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Acute sodium tungstate inhalation is associated with minimal olfactory transport of tungsten (^{188}W) to the rat brain

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ABSTRACT

Olfactory transport of represents an important mechanism for direct delivery of certain metals to the central nervous system (CNS). The objective of this study was to determine whether inhaled tungsten (W) undergoes olfactory uptake and transport to the rat brain. Male, 16-week-old, Sprague–Dawley rats underwent a single, 90-min, nose-only exposure to a $Na_2{}^{188}WO_4$ aerosol (256 mg W/m^3). Rats had the right nostril plugged to prevent nasal deposition of ^{188}W on the occluded side. The left and right sides of the nose and brain, including the olfactory pathway and striatum, were sampled at 0, 1, 3, 7, and 21 days post-exposure. Gamma spectrometry ($n = 7$ rats/time point) was used to compare the levels of ^{188}W found on the left and right sides of the nose and brain and blood to determine the contribution of olfactory uptake to brain ^{188}W levels. Respiratory and olfactory epithelial samples from the side with the occluded nostril had significantly lower end-of-exposure ^{188}W levels confirming the occlusion procedure. Olfactory bulb, olfactory tract/tubercle, striatum, cerebellum, rest of brain ^{188}W levels paralleled blood ^{188}W concentrations at approximately 2–3% of measured blood levels. Brain ^{188}W concentrations were highest immediately following exposure, and returned to near background concentrations within 3 days. A statistically significant difference in olfactory bulb ^{188}W concentration was seen at 3 days post-exposure. At this time, ^{188}W concentrations in the olfactory bulb from the side ipsilateral to the unoccluded nostril were approximately 4-fold higher than those seen in the contralateral olfactory bulb. Our data suggest that the concentration of ^{188}W in the olfactory bulb remained low throughout the experiment, i.e., approximately 1–3% of the amount of tungsten seen in the olfactory epithelium suggesting that olfactory transport plays a minimal role in delivering tungsten to the rat brain.

1. Introduction

Tungsten is a transition metal that exhibits interesting properties such as high melting and boiling points, high electrical conductivity, strength and flexibility (Koutsospyros et al., 2006). By exploiting these properties, tungsten has become widely applicable in many industries and is of great demand commercially. Tungsten and tungsten alloys are used in light bulb filaments, phonographic needles, televisions, X-ray tubes, golf clubs, turbine blades, welding electrodes, counter balance and fishing weights, and as catalysts in chemical reactions (Lagarde and Leroy, 2002). Tungsten, unlike other heavy metals, is not regulated; therefore, data on anthropogenic contribution to the environmental load is not known. As a result of these uncertainties, the Centers for Disease Control (CDC) nominated tungsten for further studies by the National Toxicology Program (Koutsospyros et al., 2006).

Due to its wide application, the potential for human exposure to tungsten is of great concern. Exposure to tungsten and its alloys occurs in the hard metal industry and for military personnel (Lagarde and Leroy, 2002; Gold et al., 2007). Tungsten has been used to replace lead and depleted uranium in bullets and armor penetrators, respectively (Koutsospyros et al., 2006). Aerosolized tungsten is generated when an armored vehicle is penetrated by kinetic penetrators containing tungsten along with other metals (Gold et al., 2007). The presence of these aerosols could place military personnel at risk from inhalation exposure to tungsten.

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While it is increasingly clear that inhalation exposure to tungsten can occur, our current knowledge of the pharmacokinetics of this metal is poorly understood. Inhalation exposure to radiolabeled tungstic oxide has been previously described in beagle dogs however, in that study tungsten burdens in visceral tissues were reported but central nervous system (CNS) tungsten concentrations were not determined (Aamodt, 1975). In contrast, there have been several studies assessing the pharmcokinetics of tungsten following oral, intravenous, or intratracheal instillation of tungsten compounds (Le Lamer et al., 2000, 2001, 2002; Lagarde and Leroy, 2002).

Olfactory neurons originate in the olfactory epithelium, pass through the lamina propria and cribriform plate of the ethmoid bone and project to the olfactory bulb. Axons in the olfactory bulb synapse onto pyramidal cells which relay information to the rest of the brain via the subcortical and cortical regions (Tjalve and Henriksson, 1999). Inhalation exposure to metals such as lead, copper, nickel, zinc, cobalt, mercury and manganese have been well documented and researchers have shown that these metals may be transported to the CNS via this olfactory pathway (Tjalve and Henriksson, 1999; Persson et al., 2003). The goal of this study was to determine whether tungsten undergoes olfactory transport to the rat brain. To test this hypothesis, we used a unilateral occlusion rat model that has been well established by our laboratory (Brenneman et al., 2000). By occluding one nostril and thus restricting tungsten delivery to the side of the nasal cavity with an unoccluded nostril, this model has the added advantage of allowing each animal to serve as its own control.

2. Materials and methods

2.1. Chemicals

Radiolabeled sodium tungstate (^{188}W) was obtained in a nominally 250 mCi batch, with a radiopurity of >99% and specific activity of 1.47 mCi/ml (Oak Ridge National Laboratories, Tennessee, USA). Unlabeled sodium tungstate, $Na_2WO_4{\cdot}2H_2O$, CAS no. 10213-10-2, was obtained from SPI (West Chester, PA). All other chemicals were purchased from Sigma Chemical (St. Louis, MO) unless otherwise stated.

2.2. Animals

Thirty-five 16-week-old male Sprague–Dawley rats were purchased from Charles River Laboratories, Inc. (Raleigh, NC). The rats were allowed a 2-week acclimation period and identified using ear tags. Animals were weighed weekly and randomized to the experimental groups according to body weight. Animal weights were also recorded at the beginning of each exposure and at each post-exposure time point. The rats were housed individually in suspended-stainless steel wire cages in biologically clean rooms with HEPA-filtered air and a 12 h light/dark photoperiod cycle. The cage rooms were maintained at 22 ± 4 °C and 50 ± 20% relative humidity. All rats received NIH-07 pelleted diet (Zeigler Brothers, Gardner, PA) and reverse osmosis water (Hydro Picosystems; HYDRO Service and Supplies, Research Triangle Park, NC) *ad libitum*. The experiments reported here were conducted in accordance with federal guidelines for the care and use of laboratory animals (NRC, 1996) and the study was approved by The Hamner Institutes for Health Sciences and the Department of Defense Institutional Animal Care and Use Committees.

2.3. Nostril-plugging procedure

Nasal plugs were made by inserting two 20 mm pieces of polyethylene tubing (OD = 0.024 in. and ID = 0.011 in.; Becton Dickinson, Parsippany, NJ) into the end of a 7 mm piece of silastic laboratory tubing (OD = 0.085 in. and ID = 0.04 in.; Dow Corning Corp., Midland, MI). The ends of the polyethylene tubing were melted to form a rounded seal on both ends of the silastic tubing (Brenneman et al., 2000). Randomly assigned rats were anesthetized briefly (<5–10 min) using isofluorane and a plastic plug lubricated with water-soluble jelly was gently inserted into the nasal vestibule. The plastic plug was held in place with nexaband liquid topical tissue adhesive (Abbott Laboratories, North Chicago, IL). The right nostril of the rats was occluded (left side unoccluded) using a nasal plug 2.5 ± 0.5 h prior to the start of the inhalation exposure.

2.4. Inhalational exposure and necropsy

Rats with their right nostril plugged (n = 7/group/time point) under went a 90 min nose-only exposure. The rats were exposed in two separate sessions (21 rats for exposure 1; 14 rats for exposure 2) in order to accommodate the necropsy and nasal plugging procedures. These exposures were conducted 5 days apart. The animals were euthanized immediately following the end of the exposure (0 hr time point), or 1 day, 3 days, 7 days, and 21 days thereafter. Rats were anesthetized using sodium pentobarbital, and rinsed with ethanol to remove excess radiolabeled compound from the fur. Cardiac puncture was performed to collect whole blood and the animal was euthanized by exsanguination. The following tissues were taken for gamma spectrometry: (right and left sides) respiratory epithelium (RE), olfactory epithelium (OE), olfactory bulb (OB), olfactory tract/tubercle (OTT), striatum (ST), cerebellum (CE), trigeminal nerve (TN), pituitary gland, and the rest of brain (ROB).

2.5. Gamma spectrometry

The amount of radioactivity (^{188}W) in a representative preweighed tissue sample was determined using a Packard Cobra Series Auto-Gamma Counting System (Meriden, CT). The amount of ^{188}W was reported as nCi/g tissue wet weight and was corrected for radioactive decay of this isotope.

2.6. Tungsten aerosol generation and exposure system

Sodium tungstate aerosol was produced using a nebulizer (3-jet Collison Nebulizer, BGI, Inc., Waltham, MA). The aerosol passed through a diffusion dryer and ^{85}Kr bipolar discharger into a nose-only exposure system (52-port, Lab Products, Seaford, DE). A continuous flow of the exposure atmosphere was drawn through an optical particle sizing spectrometer (Aerodynamic Particle Sizer, Model 3321, TSI, Inc., St. Paul, MN). The instrument measured aerodynamic particle size and provided particle size distribution parameters, geometric mean diameter (GMD) and geometric standard deviation (GSD). Mass median aerodynamic diameter (MMAD) was calculated from the GMD (Hinds, 1999).

Aerosol mass concentration was measured using gravimetric filtration. Filters were weighed on a microbalance (Cahn, Model C-35, ATI, Boston, MA). The aerosol concentration was determined by weighing a filter (Osmotics Inc., Minnetonka, MN) prior to sampling, sampling at a known flow rate for a measured time period, then re-weighing the filter after sampling and determining the mass collected per volume sampled. The gravimetric filter results were used to calculate exposure concentration by mass. The aerosol radiolabeled activity was determined by counting the filters on a gamma counter (Packard Cobra Quantum). The combination of the radiolabeled activity and the gravimetric results were used to calculate the specific activity. Two filter samples were taken, one for the first half of the study, and the second for the second half.