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The cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in leatherback sea turtle lung cells

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ABSTRACT

Hexavalent chromium [Cr(VI)] is a marine pollution of concern as recent studies show it has a global distribution, with some regions showing high Cr concentrations in marine animal tissue, and it is extensively used. Leatherback sea turtles (*Dermochelys coriacea*) are an endangered marine species that may experience prolonged exposures to environmental contaminants including Cr(VI). Human activities have led to global Cr(VI) contamination of the marine environment. While Cr(VI) has been identified as a known human carcinogen, the health effects in marine species are poorly understood. In this study, we assessed the cytotoxic and genotoxic effects of particulate and soluble Cr(VI) in leatherback sea turtle lung cells. Both particulate and soluble Cr(VI) induced a concentration-dependent increase in cytotoxicity. Next, using a chromosome aberration assay, we assessed the genotoxic effects of Cr(VI) in leatherback sea turtle lung cells. Particulate and soluble Cr(VI) induced a concentration-dependent increase in clastogenicity in leatherback sea turtle lung cells. These data indicate that Cr(VI) may be a health concern for leatherback sea turtles and other long-lived marine species. Additionally, these data provide foundational support to use leatherback sea turtles as a valuable model species for monitoring the health effects of Cr(VI) in the environment and possibly as an indicator species to assess environmental human exposures and effects.

1. Introduction

The leatherback sea turtle species (*Dermochelys coriacea*) is a long-lived marine reptile that spends the vast majority of its life in the ocean with only females coming on shore for short instances to lay their eggs. Leatherbacks are large turtles growing to over six feet long and weighing up to 1400 pounds (Eckert et al., 2012). They have a leathery shell, while all other sea turtles have hard, bony-plated shells. They have uniquely adapted (among turtles) a thick insulating layer of fat and the ability to eliminate waste gases through their skin, which allows them to dive underwater to great depths for long periods of time (Bickler and Buck, 2007; Dodge et al., 2014). However despite their charisma and immense size, the leatherback sea turtle is considered critically endangered and at risk of extinction (Spotila et al., 2000; Tapilatu et al., 2013).

It is important to study the potential effects of environmental contaminants on leatherback sea turtles in order to understand the potential impact on the health of their population. Leatherback sea turtles are an endangered species that face many pressures due to human activity including plastic in the oceans, fishing entanglement, habitat degradation, and human released pollution and contaminants (Innis et al., 2010; James et al., 2005; Kaplan, 2005; Lewison et al., 2004; Perrault et al., 2011). Leatherbacks are found throughout all of the world's oceans and commonly travel long distances during their lives.

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The extended amount of time leatherbacks spend in the ocean subjects them to exposure to any marine pollutants and contaminants that may be present (Godley et al., 1999; Guirlet et al., 2008; Guirlet et al., 2010; Perrault et al., 2013; Perrault, 2014; Stewart et al., 2011; Storelli and Marcotrigiano, 2003). These pollutants have the potential to lead to detrimental health effects including reproductive issues (Guirlet et al., 2010; Perrault et al., 2011; Stewart et al., 2011). Furthermore, leatherbacks may bioaccumulate environmental contaminants exacerbating health issues.

The health status of the oceans has recently been changing at a faster rate due to climate change leading to concerns, such as ocean acidification. This process may lead to the release of hazardous compounds, such as hexavalent chromium [Cr(VI)], that were previously deposited in ocean sediments (Ellis, 2002; Wang et al., 2015; Zeng et al., 2015). Hazardous compounds released from the sediments may suspend in the water column where leatherback sea turtles, a pelagic species, spend a majority of their time (Dodge et al., 2014).

In addition to being released from ocean sediments, Cr(VI) is also released into the environment through the burning of fossil fuels and other industrial processes. Ultimately, Cr(VI) released in this manner can travel through air currents around the world with the potential to settle in the oceans. Cr(VI) is a known human lung carcinogen inducing lung tumors characterized by genomic instability (Holmes et al., 2008; Urbano et al., 2008; Wise and Wise, 2012). However, to date, there are no studies on the effect of Cr(VI) in leatherback sea turtles. Since leatherback sea turtles may experience prolonged exposure to Cr(VI) in the marine environment through the air, water, and food sources it is important to understand the health implications from this potential exposure.

It is increasingly clear that pollution derived from anthropogenic activities has reached even the remotest ocean regions. We recently described metal pollution around the world using sperm whales (*Physeter macrocephalus*) as an indicator species (Wise et al., 2009a). One marine pollutant with particularly high levels was Cr. Data from humans and laboratory animals show Cr(VI) can damage DNA and induce reproductive and developmental toxicity (Al-Hamood et al., 1998; Bataineh et al., 1997; Chowdhury and Mitra, 1995; Mancuso, 1997; Witmer et al., 1989; Witmer et al., 1991). If such outcomes occur it could lead to disease and decreased reproductive success, which could seriously impair a critically endangered species like the leatherback.

Several studies have investigated metal levels in leatherback sea turtles around the world and found their tissues may accumulate metals such as mercury, cadmium, lead, and arsenic (Guirlet et al., 2008; Guirlet et al., 2010; Kunito et al., 2008; Mckenzie et al., 1999; Perrault et al., 2013; Perrault, 2014; Poppi et al., 2012; Stewart et al., 2011; Storelli et al., 1998). To our knowledge only one study has investigated Cr levels in leatherback sea turtles, but did not measure Cr levels in lung tissue (Poppi et al., 2012). Poppi et al. did find that Cr was present in the kidney, muscle, skin, and, at the highest concentration, in the liver. However, one study showed that in tissues of adult and young loggerhead sea turtles (*Caretta caretta*) Cr accumulated in the highest concentrations in the lung (Storelli et al., 1998). Another study found that Cr levels in the yolk of eggs from green sea turtles (*Chelonia mydas*) were considered above normal compared to levels observed in mammals and birds, however the effects of these levels remain unknown (Lam et al., 2006).

Metal exposure in sea turtle model systems has been considered in two studies: one on green turtles and one on hawksbill. The green sea turtle studies correlated carapace metal levels with adverse health markers and found that cadmium and Cr were the most cytotoxic of four metals tested for cytotoxicity in cell lines (Tan et al., 2010; Wang et al., 2015).We found Cr(VI) was both cytotoxic and genotoxic to another marine sea turtle species, the hawksbill sea turtle (*Eretmochelys imbricate*) (Wise et al., 2014; Young et al., 2015). We found no studies of metal toxicity in leatherbacks. Therefore, in this study we investigated the cytotoxic and genotoxic effects of Cr(VI) in leatherback sea turtle lung cells. We focused our study on particulate and soluble Cr(VI) compounds as the marine environment favors the hexavalent form of Cr (Geisler and Schmidt, 1991; Pettine and Millero., 1990) and because in humans the particulate Cr(VI) forms are more potent than soluble ones (Holmes et al., 2008; IARC, 1990; Wise et al., 2002, 2008).

2. Materials and methods

2.1. Chemicals and reagents

DMEM/F12 (1X), phosphate-buffered solution (PBS) 1X without calcium or magnesium, Corning glutagro supplement (200 mM), tissue culture dishes, flasks and plasticware were purchased from Corning (Corning, NY). Sodium pyruvate (100 mM) was purchased from Lonza (Allendale, NJ). Gurr's buffer and 0.5% trypsin-EDTA (10X) were purchased from Life Technologies Corp (Carlsbad, CA). Crystal violet and acetic acid were purchased from J.T. Baker (Phillipsburg, NJ). Lead chromate (CAS #7758-97-6), sodium chromate (CAS#7775-11-3), and demecolcine were purchased from Sigma-Aldrich (St. Louis, MO). Giesma stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA). Seradigm premium grade fetal bovine serum (FBS), Sodium dodecyl sulfate (SDS), and methanol were purchased from VWR International (Radnor, PA). Potassium chloride (KCl) was purchased from Alfa Aesar (Tewksbury, MA). Attachment factor was purchased from ThermoFisher Scientific (Waltham, MA). Trace-element grade nitric acid was purchased from Fischer Scientific (Hampton, NH).

2.2. Cell line development and cell culture

A leatherback sea turtle primary lung fibroblast cell line was established from explant lung tissue derived from a leatherback sea turtle embryo and were named PGDC9-1LU cells. Primary PGDC9-1LU cells (Fig. 1) were maintained as sub-confluent monolayers in DMEM/F12 media supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutagro, and 0.1% sodium pyruvate. PGDC9-1LU cells were incubated in 5% CO_2 at 26 °C and media was replaced with fresh, warm media every two to three days. Cells were subcultured every four to seven days using 0.1% trypsin-EDTA. The cell line was evaluated for numerical and structural chromosome normality through sub sequential passaging of the cells. No aneuploidy or cellular phenotypic changes were observed in untreated cells.

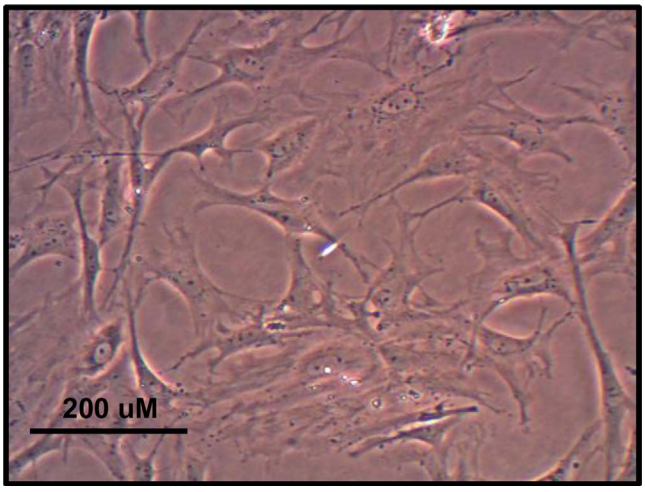

Fig. 1. Image of PGDC9-1LU Cells.
This figure shows a representative image of the leatherback sea turtle lung cells used in this study.