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Effect of nephrotoxic treatment with gentamicin on rats chronically exposed to uranium

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ABSTRACT

Uranium is a radioactive heavy metal with a predominantly chemical toxicity, affecting especially the kidneys and more particularly the proximal tubular structure. Until now, few experimental studies have examined the effect of chronic low-dose exposure to uranium on kidney integrity: these mainly analyse standard markers such as creatinine and urea, and none has studied the effect of additional co-exposure to a nephrotoxic agent on rats chronically exposed to uranium. The aim of the present study is to examine the potential cumulative effect of treating uranium-exposed rats with a nephrotoxic drug. Neither physiological indicators (diuresis and creatinine clearance) nor standard plasma and urine markers (creatinine, urea and total protein) levels were deteriorated when uranium exposure was combined with gentamicin-induced nephrotoxicity. A histological study confirmed the preferential impact of gentamicin on the tubular structure and showed that uranium did not aggravate the histopathological renal lesions. Finally, the use of novel markers of kidney toxicity, such as KIM-1, osteopontin and kallikrein, provides new knowledge about the nephrotoxicity threshold of gentamicin, and allows us to conclude that under our experimental conditions, low dose uranium exposure did not induce signs of nephrotoxicity or enhance renal sensitivity to another nephrotoxicant.

1. Introduction

Due to its civilian and military applications as well as its natural presence in the environment, uranium (U) can be found throughout the environment at low concentration. Nephrotoxicity is a well-known consequence of uranium intoxication (Diamond et al., 1989; Haley, 1982; Leggett, 1989). Additional studies have shown that chronic uranium ingestion induces organic and functional renal disturbances in rats (Gilman et al., 1998; Gueguen et al., 2006; Ortega et al., 1989) and humans (Kurttio et al., 2002; Magdo et al., 2007; Zamora et al., 1998). The specificity of uranium nephrotoxicity is apparently related to its accumulation in the renal proximal tubules, where it causes various morphologic and functional alterations (Leggett, 1989). At the present time, no work has studied the cumulative renal toxicity of chronic exposure to a non-nephrotoxic dose of uranium and acute exposure to a nephrotoxic drug such as gentamicin.

Gentamicin is a well-known nephrotoxic agent. It is an aminoglycoside antibiotic used to treat life-threatening Gram-negative bacterial infections. Gentamicin is thought to be taken up into the proximal tubular cells of the nephron, internalised via endocytosis and transported into lysosomes. This process causes renal failure in 30% of treated patients (Mingeot-Leclercq and Tulkens, 1999). Uranium and gentamicin have roughly the same nephrotoxic effects and the study of their potential cumulative toxicity will improve our ability to estimate the potential risk of daily uranium exposure for human health.

The aim of this work was to study the cumulative toxicity of these two compounds on kidney integrity and function, especially in cortical tubular section.

Abbreviations: U, uranium; DU, depleted uranium; KIM-1, kidney injury molecule 1; ICP-MS, inductively coupled plasma mass spectrophotometer; Na, sodium; Ca, calcium; Cl, chloride; K, potassium; P, phosphorus; CYP, cytochrome P450; GST, glutathione S transferase; UGT, UDP-glucuronyl transferase; ST, sulfotransferases; HPRT, hypoxanthine-guanine phosphoribosyl-transferase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ANOVA, analysis of variance; NE, non-exposed; AU, arbitrary unit; IRSN, Institut de radioprotection et de sureté nucléaire; creat, creatinine.

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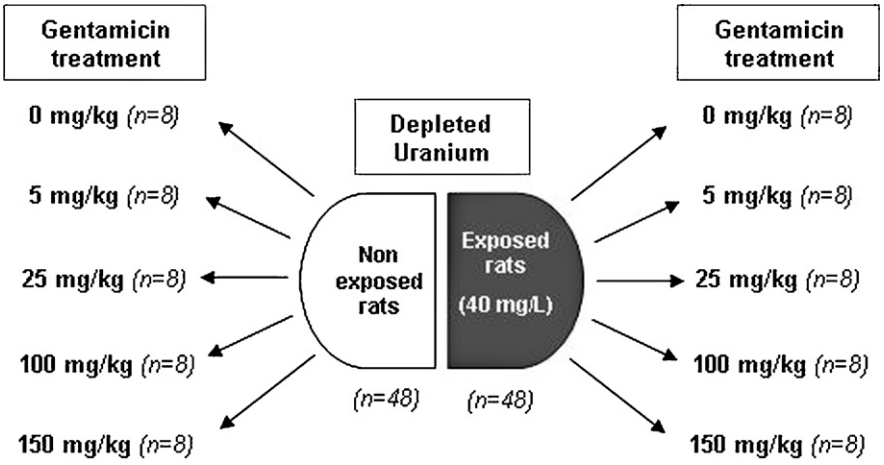

Fig. 1. Distribution of rats in gentamicin and DU groups.

Adult male rats were exposed for 9 months to a low dose (40 mg/L) of depleted uranium (DU) and were then treated with increasing doses of gentamicin (0, 5, 25,100 and 150 mg/kg).

Standard biomarkers (creatinine, urea, etc.) and histological analyses were used to assess renal alterations. The use of some novel especially sensitive and specific markers of tubular injuries, such as KIM-1, kallikrein and osteopontin, made it possible to distinguish the toxic effects of each agent. Moreover, the xenobiotic metabolizing enzymes were also studied. These enzymes are mainly localized in the renal cortex and have previously been shown to be a target of both DU (Moon et al., 2003; Pasanen et al., 1995; Souidi et al., 2005) and gentamicin (Ozaki, 2009). This work brings new information about cortical functional alterations after co-exposition to uranium and gentamicin.

2. Materials and methods

2.1. Animals

Experiments were performed with male Sprague–Dawley rats (250 g) obtained from Charles River Laboratories (L'Arbresle, France). Animals were housed at constant room temperature (21 °C ± 1) with a 12 h:12 h light–dark cycle. The Animal Care Committee of the Institute approved the experiments, which were conducted in accordance with French regulations for animal experimentation (Ministry of Agriculture Act No. 2001-464, May 2001).

2.2. Experimental design

Fig. 1 represents the animal distribution in DU and gentamicin groups.

2.2.1. Animal DU exposure

The rats in the contaminated group ($n = 48$) were exposed to uranyl nitrate (U238: 99.74%, U235: 0.26%, U234: 0.001%) via drinking water for 9 months, at a dose of 40 mg/L (about 1 mg/rat/day) (AREVA-NC, France). This dose corresponds to twice the highest uranium concentration found naturally in well-water in Finland (Salonen, 1994). Animals were carefully monitored once a week (body weight, food and water intake) throughout the DU exposure period and until the end of the experiment. Exposure to DU did not influence the rats' food consumption, body weight or general health status (data not shown). Control animals ($n = 48$) had normal drinking water. DU kidney concentration was quantified by ICP-MS (inductively coupled plasma mass spectrophotometer).

2.2.2. Animal gentamicin treatment

During the last week of uranium exposure, the rats in both groups received gentamicin treatment. The gentamicin sulfate (Sigma, G3632, Saint Quentin Fallavier, France) was administrated by subcutaneous injection for 4 consecutive days (0, 5, 25, 100 and 150 mg gentamicin per kg of body weight) in NaCl 0.9% using an injection volume of 1 mL/kg ($n = 8$ for each condition). During gentamicin treatment, rats were weighed daily.

2.2.3. Urine, plasma, serum and tissue collection

Urine was collected and quantified for 24 h immediately after the fourth injection of gentamicin. To allow the animals to adapt to the change of cage, they were placed in standard metabolic cages 24 h before urine collection. Urine was centrifuged at 3000 *g* for 10 min (4 °C), and supernatants were collected and stored at −80 °C.

After the 24-h period of urine collection following the fourth injection, rats were euthanised by terminal exsanguination (intracardiac puncture) under isofluorane anaesthesia. For each rat, both kidneys were collected and weighed. Half of each kidney was placed in formaldehyde 4% for histological analysis. The cortex and medulla sections of the other half were meticulously separated, flash-frozen in liquid nitrogen, and stored at −80 °C. The blood was centrifuged at 4000 *g* for 10 min (4 °C) to prepare plasma and serum, which were then stored at −80 °C.

2.3. Plasma and urine biochemical indicators

We used an automated Konelab 20 to measure gentamicin, creatinine, urea, calcium (Ca), chloride (Cl), potassium (K) and sodium (Na) in plasma and gentamicin, creatinine, urea, total proteins, uric acid, Ca, K, phosphorus (P) and Na in urine (biological chemistry reagents, Thermo Electron Corporation, Villebon sur Yvette, France).

2.4. Histopathology

The preserved kidney sections were cut with a microtome, stained with hematoxylin and eosin, and examined by light microscope. Damage was assessed by an expert pathology laboratory (Biodoxis, Romainville, France) according to standard criteria. The glomerular damage was estimated according to mesangial proliferation, glomerulosclerosis and glomerular cystic dilatation. The tubulointerstitial damage was estimated based on necrosis, atrophy and dilatation of the tubules and interstitial inflammation and fibrosis.

2.5. Real-time RT-PCR

Total RNA from the renal cortex was extracted with the RNeasy Total RNA Isolation Kit (Qiagen, Courtaboeuf, France) and reverse-transcribed with random hexamers, with the high-capacity cDNA reverse transcription kit (Applied Biosystems, Courtaboeuf, France). Real-time PCR was used to analyse the mRNA level of nephrotoxicity biomarkers (KIM-1, osteopontin and kallikrein) and major xenobiotic metabolising enzymes—cytochromes P450 (CYP) 3A1, 3A2 and 1A1, glutathione-S-transferase A2 (GSTA2), UDP-glucuronyl transferase 1A1 and 2B1 (UGT1A1 and UGT2B1) and sulfotransferase 1A1 (ST1A1). An AbiPrism 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France) was used with 4 ng of cDNA for each reaction. A mix of primers (Invitrogen, Cergy Pontoise, France) (2.5%, v/v), SYBR (Applied Biosystems, Courtaboeuf, France) (83%, v/v) and sterile water (14.5%, v/v) was added to each well to yield a final volume of 10 μl. Samples were normalised to hypoxanthine-guanine phosphoribosyl-transferase (HPRT) and fold induction calculated relative to the control (untreated and unexposed group). Sequences of forward and reverse primers are listed in Table 1 (Amin et al., 2004; Gueguen et al., 2007; Rekka et al., 2002; Ropenga et al., 2004; Rouas et al., 2009; Su and Waxman, 2004; Hoen et al., 2002).

2.6. Western blot

Proteins from renal cortex homogenate were loaded, separated by 10% SDS polyacrylamide gel electrophoresis, and transferred onto nitrocellulose membranes. The membranes were blocked for 1 h in 5% non-fat dry milk in TBS. The blots were incubated overnight with antibodies diluted in 2% non-fat dry milk in TBS at 4 °C. UGT1A1 (NCBI RefSeq NP_036815) and ST1A (NCBI RefSeq NP_114022) proteins were