Ecotoxicology and Environmental Safety 102 (2014) 6–11

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Kinetic response of a genotoxicity biomarker in the three-spined stickleback and implication for environmental monitoring

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ARTICLE INFO

Article history:
Received 17 October 2013
Received in revised form
8 January 2014
Accepted 8 January 2014
Available online 1 February 2014

Keywords:
Biomarker
Biomonitoring
Genotoxicity
Gasterosteus aculeatus L.
Comet assay
Kinetic response

ABSTRACT

The ultimate sink for the majority of anthropogenic compounds are the aquatic ecosystems, either through direct discharges or indirectly through hydrologic or atmospheric processes, possibly leading to long-term adverse effects in aquatic living resources. In order to assess exposure, fate and effects of chemical contaminants, aquatic ecotoxicologists have developed a large array of early-warning biomarkers proving that toxicants have entered organisms, have been distributed between organs and have triggered toxic effects regarding critical targets. However, optimal use of biomarkers in environmental studies previously requires in-depth knowledge of the kinetics of response of biomarkers. This work aimed to define as a first step of a validation process the kinetic response of a genotoxicity biomarker recently developed in the three-spined stickleback (*Gasterosteus aculeatus*). DNA damage was assessed in stickleback erythrocytes after *in vivo* exposure for 12 days to methylmethanesulfonate (MMS), an alkylating compound, followed by a 20 day-recovery period. Results show a dose-response relationship, time to maximal induction being reached after 6 days at the highest MMS concentration. No acclimation process was noticed during exposure whatever the MMS concentration, and genotoxicity decreased during the recovery phase only in fish exposed to the highest MMS concentration, suggesting more an effect of erythrocyte turn-over than of DNA repair system on the observed DNA damage level. Further field experiments are needed before including this genotoxicity biomarker in a battery of biochemical markers to monitor adverse effects of pollutants on fish health.

1. Introduction

Biomarkers have been proposed for a long time as promising tools to assess adverse effects induced by environmental stressors in aquatic organisms complementarily to chemical and ecological analyses classically used for field monitoring (Payne and Penrose 1975; Adams et al., 1990). Recently, the Marine Strategy Framework Directive (2008/56/CE) proposed to measure biomarkers – such as general stress indicators, embryotoxicity end-points, genotoxicity and endocrine disruption markers – to characterize biological status of marine water bodies. For continental waters, effect-based monitoring such as bioassays and biomarkers are not integrated in monitoring programs defined by the Water Framework Directive (2000/60/EC). Several reasons could explain the non-deployment of ecotoxicological tools in such programs. The first one is the lack of biomarker validation in freshwater organisms required for avoiding misinterpretation of field data which could lead to false positive or false negative diagnostic (Sanchez et al., 2012). Among 922 scientific papers related to biomarker responses, only 5.6 percent documented the entire response kinetics from initial induction to a partially or complete recovery (Wu et al., 2005). To bridge this gap, a validation process is required to characterize accurately biomarker response. In this context, the first step could be supported by laboratory experiments to define, in organisms exposed to reference substances, kinetic response parameters including (i) the initial induction time ("time required for a statistically significant change in biological response to become observable"), (ii) the maximum induction time ("the time required for the induced biological response to reach a plateau"), (iii) the adaptation time here called acclimation

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0147-6513/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2014.01.006

time ("the time required for the maximally induced biological response to return to background levels upon continuous exposure to a chemical stress") and (iv) the complete recovery time ("the time required for an induced biological response to return to background level following depuration"; Wu et al., 2005).

Among the available biomarkers previously developed in research laboratories and considering the increasing range of anthropogenic contaminants known as highly persistent and mutagenic in the aquatic environment, genotoxicity end-points appear as relevant indicators to address environmental risk for organisms (Waters et al., 1991; 1999; Frenzilli et al., 2009). For this purpose, the comet assay also called Single Cell Gel Electrophoresis assay (SCGE) is a relevant tool widely used in ecotoxicology to detect the level of primary DNA damage. Initially developed for human toxicology purposes (Singh et al., 1988), the comet assay has become one of the most popular assays to assess DNA damage in aquatic organisms, being described in the literature as a rapid, sensitive and relatively inexpensive method (Devaux et al., 1997; Mitchelmore and Chipman, 1998; Cotelle, Férard, 1999; Jha, 2008; Devaux and Bony, 2013). Due to these advantages, this assay has been developed in different cell types of a wide range of ecologically relevant aquatic species to assess the genotoxicity potential of environmental matrices in laboratory and in the field after collecting naturally-exposed organisms (Devaux et al., 1998; Winter et al., 2004;Frenzilli et al., 2009).

The present study was designed (i) to adapt in this fish species a classification scale to measure DNA damages detected by the SCGE assay in three-spined stickleback erythrocytes (*Gasterosteus aculeatus* L.) – a recognized sentinel fish species previously used to study environmental pollution genotoxicity (Wirzinger et al., 2007) – and (ii) to characterize kinetic profile of DNA damages including the initial induction time, the acclimation and the recovery time. For this purpose, the reference substance methylmethanesulfonate (MMS) was used as a model genotoxicant (Collins et al., 1997a; Pottenger et al., 2009; Lacaze et al., 2010; Santos et al., 2013b). Even if MMS is not an environmentally relevant pollutant, alkylating agents are thought to be the most potent and abundant genotoxic contaminants in aquatic environment (Claxton et al., 1998),used as anticancer compounds that have been recovered in surface water receiving urban waste water or hospital effluent (Buerge et al., 2006; Catastini et al., 2008; Besse et al., 2012).

2. Materials and methods

2.1. Fish origin and exposure or depuration conditions

One year old sticklebacks reared in an outdoor lotic mesocosm (INERIS, Verneuil en Halatte, France) were used in this study. In October 2010, fish were transferred indoor in 500 l tanks with continuous water renewal (13 °C^{+}/$_{-}$1 °C). Fish were daily fed *ad-libitum* with frozen bloodworms (Europrix, France) and maintained under natural photoperiod (from 10:14-h to 9:15-h light:dark between November 2010 and January 2011).

Methyl methane sulfonate [CAS number 66-27-3] and all other chemicals and reagents were purchased from Sigma-Aldrich chemicals (St Quentin Falavier, France). A total of 250 adult sticklebacks (30–50 mm total length; 310–1130 mg body weight) were randomly dispersed into fifty 4 l glass aquaria (five fish/tank). After 6 days of acclimation, fish were exposed or not through water to 0.05, 0.5 and 5 μM of MMS in semi-static conditions. Before exposure, ten fish from two tanks were sampled and then eight to ten fish (two tanks per condition) were sampled after 2, 6 and 12 days of exposure for each MMS concentration. Twelve-day exposed fish were finally maintained in uncontaminated water for 6, 12 and 20 days of recovery. A preliminary experiment showed that time for 50 percent mortality (LT50) in stickleback exposed to 100 μM MMS was 29 days with reproductive, feeding and swimming behavior changes being observed after 2 weeks of exposure. Thus, far lower MMS concentrations were used in the present experiment: 0.05, 0.5 and 5 μM, and change in fish behavior was checked daily as well as mortality. The commercial MMS solution was used to prepare every day a 50 mM stock solution in distilled water further diluted in each tank to reach the nominal concentrations. Water was renewed one hour after feeding with frozen bloodworms (0.5 g/tank) and sampled once during the experiment 2 h after water renewal. Samples were frozen (−20 °C) and actual MMS concentrations were measured by capillary gas chromatography using flame ionization detection (Li, 2004). During the course of experiment, water quality parameters were measured 5 times: pH 8.65 ± 0.03; temperature 14.8 ± 0.1 °C; 69 ± 0.8 percent dissolved O_2 content; 326 ± 4 μS cm^{-1} conductivity; 2.1 ± 0.7 mg l^{-1} NO_3^-; 0.05 mg l^{-1} NO_2^-; total ammonia being not detected (detection limit 0.25 mg l^{-1}).

2.2. Blood sampling, comet assay protocol and visual scoring

To perform the comet assay, frosted microscope slides (Labonord, France) were covered with melted normal agarose in PBS (0.8 percent) the day before

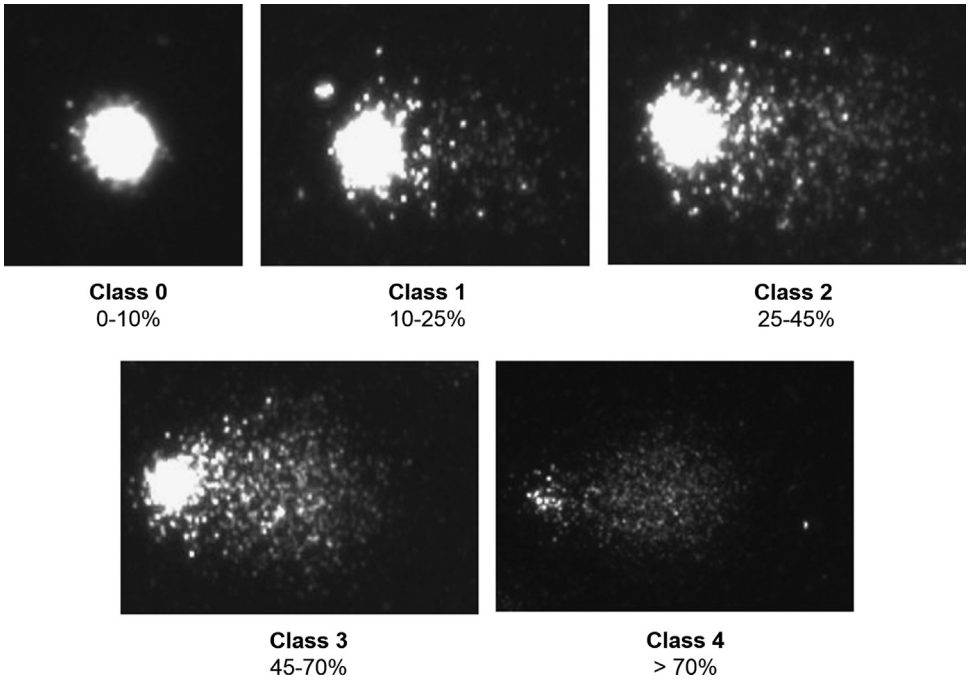

Fig. 1. Visual classification of comet figures in three-spined stickleback erythrocytes (class 0 to 4) adapted from Collins et al. (1995) with the corresponding tail intensity values measured with the Comet IV software (Perceptive Instruments Ltd).