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Metabolism of okadaic acid by NADPH-dependent enzymes present in human or rat liver S9 fractions results in different toxic effects

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ABSTRACT

The lipophilic marine biotoxin okadaic acid (OA) represents a natural contaminant produced by algae accumulating in seafood. Acute intoxications result in diarrhetic shellfish poisoning causing symptoms like nausea, vomiting and abdominal cramps.

OA was preincubated with liver enzymes present in S9 fractions from humans, rats and rats pretreated with enzyme inducers in the presence or absence of the cofactor NADPH to investigate hepatic metabolism. Cytotoxicity was examined in HepG2 cells and metabolites of OA were determined by LC-MS/MS.

Strong cytotoxicity was observed in HepG2 cells treated with OA that was preincubated in S9 fractions without NADPH. However, neither metabolites nor a decrease of OA itself were found. The addition of NADPH to the S9 fractions of rats resulted in a decreased cytotoxicity of OA, but a stronger toxicity in HepG2 cells was observed from OA preincubated in human S9 fractions with NADPH. Metabolite profiles of each S9 mix revealed that higher amounts of detoxified metabolites were formed by NADPH-dependent enzymes of rats compared to the same enzymes of humans.

These differences in OA detoxification by NADPH-dependent liver enzymes of rats and humans may be of significance in the extrapolation of toxicological data from animal models (rats to humans, for example).

1. Introduction

Dinoflagellates are microalgae known for the production of a wide variety of marine biotoxins. Out of 2000 species of dinoflagellates, almost 100 species have been identified as producers of potent toxins (Daranas et al., 2001; Hallegraeff, 1993). One important representative toxin is okadaic acid (OA), which accumulates in the hepatopancreas of filter-feeding mussels consumed by humans. Intoxication results in diarrhetic shellfish poisoning (DSP) characterised by symptoms like nausea, vomiting and abdominal cramps. DSP is therefore considered to be a public health concern. Seafood consumers can be chronically exposed to low concentrations of OA corresponding to doses below the regulatory limit of 160 µg OA eq./kg shellfish meat. A minimum dose of 50 µg OA can induce diarrhoea in human adults (EFSA, 2010, 2008), while it is assumed that concentrations of OA below this level may be harmless. However, the occurrence of DSP toxins in French coastal waters has been linked to some digestive cancers (e.g. stomach, colon, liver) (Cordier, 2000), and a chronic intake of OA has been associated with an increased risk of colon cancer in Spain (López-Rodas et al., 2006).

In vivo studies with laboratory animals indicated that OA is distributed to several organs by reaching the blood stream after passing the intestinal barrier (Berven et al., 2001; Ito et al., 2002; Matias et al., 1999; Vieira et al., 2013). The passage of OA mainly into the small intestine and liver of mice was reported by Le Hégarat et al. (2006). However, at low non-cytotoxic concentrations there appears to be an active efflux mechanism back into the intestinal lumen as shown in human small intestinal-like Caco-2 cells for doses below the regulatory limit (Ehlers et al., 2011). If OA reaches the portal vein, it is actively transported into the hepatocytes by the organic anion transporters OATP1B3, OATP1B1 and probably by further unknown transporters, suggesting that OA can act as a hepatotoxin (Ikema et al., 2015).

Abbreviations: ACN, acetonitrile; CYP, cytochrome P450 monooxygenase(s); CE, collision energy; CI, cell index; DSP, diarrhetic shellfish poisoning; EDTA, ethylenediamine-tetraacetic acid; eq, equivalent; FCS, fetal calf serum; HPLC–MS/MS, high-performance liquid chromatography-tandem mass spectrometry; HS9, 9000 × *g* supernatant fraction of human liver homogenate, iRS9, 9000 × *g* supernatant fraction of induced rat liver homogenate; MeOH, methanol; NADP, nicotinamide adenine dinucleotide phosphate; OA, okadaic acid; OATP1B1, organic anion-transporting polypeptide 1B1; OATP1B3, organic anion-transporting polypeptide 1B3; RPMI, Roswell Park Memorial Institute medium; RS9, 9000 × *g* supernatant fraction of rat liver homogenate; SPE, solid phase extraction

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The liver represents the main organ for xenobiotic-metabolising processes. In contrast to fresh hepatocytes, immortalised liver cell lines express only limited levels of metabolising enzymes, resulting in low metabolic activities for conversion of xenobiotics into less or more active intermediates – as shown for several genotoxic carcinogens requiring metabolic activation. Genotoxicity of OA has already been investigated in a number of in vitro studies, but the results vary considerably depending on cell type and assay conditions. Genotoxicity of pure OA has been shown in HepG2 cells, leukocytes and SHSY5Y cells (Valdiglesias et al., 2010). To overcome the limitation of reduced metabolic activity in immortalised cell lines of this type, the addition of an exogenous metabolic activation system is often included in genotoxicity assays, e.g. in the form of liver S9 fractions. S9 fractions from male rats (RS9) reduced the genotoxic effects of OA in SHSY5Y cells in the presence of an NADPH-regenerating system, which is required for NADPH-dependent enzyme activity such as cytochrome P450 monoxygenases (CYP). It was therefore assumed that the metabolic products of OA are less active than OA itself (Valdiglesias et al., 2010). However, OA produced chromosomal damage in Chinese hamster ovary cells in the presence of S9 fraction derived from rats pretreated with phenobarbital/β-naphthoflavone (iRS9 mix). These chemicals induce xenobiotic metabolising enzymes like Cyp1a1, 1a2, 3a1, 3a2 or 2b6 (Martignoni et al., 2004; Waxman and Azaroff, 1992). Genotoxic effects of OA have been associated with a metabolism-dependent formation of reactive intermediates by induced enzymes (Le Hegarat et al., 2003). So far, there are no experimental genotoxicity studies using human liver S9 fraction. Cell culture experiments with human CYP1A2-overexpressing HepG2 cells resulted in the formation of micronuclei, demonstrating DNA alterations in the genome of the cells due to the bioactivation of OA (Hashizume et al., 2009).

Although no data is available on comparison of OA metabolism by NADPH-dependent enzymes present in human and rat S9 liver fractions, some species differences in metabolite formation between specific CYP3A enzymes of humans and rats have recently been reported. Co-incubation of HepG2 cells with recombinant CYP enzymes revealed that human CYP3A4/3A5 produced seven OA metabolites with a lower cytotoxic potential than OA itself, whereas a toxification of OA was induced by both human CYP1A2 and rat Cyp1a2 (Kolrep et al., 2016). The molecular structures of oxidative OA metabolites generated by liver CYP enzymes were elucidated only for three OA metabolites (Fig. 1) and described as two monohydroxylated OA metabolites and one OA aldehyde (Liu et al., 2012). The two monohydroxylated metabolites, the aldehyde as well as other oxidative metabolites were detected after incubation of OA with iRS9 mix (Kittler et al., 2010) or with recombinant CYP3A4/3A5 (Guo et al., 2010). Monohydroxylated OA metabolites were also detected after incubation of OA with rat Cyp3a1/3a2 enzymes, but formation was lower when compared to human CYP3A4/3A5 enzymes (Kolrep et al., 2016). Apparently, the toxicity of OA depends not only on cell type, concentration or assay but also on the species-specific metabolic capacity of liver enzymes to detoxify OA into oxidative metabolites.

In general, toxicological data obtained from rodents used as laboratory animals or liver preparations from these animals do not always correctly predict human outcomes due to species differences in the expression of xenobiotic-metabolising enzymes. In our study, we therefore focused on the evaluation of species differences in metabolic activation of OA by using S9 fractions from livers of rats, rats pretreated with inducers of xenobiotic-metabolising enzymes and humans. Cytotoxicity of OA represents an appropriate endpoint to determine species differences in terms of metabolism. Several toxicity tests were chosen to analyse the cellular effects of species-specific metabolic activation of OA by S9 fractions in the absence and presence of the cofactor NADPH in HepG2 cells. NADPH is required for phase I enzyme activity such as cytochrome P450 monooxygenases. The toxicity data will be linked to the metabolite profiles generated after incubation of OA with each liver S9 fraction in the presence and absence of the cofactor NADPH by analysing supernatants of incubations using mass spectrometry. Our study will contribute to a better understanding of the often contradictory results regarding NADPH-dependent bioactivation of OA by different liver S9 fractions serving as an external metabolising system, and it will also increase our knowledge with regard to the extrapolation of data from animal studies to humans, thereby improving the accuracy of risk assessments of OA and its metabolites.

2. Material and methods

2.1. Chemicals

Okadaic acid (OA) was purchased from Enzo Life Science (Lörrach, Germany) and dissolved in methanol (MeOH). 36-Hydroxy-okadaic acid, 43-hydroxy-okadaic acid and 43-oxo-okadaic acid standards were kindly provided by Kathleen Rein of the Florida International

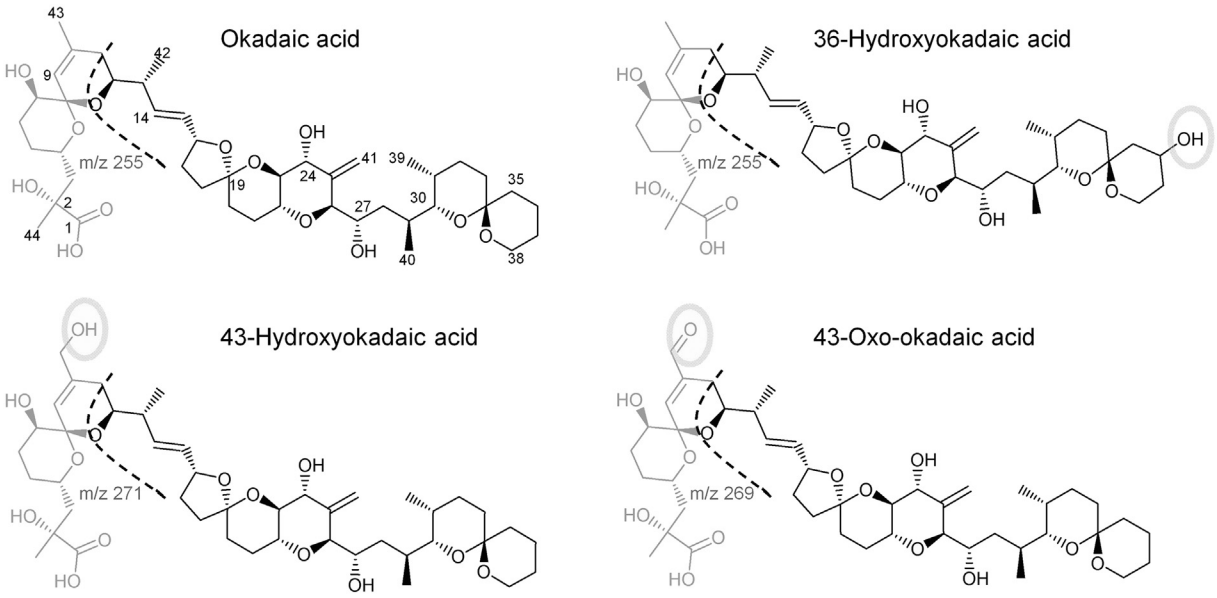

Fig. 1. Structures of okadaic acid and its main oxidative metabolites formed by human and rat hepatic phase I enzymes adopted from Liu et al. (2012). Fragmentations of OA and metabolites monitored during MS detection are included. The region of each molecule marked in grey is detected as fragment. The region marked in black leaves as neutral.