

## STUDY OF URANIUM TRANSFER ACROSS THE BLOOD-BRAIN BARRIER

V. Lemerçier†, X. Millot‡, E. Ansoborlo§, F. Ménétrier||, A. Flury-Hérard||,

Ch. Rousselle† and J. M. Schermann†

†INSERM-U26, Hôpital Fernand Widal  
75475 Paris Cedex 10, France

‡CEA/DSPG/LABM, BP 12

91680 Bruyères-le-Châtel, France

§CEA/DEN/DRCP/CETAMA, BP 17171

30200 Bagnols-sur-Cèze, France

||CEA/DSV/CARMIN, BP 6

92260 Fontenay-aux-Roses, France

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**Abstract** — Uranium is a heavy metal which, following accidental exposure, may potentially be deposited in human tissues and target organs, the kidneys and bones. A few published studies have described the distribution of this element after chronic exposure and one of them has demonstrated an accumulation in the brain. In the present study, using inductively coupled plasma mass spectrometry (ICP-MS) for the quantification of uranium, uranium transfer across the blood-brain barrier (BBB) has been assessed using the *in situ* brain perfusion technique in the rat. For this purpose, a physiological buffered bicarbonate saline at pH 7.4 containing natural uranium at a given concentration was perfused. After checking the integrity of the BBB during the perfusion, the background measurement of uranium in control rats without uranium in the perfusate was determined. The quantity of uranium in the exposed rat hemisphere, which appeared to be significantly higher than that in the control rats, was measured. Finally, the possible transfer of the perfused uranium not only in the vascular space but also in the brain parenchyma is discussed.

### INTRODUCTION

There is an extensive literature available on the toxicity of uranium in animal experiments and human studies after inhalation or ingestion<sup>(1,2)</sup>. Numerous studies have described the biokinetics of this element, and a number of biokinetic models exist that describe and model its distribution, retention and excretion<sup>(3-5)</sup>. Bone is the principal reservoir and kidney the target organ<sup>(6)</sup>. The chemical form of administered uranium and the transport of uranium in different biological media bonded to biological ligands are generally not completely characterised. Nevertheless it is generally accepted that uranium is present in body fluids in the form of the uranyl cation ( $UO_2^{2+}$ ) and associated with ligands such as carbonate, citrate or proteins<sup>(7)</sup>. Whilst these models describe the distribution of uranium amongst major organs, they have not addressed recent data relating to the distribution of uranium in the brain or testes.

Only a few studies<sup>(8,9)</sup> have shown a significant distribution of uranium in the brain after chronic exposure and some neurological effects<sup>(11,10)</sup>. Numerous studies on lead, which is an analogous heavy metal in  $Pb^{2+}$  cation form, have been performed<sup>(11)</sup> and have shown that lead can exert neurotoxic effects by altering certain membrane-bound enzymes. Studies conducted by Pellmar *et al.*<sup>(8)</sup> on rats implanted surgically with depleted uranium

metal pellets have shown that uranium can accumulate within the central nervous system.<sup>(1)</sup>

The aim of this study is to investigate the ability of uranium to cross the blood-brain barrier (BBB) and reach brain parenchyma. For this purpose, the *in situ* brain perfusion technique was used in the rat: this sensitive method has been developed in order to study the cerebrovascular transfer of solutes across the BBB<sup>(12)</sup>. This method allows absolute control of the perfusate composition and its infusion into the cerebral microvasculature over a short time.

The transfer of uranium across the BBB has been assessed for concentrations between the detection limit of the analytical method and a dose close to the LD50 in the rat. The assessment of BBB integrity was performed by co-perfusion with <sup>14</sup>C-sucrose which is a vascular volume marker.

### MATERIAL AND METHODS

#### Animals

Male Sprague-Dawley rats (200–250 g; 8 weeks of age) were obtained from Iffa-Credo (L'Arbresle, France). Animals were maintained under standard conditions of temperature and lighting with an *ad libitum* access to food and water. Rats were anaesthetised with an intraperitoneal injection of combined ketamine hydrochloride (Parke-Davis, Courbevoie, France; 50 mg ml<sup>-1</sup>, 70 mg kg<sup>-1</sup>) and diazepam (Roche, Neuilly-sur-Seine, France; 5 mg ml<sup>-1</sup>, 7 mg kg<sup>-1</sup>). Ethical rules of the French Ministry of Agriculture for experimentation

Contact author E-mail: valerie.lmercier@voila.fr

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28 NaCl, 24 NaHCO<sub>3</sub>, Cl<sub>2</sub>, 0.9 MgSO<sub>4</sub>) and 9 usior : solution was CO<sub>2</sub> ... pH control (= r ...<sup>-1</sup>) was from Per- r ... uranium solution l<sup>-1</sup> (4.2 × 10<sup>-3</sup> M) was de (U<sub>3</sub>O<sub>8</sub>) suspended in ion were directly added l in order to have a final : 10<sup>-6</sup> M. Under these tially the uranyl tricar- according to the predic- s software) which does e.

ned at 450°C then min- Uranium mass was mea- plasma mass spec- sient <sup>238</sup>U mass was arded by subtracting the : reaction mixture. The the cerebral hemisphere et weight.

in value of total uranium istical comparisons con- t-test or ANOVA. Stat- at th ... 0.05 signifi- the mean value ± one

for uranium exposure

M to evaluate the permeability of uranium across the BBB.

As a preliminary experiment, the background amount of uranium was determined after perfusion of buffer only. In the control rats, uranium was measured at about 40 ± 20 pg per g of wet weight cerebral hemisphere. When uranium was perfused at 5 × 10<sup>-6</sup> M (10 ml min<sup>-1</sup> perfusion rate) for 2 min, the amount of uranium in brain tissue was 52 ± 20 ng g<sup>-1</sup>.

## DISCUSSION

The *in situ* brain perfusion method described by Takasato *et al*<sup>(12)</sup> is the most sensitive method for the evaluation of BBB kinetic transport. For the first time, this technique has been used to study the transfer of uranium across the BBB to the brain. This method enables the BBB to be exposed for a short time to a drug under infusion conditions. In this case, the fluid composition and infusion rate are strictly controlled. On the other hand, the ICP-MS technique has been validated for the quantification of uranium in the brain: this analytical method is sensitive enough for the determination of background uranium levels in the brain samples.

In the rats exposed to uranium, the non-alteration of the BBB has been demonstrated. The measurement of

uranium in the control rats showed that their brains spontaneously contained traces of this element, probably initially present in water and food. Pellmar *et al*<sup>(8)</sup> demonstrated an accumulation of uranium in different areas of the brain after surgical implantation of depleted uranium pellets within muscle: this accumulation was described as a dose-response relationship.<sup>3</sup> This observation was significant from 1 to 18 months.

In our study, the concentration of this toxic metal significantly increased in the exposed group after one perfusion. However, the concentration of uranium measured was 52 ± 20 ng g<sup>-1</sup> corresponding to total uranium present both in the vascular volume and extravascular brain parenchyma. By subtraction of the estimated quantity of uranium present in the vascular space (17 ± 3 μl g<sup>-1</sup>), corresponding to 20 ng, it has been possible to estimate the quantity in the vessels and/or nervous parenchyma at about 32 ng g<sup>-1</sup>.

## CONCLUSION

Our study reveals that a significant amount of uranium in the form of uranyl tricarbonate is measured in the brains of exposed rats. Following this primary investigation, other studies should be made to determine the exact location of uranium in the different cellular components of the BBB and to elucidate potential mechanisms of transfer.

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