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Uranium deposition in bones of Wistar rats associated with skeleton development

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HIGHLIGHTS

- Uranium deposited in bones increases faster in younger animals saturating in older.
- *U* data were fitted by a sigmoid curve, suggesting that it mimics calcium metabolism.
- Bone mineral density indicates that even minute *U* could induce death of bone cells.

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ABSTRACT

Sixty female Wistar rats were submitted to a daily intake of ration doped with uranium from weaning to adulthood. Uranium in bone was quantified by the SSNTD (solid state nuclear track detection) technique, and bone mineral density (BMD) analysis performed. Uranium concentration as a function of age exhibited a sharp rise during the first week of the experiment and a drastic drop of 70% in the following weeks. Data interpretation indicates that uranium mimics calcium. Results from BMD suggest that radiation emitted by the incorporated Uranium could induce death of bone cells.

1. Introduction

Uranium is heavily present in rock phosphate, which is used as a source of phosphorus in the making of fertilizers and livestock feed supplements, as dicalcium phosphate (DCP). The prolonged utilization of such fertilizers leads to absorption of substantial amounts of uranium by plants, contributing to the increase of this element in the human diet (Yamazaki and Geraldo, 2003). Furthermore, DCP is extensively used in broilers diet, another important consumption item by humans (Sebastian et al., 1996; Lima et al., 1995). It is important to note, however, that DCP can present concentrations of uranium as high as 200 ppm (Arruda-Neto et al., 1997). Also, insoluble forms of uranium are greatly solubilized during digestion giving rise to uranyl compounds, particularly uranyl nitrate.

Health hazards generated by uranium are of two categories: toxicological and radiobiological. It is well documented from toxicity studies that the kidney is the target organ and that chronic ingestion of uranium may cause kidney lesions and malfunction

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(Zamora et al., 1998). This element also causes damage to the microvasculature of the liver and induces hepatitis (Alpen, 1990).

However, the radiobiological issue is a matter of much greater concern, since (1) uranium has three natural isotopes which are long-life α-emitters, and (2) the uranyl radical $^{++}UO_2$ produced in the gastrointestinal tract seems to mimic ^{++}Ca. Additionally, uranium belongs to the category of "bone-seeker radionuclides", and about 80% of incorporated uranium is accumulated in the skeleton (ICRP 69, 1995).

In an earlier experiment performed at this Laboratory with Beagle dogs, uranium mixed with food was administered to the animals. It was observed that this element accumulates similar amounts in both mineral bone and marrow (Arruda-Neto et al., 2004b). Thus, doses from α-emitters are imparted to the entire bone marrow volume and, consequently, primitive hematopoietic stem cells, concentrated in the central marrow (Lord, 1990), are subject to radioactive burdens as intense as those in mineralized bone. Thus, possible radiobiological risks need to be taken into account, even for small amounts of uranium. Regarding consequences to humans, children are of much greater concern because of the higher absorption rate of essential elements in their growing skeleton (Tandon et al., 1998).

This circumstance motivated the need to measure the content of uranium in the bones of Wistar rats, following chronic ingestion, starting in the postweaning phase and at intervals of three days. This data could prove to be very useful for extrapolations to humans, particularly children, since the vast majority of studies has been conducted with adult animals following single administration of acute dosages (Tandon et al., 1998; Ubios et al., 1998) as far as prolonged intake (Arruda-Neto et al., 2001). In fact, with an ad-hoc multiple compartment model (Garcia et al., 1999) it would be possible to estimate the content of uranium transferred to organs and milk by using uranium accumulation data in bones as input.

The experiment described in this study is part of an ongoing comprehensive project on teratogeny, dealing particularly with trans-placental biokinetics of uranium, a circumstance motivating the desire to use female animals also in this study.

2. Materials and methods

2.1. Animals

Sixty female Wistar rats, 21 days old (so far breastfed) were separated into 8 groups with the following composition: (a) a first group with 3 animals (control group); (b) 6 groups having 8 animals each, with 5 submitted to Uranium treatment (treated animals) and 3 as control; and (c) one group having 9 animals, with 5 submitted to Uranium treatment and 4 as control. Natural Uranium was administered as Uranyl nitrate. Naturally occurring uranium is composed of three major isotopes, uranium-238 (99.275% natural abundance), uranium-235 (0.72%), and uranium-234 (0.006%). This isotopic composition is routinely verified in our Laboratory by means of neutron activation spectroscopy analysis—see details in Arruda-Neto et al. (2004b).

The animals were maintained with temperature and ventilation under control with light cycle of 12 h/12 h, fed with controlled amounts of ration, and water "ad libitum".

Uranyl nitrate, homogeneously mixed into the ration at a concentration of 50 ppm (parts per million), was administered to the treated animals. These animals were sacrificed at the ages of 25, 29, 33, 36, 43 and 50 days. The animals in the control group were fed with noncontaminated ration, and were sacrificed at the ages of 22, 25, 29, 33, 36, 40, 43 and 50 days, respectively. The animals were clinically checked throughout experiment duration, and their weight and amount of ration ingested verified daily.

2.1.1. Euthanasia and necropsy

The animals were anesthetized with ketamine at a dose of 80 mg/kg associated with xylazine in a dose of 10 mg/kg intraperitoneally. While they were still alive, blood was collected via intracardiac puncture with 3 ml syringes, using 30×7 needles in the fourth left lateral intercostal space. About 2 ml of blood was collected and stored in collection tubes with and without heparin. The animals died of hypovolemia; autopsy followed by external and cavity inspection was then performed. Organs were removed and stored in 10% formalin. Femora were dismembered and stored under freezing conditions for posterior processing (next paragraph).

2.2. Quantification of uranium in femora

To determine *U* concentrations in bone samples of Wistar rats the SSNTD (solid state nuclear track detection) technique was employed using a polycarbonate plastic named PCLIGHT (1 mm thickness), produced by Policarbonatos do Brasil S/A (Yamazaki and Geraldo, 2003).

Initially, the bones were stored in an incubator at 70 °C for three hours to obtain dry weight. These samples were placed in a muffle furnace for one hour for the carrying out of calcination, reaching a maximum temperature of 800 °C. Following this, each bone was macerated under a 250 W lamp, intended to minimize absorption of water from the environment, and were weighed again to establish the weight after calcination in a muffle furnace.

The calcinated samples were oven-dried at 90 °C until attaining a constant weight ($\pm$ 1%). About 40 mg dry weight from each bone powder was treated with acqua-regia solution (1HNO_3: 3HCl), at a temperature around 115 °C, for approximately 1 hour, in order to eliminate organic compounds and to obtain a homogeneous solution. The resulting residue was diluted to a total volume of 10 mL. Aliquots of 10 μL were deposited on plastic detectors (area of 1.2 cm^2) followed by 5 μL of a Cyastat detergent solution (5%, Cytec Industrias). Evaporation of water and volatile compounds were accomplished by exposing the set to an infrared lamp (250 W) at a temperature around 75 °C. The Cyastat detergent solution works as an electrostatic neutralizer reducing the droplet surface tension and making it possible to obtain deposits with better homogeneity. The deposits were covered with an extremely thin (about 20 μg/cm^2) collodion film (isoamile acetate plus elastic collodion 1:1 in volume) in order to prevent contamination and water absorption from room humidity.

A standard solution having a precisely known uranium content was prepared in the same way as described above for bone samples, to be used as neutron flux monitor during irradiations. A solution of the reference bone sample IAEA-A12 was also prepared, so as to check the analytical accuracy of the method.

Plastic films corresponding to bone samples from three animals were sandwiched between two *U* standard samples, and this set was accommodated inside an aluminum *rabbit* (22 mm diameter by 70 mm height), a container usually employed for irradiations at IPEN-IEA-R1 (3.5 MW) pool type research nuclear reactor—over 20 *rabbits* were used. With the *U* standards in each *rabbit* a much better neutron flux monitoring was achieved.

The *rabbits* were placed near the reactor core for irradiation at the position EIRA 24B, where the estimated thermal neutron flux was 1.2×10^{13} n/cm^2 s. The irradiation time employed for all samples was about 3 min.

After irradiations, chemical etching of the plastic detectors was carried out at approximately 60 °C, for a period of 65 min, in a NaOH (6 N) solution. For these chemical etching conditions the fission tracks produced in the detectors presented the best visibility condition when observed under a conventional optical microscope. The fission tracks were counted by scanning the entire