for acute uranium exposure, and the ability of these compounds to prevent or counteract the effects of uranium on target organs. As was described in the second section, ultrafine uranium particles enter the body mainly by inhalation, and depending on its solubility they remain in the lungs or slowly dissolved and are transported into the blood, getting distributed to bone, kideny, brain, liver, lymph, spleen, testes and other organs. Uranium remains in plasma for a very short period of time (Walinder et al., 1967) while instead in kidney and bone, the highest concentration of this heavy toxic metal can be found (Hursh & Sopor, 1973). In the kidneys, it causes destruction of the proximal convoluted tubules resulting in potentially life threatening renal failure (Adams & Spoor, 1974; Domingo et al., 1987). As regards its effect on bone, acute exposure to uranium was found to inhibit endochondral ossification (Guglielmotti et al., 1984), alveolar wound healing (Guglielmotti et al, 1985), bone formation in the tooth alveolus (Ubios et al., 1991), and maxillary growth (Ubios et al., 1998).

The first detailed study of the action of the salts of uranium was made by Chittenden & Hutchinson in two publications which appeared in 1887 and 1888. The first of these publications was concerned with the effect of various soluble salts of uranium. The authors showed that in very high dilutions especially the uranium nitrate, exerted an inhibitory effect upon the action of saliva on the amylolytic action and the proteolytic action of pepsin and trypsin. The second paper, although it was chiefly concerned with the effect of uranium salts upon protein metabolism, gave a brief account of the toxic effect of uranium in liver and kidney. The ability of uranium to induce a glycosuria was recorded, and the observation was made that the glycosuria usually did not occur until after the appearance of

albumin in the urine. The toxic effect of uranium for the liver and for the kidney is usually attributed to the action of the metal as such. Nevertheless, back in 1916, MacNider showed that the toxicity of uranium runs parallel with its ability to lead to the formation of various acid bodies, and that if the appearance of these substances in the urine is delayed and their amount diminished there is less evidence of the toxic action of the metal. Even more, MacNider (1916) showed that uranium nitrate toxic action may be inhibited by the use of an alkali. Since then several has been the chemicals studied in order to prevent the effects of uranium poisoning in its target organs, mainly kidneys and bones.

During the Manhattan Project in the early 1940s, the toxic properties of uranium were thorouly investigated finding out that uranium oxides stick very well to cotton cloth, but did not wash out with soap or laundry detergent (Orcutt, 1949) but, with a 2% solution of sodium bicarbonate. Donnelly & Holman (1942) found that giving sodium citrate to animals protected them from an otherwise lethal toxic dose of uranium. Sodium citrate is a neutralized form of citric acid. The sodium citrate caused the animals to excrete uranium faster, resulting in less uranium deposited in the body. Citrate salts, such as potassium citrate and magnesium citrate, are available for treatment of kidney stones and would probably be useful in the treatment of suspected uranium poisoning. There have been no clinical trials done with this type of treatment, but evidence for the efficacy of citrate treatment from animal studies when intoxicated with uranium are very convincing. As citrate salts and citric acid are readily available simple treatments would offer the best protection for those exposed to uranium with v ery few side effects (possibly nausea, diarrhea, gas). Several compounds ranging from natural and simple such as sodium citrate, to more sophisticated synthetic chelators agents have been tested in order to treat uranium contamination. To date, several studies have been performed to determine the effectiveness of different chelating agents in preventing the toxic effets of uranium on the body (Domingo et al., 1990; Cabrini et al., 1984; Stradling et al., 1991; Martinez et al., 2000; Bozal et al., 2005). However, not all chelating compound tested showed to be effective to counterbalance the action of uranium. In animal models tetracyclines, proved to be effective in neutralizing the uranium inhibition of bone formation but were unable to prevent nephrotoxic side effects (Guglielmotti et al., 1989). For acute uranium contamination Ortega et al. (1989) assayed 16 different chelating agents administered intraperitoneally (ip). They showed that Tiron (sodium 4,5-dihydroxybenzene-1,3-disulfonate) was the most effective from all the compounds tested increasing urinary and faecal excretion of uranium 24 hours post-administration and lowering the toxic concentration in bone and kidney, thus allowing survival of 100 and 70% depending on the interval between the administration of the toxic and the antidote (Domingo et al., 1992). The increase in the interval between exposure to uranium and the administration of chelating agent drastically reduced the mobilization of uranium from the target organs, proving to be a critical point that must be taken into account. Thus, the faster the chelator is administered after lethal uranium intoxication the greater the likelihood of increased survival. Therefore, if time frame for the administration of any chelating compounds is beyond 24 hours post-intoxication, none of them will be able to reverse the deposition of uranium in the bones. Durbin et al. (1997) found that daily injections of 5-Li or TREN-(Me-3.2-HOPO) were able to reduce by 50% retention of uranium in the tissues, accelerating the renal excretion of the poison. This compound is able to greatly reduce the damage at the renal tubular epithelium but not proved to be effective in removing uranium on skeletal tissues. In 2000, Martinez et al.

showed that ethane-1-hydroxy-1,1-biphosphonate (EHBP) administered both orally or subcutaneously, was highly effective as an antidote in uranium contamination increasing survival rate from 45% up to 100% of success, depending on the age of the animal. The longest survival time was observed for suckling animals (Ubios et al., 1994) when compared to adult animals (Martinez et al., 2000). Again, the efficiency of the treatment depended on the time lapse between exposure to the toxicant and the administration of the bisphosphonate proving that efficency is inversely proportional to the time interval (Ubios et al., 1998).

The effectiveness of EHBP focuses on the prevention of the adverse effects of uranium on renal function and the uranium-inhibition reversal on bone formation. Therefore, after uranium contamination, EHBP can not only act as a therapeutic agent to improve survival but, as an agent capable to prevent uranium toxicity on target organs.

Once ingested, EHBP is absorbed mainly through the small intestine. From the absorbed EHBP, 60% is deposited in bone and the remaining 40% excreted in urine (Fleisch, 1995). Also in 1995, Henge-Napoli et al. showed that the chelating effect of EHBP prevents uranium from reaching the kidney. Therefore it was assumed that EHBP could "intercept" uranium while in the circulatory system, preventing tissue damage of the target organs (Martinez et al., 2003). Bioavailable uranium could link to EHBP in blood conforming chelates that allow uranium to be excreted supressing its effect as a life-threatening compound. Our group has extensive evidence on the preventive effect of EHBP for acute

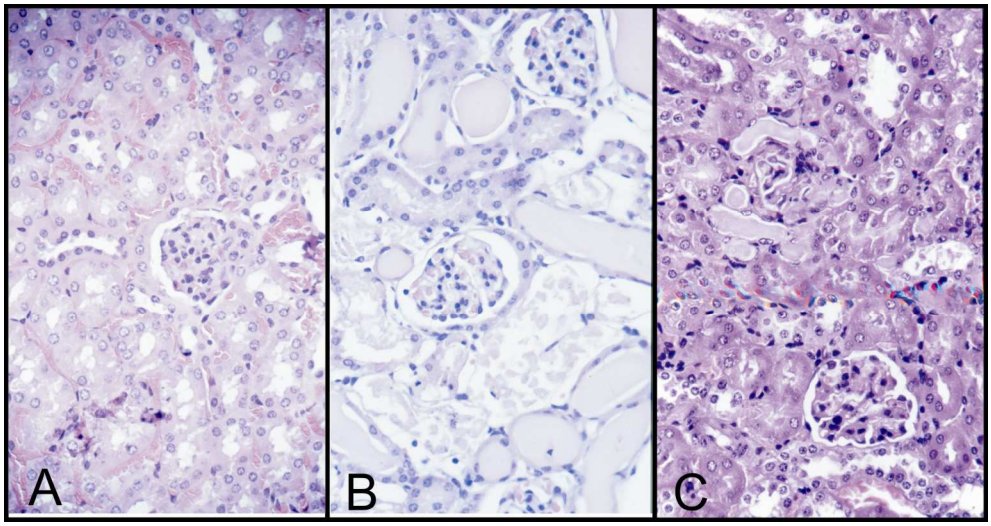

Fig. 2. **Histologic sections of renal cortex**. A): control, B): exposed to a lethal oral dose of uranyl nitrate 48 h post-intoxication, and C) intoxicated with uranyl nitrate and treated-EHBP after 14 days. A) Note integrity of tubule and glomerular structure. B) Note the marked vacuolization of the tubules, abundant hyaline cylinders, and extensive areas of necrosis. Widening of the uriniferous tubule and Bowman´s capsule is also evident. C) Note that although areas presenting necrosis and hyaline cylinders are evident, the proportions of these areas are much lesser than in B). (HE 400X)

uranium intoxications administered intraperitoneally (Ubios et al., 1994), subcutaneously (Ubios et al., 1998; Martinez et al., 2000) or orally (Martinez et al., 2000). In all cases we demonstrated that EHBP is able to ameliorate the structural and functional damages induced by uranium. On kidneys EHBP causes a decrease in the renal concentration of uranium (Henge-Napoli et al., 1999) and prevents from functional alterations resulting in urea and creatinine values similar to those of control non-intoxicated animals (Martinez et al., 2003). At 14 days post- uranium intoxication, kidney lesions from EHBP treated animals (orally or subcutaneously), showed clear signs of tissue recovery featuring areas of apparent normal parenchyma (figure 2) and urea and creatinine levels remained within normal range. A marked reduction in hyaline casts in kidney sections might suggest that uranium-induced damage could be reversible (Martínez et al., 2003). In the same work we demonstrated that the efficacy of EHBP in non-fasted animals.

The effect of EHBP on bone was similar to the observed in kidney. EHBP reverses induced-uranium alterations on the metaphyseal cartilage and endochondral ossification. Although EHBP causes a decrease in the height of the metaphyseal cartilage, it does not alter the activity or the formation of bone subchondral trabeculae indicating that though at a slower rate, the endochondral ossification process continues (figure 3) (Bozal et al., 2005). During growth, catch-up growth has been defined as growth with a velocity above the statistical limits of normality for age during a defined period of time, which follows a period of impaired growth (Williams et al., 1974). We reported that tooth eruption, dental development and mandibular growth of suckling rats, impaired by acute uranium exposure

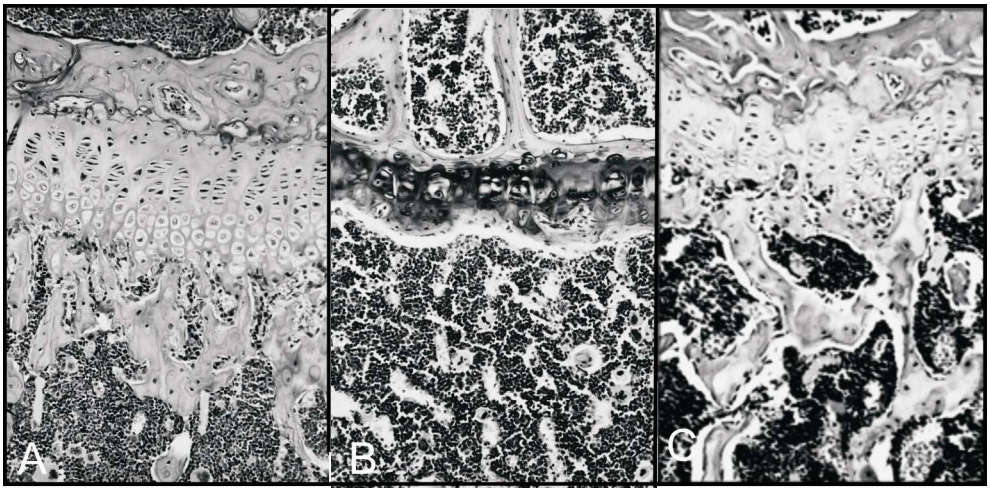

Fig. 3. **Histologic sections of the metaphyseal bone.** A): control, B) exposed to an oral dose of 350 mg/kg of uranyl nitrate 48 h-post intoxicaton. Note the marked diminution in cartilage width and the absence of proliferation cells in the animal exposed to uranyl nitrate, as compared to A). The absence of trabeculae and formation of sealed bone in uranyl nitrate exposed animals can be observed, and the diminution in subchondral bone volume is evident. C): intoxicated with uranyl nitrate and treated-EHBP after 14 days (C). Note that there is some reduction in growth cartilage width but, no differences in subchondral bone volume are observed when comared to A). (HE 200X)

catch up to controls 27 days post - EHBP treatment (Pujadas Bigi & Ubios, 2007). As for metaphyseal cartilage, a decrease in mandibular growth in uranium-exposed animals was observed. This finding is in agreement with the inhibition on bone formation and endochondral ossification (Díaz Sylvester et al., 2002; Bozal et al., 2005). The reduction in dental development is associated to cementum and dentin of the dental root. Therefore, the delay in dental growth in uranium-exposed animals and the fact that we observed catch-up growth after 27 days, may indicate that osteoblast and odontoblast precursor cells, which are genetically determined to form bone and dentin respectively, did not suffer irreversible damage during uranium exposure or that uranium affected only the cells that were determined at the time of the exposure (Pujadas Bigi & Ubios, 2007).

It must be kept in mind that longterm oral EHBP therapy was widely used to treat osteopenia and is still used to treat Paget´ s disease and hypercalacemia. It is worth to note that oral intake is a simple and fast route of entry to the body, which is essential in case of accidental uranium ingestion.

5. Conclusion

As is known uranium, natural and depleted (DU) present similar chemical and radiological properties. Although DU is not classified as a dangerous substance radiologically, for its emissions are very low, it is, in large quantities, a potential toxicological hazard. Despite much study, epidemiologycal, in vivo on experimental animal models and in vitro on cultured cells, uranium impact on health remains controversial. As uranium decay proceeds its final product, lead, increases in relative abundance in nature. Both elements have similar chemical toxicity, so inhaled fume or ingested particles of any of them is considered a health hazard. In this context, in this chapter we made an attempt to consider uranium chemotoxicity and its intracellular mechanism of action similar to that of lead, a heavy persistent metallic toxic particle. The current knowledge in the field of metallo-biochemistry of oxidative stress indicates that metal-induced and metal-enhanced formation of free radicals and other reactive species can be regarded as a common factor in determining metal-induced toxicity and carcinogenicity. Metals interfere with cell signalling pathways and affect growth receptors, tyrosine and serine/threonine kinases, and nuclear transcription factors by ROS-dependent and ROS-independent mechanisms. Many of the DNA base modifications caused by free radicals are pro-mutagenic, pointing to a strong link between oxidative damage, declined antioxidant mechanisms and carcinogenesis. As occupational exposures to heavy metals (lead, arsenic) primarily by inhalation are causally associated with lung cancer, natural uranuim and DU could follow the same path. Neverhteless, further studies have to be done in order to conclude uranium implication in this matter. Different compounds are used to treat heavy metal poisoning and chelate redox active metals. For this last purpose, antioxidants are the selected substances while biophosphonates seem to be the to promissory compounds able to prevent or reverse health problems of individuals exposed to uranium.

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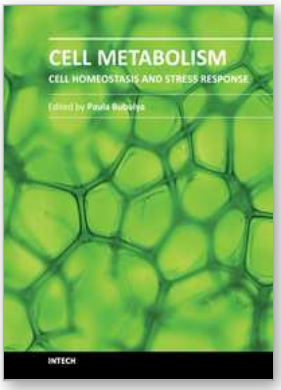

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A global research community of scientists is teasing out the biochemical mechanisms that regulate normal cellular physiology in a variety of organisms. Much of current research aims to understand the network of molecular reactions that regulate cellular homeostasis, and to learn what allows cells to sense stress and activate appropriate biochemical responses. Advanced molecular tools and state-of-the-art imaging techniques discussed in this book continue to provide novel insights into how environmental changes impact organisms, as well as to develop therapeutic interventions for correcting aberrant pathways in human disease.

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