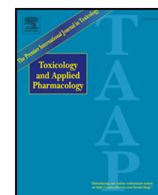
Toxicology and Applied Pharmacology 279 (2014) 113–118

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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



Hexavalent chromium is cytotoxic and genotoxic to hawksbill sea turtle cells

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ARTICLE INFO

Article history:
Received 8 May 2014
Revised 30 May 2014
Accepted 8 June 2014
Available online 19 June 2014

Keywords:
Chromate
Chromium
Hexavalent chromium
Genotoxicity
Hawksbill sea turtle

ABSTRACT

Sea turtles are a charismatic and ancient ocean species and can serve as key indicators for ocean ecosystems, including coral reefs and sea grass beds as well as coastal beaches. Genotoxicity studies in the species are absent, limiting our understanding of the impact of environmental toxicants on sea turtles. Hexavalent chromium (Cr(VI)) is a ubiquitous environmental problem worldwide, and recent studies show it is a global marine pollutant of concern. Thus, we evaluated the cytotoxicity and genotoxicity of soluble and particulate Cr(VI) in hawksbill sea turtle cells. Particulate Cr(VI) was both cytotoxic and genotoxic to sea turtle cells. Concentrations of 0.1, 0.5, 1, and 5 μg/cm^2 lead chromate induced 108, 79, 54, and 7% relative survival, respectively. Additionally, concentrations of 0, 0.1, 0.5, 1, and 5 μg/cm^2 lead chromate induced damage in 4, 10, 15, 26, and 36% of cells and caused 4, 11, 17, 30, and 56 chromosome aberrations in 100 metaphases, respectively. For soluble Cr, concentrations of 0.25, 0.5, 1, 2.5, and 5 μM sodium chromate induced 84, 69, 46, 25, and 3% relative survival, respectively. Sodium chromate induced 3, 9, 9, 14, 21, and 29% of metaphases with damage, and caused 3, 10, 10, 16, 26, and 39 damaged chromosomes in 100 metaphases at concentrations of 0, 0.25, 0.5, 1, 2.5, and 5 μM sodium chromate, respectively. These data suggest that Cr(VI) may be a concern for hawksbill sea turtles and sea turtles in general.

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Introduction

Sea turtles are a charismatic species that inhabit both coastal and pelagic ecosystems. They have long lives, up to 80 years, and have the potential to bioaccumulate pollutants from food, sediment, and water, as well as air. The hawksbill sea turtle (*Eretmochelys imbricata*) is considered critically endangered and at risk of extinction (IUCN, 2013). Hunting and loss of egg-laying habitat have been key factors in their decline (Meylan and Donnelly, 1999). Efforts have been undertaken to reduce these factors by banning the trade of materials from hawksbills and protecting some egg laying sites. However, illegal trade and continued coastal development continue to impair their recovery.

Superimposed on these two factors is the concern that ocean pollution may put the struggling hawksbill population at further risk. It is increasingly clear that ocean pollution has reached even the remotest regions and, if they are sufficiently exposed, pollution could impair the ability of the hawksbill and other similarly endangered sea turtles and sea life to survive, reproduce and thrive. For example, we recently identified chromium (Cr) as a global marine pollutant using sperm whales (*Physeter macrocephalus*) as an indicator species. Human and rodent studies indicate that Cr 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, 1991). Such outcomes could lead to disease in an individual and a reduction in reproductive success for a population, both of which could seriously impair a critically endangered species like the hawksbill.

Metal pollutants have been found in tissue from several sea turtle species (Storelli et al., 1998, 2008; Anan et al., 2002; Franzellitti et al., 2004; Maffucci et al., 2005; Gardner et al., 2006; Frias-Expericuet et al., 2006; Andreani et al., 2008; Garcia-Fernandez et al., 2009; Jerez et al., 2010). Only two of these studies measured Cr. One study reported low Cr levels (0.039 μg/ml) in the plasma of captive hawksbills (Suzuki et al., 2012). The other reported average Cr levels from 12 stranded loggerhead turtles in the Mediterranean of 1.05, 1.57, and 1.43 mg/kg dry weight in liver, kidney and muscle, respectively, with the highest levels found in lung tissue (2.29 mg/kg) (Storelli et al., 1998).

Only two studies considered the impact of metal exposure in sea turtle model systems. Both were focused on green turtles. One study

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http://dx.doi.org/10.1016/j.taap.2014.06.008
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correlated increased carapace metal levels with adverse health markers in green turtles from San Diego Bay (Komoroske et al., 2011). The other study considered the cytotoxic effects of four metals, including Cr, in green sea turtle cell lines and found that cadmium and Cr were the most cytotoxic (Tan et al., 2010). It appears none have considered impacts in hawksbill turtles.

Presumably, one reason for this lack of effects data is the intent to avoid losing any individual turtles to scientific studies and thereby preserve as many as possible. While such reasoning makes sense, it remains possible to conduct controlled toxicology studies and gain valuable species-specific insights into the potential toxicological response and impact by using cells cultured from sea turtle tissues in a manner that does not harm the animal or is collected from a recently deceased animal. Accordingly, to begin developing a better understanding of ocean pollution impacts on the hawksbill in particular and sea turtles in general, we investigated the cytotoxicity and genotoxicity of Cr in skin cells developed from a hawksbill sea turtle. Because 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 (IARC, 1990; Holmes et al., 2008; Wise et al., 2008a,b), we focused our study on particulate and soluble Cr(VI) compounds.

Methods

Chemicals and reagents. RPMI was purchased from Mediatech (Manassas, VA). Penicillin/streptomycin, Gurr's buffer, and trypsin/EDTA were purchased from Invitrogen Corporation (Grand Island, NY). Crystal violet, acetic acid, and methanol were purchased from J.T. Baker (Phillipsburg, NJ) and fetal bovine serum (FBS) was purchased from Gibco Life Technologies (Grand Island, NY). Tissue culture dishes, flasks, and plasticware were purchased from BD (Franklin Lakes, NJ). Lead chromate, sodium chromate, potassium chloride (KCl) and demecolchicine were purchased from Sigma/Aldrich. Giemsa stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA).

Cells and cell culture. Hawksbill sea turtle fibroblast cells were established from a skin biopsy of a healthy juvenile hawksbill sea turtle (Fukuda et al., 2012). The cells were grown in RPMI with 10% FBS and maintained in 5% CO_2 at 26 °C. Cell cultures were maintained and all experiments performed as subconfluent monolayers. They were fed at least twice a week and subcultured at least once a week. The cells were tested routinely for mycoplasma contamination. All experiments were conducted on logarithmically growing cells.

Preparation of chemicals. Sodium chromate (CAS #7775-11-3, ACS reagent minimum 98% purity), a soluble hexavalent Cr compound was administered as a solution in water as previously described (Wise et al., 2002). Lead chromate (CAS# 7758-97-6, ACS reagent minimum 98% purity), a particulate Cr(VI) compound was administered as a suspension in water as previously described (Wise et al., 2002). Lead chromate does not fully dissolve in tissue culture while sodium chromate does (Holmes et al., 2005; Wise et al., 2005). Thus, direct comparisons of the two chemicals using a common unit of measure are difficult. If dissolution had been complete the concentrations for lead chromate (0.1, 0.5, 1, and 5 μg/cm^2) would be 0.42, 2.1, 4.2 and 21 μg/ml, and the concentrations for sodium chromate (0.25, 0.5, 1, 2.5, and 5 μM) would be 0.04, 0.08, 0.16, 0.4, and 0.8 μg/ml.

Cytotoxicity. Cytotoxicity was established using a clonogenic assay based on our published methods (Wise et al., 2008b). Briefly, cells were seeded in each well of a 6-well tissue-culture dish and treated for 24 h with either lead chromate or sodium chromate. After treatment, the cells were resuspended in fresh medium and reseeded at 1000 cells in four 100 mm dishes per treatment group. Once colonies formed (about 14 days), dishes were fixed and stained with crystal violet and colonies counted. Each experiment was repeated at least three times.

Clastogenicity. Clastogenicity was determined using a chromosomal aberration assay based on our published methods (Wise et al., 2008b). Briefly, log phase cells were seeded into 100 mm dishes and treated for 24 h with either lead chromate or sodium chromate. Demecolcine (0.1 g/ml) was added 1 h before the end of treatment to arrest the cells in metaphase. The cells were then collected by trypsinization, spun down and resuspended in a 0.075 M KCl hypotonic solution for 20 min followed by fixation with 3:1 methanol:acetic acid. The fixative was changed twice then cells were dropped onto clean wet slides and stained with 5% Giemsa stain in Gurr's buffer. One hundred metaphases per treatment were analyzed in each experiment and chromosome aberrations were scored by standard criteria (Wise et al., 2008b). All experiments were repeated three times.

Statistics. Dose–response curves were estimated using regression analysis. Ninety-five percent Wald confidence intervals were calculated, as were Wald chi square tests of statistical significance. Differences between pairs of dose levels were assessed using t-tests.

Results

Cytotoxicity

Sodium chromate induced a concentration-dependent decrease in cell survival (Fig. 1). Specifically, 0.25, 0.5, 1, 2.5, and 5 μM sodium chromate induced 84, 69, 46, 25, and 3% relative survival, respectively, in hawksbill sea turtle cells after a 24 h treatment. Lead chromate also induced a concentration-dependent decrease in cell survival (Fig. 2). Specifically, concentrations of 0.1, 0.5, 1, and 5 μg/cm^2 induced 108, 79, 54, and 7% relative survival, respectively, in hawksbill sea turtle cells after 24 h exposure. The estimated LC50s for sodium chromate and lead chromate were 1.2 μM (95% confidence interval: 0.9 to 1.5) and 1.1 μg/cm^2 (95% confidence interval: 0.9 to 1.3), respectively.

Clastogenicity

Clastogenicity was used as a measure of genotoxicity and expressed as percent of damaged metaphases and as the total damage observed in 100 metaphases. Sodium chromate induced a concentration-dependent

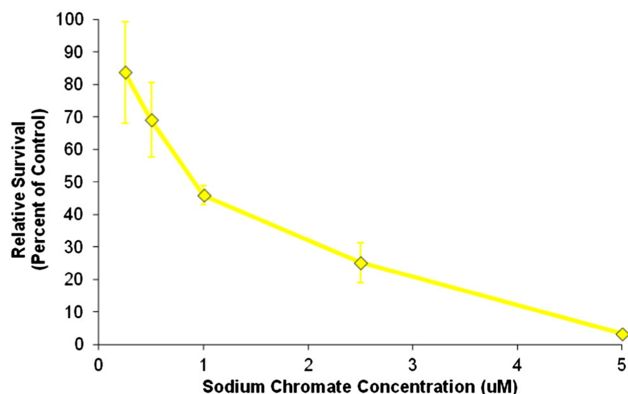

Fig. 1. Sodium chromate is cytotoxic to hawksbill sea turtle cells. This figure shows that soluble sodium chromate is cytotoxic to hawksbill sea turtle cells. Data represent 3 experiments ± the standard error of the mean. All concentrations were significantly different from the control, except for 0.25 and 0.5 μM ($p < 0.0001$ for 1, 2.5 and 5 μM). 0.25 μM was significantly different from 2.5, and 5 μM ($p < 0.02$ and $p < 0.01$, respectively). 0.5 μM was significantly different from 2.5, and 5 μM ($p < 0.02$ and $p < 0.01$, respectively). 1 μM was significantly different from 2.5, and 5 μM ($p < 0.03$ and $p < 0.0003$, respectively). 2.5 μM was also significantly different from 5 μM ($p < 0.03$).

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