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The ex-vivo intestinal absorption rate of uranium is a two-phase function of supply

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ABSTRACT

The concentration-dependent absorption behaviour of uranium was investigated with surviving intestinal segments of rat jejunums, using an ex-vivo model. The results showed a monotonic slightly nonlinear increase in absorption as uranium concentrations increased. This trend was observed over the entire concentration range tested. In the lower concentration range a slower linear ascent was observed while a steeper linear ascent was found for the higher concentration range. Statistical fit was only slightly poorer for an exponential function in the range of lower values and a logarithmic function in the range of higher values. The proportion of uranium absorbed expressed as percent of uranium concentrations in the perfusion solutions followed a monotonically increasing trend from 20 to around 200 μg/l uranium in the perfusion solutions, which thereafter appears to reach a plateau, as further increase towards concentrations around 400 μg/l is not substantial. The uranium concentration administered had no effect on the vitality and consequently the functionality of the intestinal segments, measured in terms of active glucose transport. The results imply that uranium concentrations of more than 20 μg/l in drinking water, for example, could lead to elevated absorption rates and thus to higher internal exposures to consider when setting of Guideline values in this concentration range.

1. Introduction

Uranium is a natural radioactive element which can be present in the ground water in concentrations of a few hundred μg/l, depending on the geological conditions. In the lower concentration range, health effects are expected from its chemical toxicity as a heavy metal rather than from its radioactivity (Tasat et al., 2012). The chemical toxicity of uranium depends on the chemical species as which it occurs, particularly on its water solubility (Harrison and Stather, 1981). Uptake can be through the lung, the skin or the gastrointestinal tract and results of a study with volunteers in Canada (Zamora et al., 2002) show the relevance of uptake through drinking-water. As highlighted in the World Health Organisation's (WHO) Guidelines for Drinking-water Quality, "in circumstances in which uranium is present in a drinking-water source, the majority of intake can be through drinking-water" (WHO, 2011, p. 3). While concentrations are low in many areas, more than 20 μg/l have particularly been reported from a range of small drinking-water supplies in a number of regions of the world, and the WHO is including uranium in its plan of work of the rolling revision of the WHO Guidelines for Drinking-water Quality (WHO, 2012). Germany introduced a limit of 10 μg/l in its Drinking Water Ordinance in 2011 (TrinkwV, 2011).

Uranium mainly damages the kidneys, especially the glomeruli and proximal tubuli. It causes histological changes of the epithelial cells in the lower segment of the proximal tubuli, which functionally lead to increased excretion of glucose, amino acids and proteins (e.g., β-micro globulin). Glomerulus damage can lead to a decrease of the glomerular filtration capacity, recognizable through changes in clearance rates and through proteinuria (EFSA, 2009; WHO, 2012). Furthermore moderate effects on liver function were demonstrated experimentally (Gueguen et al., 2006), and Dublineau et al. (2006) report indication for altered intestinal immunological reactions after repeated exposure.

Data on the various absorption sites along the gastrointenstinal tract as well as for cellular pathways (para- or trans-cellular) and the transport systems involved in uranium absorption are limited (Dublineau et al., 2005). Experimental results obtained with rats indicate the trans-cellular pathway to be the primary one for gastrointestinal absorption of dissolved uranium compounds in the small intestine (Dublineau et al., 2006). As in-vivo experiments are under ethical debate, cost-intensive and the information they provide is limited, it is essential to include ex-vivo and in-vitro

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methods to clarify specific questions on e.g. absorption concentration relations. Ex-vivo investigations of the intestinal lining take the interaction between natural cell populations into account and can thus provide insights in addition to those gleaned from in-vitro investigations using intestinal cell cultures. Data available to date on dose-dependent uranium (U) absorption are inconsistent (Konietzka, submitted for publication). The collation of percentage absorption levels for various species published by Leggett and Harrison (1995) shows no increase with increasing amounts of soluble uranium compounds administered. However, among the reports used by these authors, a trend is only clear in the data from La Touche et al. (1987), and the increase of absorption rates they observed following a hundredfold increase in the dose administered to rats was only barely fourfold. This may be due to exhaustion of the capacity of active transport systems (as known for other divalent metals, Illing et al. (2012) and which are likely to be relevant for uranium as well), eg. the exhaustion of transport proteins or their expression.

The question therefore arises as to whether uranium that occurs naturally in the environment, for example in ground, drinking and mineral water, follows the same absorption pattern at higher concentrations, or whether there is no need to regulate higher environmentally-relevant concentrations as they do not lead to higher intake. The results presented here targeted experimental clarification of this issue by examining the absorption of uranium from a range of uranium concentrations using an ex-vivo model consisting of isolated surviving jejunal segments of rats, as part of the small intestine where the uranium adsorption takes place (Dublineau et al., 2005).

2. Material and methods

2.1. Experimental animals

The tests, which were notified to Chemnitz Regional Council in accordance with animal protection legislation, were conducted on male Sprague–Dawley rats (supplier: Charles River, Sulzfeld) with 220–250 g body weight. The animals were kept in a filter cabinet (supplier: Ehret, Emmendingen), five animals per type IV macrolon cage, in a dark/light cycle of 12 h darkness alternating with 12 h light, and at a temperature of 24 ± 2 °C. Unlimited drinking water and feed (supplier: Altromin, Lage) was available. After a one-week familiarisation phase, feed was withdrawn from the animals 12 h before intestinal preparation, but they continued to have unlimited access to drinking water.

2.2. Preparation of the surviving jejunal segments

The rats were killed by cervical dislocation while under ether anaesthesia. The abdominal cavity was opened along the median line, the upper small intestine segment was released and severed behind the Flexura duodenojejunalis. A glass capillary tube was inserted into the intestinal lumen and made liquid-tight. An intestinal segment approximately 10 cm long lying in the caudal direction was severed and flushed through via the capillary tube in place with an electrolyte solution (Tyrode's solution, pH 7.2) at a temperature of 37 °C that had been enriched with oxygen by means of carbogen gasification (95% O_2, 5% CO_2). Another glass capillary tube was then attached at the other end of the segment. Finally the blood vessels of the intestine were severed, and the segment was placed in a vessel and flushed internally and externally with oxygen-enriched Tyrode's solution at a temperature of 37 °C before being transferred to the perfusion equipment.

2.3. Perfusion equipment

The intestinal segments were placed in perfusion equipment constructed according to the design devised by Fisher and Parson (1949), with the modifications introduced by Schümann et al. (1986). The equipment (Fig. 1) consisted of two double-walled sealable glass vessels placed one on top of the other, with water at 37 °C flowing through the space between them, as described by Richter and Strugala (1985). The upper glass vessel, which acted as the reservoir for the perfusion solution, ran into a small glass tube at its bottom end leading into the lower vessel via a Teflon tube fitted with a three-way valve. It was connected to one end of the prepared intestinal segment at this point, under liquid-tight conditions. The other end of the intestinal segment was connected with the small glass tube used to supply carbogen, also under liquid-tight conditions. As a result, the intestinal segment hung free in the space of the lower glass vessel, which was kept moist by means of blotting paper soaked in Tyrode's solution placed on

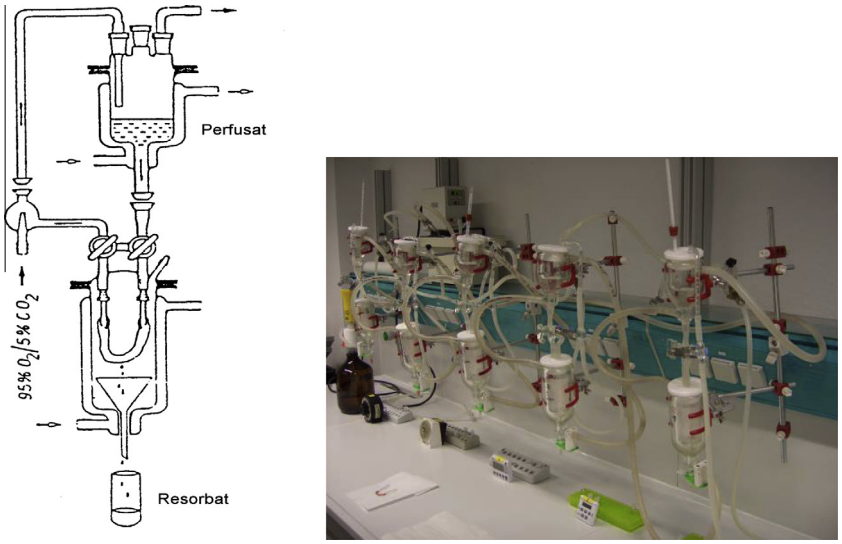

Fig. 1. Ex-vivo perfusion equipment constructed according to the design devised by Fisher and Parson (1949), modified from Schümann et al. (1986), (left, Perfusat = perfusate, Resorbat = absorbate; Fig. from Richter and Strugala, 1985) and the laboratory set-up of five sets of perfusion equipment forming an ex-vivo perfusion battery (right).