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Chronic uranium contamination alters spinal motor neuron integrity *via* modulation of SMN1 expression and microglia recruitment

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HIGHLIGHTS

- Uranium contamination induces a decrease of motor neuron number in rat spinal cord.
- Uranium contamination induces a decrease of expression of the survival protein SMN1.
- Uranium contamination increases the number of microglial cells in rat spinal cord.
- Results suggest potential overexpression of MCP-1 by motor neurons themselves.
- Results suggest the trigger of a neuroinflammation process.

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ABSTRACT

Consequences of uranium contamination have been extensively studied in brain as cognitive function impairments were observed in rodents. Locomotor disturbances have also been described in contaminated animals. Epidemiological studies have revealed increased risk of motor neuron diseases in veterans potentially exposed to uranium during their military duties. To our knowledge, biological response of spinal cord to uranium contamination has not been studied even though it has a crucial role in locomotion. Four groups of rats were contaminated with increasing concentrations of uranium in their drinking water compared to a control group to study cellular mechanisms involved in locomotor disorders. Nissl staining of spinal cord sections revealed the presence of chromatolytic neurons in the ventral horn. This observation was correlated with a decreased number of motor neurons in the highly contaminated group and a decrease of SMN1 protein expression (Survival of Motor Neuron 1). While contamination impairs motor neuron integrity, an increasing number of microglial cells indicates the trigger of a neuroinflammation process. Potential overexpression of a microglial recruitment chemokine, MCP-1 (Monocyte Chimioattractant Protein 1), by motor neurons themselves could mediate this process. Studies on spinal cord appear to be relevant for risk assessment of population exposed via contaminated food and water.

1. Introduction

Natural uranium is a radioactive heavy metal ubiquitously present in the environment. Human activities can also be at the origin of uranium dispersal as it is widely used in the nuclear industry or for military purposes. As a consequence, potential chronic exposition of general population *via* contaminated food or drinking water raises some issues in terms of risks assessment on human health (ATSDR, 2013).

In humans, evidences of behavioural consequences potentially linked to uranium exposure do exist. Gulf War veterans were potentially exposed to depleted uranium *via* dust inhalation and embedded shrapnel fragments. First epidemiological studies revealed alteration of cognitive performances as well as disturbances of some sensorimotor functions associated with higher uranium concentration in urine in comparison to a control cohort

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(McDiarmid et al., 2000). However, twenty years after exposure, neurological signs were transitory in the same cohort and even if uranium concentrations in urine remains high, they were not linked with deleterious effects in target organs (McDiarmid et al., 2015). Nevertheless, studies on other cohorts of Gulf War veterans revealed increased risks of neurodegenerative diseases such as Alzheimer disease affecting brain (Veitch et al., 2013) or Amyotrophic Lateral Sclerosis (ALS) affecting locomotor functions (Horner et al., 2003). Interestingly, concentrations of several metals have been measured in ALS patient cerebrospinal fluid. Uranium concentrations were increased in comparison to a control population but also in comparison to the other metals in the ALS cohort (Roos et al., 2013). All together, these observations might reveal a potential link between uranium exposure and the development of neurodegenerative processes.

In the literature, experimental evidences of uranium induced cognitive deficits are well documented. In particular, spatial memory impairments have been demonstrated in rats chronically exposed to uranium contaminated drinking water (Houpert et al., 2007; Houpert et al., 2005). More recently, ApoE−/− mice, currently used as a model of Alzheimer disease, have been shown to exhibit an acceleration of cognitive loss (Lestaevel et al., 2013). To explain the origin of these deficits, modulation of anti-oxidant enzyme expression has been studied as they appeared to be potential targets of uranium. An increase in gene expression of superoxide dismutase (SOD) and catalase (CAT) has been demonstrated in response to uranium contamination in rat brain (Lestaevel et al., 2009; Linares et al., 2007). Metabolism and levels of some neurotransmitters have also been investigated. In particular, the dopaminergic and cholinergic systems have shown modulation after uranium chronic contamination (Bensoussan et al., 2009; Bussy et al., 2006).

In parallel to cognitive function studies, other behavioural analyses linked to central nervous system functions have been performed. A decreased locomotor activity has been shown in 3 week old pups after contamination of their mothers 2 weeks prior mating and carried on until pup weaning (Briner and Abboud, 2002). In adult or juvenile rodents, locomotor dysfunctions such as sensorimotor coordination impairments or significant modification of locomotor activity have been observed in uranium contaminated animals (Abou-Donia et al., 2002; Briner and Murray, 2005; Lestaevel et al., 2015). Modulations of the cholinergic system, nitric oxide production, and cerebral lipid peroxidation have been linked to these behavioural impairments.

The interesting point about most of the mechanistic studies performed to explain the locomotor effects observed in experimental models is that they have focused on brain function. To our knowledge, spinal cord and medullar cells, even though involved in conveying motor and sensory information between brain and the peripheral nervous system, have not been studied.

In the light of these observations, we designed a study to contaminate adult rats with increasing concentrations of natural uranium (NU) *via* the drinking water for 9 months. The consequences of uranium contamination on spinal cord global morphology and on neurons in the ventral horn were analysed. We also focused on the cellular integrity of a specific neuronal population using specific immunohistochemical markers: the motor neurons of the ventral horn. Pathological characteristics of neurodegenerative diseases affecting motor neurons were used to identify potential targets of uranium contamination. We indeed investigated the implication of the redox balance and, in particular, gene expression of Super Oxide Dismutase 1 (SOD1) known to be involved in ALS onset (Chen et al., 2013) and the expression of a protein called Survival of Motor Neuron 1 protein (SMN1) whose expression is known to be affected in Spinal Muscular Atrophy (SMA) disease (Crawford and Pardo, 1996).

At last, we also studied the implication of the neuroinflammation process which plays a critical role when it is about central nervous system response to a toxic stress. We studied gene expression of a selection of cytokines and chemokines and used specific markers of microglial cells.

2. Materials and methods

2.1. Animals and contamination protocol

Fifty Sprague-Dawley male rats (Charles River, France) were housed in pairs under standard conditions (12-h light/12-h dark cycle, light on from 8 a.m to 8 p.m) at a constant room temperature (21 ± 1 °C). Animals were contaminated *via* the drinking water with increasing concentrations of NU for 9 months (chemical form: uranyl nitrate, $UO_2(NO_3)_2$, $6H_2O$; isotopic composition: ^{238}U: 99%, ^{235}U: 0.76%, ^{234}U: 0.051%, specific activity: 25000 Bq/g, AREVA-NC, France) at four concentrations: 1, 40, 120 or 600 mg/L as described previously (Poisson et al., 2014) (respective uranium activities are 25, 1000, 3000 and 15000 Bq/L). Control animals received non contaminated drinking water (Evian®). Concentrations were chosen from previous studies performed in the laboratory (Lestaevel et al., 2009, 2015). These doses are approximately equal to 1/4000, 1/100, 1/33 and 1/7 of acute oral LD50 of U in adult rats (Domingo et al., 1987). Animal food intake, drinking consumption and body weight were measured weekly. Thirty animals were used for uranium concentration analysis by ICP-MS and qPCR analysis (n = 6 per group) and twenty animals were used for immunohistochemistry experiments (n = 4 per group). All animal procedures were approved by the IRSN Animal Care Committee and in accordance with French (Ministry of Agriculture Act No. 2011-110, June 2011) and European (Act No. 87-848) regulations for animal experimentation.

2.2. Sample collection

After 9 month of contamination, rats were anesthetised by inhalation of 95% air/5% isoflurane and euthanised by intracardiac puncture. The cervico-thoracic spinal cord was collected and weighed for each animal. The first half of spinal cord was used for uranium quantification by ICP-MS and stored at −20 °C and the second half was put in cryotubes for reverse transcription and qPCR experiments, flash-frozen in liquid nitrogen and stored at −80 °C. Each half of spinal cord was weighed. Rats used for immunohistochemistry experiments were perfused transcardially with 500 mL of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (with pH 7.4). Spinal cord was dissected out and post-fixed for 1 h in 4% PFA at room temperature and left overnight in 30% sucrose solution at 4 °C. Spinal cord tissue was then embedded in Tissue-Tek® O.C.T™ compound, kept in liquid nitrogen/isopentane and conserved at −80 °C until experiments.

2.3. Nissl staining

Spinal cord frozen sections were cut on a cryostat (14 μm thick), placed on superfrost slides (Dutscher, France) and kept at −80 °C. Sections were hydrated with decreasing concentrations of alcohols for 2 min (95–80–70%) and stained with cresyl violet solution for 45 s. Tissues are then differentiated with acetic formalin solution for 2 min. Spinal cord sections were then dehydrated with increasing concentrations of alcohols for 2 min (80–95–100%). Finally, the sections are fixed in xylene with two successive baths for 2 min and were coverslipped with Permount (Fishier Scientific, New Jersey).

The chromatolytic and the healthy neurons were quantified blinded in the ventral horn of the spinal cord. The percentage of