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STUDY OF URANIUM TRANSFER ACROSS THE BLOOD–BRAIN BARRIER

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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[1,2]. 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[3–5]. Bone is the principal reservoir and kidney the target organ[6]. 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[7]. 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[8,9] have shown a significant distribution of uranium in the brain after chronic exposure and some neurological effects[1,10]. Numerous studies on lead, which is an analogous heavy metal in Pb^{2+} cation form, have been performed[11] and have shown that lead can exert neurotoxic effects by altering certain membrane-bound enzymes. Studies conducted by Pellmar *et al*[8] on rats implanted surgically with depleted uranium metal pellets have shown that uranium can accumulate within the central nervous system.

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[12]. 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 ^{14}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^{-1}, 70 mg kg^{-1}) and diazepam (Roche, Neuilly-sur-Seine, France; 5 mg ml^{-1}, 7 mg kg^{-1}). Ethical rules of the French Ministry of Agriculture for experimentation

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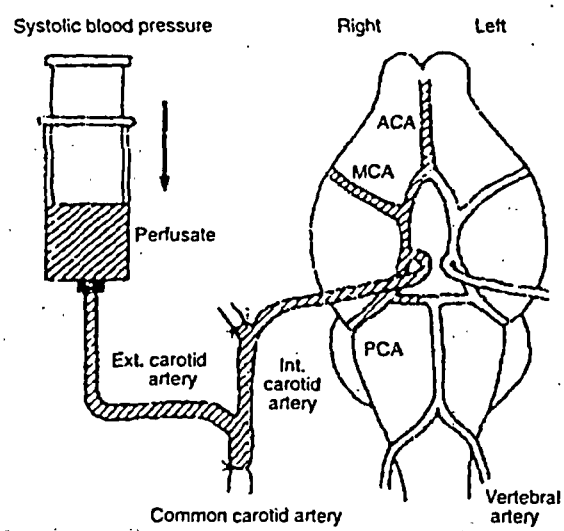
with laboratory animals (law no 87-848) have been followed.

In situ brain perfusion

The *in situ* brain perfusion technique described in previous studies[12-14] was used. Briefly, the right common carotid artery (CCA) of anaesthetised rats was exposed and ligated at the heart side. The external carotid artery (ECA) was ligated where the internal carotid (ICA) bifurcates before the occipital artery (Figure 1). The common carotid was then catheterised with polyethylene tubing (Biotrol Diagnostic, Chennevières-les-Louvre, France) filled with 0.9% sodium chloride and adapted on a 23-gauge needle. The syringe containing the perfusion fluid was placed in an infusion pump (Harvard pump PHD 2000; Harvard Apparatus, Holliston, MA) and connected to the catheter. Immediately before the perfusion, the heart was stopped by cutting the ventricles to eliminate the contribution of contralateral blood flow. Brains were then perfused for 2 min at a flow rate of 10 ml min^{-1} which is adequate to perfuse the ipsilateral hemisphere at a reasonable pressure. In the control group (n = 5-6), the buffered solution without added uranium was perfused for measuring the background level of this metal in the brain. In the other groups (n = 5-8), uranium with or without ^{14}C-sucrose was perfused. At the end of the perfusion, the rat was sacrificed and the brain quickly removed. The right cerebral hemisphere was dissected on ice.

Reagents

The perfusion fluid was a bicarbonate-buffered physiological saline (in mM: 128 NaCl, 24 $NaHCO_3$, 4.2 KCl, 2.4 $NaHPO_4$, 1.5 $CaCl_2$, 0.9 $MgSO_4$) and 9 mM D-glucose added before infusion. The solution was bubbled with 95% O_2 and 5% CO_2 for pH control (= 7.4). The ^{14}C-sucrose (15 MBq $mmol^{-1}$) was from Perkin Elmer (Paris, France). The original uranium solution at a concentration of about 1 g l^{-1} (4.2 × 10^{-3} M) was made of natural uranium octoxide (U_3O_8) suspended in nitric acid. 23.8 µl of this solution were directly added to the perfusate solution (20 ml) in order to have a final exposure concentration of 5 × 10^{-6} M. Under these conditions, uranium was essentially the uranyl tricarbonate species ($UO_2(CO_3)_3^{4-}$) according to the predictive speciation model (JCHESS software) which does not alter the pH of the perfusate.



ıre 1. Diagram of the *in situ* brain perfusion technique in the rat according to Smith[14]. ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery.

Uranium measurement

Brain hemispheres were calcined at 450°C then mineralised with HNO_3 and H_2O_2. Uranium mass was measured using an inductively coupled plasma mass spectrometer (ICP-MS). The present ^{238}U mass was calculated using an external standard by subtracting the amount of ^{238}U already in the reaction mixture. The quantity of uranium present in the cerebral hemisphere was expressed in pg per g of wet weight.

Statistical analysis

Data are expressed as the mean value of total uranium in the right hemisphere. The statistical comparisons conducted here used the Student's t-test or ANOVA. Statistical difference was accepted at the $p < 0.05$ significance level. Data are given as the mean value ± one standard deviation.

RESULTS

The integrity of the BBB for uranium exposure between 5 × 10^{-8} and 5 × 10^{-5} M was explored by co-perfusing labelled ^{14}C-sucrose as a vascular marker which does not measurably penetrate the BBB during brief periods of perfusion. After 2 min of perfusion with a buffered physiological bicarbonate solution (pH 7.4) at a rate of 10 ml min^{-1}, the vascular volumes were not significantly different from those found in the rats with non-altered BBB. They ranged between 15 and 20 µl per g of brain; these values are similar to the values obtained in previous reports using the *in situ* brain perfusion method in the rat[13]. For the study of transfer of uranium across the BBB, a final 5 × 10^{-6} M concentration was selected as being 10-fold less than LD50.

In this case, the vascular volume of ^{14}C-sucrose was about 16.0 ± 2.8 µl g^{-1} for the exposed rats and about 17.1 ± 3.4 µl g^{-1} in control rats. No significant difference has been observed between these two groups. This allowed us to use a uranium concentration of 5 × 10^{-6}

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DISCUSSION

The *in situ* brain perfu
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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^{-6} M (10 ml min^{-1} perfusion rate) for 2 min, the amount of uranium in brain tissue was 52 ± 20 ng g^{-1}.

DISCUSSION

The *in situ* brain perfusion method described by Takasato *et al*[12] 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*[8] 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. 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^{-1} 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^{-1}), corresponding to 20 ng, it has been possible to estimate the quantity in the vessels and/or nervous parenchyma at about 32 ng g^{-1}.

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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