Aquatic Toxicology 177 (2016) 171–181

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

The fatty acid profile of rainbow trout liver cells modulates their tolerance to methylmercury and cadmium

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ARTICLE INFO

Article history:
Received 15 November 2015
Received in revised form 12 May 2016
Accepted 22 May 2016
Available online 23 May 2016

Keywords:
Methylmercury
Cadmium
Polyunsaturated fatty acids
Fish
RTL-W1 cells
Cytotoxicity

ABSTRACT

The polyunsaturated fatty acid (PUFA) composition of fish tissues, which generally reflects that of the diet, affects various cellular properties such as membrane structure and fluidity, energy metabolism and susceptibility to oxidative stress. Since these cellular parameters can play an important role in the cellular response to organic and inorganic pollutants, a variation of the PUFA supply might modify the toxicity induced by such xenobiotics. In this work, we investigated whether the cellular fatty acid profile has an impact on the *in vitro* cell sensitivity to two environmental pollutants: methylmercury and cadmium. Firstly, the fatty acid composition of the rainbow trout liver cell line RTL-W1 was modified by enriching the growth medium with either alpha-linolenic acid (ALA, 18:3*n*-3), eicosapentaenoic acid (EPA, 20:5*n*-3), docosahexaenoic acid (DHA, 22:6*n*-3), linoleic acid (LA, 18:2*n*-6), arachidonic acid (AA, 20:4*n*-6) or docosapentaenoic acid (DPA, 22:5*n*-6). These modified cells and their control (no PUFA enrichment) were then challenged for 24 h with increasing concentrations of methylmercury or cadmium. We observed that (i) the phospholipid composition of the RTL-W1 cells was profoundly modulated by changing the PUFA content of the growth medium: major modifications were a high incorporation of the supplemented PUFA in the cellular phospholipids, the appearance of direct elongation and desaturation metabolites in the cellular phospholipids as well as a change in the gross phospholipid composition (PUFA and monounsaturated fatty acid (MUFA) levels and *n*-3/*n*-6 ratio); (ii) ALA, EPA and DPA enrichment significantly protected the RTL-W1 cells against both methylmercury and cadmium; (iv) DHA enrichment significantly protected the cells against cadmium but not methylmercury; (v) AA and LA enrichment had no impact on the cell tolerance to both methylmercury and cadmium; (vi) the abundance of 20:3*n*-6, a metabolite of the *n*-6 biotransformation pathway, in phospholipids was negatively correlated to the cell tolerance to both methylmercury and cadmium. Overall, our results highlighted the importance of the fatty acid supply on the tolerance of fish liver cells to methylmercury and cadmium.

1. Introduction

The release of various pollutants in the environment, the degradation and fragmentation of habitats, the overexploitation of ecosystems as well as global warming have increased the number and intensity of external stressors on aquatic organisms (Dudgeon, 2010; Dudgeon et al., 2006). Shifts in water quality, flow, temperature and nutrient availability are relevant examples of such stressors. By moving an organism away from its optimum performance in terms of growth and reproduction, these stressors may influence survival and fitness (Van Straalen, 2003). While many studies focus on the effect of one stressor in otherwise optimal conditions, responses to a stressor such as chemical pollution are known to be influenced by environmental conditions (*e.g.* temperature, nutritional status) (Holmstrup et al., 2010).

Chemical pollutants are ubiquitous stressors that can strongly degrade aquatic ecosystems (Peters et al., 2013). Among these, methylmercury (MeHg) and cadmium (Cd) occur at elevated concentrations in many aquatic environments and can pose significant

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http://dx.doi.org/10.1016/j.aquatox.2016.05.023
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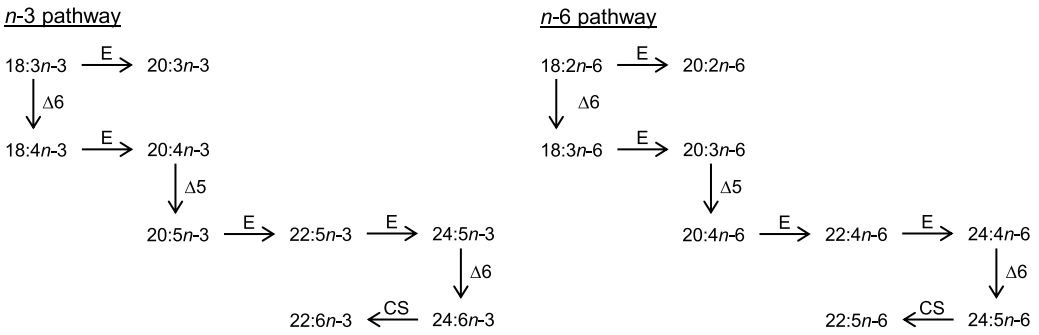

Fig. 1. Alpha-linolenic acid (left) and linoleic acid (right) biotransformation pathways in fish hepatocytes. Abbreviation used: Δ5 and Δ6, microsomal fatty acid desaturase enzyme mediated reactions; E, hepatic microsomal elongase mediated reactions; CS, peroxisomal fatty acid chain shortening.

problems to organisms (Boening, 2000; Pan et al., 2010; Rytuba, 2003). For example, anthropic release of MeHg and Cd in the environment was historically associated with human health and ecological effects in the Japanese Minamata Bay (Harada, 1995) and in the Japanese prefecture of Toyama (Sebastian and Prasad, 2014), respectively. However, the current stock of MeHg and Cd in the environment is mainly due to their direct and indirect use in numerous industrial, agricultural, medical and artistic applications, the combustion of fossil fuels and the incineration of waste (Kidd and Batchelar, 2011; McGeer et al., 2011; Pan et al., 2010; Rytuba, 2003).

MeHg and Cd are persistent in the environment and can bioaccumulate in most aquatic organisms (Kidd and Batchelar, 2011; McGeer et al., 2011). Whilst the aqueous route is most important for Cd exposure, the dietary route accounts for 90% of the MeHg exposure (Hall et al., 1997). MeHg is biomagnified throughout aquatic trophic webs (Lavoie et al., 2013; Rolfhus et al., 2011). In fish, Cd is mainly accumulated in liver and kidneys (McGeer et al., 2011), whereas MeHg is stored in a wide variety of organs including muscle, liver, kidney, spleen, gills, intestine and brain (McGeer et al., 2011). While Cd^{2+} has been shown to enter cells through facilitated diffusion (McGeer et al., 2011), both passive and active transport have been suggested for MeHg cellular uptake (depending on the cell type and on the metal speciation) (Heggland et al., 2009; Kidd and Batchelar, 2011). The two metallic compounds impair growth, reproduction and survival (Kidd and Batchelar, 2011; McGeer et al., 2011; Sevcikova et al., 2011). They alter divalent ions metabolism, induce oxidative stress and trigger apoptosis (Kidd and Batchelar, 2011; McGeer et al., 2011). For instance, MeHg and Cd can alter the activity of many antioxidant enzymes, decreasing the cell ability to cope with reactive oxygen species (Kidd and Batchelar, 2011; Matović et al., 2015; McGeer et al., 2011). Oxidative attacks to lipids may lead to uncontrolled chain-reactions propagating the oxidative damage throughout cell membranes (Catalá, 2012). This is particularly the case with fatty acids with a high degree of unsaturation (polyunsaturated fatty acids (PUFAs) and highly unsaturated fatty acids (HUFAs)) as they are very prone to oxidation.

In fish, the cellular fatty acid profile of tissues such as muscle and liver is highly influenced by both dietary and environmental parameters. Firstly, the fatty acid profile globally reflects that of the diet (Bell et al., 2004; Bell et al., 2001; Bell et al., 2006; Caballero et al., 2002; Fonseca-Madrigal et al., 2005; Mourente and Bell, 2006; Petropoulos et al., 2009). Secondly, environmental conditions such as temperature have also been reported to modulate the fish tissue fatty acid composition (Caballero et al., 2002; Snyder et al., 2012; Wijekoon et al., 2014). These shifts may alter the organism fitness and ability to cope with additional stressors, such as chemical pollutants, as lipids play a central role in fish metabolism. Indeed, they provide metabolic energy for maintenance, growth, reproduction and locomotion, maintain the structure and function of biological membranes and are the precursors of highly bioactive molecules such as the eicosanoids (Tocher, 2003).

As suggested by previous literature, the toxicity of MeHg and/or Cd could target cell properties modulated by PUFA content such as susceptibility to oxidative stress or membrane characteristics. The cellular lipid profile is thus likely to affect sensitivity to Cd and MeHg, particularly in animals with variable lipid profiles such as fish. Despite the potential sites of interaction between lipids and pollutants, the influence of dietary lipids on metallic compounds toxicity has received little attention. A few authors have assessed whether the quality of the lipid supply can modulate the biological response to MeHg (Grotto et al., 2011; Jayashankar et al., 2012; Jin et al., 2008; Jin et al., 2007; Kaur et al., 2008; Kaur et al., 2007; Nøstbakken et al., 2012a; Nøstbakken et al., 2012b; Olsvik et al., 2011; Pal and Ghosh, 2012b; Remø et al., 2011) and Cd (Amamou et al., 2015). Overall, these authors pointed out a protective effect of fish oil and *n*-3 PUFAs against MeHg or Cd toxicity. In general those studies focussed on only few selected *n*-3 PUFAs and on one metallic compound. For instance, the impact of alpha-linolenic acid (ALA, 18:3*n*-3), eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) has been assessed on MeHg toxicity *in vitro* (mouse and rat neural cells, fish and human renal cells) and *in vivo* (brain, plasma, liver and kidneys of rats) but not on Cd toxicity (Kaur et al., 2008; Kaur et al., 2007; Nøstbakken et al., 2012a; Nøstbakken et al., 2012b; Pal and Ghosh, 2012a,b). To date, the impact of *n*-6 PUFAs on metallic compounds sensitivity has received very little attention as only one study investigated the impact of arachidonic acid (AA, 20:4*n*-6) on the cell resistance to MeHg in fish and human renal cells (Nøstbakken et al., 2012a). Comparisons between these studies are difficult as the models and the tested PUFAs and concentrations varied between studies.

In this study, we investigated the effect of *n*-3 and *n*-6 PUFAs on the toxicity induced by both MeHg and Cd, using a cellular model. Liver being an important site of interaction between lipids and metallic compounds as it is the key organ for both detoxification and fatty acid metabolism, we conducted an *in vitro* experiment based on rainbow trout liver cells (RTL-W1 cell line (Lee et al., 1993)). Cells were artificially enriched with a specific PUFA from either the *n*-3 (ALA, EPA or DHA) or the *n*-6 (linoleic acid (LA, 18:2*n*-6), AA or docosapentaenoic acid (DPA, 22:5*n*-6)) pathway (Fig. 1) before being exposed to MeHg or Cd during 24 h. After exposure, cell viability was assessed and linked to the fatty acid profile of the cells.

2. Material and methods

2.1. Cells, culture media and growth conditions

The RTL-W1 cell line derived from the liver of a 4 year old male rainbow trout (Lee et al., 1993) was kindly provided by S. Bony