Toxicity of Polycyclic Aromatic Compounds in Oil Sands: A Metabolomics Study

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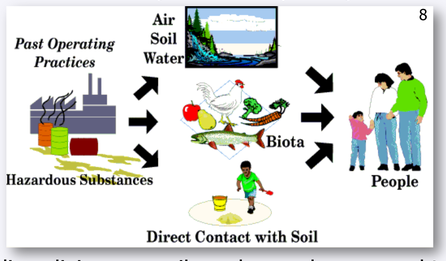
Introduction

- The Alberta bituminous sands naturally contain Polycyclic Aromatic Compounds (PACs): organic, colorless molecules composed of 2-100 linked rings.
- PACs are the by-products of the incomplete combustion of coal, petroleum, wood, and used tires.
- PACs are also associated with increased risk of chronic obstructive pulmonary disease and lung cancer[1] (Yang et al., 2017), oxidative stress with increased disease susceptibility in liver[2] (Bravo et al., 2011), and also behavioural deficits[3] (Brown et al., 2016).



- Canadians living near oil sands may be exposed to PACs through contaminated water, air, and soil, especially the aboriginal population who hunt and fish from the Athabasca River.
- The mechanism of PAC-induced damage is not well understood at the molecular level, let alone the synergistic effect of mixtures of PACs
- The goal of this study is to determine the cellular pathways of toxicity of acute exposure to a mixture PACs extracted from Fort MacKay, AB.

Methods

1. Cell culture

Human lung adenocarcinoma (A549) and human liver cancer (HepG2) cells were maintained in Dulbecco's media supplemented with FBS, penicillin, and streptomycin. The human neuroblastoma SK-N-SH cells were cultured on poly-D-lysine coated flasks and underwent differentiation through the addition of retinoic acid. Media was renewed every two or three days and cells were passaged every 7 days.

2. Dosage and Metabolite Extraction

5.0×10^6 SK-N-SH, A549 cells, and HepG2 cells were each plated on individual 100 mm dishes. The cells were treated with oil sand extract for 24 h. PAC mixtures were sourced from three soil samples from Fort MacKay, AB. Cell numbers were equalized between all groups. Metabolites were extracted into solvent using probe sonication. Solvent was dried under nitrogen flow and re-suspended with 500 µl of Acetonitrile: Methanol: Water (2:2:1) solvent for analysis.

3. Mass spectrometry

Metabolites were analysed using liquid chromatography coupled to the Orbitrap Q Extractive plus mass spectrophotometer for HPLC-MS analysis of peptides in a 100 mm length separation column. 20 µl of a sample were injected and separated by formic acid in a water to methanol gradient with a flow of 250 µl/min.

4. Metabolite Identity

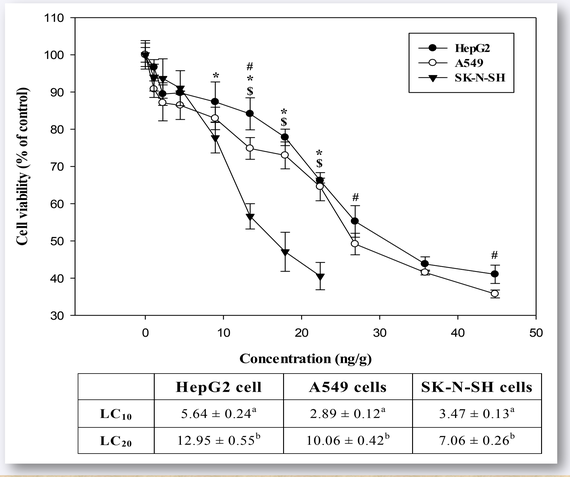
The masses of the detected metabolites were used to determine their molecular formula and were then matched to metabolites using Metlin, human metabolome database, and Chemspider databases. They were then compared with metabolites found in known metabolic pathway maps.

5. Statistical analysis

Using online tools MetaboAnalyst and XCMS, significantly changed metabolites were identified by $p < 0.05$ in t-test and partial least squares-discriminant analysis. Variables that had significant contributions between groups were considered as potential biomarkers.

Results

Chemical Treatment Concentrations



	HepG2 cell	A549 cells	SK-N-SH cells
LC_{10}	5.64 ± 0.24^a	2.89 ± 0.12^a	3.47 ± 0.13^a
LC_{20}	12.95 ± 0.55^b	10.06 ± 0.42^b	7.06 ± 0.26^b

Fig. 1. HepG2, A549 and SK-N-SH cells were treated with different concentrations of oil sand extracts for 24h. MTT assay were performed and lethal concentrations (LC) were calculated using a dose-response curve. Results were expressed as percentage of corresponding controls.

HepG2 Metabolic Pathways

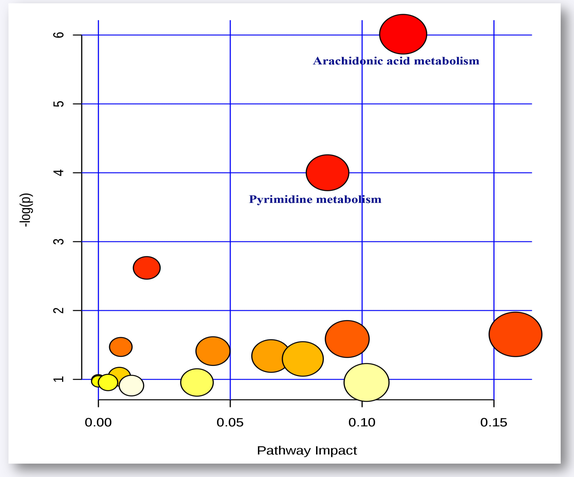


Fig. 3. Metabolomic pathway regulation in HepG2 cells. The cells were treated with the different concentration of PACs for 24h. Data are expressed as $p<0.001$ versus control.

SK-N-SH Metabolic Pathways

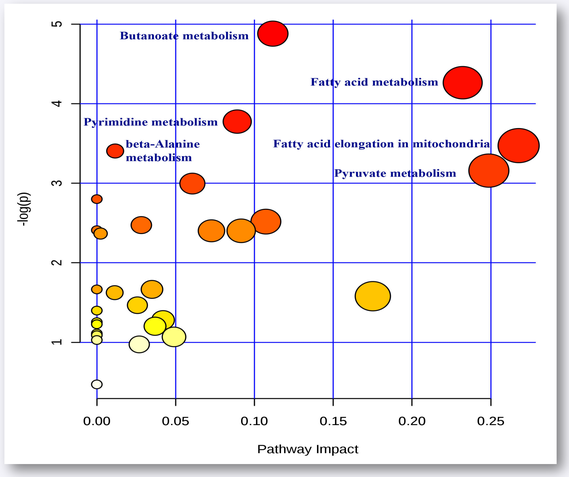


Fig. 2. Metabolomic pathway regulation in SK-N-SH cells. The cells were treated with the different concentration of PACs for 24h. Data are expressed as $p<0.001$ versus control.

A549 metabolic pathways

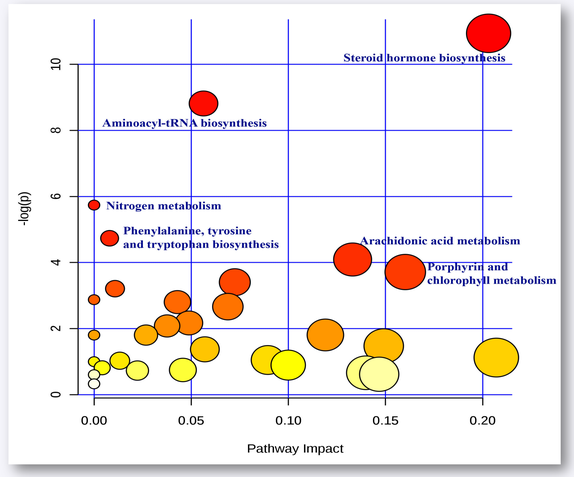


Fig. 4. Metabolomic pathway regulation in A549 cells. The cells were treated with the different concentration of PACs for 24h. Data are expressed as $p<0.001$ versus control.

Key Findings

- Cytotoxicity results show that alveolar cells had the lowest LC10, at 2.89 ng/g.
- The majority of the upregulated metabolites are amino acids : L-cysteine, L-leucine, L-glutamine, L-lysine, L-histidine, L-phenylalanine, L-tyrosine.
- In the hepatic cells, significant alterations were found in the arachidonic acid metabolism. Arachidonic acid is activated by the cytochrome P450 family enzymes in the liver, and is transformed into a variety of metabolites to modulate inflammatory reactions.[4]
- Exposure to PACs in SK-N-SH cells was mainly involved with the butanoate and the fatty acid metabolism. Neurons have a unique lipid composition, which are associated with neurological diseases through dysregulated lipid metabolism.[5]
- With 8 potential metabolites significantly up-regulated in the steroid hormone biosynthesis, this pathway was the most affected in alveolar cells. Sex hormones are increasingly recognized as regulators of lung development[6] and responsible for major lung diseases[7].
- Further studies should focus on the novel pathways employed by estrogens, progestogens, androgens, and their metabolites in normal lung function.
- These findings provide biochemical pathways for PAC-induced toxicity in humans as well as potential biomarkers for its detection.

Steroid hormone biosynthesis

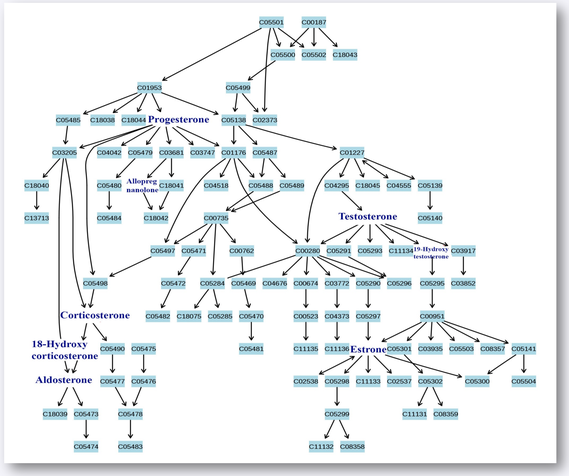


Fig. 5. Steroid hormone biosynthesis pathway in A549 cells. Significantly regulated metabolites by acute PAC exposure ($p<0.001$ versus the control) are in blue.

References

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