Potential human health effects of phycotoxins in marine bioaerosols

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Oceans and mankind are unequivocally linked as oceans contribute, both directly and indirectly, to human welfare. Owing to their sheer size and productivity, oceans play a significant role in the functioning of planet Earth (Fleming et al. 2014; Moore, Baker-Austin, et al. 2013). On a global scale, oceans drive the climate, weather systems and work as a buffer against the rising atmospheric concentrations of carbon dioxide (CO2). They provide mankind with key services such as the production of food, material resources and novel pharmaceuticals (Moore, Baker-Austin, et al. 2013; Fleming et al. 2014; Moore, Depledge, et al. 2013). An intangible benefit credited to oceans, related to coastal proximity, is a general sense of wellbeing and good health. Moore (2015) recently proposed an immuno-regulatory mechanism to explain the ocean's influence on human wellbeing. He suggests that the wide variety of airborne biogenic compounds in blue and green environments (i.e. polyphenolics, antibiotics, algal and bacterial toxins) have an inhibitory effect on the activity of the phosphatidylinositol-3 kinase/protein kinase B/mechanistic target of rapamycin complex 1 (PI3K/Akt/mTORC1) cell signalling pathway. This should exert beneficial health effects since the augmented activity of this pathway is related to several pathological conditions (i.e. cancers, diabetes, inflammation, immunosuppression, and neurodegenerative diseases).

Phycotoxins, toxins produced by microalgae, are one the many groups of biogenic compounds that are incorporated in marine aerosols. Depending on the type and dosage, these bioaerosols may contribute to positive or negative human health effects. Ingestion of phycotoxin-contaminated shellfish, also known as shellfish poisoning, is the best known exposure route in case of adverse effects (James et al. 2010). Literature also confirms that inhalation of high doses aerosolised phycotoxins, particularly brevetoxins, from sea spray may cause respiratory irritation and other adverse health effects in humans and mammals (Pierce et al. 2003; Kirkpatrick et al. 2008). However, no knowledge is available on the potentially positive health effects caused by exposure to low doses of these aerosolized toxins.

This research aims to explore and assess whether phycotoxins, in marine bioaerosols, positively affects human health through the inhibition of the PI3K/Akt/mTORC1 system. We hypothesised that low concentrations of phycotoxins elicit a positive human health effect by downregulating the PI3K/Akt/mTORC1 pathway activity. As a first step, a broad concentration range was first examined with cell viability assays (43 h). In this way cytotoxicity and the effects on cell proliferation of two specific phycotoxins, yessotoxin (YTX) and homoYTX (hYTX), on two human lung cell lines were assessed. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was used as a marker for cell viability by measuring mitochondrial activity. The test is based on the colorimetric reaction of viable cells converting MTT (yellow reagent) to formazan (purple crystals). Since this research is concerned with realistic environmental doses humans encounter along the coast, both low and high concentration levels were tested. This was to determine test concentrations where an adverse effect would be experienced (higher test concentrations) and concentrations that could potentially benefit human health (lower test concentrations). Upon determination of the adverse effect concentrations in terms of viability, Western blot analysis was used to assess the PI3K/Akt/mTORC1 pathway activity by studying the effects of low YTX concentrations on four downstream effector proteins of mTORC1. The phosphorylation of these four downstream effectors (i.e. S6RP, Akt, S6K and 4E-BP1) was used as a proxy for the pathway activity. Demonstrating the bioactivity of YTX on these effector proteins, specifically the downregulation of the pathway, may give insights into the potential health benefits accrued from exposure to low concentrations of aerosolised phycotoxins in coastal environments.

YTX and its analogue hYTX exhibited a reduction in cell viability in the two lung cells lines. A 43 h exposure of the alveolar epithelial cell line (A549) resulted in EC50 values of 4.34 ± 0.75 μg.L-1 (YTX) and 2.48 ± 1.15 μg.L-1 (hYTX). The bronchial epithelial cells (BEAS-2B) exhibited a higher sensitivity to YTX (43 h EC50 = 3.40 ± 0.63 μg.L-1). To predict environmental effect concentrations, these in vitro effect concentrations were extrapolated to real air concentrations. Since the median effect concentration (EC50) is an extreme endpoint where viability is already affected, 43 h EC10 values were used instead for this purpose. The bronchial epithelial tissue should show a higher sensitivity

to YTX (81.52 ng.m-3) than alveolar tissue (90.68 μg.m-3). This is mainly because the bronchial surface area in human lungs is much smaller than the alveolar surface area. The calculated environmental effect concentrations for the bronchial tissue are of the same magnitude as brevetoxin (PbTx-2) concentrations in the sea air measured during harmful algal blooms, found to be roughly 180 ng.m-3 (Pierce et al. 2003). Following on the cell viability assays, two major experiments were conducted using Western blotting to examine the effect on the PI3K/Akt/mTORC1 pathway activity. The first experiment looked at the effect of YTX on the downstream effector proteins in the pathway for both cell lines, BEAS-2B and A549. The highest YTX concentration (1 μg.L-1) used in these experiments was approximately equal to the 43 h EC50 found in the cell viability tests. The second major experiment investigated the effects of pure hYTX and of an experimentally generated aerosol hYTX extract (Baelus 2017) on the previously described effector proteins of the PI3K/Akt/mTORC1 pathway for the A549 cell line. The highest toxin concentration used in this experiment was 0.5 μg.L-1 since a limited amount of the hYTX aerosol extract was available. The phosphorylation of the downstream effector proteins as a consequence of active mTORC1, was used as a proxy for PI3K/Akt/mTORC1 activity.

When compared to the negative control, lower PI3K/Akt/mTORC1 activities ($p<0.05$) were observed for all toxins with three biomarkers, for at least one concentration treatment. One biomarker, Akt, exhibited an upregulation in phosphorylation when treated with YTX for the BEAS-2B cell line. This could be due to the ability of mTORC2 to positively regulate Akt (Dalle Pezze et al. 2012) or disrupted feedback regulation as noted by Korets et al. (2014). This research, as well as prior work within this specific research by Van Hal (2016), indicates that phycotoxins such as YTX and hYTX indeed have the capability of downregulating parts of the PI3K/Akt/mTORC1 pathway.

Most interesting, the concentration where the highest PI3K/Akt/mTORC1 downregulation effect is observed, only induces a partial decrease in cell viability (60-80%). This partial effect on cell viability is different as compared to prior in-house cell viability experiments on phycotoxins other than YTX, where there was a full cytotoxic effect (i.e.100% mortality) at the highest concentration treatments. Both inhibition in cell proliferation or an augmented induced cell death related to an inhibited PI3K/Akt/mTORC1 pathway could explain this observation. Further research is however needed to show as from which concentration this downregulation begins to have a significant effect, to ultimately support the biogenics hypothesis for the first time with this kind or experimental data.

Keywords: bioaerosols; biogenics hypothesis; yessotoxin; PI3K/Akt/mTORC1; harmful algal blooms