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Inhibition of ABC transport proteins by oil sands process affected water

Hattan A. Alharbi [a], David M.V. Saunders [a], Ahmed Al-Mousa [b], Jane Alcorn [a,b], Alberto S. Pereira [c], Jonathan W. Martin [c], John P. Giesy [a,d,e,f,g,h,*], Steve B. Wiseman [a,*]

[a] Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada
[b] College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada
[c] Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, Edmonton, AB, Canada
[d] Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada
[e] Zoology Department, Center for Integrative Toxicology, Michigan State University, East Lansing, MI, USA
[f] School of Biological Sciences, University of Hong Kong, Hong Kong Special Administrative Region, People's Republic of China
[g] Department of Biology, Hong Kong Baptist University, Kowloon, Hong Kong Special Administrative Region, People's Republic of China
[h] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China

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ABSTRACT

The ATP-binding cassette (ABC) superfamily of transporter proteins is important for detoxification of xenobiotics. For example, ABC transporters from the multidrug-resistance protein (MRP) subfamily are important for excretion of polycyclic aromatic hydrocarbons (PAHs) and their metabolites. Effects of chemicals in the water soluble organic fraction of relatively fresh oil sands process affected water (OSPW) from Base Mine Lake (BML-OSPW) and aged OSPW from Pond 9 (P9-OSPW) on the activity of MRP transporters were investigated in vivo by use of Japanese medaka at the fry stage of development. Activities of MRPs were monitored by use of the lipophilic dye calcein, which is transported from cells by ABC proteins, including MRPs. To begin to identify chemicals that might inhibit activity of MRPs, BML-OSPW and P9-OSPW were fractionated into acidic, basic, and neutral fractions by use of mixed-mode sorbents. Chemical compositions of fractions were determined by use of ultrahigh resolution orbitrap mass spectrometry in ESI^+ and ESI^- mode. Greater amounts of calcein were retained in fry exposed to BML-OSPW at concentration equivalents greater than 1× (i.e., full strength). The neutral and basic fractions of BML-OSPW, but not the acidic fraction, caused greater retention of calcein. Exposure to P9-OSPW did not affect the amount of calcein in fry. Neutral and basic fractions of BML-OSPW contained relatively greater amounts of several oxygen-, sulfur, and nitrogen-containing chemical species that might inhibit MRPs, such as O^+, SO^+, and NO^+ chemical species, although secondary fractionation will be required to conclusively identify the most potent inhibitors. Naphthenic acids (O_2^-), which were dominant in the acidic fraction, did not appear to be the cause of the inhibition. This is the first study to demonstrate that chemicals in the water soluble organic fraction of OSPW inhibit activity of this important class of proteins. However, aging of OSPW attenuates this effect and inhibition of the activity of MRPs by OSPW from Base Mine Lake does not occur at environmentally relevantconcentrations.

1. Introduction

In the surface mining oil sands industry extraction of bitumen from oil sands generates oil sands process affected water (OSPW) that is retained on-site in tailings ponds and settling basins that, as of 2009, covered an area of approximately 170 km^2 (Government of Alberta, 2011). Because oil sands mining companies do not discharge OSPW to the wider environment, the volume of OSPW

* Corresponding authors at: Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada. Fax: +1 306 966 4796.
E-mail addresses: jgiesy@aol.com (J.P. Giesy), steve.wiseman@usask.ca (S.B. Wiseman).

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stored in tailings ponds will increase as surface mining operations expand. Therefore, methods to remediate OSPW are needed. One strategy companies are exploring for remediation and reclamation of OSPW is use of end pit lakes (EPLs) constructed by filling mined-out pits with products of the extraction of bitumen, including OSPW (Gosselin et al., 2010). The expectation is that toxicity of OSPW in EPLs will decrease because of biodegradation of chemicals in the water soluble organic fraction of OSPW (Del Rio et al., 2006; Han et al., 2008, 2009), and that EPLs will eventually be capable of sustaining life.

The chemistry of tailings ponds is complex. Liquid tailings are a mixture of water, residual bitumen, sand, silt, and inorganic and organic compounds. Over time particulates (silt and clay fractions) settle to form a layer of mature fine tailings (MFTs), leaving behind an aqueous layer of OSPW. Two constituents of tailings ponds that have the potential to cause toxicity to aquatic organisms are polycyclic aromatic hydrocarbons (PAHs) and acid-extractable organic compounds, including naphthenic acids (NAs; $C_nH_{2n+z}O_2$), in the water soluble organic fraction of OSPW. Concentrations of individual lower molecular mass PAHs range from 10 to 330 ng/L in porewater of MFTs (Madill et al., 1999) and total concentrations of PAHs range from 1150–1600 ng/L in the upper clarified zone of OSPW (Rogers et al., 2002; Galarneau et al., 2014). The water-soluble organic fraction of OSPW has been described as a "supercomplex mixture" (Jones et al., 2012). Much of the characterization of the water-soluble organic fraction has focused on NAs, but advances in ultra-high-resolution mass spectrometry have identified a variety of oxygen-, sulphur- and nitrogen-containing compounds in this mixture (Barrow et al., 2010; Pereira et al., 2013; Morandi et al., 2015).

Several mechanisms by which OSPW could cause toxicity have been identified. OSPW that is fresh causes acute lethality, and it has been proposed that the mechanism of this effect is narcosis (Frank et al., 2008; Scarlett et al., 2013; Morandi et al., 2015). Also, OSPW causes a variety of sub-lethal effects, including endocrine disruption (Lister et al., 2008; He et al., 2010, 2011, 2012a; Van den Heuvel et al., 2012; Kavanagh et al., 2011, 2012, 2013; Leclair et al., 2015), oxidative stress (He et al., 2012b; Wiseman et al., 2013a,b), and alterations to immune function (Garcia-Garcia et al., 2011; McNeill et al., 2012; MacDonald et al., 2013; Hagen et al., 2014). In many of these studies effects were caused by the water-soluble organic fraction of OSPW.

A variety of natural and synthetic chemicals can inhibit members of the ATP (energy-dependent efflux pumps)-binding cassette (ABC) superfamily of transporter proteins. These inhibitors might not be toxic themselves, but might cause toxicity of other chemicals by inhibition of these transporter proteins, a process known as chemosensitization (Smital and Kurelec, 1997; Kurelec et al., 2000; Ferreira et al., 2014; Kurth et al., 2015). ABC proteins are important for detoxification of xenobiotics because they actively transport a variety of structurally diverse chemicals, and their metabolites, from cells thereby protecting organisms from adverse effects (Leslie et al., 2005; Klaassen and Lauren, 2010; Hessel et al., 2013). In teleost fishes, PAHs and their metabolites are transported from cells by multidrug resistance-associated proteins (MRP) 1–6 (ABCC1–6) (Bard, 2000; Ferreira et al., 2014; Luckenbach et al., 2014). Although it is not known if constituents of OSPW inhibit ABC proteins, water-soluble fractions of crude oil inhibit ABC transporters in larvae of the marine invertebrate, the fat innkeeper (*Urechis caupo)* (Hamdoun et al., 2002). If constituents of OSPW inhibit activity of MRPs it could exacerbate accumulation and effects of PAHs or their bio-activated metabolites on aquatic organisms. Therefore, the objective of this study was to determine if the water soluble organic fraction of OSPW affects the activity of MRPs by use of a model species of teleost fish, the Japanese medaka (*Oryzias latipes*). Also, semi-quantification of chemicals in fractions by use of ultra-high resolution orbitrap mass spectrometry, were performed to identify classes of chemicals in OSPW that might cause effects on activity of MRPs.

2. Materials and methods

2.1. Chemical, reagents, and OSPW

MK-571, an inhibitor of MRPs (Fischer et al., 2013; Zaja et al., 2007), was purchased from Cayman Chemical Company (Anne Arbor, MI, USA) and calcein-AM was from AAT Bioquest (Sunnyvale, CA, USA). Dimethylsulfoxide (DMSO) and trypan blue were from the Sigma Chemical Company (St. Louis, MO, USA). All solvents used were of analytical grade. Two samples of OSPW were collected on the site of Syncrude Canada, Ltd. (Fort McMurray, AB, Canada). One sample was from Base Mine Lake (BML-OSPW), which is an end-pit-lake constructed from the West-In-Pit settling basin that received input of tailings from the main extraction facility. The other sample was from an experimental reclamation pond called Pond 9 (P9-OSPW) that was constructed in 1993 and has not received input of OSPW since that time. Both samples were collected in September of 2012, shipped to the University of Saskatchewan (Saskatoon, SK, Canada), and used for fractionation immediately upon arrival.

2.2. Fractionation of OSPW

Both samples of OSPW were fractionated into acidic, basic, and neutral fractions of polar organic compounds by use of mixed-mode sorbents (MMS). Prior to fractionation 500 ml of each sample of OSPW was passed through a glass microfiber filter (GF/D 0.47 mm, Whatman) to remove any particulate matter, then acidified to pH 2 by use of concentrated HCl (37%). Next, for isolation of basic fractions, pre-concentration was performed in one step by use of 500 mg of mixed-mode Strata®-X Polymeric-C solid-phase sorbent in plastic cartridges (Phenomenex, Milford, MA, USA). This matrix is a porous copolymer with a weak mixed-mode cation that provides dual modes for the retention and adsorption of lipophilic and hydrophilic compounds was well as ionic compounds. Before addition of OSPW cartridges were conditioned with 6 mL of methanol and 6 ml of acidified water. The 500 ml of filtered and acidified OSPW was passed through the cartridges under vacuum. Next, cartridges were washed with 2% (v/v) of formic acid and were allowed to dry under vacuum for 30 min. The first elution was performed with 100% of methanol and this extract contained acidic and neutral compounds. The second elution was performed with 5% (v/v) of NH_4OH in methanol and this fraction contained basic compounds. To separate acidic and neutral compounds a pre-concentration of samples was performed by use of Strata®-X-A 500 mg solid-phase matrix in plastic cartridges (Phenomenex). This polymeric sorbent is water wetable and provides dual modes of retention – anion exchange and reversed phase. Prior to use the cartridge was conditioned by washing with 100% methanol followed by 5% (v/v) of NH_4OH (aq). Next, elutant I from the Strata®-X Polymeric-C sorbent was evaporated to approximately 0.5 ml, adjusted to a pH of 10–11 with NaOH, and then passed through the cartridge without vacuum. Cartridges were washed with 5% (v/v) of NH_4OH (aq) and left to dry under vacuum for 30 min. Finally, the fraction containing neutral compounds was eluted with 100% methanol and a fraction containing acidic compounds was eluted with 2% (v/v) of formic acid in methanol. Fractions were dried, and reconstituted in ethanol for bioassays. A pooled sample representative of the organics fraction was generated by pooling equal volumes of the acidic, neutral, and basic fractions. Blank samples, which were city of Saskatoon municipal tap water, were extracted by use of this method.