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Cellular distribution of uranium after acute exposure of renal epithelial cells: SEM, TEM and nuclear microscopy analysis

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Abstract

The major health effect of uranium exposure has been reported to be chemical kidney toxicity, functional and histological damages being mainly observed in proximal tubule cells. Uranium enters the proximal tubule as uranyl-bicarbonate or uranyl-citrate complexes. The aim of our research is to investigate the mechanisms of uranium toxicity, intracellular accumulation and repartition after acute intoxication of rat renal proximal tubule epithelial cells, as a function of its chemical form.

Microscopic observations of renal epithelial cells after acute exposure to uranyl-bicarbonate showing the presence of intracellular precipitates as thin needles of uranyl-phosphate localized in cell lysosomes have been published. However the initial site of precipitates formation has not been identified yet: they could either be formed outside the cells before internalization, or directly inside the cells.

Uranium solubility as a function and initial concentration was specified by ICP-MS analysis of culture media. In parallel, uranium uptake and distribution in cell monolayers exposed to U-bicarbonate was investigated by nuclear microprobe analyses. Finally, the presence of uranium precipitates was tested out by scanning electron microscopic observations (SEM), while extracellular and/or intracellular precipitates were observed on thin sections of cells by transmission electron microscopy (TEM).

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1. Introduction

Uranium is a naturally occurring element, whose use in the nuclear cycle has raised concerns regarding the risk associated to a potential contamination. Its health effects have been largely studied in vivo [1–4]. After an internal exposure, uranium rapidly transits by the blood to target organs, namely bones and kidneys and 80% of the initial dose is excreted in the urine during the first 24 h after contamination. It is particularly toxic to the epithelial cells of renal proximal tubules [4]. The only uranium chemical forms that reach these tubules in vivo are uranyl-citrate and -bicarbonate complexes, which then dissociate as a consequence of pH drop in this tubule portion. Uranium then interacts with other complexing agents, including cell membrane components, and cell death occurs [4,5]. The real site of cell association and the exact uranium chemical form able to interact with cell membranes and then be internalized in cells are not known.

In vitro, uranium is toxic when prepared by dissolution of uranyl crystals in a concentrated bicarbonate solution [6] or a concentrated citrate solution [7]. These uranyl solutions, when diluted in cell culture medium, lead to multiple uranium complexes, whose more toxic ones are assumed to be $UO_2(CO_3)_3^{4-}$ and $UO_2CIT_2^{4-}$ [7]. It is not known whether these complexes are directly the toxic ones or if they are the more efficiently dissociated to permit uranium association with cell membranes.

Microanalytic studies show that uranium enters renal cells to a significant extent both in vivo and in vitro [8–12]. It is supposed to be internalized in cells as a soluble form and precipitate intracellularly with cell-incoming phosphates. The precipitates are then excluded from the cell, leading to intracellular and extracellular uranium precipitates [6,8].

Association of microanalytic and biochemistry methods allowed us to correlate submicronic observation of uranium cellular accumulation (nuclear microprobe, transmission electron microscopy), chemical analysis of the elements co-precipitating with uranium (scanning electron microscopy), precise and localized uranium quantification (nuclear microprobe) and determination of uranium toxicity (MTT assay).

2. Materials and methods

2.1. Cell culture and uranium exposure

NRK-52^E cell line was used (American Type Culture Collection, CRL-1571), a rat proximal tubule model [13]. Cells were grown in DMEM (Sigma) supplemented with 2 mM L-glutamine, penicillin/streptomycin (50 U/ml and 50 μg/ml, respectively) and 10% (v/v) fetal bovine serum, at 37 °C in a 5% CO_2/air incubator. Uranyl stock solutions (10 mM) were prepared by dissolution of uranyl nitrate (Labosi) in 100 mM $NaHCO_3$, stirred for 30 min and filtered through 0.22 μm filter units after pH adjustment to 7.2. This stock solution was diluted in MEM (Sigma). Uranium toxicity was evaluated using the MTT assay [14].

2.2. Uranium solubility evaluation

For in vitro solubility experiment, uranium solutions were placed at 37 °C, 5% CO_2/air. After 24 h, solutions were filtered through 0.45 μm filter units. Samples were acidified with 65% nitric acid (NORMATOM quality grade, VWR Prolabo), diluted in ultra-pure water and uranium concentration was measured by inductively coupled plasma-mass spectroscopy (ICP-MS) using a quadrupole apparatus (Thermo Electron Corporation).

2.3. Microscopy

For transmission electron microscopy (TEM), NRK-52^E were exposed 24 h to uranyl-bicarbonate and then rinsed with 10 mM $NaHCO_3$. Cells were fixed as described elsewhere [15], pre-embedded in 2% agar and embedded in epon after dehydration in graded concentrations of ethanol. Thin sections were cut, counterstained with Reynold's lead citrate and observed with a CM 12 Philips electron microscope at 80 kV.

For scanning electron microscopy (SEM), semi-permeable transwell-clear membranes were exposed to uranyl solutions both on the apical and