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Chemically dispersed oil is cytotoxic and genotoxic to sperm whale skin cells

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ABSTRACT

Two major oil crises in United States history, the 1989 Exxon-Valdez oil spill in Alaska and the 2010 Deepwater Horizon Oil Rig explosion in the Gulf of Mexico, drew attention to the need for toxicological experiments on oil and chemically dispersed oil. We are still learning the effects these spills had on wildlife. However, little data is known about the toxicity of these substances in marine mammals. The objective of this study is to determine the toxicity of Alaskan oil, as well as chemically dispersed oil. Oil experiments were performed using the water accommodated fraction of Alaskan oil (WAF) and the chemically enhanced water accommodated fraction of Alaskan oil (CEWAF). The Alaskan WAF is not cytotoxic to sperm whale skin cells though it did induce chromosome damage; S9-mediated metabolism did not affect the cytotoxicity of WAF but did increase the levels of chromosome damage. Alaskan CEWAF is more cytotoxic and genotoxic than the WAF; S9 mediated metabolism increased both cytotoxicity and genotoxicity of CEWAF. Analysis of the PAH content of Alaskan WAF and CEWAF revealed a forty-fold increase in the total levels of PAHs in CEWAF compared to WAF. These findings show that chemically dispersed oil leads to higher levels of PAH exposure which are more toxic and likely to lead to longer and more persistent health effects.

1. Introduction

The Exxon-Valdez oil spill (EVOS) in 1989 was the first major oil spill in US territory. This spill resulted in over 11 million gallons of crude oil released into the relatively pristine environment of Prince William Sound (Deep water, 2011). Attempts to clean up the oil included mechanical collection, burning and chemical dispersants. Chemical dispersants were used on a limited basis but were determined to be fairly ineffective due to ocean conditions, however at the time only small quantities of dispersant were available (EVOSTC, 2015). The difficulties in the EVOS clean-up lead to programmatic changes to oil spill response including the congressional implementation of the Oil Spill Recovery Institute (OSRI) which included stockpiling of chemical dispersants.

In 2010, the Deepwater Horizon Oil Rig exploded resulting in the largest crude oil spill in US history releasing over 168 million gallons (4 million barrels) of crude oil into the Gulf of Mexico (Deep water, 2011). To address the crisis millions of gallons of chemical dispersants were used to break up the oil (Kujawinski et al., 2011). While dispersant use was limited in response to EVOS, there were at least 1.8 million gallons (7 million liters) of dispersant used in response to the Deepwater Horizon Crisis (Wise and Wise, 2011). This raised key questions about the effects of dispersants which had remained understudied since the EVOS incident; in particular, how does dispersing oil impact its longer-term toxicity with concerns that the dispersion makes the oil more bioavailable and thus, more toxic, particularly for marine species in the path of a spill?

In the wake of this crisis, this question has garnered some new insights with data indicating that dispersing oil can indeed increase its longer-term toxicity. For example, oil-dispersant mixtures increased lethality up to 52-fold in rotifers (Rico-Martínez et al., 2013). In a different aquatic invertebrate model, Almeda et al. (2014) found that oil-dispersant mixture (Corexit 9500) was 1.6 times more toxic than crude oil alone to copepods in regards to egestion and fecundity. Similarly, a study by Goodbody-Gringley et al. (2013) reported that oil-dispersant mixtures using Corexit 9500 significantly decreased settlement ability and survival for two species of coral larvae. Dispersed oil also decreased innate immunity in a variety of tissues from sea bass with effects on lysosome concentration and inhibited superoxide dismutase (Dussauze et al., 2015). Chemically dispersed oil (CEWAF) also reduced larval spotted seatrout growth more than the water accommodated fraction of oil alone (Brewton et al., 2013). In a human health

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model, oil-dispersant mixtures using Corexit 9500 and Corexit 9527 induced more cytotoxicity in human lung epithelial (A549) cells than oil alone (Wang et al., 2012).

By contrast, while data are emerging for humans and various lower order aquatic species, no studies have considered dispersed oil toxicity in marine mammal models despite their known presence in the area of both the EVOS and the Deepwater Horizon incidents. Furthermore, there is a suggestion that marine mammals may suffer long term population impacts from oil spills. For example, photographs from the EVOS incident show orcas from two distinct pods, one a resident population and the other a transient population, swimming in the oil. In the year after the EVOS these two pods had suffered a loss of 33 and 41% of their populations, respectively (Matkin et al., 2008). In a population study, sixteen years later, these populations had still not recovered to their pre-spill numbers. In fact, the transient pod has not successfully reproduced since the spill and is now listed as a depleted population under the Marine Mammal Protection Act (Matkin et al., 2008). Although, there is no way to definitively contribute these deaths and reproductive effects directly to the oil spill, it highlights the need to better understand the impacts of oil and dispersed oil on whales.

The challenge to studying whale toxicity is the access to appropriate species specific models for controlled toxicity studies as it is not technically feasible to expose groups of large whales to different doses of chemicals. Species specific data can, however, be gleaned from cell culture models as we have demonstrated in a series of papers considering dispersant (Wise et al., 2014) and metal toxicity in cells from large whale species (Li Chen et al., 2009a, 2009b; Wise et al., 2015; Wise et al., 2011). This approach is also commonly used in human studies and for insights into the impact of dispersed oil on humans as shown in the Wang et al. study of cultured human lung cells (Wang et al., 2012). Thus, in our study, we investigate the genotoxic and cytotoxic impacts of Alaskan crude oil and chemically dispersed Alaskan crude oil on sperm whale skin cells. Our data show that chemical dispersants increase the toxicity of Alaskan crude oil to sperm whale cells.

2. Materials and methods

2.1. Materials

All plasticware was manufactured by BD Falcon. Thermo scientific manufactured the microscope slides. Dulbecco's Phosphate-Buffered Saline (DPBS), Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM-F12), Glutagro supplement and Nicotinamide Adenine Dinucleotide Phosphate solutions (NADPH) were purchased from Corning. Potassium chloride, demecolcine and sodium chromate were produced by Sigma/Aldrich. Crystal violet, methanol and acetic acid were purchased from JT Baker. Gurr's Buffer, trypsin, penicillin-streptomycin and sodium pyruvate were purchased from GIBCO Invitrogen Corporation. Cosmic calf serum was purchased from Hyclone. Giemsa Stain was purchased from Rica Chemical Co. S9 fractions were purchased from Celsis In Vitro Technologies. Alaskan crude oil was generously provided by the Prince William Sound Regional Citizens Advisory Council (PWSRCAC). Corexit 9527A was generously provided by the Nalco Holding Company.

2.2. Cell culture

Primary skin fibroblast cells were obtained from a skin biopsy of a free ranging adult female sperm whale off the coast of North Carolina. Tissue explants were cut into small pieces using a scalpel then placed in a T-25 flask with media. Media was prepared with DMEM/F-12 containing 15% Cosmic calf serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.1 mM sodium pyruvate. Cells were grown at 33 °C in a humidified incubator with 5% CO_2. Fibroblast cells begin growing on the flask from the tissue explants within a couple of weeks. Cells were fed three times per week until they grew to a sub-confluent monolayer; they were sub-cultured weekly.

2.3. Chemical and S9 fraction preparation

2.3.1. Water accommodated fraction of crude oil and chemically enhanced water accommodated fraction of crude oil

The water accommodated fraction (WAF) of crude oil and chemically enhanced water accommodated fraction (CEWAF) of crude oil were prepared using the Hodson Lab SOP, which followed the method of Singer et al. (2000). Briefly, 10 mL of Alaskan crude oil and 90 mL of water (1:9 oil:water ratio) were added into a 250 mL side-arm flask. The flask was placed on a magnetic stir plate with a 0.5 in Teflon coated magnetic stir bar. The WAF was spun for 18 h in the dark, with speed such that the vortex formed in the oil was 1/3 the height of the water. After 18 h the stir plate was turned off and the oil/water mixture was allowed to settle for 1 h; the bottom layer of the WAF was collected. CEWAF was prepared the same way as WAF, with the exception that after 18 h of stirring, 1 mL of Corexit® 9527 (Nalco Holding Company, Naperville, Illinois) was added. After 1 h of additional stirring, the stir plate was shut off and the CEWAF was allowed to settle for 1 h, after which time the bottom layer was collected in the same way as the WAF. All treatments were made from the same sample of Alaskan crude oil. Both WAF and CEWAF were prepared fresh for each independent experiment. Cells were treated with WAF and CEWAF based on a percent of total volume of extracellular media; concentrations of 0, 0.5, 1, 5, 10 or 20% were used.

2.3.2. S9 fractions

Since fibroblast cells may not express cytochrome P450 to metabolize organic compounds, we induced phase one metabolism using S9 fractions to determine if there is a difference in toxicity between the parent compound and the metabolite. S9 fractions were prepared with 1 × Tris buffer, NADPH regenerating system solution A, NADPH regenerating system solution B, and minipig liver S9 fractions. The mixture was prepared just prior to each treatment. Sodium chromate was used as a positive control for all experiments. It was dissolved in water and filter sterilized.

2.4. Cytotoxicity

We determined the cytotoxicity of WAF and CEWAF using our published methods for a clonogenic assay (Wise et al., 2011). Briefly, we plated cells into 6 well tissue culture plates. Each treatment dose was applied with and without S9 fractions 48 h after initial seeding. After 24 h exposure, cells were reseeded at colony forming density into each of four 100 mm tissue culture dishes coated with 0.01% gelatin. Dishes were fed every 5 days until cell colonies formed. Colonies were stained after a 1 × phosphate-buffered saline rinse and subsequent methanol fixation, followed by crystal violet staining. The number of colonies per dish was counted then averaged per dose and treatments were compared to controls. A minimum of three independent experiments for each treatment were performed.

2.5. Clastogenicity

To determine the genotoxicity of the WAF and the CEWAF we used a chromosomal aberration assay based on our published methods (Wise et al., 2011). Briefly, cells were seeded in 100 mm tissue culture dishes. After 48 h they were treated with either WAF or CEWAF for 24 h with or without S9 fractions. Cells were arrested in metaphase using 0.1 g/ml demecolcine applied 5 h before the end of the treatment period. After the treatment period, cells were collected and resuspended in a 0.075 M potassium chloride hypotonic solution (KCl) for 17 min then fixed with 3:1 methanol:acetic acid. The fixative was changed twice. Then cells were dropped onto microscope slides and solid stained with 5% Giemsa stain in Gurr's Buffer. 100 metaphases per dose were