Immunotoxicity of benzothiazole on Mytilus edulis following in vitro exposure of hemocytes

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Introduction

In recent years, a wide array of industrially-made substances have been classified as emerging organic pollutants due to their omnipresence in the aquatic environment.¹,² Many environmental pollutants are the result of improper use and disposal and they are not properly eliminated by sewage treatments.¹,² Benzothiazole (C₇H₇NS) is an aromatic heterocyclic molecule formed with a 1,3-thiazole ring linked to a benzene group.¹,³ Benzothiazole (BTs) possess a vast array of derivate molecules used in many consumer products ranging from rubber (tires), anticorrosive agents, as fungicide in the paper and leather industry and it is also commonly found in a wide range of household products.¹,²,³,⁵ BTs are not very soluble in water and are thus found in large quantities in urban particulate matter.³ The remarkably stable chemical properties and the slow degradation rate of BTs contribute to their popularity and ubiquity in the aquatic environment.¹,² Human exposure to benzothiazole is widespread, as it is also been documented bottled water, food flavors, tea leaves and tobacco smoke.⁴ Some benzothiazole derivate molecules have been identified as carcinogenic agents in humans or have been associated with mutagenicity in different microorganisms.⁵,⁶ Some studies have even shown that BTs derivate molecules can accumulate in the human adipose tissues.⁴ There is a clear lack of knowledge on the toxicity levels of benzothiazole and its derivate on aquatic environments. The goal of this study is to monitor the immunotoxicity of benzothiazole on the hemocytes of the blue mussel (Mytilus edulis) following an in vitro exposure.

Materials and Methods

Hemocytes collection

A total of 14 blue mussels (M. edulis) were collected in Mitis, 900 Route de la Mer, Ste-Flavie, Québec, Canada, G5H 3Z4, ISMER - Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, Québec, Canada, G5L 3A1. E-mail: Catherine.cote02@uqar.ca

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Key words: Immunotoxicity; benzothiazole; Mytilus edulis.

Conference presentation: part of this paper was presented at ECOBIM meeting, 2015 May, Québec City, Québec, Canada.

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Journal of Xenobiotics 2015; 5:5771
doi:10.4081/exeno.2015.5771

Results

For the ethanol dose-response curve, results are presented in Figure 1. There are no significant differences in viability except for hemocytes exposed to 7.5 and 10% (V/V) of ethanol when compared to hemocytes from control group. Phagocytic activity and efficiency did not appear to be significantly affected by ethanol. For the benzothiazole response-curve, the results are presented in Figure 2. Due to the poor solubility of BT, the first working dilution of BT (10⁻³ M) contained 10% final concentration of ethanol. Due to poor hemocyte viability at this con-
concentration, the phagocytosis was not measured. For the others concentrations tested, the ethanol content was 0.1% and lower. No significant differences could be observed in phagocytic activity and efficiency as well as in viability of the hemocytes exposed from $10^{-9}$ up to $10^{-3}$ M of benzothiazole for a period of 21 h.

Discussion and Conclusions

Benzothiazole has been quantified in aquatic environments by many laboratories. Herrero et al., analyzed the occurrence of benzothiazole and its derivatives in a few locations such as Greece, Germany, Spain and China. The highest level of contamination was found in effluent sewage water at a concentration of 12 $\mu$g/L in Germany. The exposure concentrations in this study ranged from concentrations below the values found by Herrero et al., to 100 times higher than the concentration of benzothiazole found in the environment and no effect could be found on the phagocytic capacity and viability of hemocytes in blue mussels. The only effects observed were a very high mortality rate of hemocytes exposed to $10^{-3}$ M of benzothiazole. However, because the solution contained 10% V/V of ethanol and the dose-response curve showed similar cytotoxicity at the same concentration, it is impossible to discriminate between the effects related to the ethanol or the possible effects of benzothiazole. Benzothiazole has low solubility and most studies only analyze the dissolved fraction of benzothiazole in waste water. Some studies have shown that BTs compounds are found at higher concentration in the sewage sludge (up to 265 ng/g d.w.) than in the water column. Those information suggest that although the molecules are very stable, once released in the environment, BTs can undergo processes; such as adsorption to sediment. More studies need to be done to confirm this theory because data on the behavior of benzothiazoles in the water column are scarce. Some studies suggest that BTs are not prone to bioaccumulate but recent studies have shown that derivate from benzothiazoles have been found in human adipose tissues suggesting otherwise. Due to the high concentrations of those pollutants found in the sewage sludge, studies should be done to determine whether or not benthic marine organisms could be affected by benzothiazole including its metabolites.

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