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In vivo effects of chronic contamination with depleted uranium on vitamin D_3 metabolism in rat

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Abstract

The extensive use of depleted uranium (DU) in today's society results in the increase of the number of human population exposed to this radionuclide. The aim of this work was to investigate *in vivo* the effects of a chronic exposure to DU on vitamin D_3 metabolism, a hormone essential in mineral and bone homeostasis. The experiments were carried out in rats after a chronic contamination for 9 months by DU through drinking water at 40 mg/L (1 mg/rat/day). This dose corresponds to the double of highest concentration found naturally in Finland. In DU-exposed rats, the active vitamin D ($1,25(OH)_2D_3$) plasma level was significantly decreased. In kidney, a decreased gene expression was observed for *cyp24a1,* as well as for *vdr* and *rxrα*, 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 kidney. In the brain lower levels of messengers were observed for *cyp27a1* as well as for *lxrβ*, involved in its regulation. In conclusion, this study showed for the first time that DU affects both the vitamin D active form ($1,25(OH)_2D_3$) level and the vitamin D receptor expression, and consequently could modulate the expression of *cyp24a1* and vitamin D target genes involved in calcium homeostasis.

Keywords: Depleted uranium; Cytochrome P450; Vitamin D3; Vitamin D receptor; Chronic contamination

1. Introduction

Uranium is a naturally occurring heavy metal found in Earth's crust. Recently, it has been observed that concentrations of uranium were increasing in the environment as a consequence of the use of depleted uranium (DU) in both civil and military industries, leading to possible exposure of human population [1,2] either through drinking water or the food chain [3]. The chemical and radiological toxicities of this radionuclide have been demonstrated in a variety of organs such as bone, kidney, liver and brain [4–6]. To date, little attention was paid to cellular and molecular effects of chronic ingestion of low uranium quantities on important metabolic functions in these organs. Few studies [7–10], however, report that DU could affect cytochromes P450 (CYPs). Indeed, this enzyme family is involved in the regulation of testosterone [7] and cholesterol metabolism [8] as well as xenobiotic detoxification [9,10]. Cytochromes P450 consist of a superfamily of heme-containing monooxygenases and participates in the metabolism of many drugs as well as endogenous substances including steroids [11]. Among them, vitamin D is a secosteroid hormone

Abbreviations: $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D3; CaBP-D28k, Calbindin-D28k; CYP, cytochrome P450; DU, depleted uranium; ECaC1, Epithelial Ca^{2+} channel 1; HNF, hepatocyte nuclear factor; LXR, liver X receptor; NCX1, Na^+/Ca^{2+} exchanger 1; NT3, neurotrophin 3; PMCa1b, Ca^{2+}-ATPase 1b; PPAR, peroxisome proliferator activated receptor; PTH, parathyroid hormone; RXR, retinoid X receptor; VDR, vitamin D receptor

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playing an essential role in mineral homeostasis and bone metabolism [12]. To carry out these functions, vitamin D must be metabolized to its biologically active form, 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), by two sequential hydroxylation steps catalyzed by CYPs. The first step occurs in liver and involves the mitochondrial CYP27A1 or the microsomal CYP2R1, which synthesizes the major circulating and inactive form of vitamin D_3, 25-hydroxyvitamin D_3 or $25(OH)D_3$. In the kidney, the active form $1,25(OH)_2D_3$ is synthesized by the mitochondrial CYP27B1, whereas hormone inactivation is initiated by CYP24A1 [13,14]. The kidney is also an important target organ for $1,25(OH)_2D_3$, responsible for the handling of calcium and phosphate in kidney. In target organs of the vitamin D such as intestine, kidney and bone, the active metabolite 1,25(OH)2D3 exerts its effects by binding to the nuclear vitamin D receptor (VDR) which could form a heterodimeric complex with the retinoic acid X receptor alpha (RXRα), the receptor for the 9-*cis* retinoic acid, and acts as a ligand-activated transcription factor by altering the transcription rates of target genes. Among the targets, osteocalcin, an abundant, highly conserved bone-specific protein that is synthesized by osteoblasts, plays an important role in bone mineralization [15]. Calbindin is also an important target since it acts as a calcium binding protein in intestine and kidney, allowing calcium to cross the epithelia [16]. Expression of *cyp24a1* in the kidney is induced by VDR leading to a feedback loop of regulation [17] while *cyp27a1* expression in liver is regulated by other members of the nuclear receptors family such as the peroxisome proliferated activated receptors (PPAR) γ [18], the hepatocyte nuclear factor (HNF) 4α [19] and the liver X receptor (LXR) [20]. Oppositely, PPAR α is an inhibitor of *cyp27a1* transcription [21]. In addition, HNF1α, a non-nuclear receptor transcription factor, acts positively on *cyp27a1* transcription [22]. Today, it is assumed that the various actors involved in vitamin D metabolism are present in the brain leading to a locally biosynthetic and degradation pathway [23].

Recently, we have reported that acute contamination by high doses of DU modulates both mRNA levels and activities of CYPs enzymes involved in vitamin D metabolism [24]. We hypothesized that a long-term exposure with environmental doses of DU may induce alterations of vitamin D metabolism. The aim of this study was to investigate the biological effects of chronic exposure to DU on vitamin D metabolism using metabolic and physiological approaches. Vitamin D metabolism was investigated by the mRNA measurements of *cyp27a1*, *cyp2r1*, *cyp27b1* and *cyp24a1*, as well as those of the nuclear receptors regulating them, in liver and kidney of rat chronically exposed to DU. Physiological effects was explored by the analysis of the accumulation of VDR-target messengers encoding the epithelial Ca channel (*ecac1*) [25], the calbindin-D28K (*cabp-d28k*) [26], the Na^+/Ca^{2+} exchanger (*ncx1*), the Ca^{2+}-ATPase (*pmca1b*) [27] and the expression of the neurotrophin 3 (*nt-3*), a target gene in the brain [28]. Besides, levels of $25(OH)D_3$, $1,25(OH)_2D_3$, parathyroid hormone (PTH), calcium and phosphate as well as osteocalcin were measured in plasma of DU-exposed rats.

2. Materials and methods

2.1. Chemicals and materials

Depleted uranyl nitrate hexahydrate (DU) was obtained from V.W.R. (Fontenay-sous-Bois, France). Hydroxypropyl-β-cyclodextrin (HPβCD) was provided by Dr Michel Riottot (Université Paris-Sud, Orsay, France). [4-^{14}C] cholesterol was obtained from NEN Products (Les Ulis, France). Cholesterol and 27-hydroxycholesterol were obtained from Sigma Diagnostics (Isle d'Abeau Chesnes, France).

2.2. Animals

Twenty Sprague–Dawley male rats, 12 weeks old, weighing about 250 g and obtained from Charles River Laboratories (L'Arbresle, France) were used and divided into two groups of ten rats (control and exposed). The rats were housed in pairs, with a 12-h light/12-h dark cycle (light on: 08:00 h/20:00 h) and a temperature of 22 ± 1 °C. Water and standard rat pellets (R03 from SAFE, Augy, France) were delivered *ad libitum*. This diet contains 9000 mg/kg calcium, 6000 mg/kg phosphorus and 1500 IU/kg vitamin D_3. All experimental procedures were approved by the Animal Care Committee of the Institute of Radioprotection and Nuclear Safety and complied with French regulations for animal experimentation (Ministry of Agriculture Act No. 87-848, October 19, 1987, modified May 29, 2001).

2.3. Contamination procedures

The rats in the experimental group were exposed to DU in their drinking water for 9 months. DU contained 0.26% ^{235}U and was diluted in mineral water to obtain a dosage of 40 mg U l^{-1} (1 mg/rat/day). This dose was the double of highest concentration found naturally on Earth, in the drinking water of Finland [29]. The rats in control group drank uncontaminated mineral water. After 9 months of contamination, animals were anaesthetized by inhalation (TEM anaesthesia, France) of 95% air/5% isoflurane (Forène®, Abbott France, Rungis, France) and euthanized by intracardiac puncture with a 2-ml insulin syringe to collect blood. Organs were rapidly removed, chilled in ice-cold buffer for enzymatic analyses or put in liquid nitrogen for RNA analyses and stored at −80 °C. Accumulation of uranium in all tissues and urine was previously reported by Paquet et al. [30]. Note that the exposure to DU had no influence on food consumption, body weight or general health status of rats (data not shown).

2.4. Biochemical assays

Plasma calcium and inorganic phosphate (biological chemistry reagents, Thermo Electron Corporation, France) were measured on an automated Konelab 20 (Thermo Electron Corporation, France) system. Plasma $1,25(OH)_2D_3$ (active Vitamin D) and $25(OH)D_3$ (the major circulating metabolite) were assayed with a ^{125}I radioimmunoassay kit (IDS, Paris, France). Parathyroid hormone (PTH) and osteocalcin were determined in plasma using the rat PTH IRMA Kit (DSL, Cergy Pontoise, France) and the rat osteocalcin EIA Kit (IDS, Paris, France) respectively.

2.5. Determination of CYP27A1 activity in the liver

The preparation of liver mitochondria was carried out as described elsewhere [31]. Sterol 27-hydroxylase (CYP27A1) in the mitochondrial fractions was assayed with a radioisotopic method that used [4-^{14}C]Cholesterol, solubilized in hydroxypropyl-β-cyclodextrin, as previously reported [32].

2.6. Real-time PCR

Total RNA from the kidney, liver and brain (cortex) was isolated using RNeasy total RNA isolation Kit or RNeasy Lipid Tissue kit (Qiagen, Courtaboeuf, France) and reverse transcribed with random hexamers using Superscript II First Strand Synthesis System (Invitrogen, Cergy Pontoise,