Comparative Biochemistry and Physiology, Part C 155 (2012) 143–150

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Comparative cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human and sperm whale (*Physeter macrocephalus*) skin cells ☆

Tânia Li Chen [a,b,c], Carolyne LaCerte [a,b,c,d], Sandra S. Wise [a,b,c,d], Amie Holmes [a,b,c], Julieta Martino [a,b,c], John Pierce Wise, Jr. [a,b,d], W. Douglas Thompson [b,c], John Pierce Wise Sr. [a,b,c,d,*]

[a] Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, 96 Falmouth St., Portland, ME 04104, USA
[b] Maine Center for Toxicology and Environmental Health, University of Southern Maine, 96 Falmouth St., Portland, ME 04104, USA
[c] Department of Applied Medical Sciences, University of Southern Maine, 96 Falmouth St., Portland, ME 04104, USA
[d] Ocean Alliance, 191 Weston Rd., Lincoln, MA 01773, USA

ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 27 March 2011
Accepted 28 March 2011
Available online 3 April 2011

Keywords:
Clastogenicity
Hexavalent chromium
Human
Skin cells
Sperm whale

ABSTRACT

Chromium (Cr) is a global marine pollutant, present in marine mammal tissues. Hexavalent chromium [Cr(VI)] is a known human carcinogen. In this study, we compare the cytotoxic and clastogenic effects of Cr(VI) in human (*Homo sapiens*) and sperm whale (*Physeter macrocephalus*) skin fibroblasts. Our data show that increasing concentrations of both particulate and soluble Cr(VI) induce increasing amounts of cytotoxicity and clastogenicity in human and sperm whale skin cells. Furthermore, the data show that sperm whale cells are resistant to these effects exhibiting less cytotoxicity and genotoxicity than the human cells. Differences in Cr uptake accounted for some but not all of the differences in particulate and soluble Cr(VI) genotoxicity, although it did explain the differences in particulate Cr(VI) cytotoxicity. Altogether, the data indicate that Cr(VI) is a genotoxic threat to whales, but also suggest that whales have evolved cellular mechanisms to protect them against the genotoxicity of environmental agents such as Cr(VI).

1. Introduction

Chromium is a marine concern. Chromium enters the marine ecosystem through the discharge of contaminated waste waters and the release of contaminated air from coastal industry (Agency for Toxic Substances and Disease Registry, "ATSDR", 2008; Neff, 2002). Recently, we identified chromium (Cr) as a global marine pollutant, reporting that sperm whales from around the world had measurable chromium levels. Some were remarkably high, reaching levels previously only seen in workers that died of chromate-induced lung cancer (Wise et al., 2009a). In the ocean, Cr(VI) is the predominant state for Cr (Geisler and Schmidt, 1991; Pettine and Millero, 1990), and thus, Cr(VI) is a health threat for marine species, such as the sperm whales.

In humans, Cr(VI) is a well known health threat that damages DNA leading to lung cancer and reproductive effects (Agency for Toxic Substances and Disease Registry, "ATSDR", 2008). In marine mammals, the health impacts are uncertain and little studied. We recently demonstrated that both particulate and soluble Cr(VI) are cytotoxic and genotoxic to whale and sea lion cells (Wise et al., 2008a; Li Chen et al., 2009a; Wise et al., 2009b; Wise et al., 2010; Wise et al., 2011). Considered in conjunction with Cr levels in the animals, the data suggest that the whales may be exposed to potentially genotoxic levels of Cr (Wise et al., 2008a; Wise et al., 2009b; Li Chen et al., 2009a; Wise et al., 2011).

Cancer incidence in marine mammals is underestimated due to unnoticed deaths in the wild and incomplete necropsies (Newman and Smith, 2006). Tumors have been reported in marine mammals, especially in California sea lions (*Zalophus californianus*) and St. Lawrence beluga whales (*Delphinapterus leucas*) (Newman and Smith, 2006). Exposure to environmental chemicals is the primary suspected cause for tumors found in these two species (Martineau et al., 2002; Newman and Smith, 2006; Ylitalo et al., 2005), although the potential role of heavy metals including Cr was not investigated.

One of the two whale species high in Cr, the North Atlantic right whale (*Eubalaena glacialis*) has been intensively studied on an annual basis for more than thirty years (Kraus and Rolland, 2007). Cancers have generally not been reported in this population, which may reflect either the very small population size, the potential for diseased animals to die at sea, or perhaps a resistance of these animals to the genotoxic effects of Cr. Consistent with the latter possibility, we conducted a comparative study of right whale and human cells and found that, after correcting for differences in uptake, Cr(VI) induced significantly less genotoxicity in the whale cells compared to the human cells (Li Chen et al., 2009b).

☆ This paper is based on a presentation given at the *5th Aquatic Annual Models of Human Disease* conference: hosted by Oregon State University and Texas State University-San Marcos, and convened at Corvallis, OR, USA September 20–22, 2010.

* Corresponding author at: 178 Science Building, 96 Falmouth St., Portland, ME 04103, USA. Tel.: +1 207 228 8050; fax: +1 207 228 8518.
E-mail address: john.wise@usm.maine.edu (J.P. Wise).

1532-0456/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2011.03.011

The sperm whale is much less understood and studied than the right whale in part because they are more nomadic with a larger geographic range and in part because they live in deeper waters. Tumors occur in sperm whales, but with unknown etiology (Newman and Smith, 2006). The Cr levels observed in sperm whales were much higher than those of the right whale, both as a mean level (8.8 μg/g versus 7.1 μg/g) and as a range (high 122.6 μg/g versus 10 μg/g) (Wise et al., 2008a; Wise et al., 2009a). These high levels raise questions about whether sperm whales have adapted cellular and molecular responses to protect them from the genotoxicity of agents like Cr. The possibility has not been studied before for sperm whale cells. Accordingly, in this study, we compared the cytotoxic and genotoxic effects of Cr(VI) in primary sperm whale and human skin cells.

2. Materials and methods

2.1. Chemicals and reagents

Lead chromate ($PbCrO_4$), sodium chromate (Na_2CrO_4), demecolchicine and potassium chloride (KCl) were purchased from Sigma/Aldrich (St. Louis, MO, USA). Giemsa stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA, USA). Cytoseal 60 slide mounting medium was purchased from VWR (Bridgeport, NJ, USA). Gurr's buffer, trypsin/EDTA, sodium pyruvate, penicillin/streptomycin, and L-glutamine were purchased from Invitrogen Corporation (Grand Island, NY). Crystal violet, methanol and acetone were purchased from J.T. Baker (Phillipsburg, NJ, USA). Dulbecco's minimal essential medium and Ham's F-12 (DMEM/F-12) 50:50 mixture was purchased from Mediatech Inc. (Herndon, VA, USA). Cosmic calf serum (CCS) was purchased from Hyclone, (Logan, UT, USA). MycoAlert detection kits were purchased from Lonza Rockland, Inc (Rockland, ME, USA). Tissue culture dishes, flasks, and plastic ware were purchased from BD Biosciences (Franklin Lakes, NJ, USA).

2.2. Cells and cell culture

We used primary sperm whale skin cells isolated from a free-ranging female sperm whale off the coast of North Carolina previously described in Wise et al. (2011), and primary human skin cells (BJ cells) previously described in Vaziri and Benchimol (1998). All cells were cultured as adherent monolayers of cells and were subcultured at least once a week. Cells were maintained in Dulbecco's minimal essential medium and Ham's F-12 (DMEM/F-12) 50:50 mixture, supplemented with 15% cosmic calf serum, 2 mM L-glutamine 100 U/mL penicillin/100 μg/mL streptomycin, and 0.1 mM sodium pyruvate. Cells were maintained in a humidified incubator with 5% CO_2 set at 33 °C for sperm whale cells and 37 °C for human cells. Cells were regularly tested for mycoplasma contamination. All experiments were conducted on logarithmically growing cells with a doubling time of 24 h for human cells and 36 h for sperm whale cells.

2.3. Preparation of chromium compounds

Lead chromate ($PbCrO_4$), a representative particulate Cr(VI) compound (CAS# 7758-97-6, ACS reagent minimum 98% purity), was administered as a suspension of particles as previously described, to ensure that cells were exposed to intact particles (Wise et al., 2002). Briefly, $PbCrO_4$ was weighed, suspended in deionized water and desired treatment concentrations were made from this stock suspension. Sodium chromate (Na_2CrO_4), a representative soluble Cr(VI) compound (CAS #7775-11-3, ACS reagent minimum 98% purity), was administered as a solution in water as previously described (Wise et al., 2002). Briefly, Na_2CrO_4 was weighed and dissolved in deionized water. Desired treatment concentrations were made from a sterile filtered sample of the stock solution.

In accordance with the majority of the published Cr(VI) literature, treatments with particulate Cr(VI) are presented in μg/cm^2 and treatments with soluble Cr(VI) in μM. These units reflect the fact that the lead chromate particles only partially dissolve while sodium chromate fully dissolves. Thus, these chemicals cannot accurately be compared based on administered dose. Lead chromate treatment concentrations were 0.1, 0.5, 1, 5 and 10 μg/cm^2, which correspond to 0.068, 0.34, 0.68, 3.4 and 6.8 μg Cr/mL, respectively, if dissolution was complete. Sodium chromate treatment concentrations were 0.1, 0.5, 1, 2.5, 5 and 10 μM, which correspond to 0.005, 0.026, 0.05, 0.13, 0.26 and 0.5 μg Cr/mL, respectively. The best way to compare them both practically and functionally is by the amount of intracellular Cr, which is provided in the Results section.

2.4. Cytotoxicity

Cytotoxicity was measured using a clonogenic assay that determines the ability of the cell to form colonies in a culture dish following chemical treatment, as described previously (Wise et al., 2002). Briefly, logarithmically growing cells were seeded into each well of 6-well tissue culture plates, cells were allowed to rest for 48 h, and then treated for 24 h with lead chromate or sodium chromate. At the end of the treatment time, cells were collected, counted and re-seeded into 100 mm tissue culture dishes at a density of 1000 cells/dish, four dishes were seeded for each concentration. These dishes were grown for about two weeks until colonies formed, stained and counted. Experiments were repeated at least three times. All treatment groups were compared to the control and expressed as a percentage of the control.

2.5. Clastogenicity

Clastogenicity was measured using the chromosome damage assay that determines the ability of Cr to induce chromosomal structural aberrations in metaphase chromosomes, as described previously (Wise et al., 2002). Briefly, logarithmically growing cells were seeded into 100 mm tissue culture dishes, cells were allowed to rest for 48 h, and then treated for 24 h with lead chromate or sodium chromate. Near the end of the treatment time, demecolchicine was added to arrest cells in metaphase. At the end of the treatment, cells were collected, resuspended in a hypotonic solution of 0.075 M KCl, and then fixed with 3:1 methanol:acetic acid. Fixative was changed twice, and then cells were dropped onto wet clean slides, stained and coverslipped. Experiments were repeated at least three times. Chromosomal structural aberrations were scored in 100 metaphases per each treatment dose, according to standard criteria previously described (Wise et al., 2008a). Results were expressed as a percentage of metaphases with chromosome damage and as the total chromosome damage observed in the 100 metaphases analyzed.

2.6. Determination of intracellular chromium Ion levels

2.6.1. Cell preparation

Intracellular ion levels were measured using the ion uptake assay, as described previously (Holmes et al., 2005), with minor changes. Briefly, logarithmically growing cells were seeded into 100 mm tissue culture dishes, allowed to rest for 48 h and then treated for 24 h. At the end of the treatment, 3 mL of treated culture media was saved for extracellular chromium analysis; cells were collected and the number and volume of cells were determined. Cells were washed twice with PBS, resuspended in 1 mL hypotonic solution followed by 1 mL 2% SDS. Finally, the solution was sheered and filtered. Samples were stored at −20 °C until analysis.

2.6.2. Ion level measurements

Intracellular Cr ion levels were determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), equipped with a gem cone low flow nebulizer, according to previously published