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Endocrine effects of lifelong exposure to low-dose depleted uranium on testicular functions in adult rat

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ABSTRACT

Environmental toxicant exposure can induce disorders in sex steroidogenesis during fetal gonad development. Our previous study demonstrated that chronic adult exposure to a supra environmental concentration of depleted uranium (DU) does not impair testicular steroidogenesis in rats. In this study, we investigated the effects of lifelong exposure (embryo – adult) to low-dose DU (40 or 120 mg L^{-1}) on adult rat testicular steroidogenesis and spermatogenesis. A significant content of uranium was detected in testis and epididymis in the DU 120 mg L^{-1} group and the assay in epididymal spermatozoa showed a significant content in both groups. No major defect was observed in testicular histology except a decrease in the number of basal vacuoles in the DU groups. Moreover, plasma Follicle-Stimuling Hormone [FSH] and Luteinizing Hormone [LH] levels were increased only in the DU 120 mg L^{-1} group and intratesticular estradiol was decreased in both groups. Testosterone level was reduced in plasma and testis in the DU 40 mg L^{-1} group. These modulations could be explained by an observed decrease in gene expression of luteinizing hormone receptor (*LHR*), and enzymes involved in steroid production and associated signal transduction (*StAR, cyp11a1, cyp17a1, 3βhsd, 17βhsd, TGFβ1, AR*). Several genes specific to germ cells and cell junctions of the blood-testis barrier were also modulated. In conclusion, these data show that fetal life is a critical window for chronic uranium exposure and that the endocrine activities of low-dose uranium could disrupt steroidogenesis through the hypothalamic-pituitary-testicular axis. Further investigation should be so useful in subsequent generations to improve risk assessment of uranium exposure.

1. Introduction

In recent decades, observations of the decline of quality and quantity of human semen have been closely associated with increasing concern about the role of environmental pollutants on human male reproduction (Campion et al., 2012). Development of the male phenotype and adulthood reproductive function are highly dependent on androgens and estrogens (Carreau et al., 2008; Macleod et al., 2009). Consequently, disorders in sex steroid hormone balance during fetal development may interfere with male reproductive success.

In the environment, uranium may be present in drinking water contaminated naturally, by military activities or by human activity in nuclear power stations, mining sites, and farmland (use of phosphate fertilizers). For example, 90 water sources near mining sites in the Navajo Nation contained uranium above the World Health Organization (WHO) safe drinking water level (30 μg L^{-1}, (WHO, 2011)). Consequently, populations are exposed to unsafe levels, because they use water from the nearest source for household use (*i.e.* drinking water, cooking, laundering . . .) (Brugge, 2016). The scientific community and society in general are thus paying increasing attention to the consequences of low-dose uranium exposure.

Few reports have dealt with the effects of environmental uranium exposure on reproductive health outcomes. Some epidemiological studies report a significant decrease in the sex ratio of offspring of male uranium miners (Muller et al., 1967) and in serum testosterone levels in Namibian miners (Zaire et al., 1997).

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A significant relationship was also found between birth defects and the mother's proximity to uranium tailings in the Navajo Nation (Shields et al., 1992). In contrast, Squibb and McDiarmid showed no effect on reproductive hormone levels (testosterone, Follicle-Stimulating Hormone [FSH], Luteinizing Hormone [LH]), on Thyroid-Stimulating Hormone [TSH]) level and sperm parameters (sperm production and motility, sperm chromatin stability) in the veterans wounded by depleted uranium fragments as a result of the First Gulf War (Squibb and McDiarmid, 2006).

Uranium toxicity is due to both its chemical and radiological properties (Katz, 2013), but depleted uranium (DU), which is a by-product of natural uranium enrichment, has a lower proportion of ^{235}U. The chemical toxicity effects of DU have been studied in kidney (Poisson et al., 2014), bone (Wade-Gueye et al., 2012) and brain (Dinocourt et al., 2015) after chronic exposure in experimental models. But data on the effects of DU on male reproductive function are scarce. Rodents chronically exposed to 10–80 mg/kg/day of uranium presented few testicular histopathological abnormalities and decreases in pregnancy rate and spermatid number (Linares et al., 2005; Llobet et al., 1991). Acute exposure resulted in embryotoxicity (Bosque et al., 1993; Kundt et al., 2000), though no effect was observed after chronic exposure to a low dose of uranium (Paternain et al., 1989). There is no evidence for steroidogenesis effects of low-dose [2.5 mg/kg/day] DU after adult male chronic exposure (Grignard et al., 2008), except after relatively low dose exposure at 4–40 mg/kg/day (Hao et al., 2012). An *in vitro* study has demonstrated that human fetal testes are more sensitive to low-dose uranium than mouse fetal testes, without any effect on testosterone production (Angenard et al., 2011). However, to our knowledge, there is no report on the effect of *in vivo* chronic exposure from the embryo until adulthood (*i.e.* lifelong) on male reproductive function. Low concentrations ($0.5\,\mu g\,L^{-1}$) of DU have estrogenic effects in female mice (Raymond-Whish et al., 2007), and we hypothesized that low-dose DU could have endocrine effects in male rat exposed from gestation.

We aimed to elucidate the influence of uranium on steroidogenesis and spermatogenesis in mature rats subject to lifelong exposure to low-dose DU. We investigated the DU content in reproductive organs, and its effects on testis morphology, sex hormone levels, and mRNA expression of genes involved in sperm and steroid production and the blood-testis barrier, which protects the germ cells.

2. Materials and methods

2.1. Radionuclide

DU was from Areva NC (Areva-Cogema, France). The specific activity of DU is 14.103 Bq/g and its isotopic composition is ^{238}U: 99.73%, ^{235}U: 0.255%, ^{234}U: 0.01%. Uranium was administered as uranyl nitrate hexahydrate ($UO_2(NO_3)_2{\cdot}6H_2O$), because uranyl ions $(UO2)^{2+}$ are the most stable types of uranium in solution and mammalian body fluids (ATSDR, 1999). Two concentrations of uranium were prepared, 40 and $120\,mg\,L^{-1}$, by dissolution in standardized mineral water (which naturally contains $1.42\,\mu g\,L^{-1}$ uranium). These low concentrations were not nephrotoxic and were chosen from previous studies (Grignard et al., 2008; Legrand et al., 2016).

2.2. Animals and study design

Seventeen pregnant female 12-week-old Sprague-Dawley rats from Charles River (L'Arbresle, France) were housed alone under standard conditions with food and mineral water provided *ad libitum* (light on: 8.00 am/8.00 pm, temperature: $22\,^{\circ}C \pm 1\,^{\circ}C$, relative humidity of 44.1%). Body weight, food (which naturally contains $1.46\,\mu g.L^{-1}$ uranium) and water consumption were measured weekly. Pregnant females were randomly divided into three groups (n = 9 per group): control (CTRL), DU $40\,mg\,L^{-1}$ (DU40) and DU $120\,mg\,L^{-1}$ (DU120). Control rats were watered with mineral water. Pregnant females were exposed to DU *via* drinking water from the first day after mating (gestational day 1: GD1). At lactation day 21, all male pups were weaned and removed from the maternal cages. At 6 months old, ten rats from each group were used to measure content of DU in different organs. Twenty other rats were used to determine the effect of DU on reproductive function (N = 10 per group for histology, and N = 10 per group for blood and biochemical parameters and molecular analyses). All animal procedures were approved by the Animal Care Committee of the IRSN and were conducted in accordance with French legislation and European legal requirements (Decree 86/609/EEC) concerning the protection of animals used for experimental purposes. Scientists certified by the French Ministry of Agriculture performed all procedures in animals.

2.3. Uranium content

Kidney, testis and epididymis were removed in order to analyze uranium concentrations. All samples (N = 10 per group) were weighed and stored at $-20\,^{\circ}C$. Epididymal spermatozoa were extracted as described below.

For kidney, testis and epididymis, samples were prepared by adding 8 mL of ultrapure 69% nitric acid (ARISTAR, VWR, France) and 2 mL of hydrogen peroxide 30% and mineralizing them in a 1000 W microwave (Ethos Touch, Milestone Microwave Laboratory Systems, Italy) with a 20-min ramp to $180\,^{\circ}C$ and then 10 min at $180\,^{\circ}C$. Uranium content of mineralized samples was determined with an inductively coupled plasma mass spectrometer (ICP-MS X-series 2, ThermoElectron, France) with bismuth ($1\,mg\,L^{-1}$) as the internal standard. The ICP-MS limit of detection for uranium is $1\,ng\,L^{-1}$. Values were expressed as $ng\,U\,g^{-1}$ tissue.

For epididymal spermatozoa, 3 mL of sample was added to 3 mL of 69% nitric acid. The sample was put in a muffle furnace (Nabertherm SARL, France) at 110° C until fully set and the mineral deposit was taken up in 6 mL of 11% nitric acid. Samples were diluted ten-fold before assay by ICP-MS. Values were expressed as $ng\,U\,L^{-1}$ homogenate.

2.4. Blood sampling and biochemical parameter analysis

Rats were deeply anesthetized with isoflurane 5%/air 95% inhalation. Rats were terminally anesthetized by intraperitoneal injection of ketamine/xylazine. Blood was sampled by intracardiac puncture with a heparinized syringe and centrifuged (950*g*, 5 min), and plasma supernatants were immediately frozen at $-80\,^{\circ}C$ for subsequent analysis. Plasma biochemical parameters were determined with an automated spectrophotometric system (Konelab 20, Biological Chemistry Reagents, ThermoElectron Corporation, Cergy-Pontoise, France). Kits from Brahms (Asnières sur Seine, France) were used to measure triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, iron, calcium, phosphorus, chloride, potassium, sodium, magnesium, ferritin, and transferrin. Ferritin from Diagam (Lille, France) was adapted to the automated system.

2.5. Hormonal measurements

Testicular extracts were used to measure intratesticular testosterone and estradiol concentrations by radioimmunoassay (RIA) and ELISA, respectively. Testes were thawed and homogenized in cold phosphate-buffered saline (PBS) without Ca^{2+} and