Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part C 147 (2008) 61–68
CBP
www.elsevier.com/locate/cbpc

Biochemical biomarkers in chronically metal-stressed daphnids

Anita Jemec [a,*], Tatjana Tišler [a], Damjana Drobne [b], Kristina Sepčić [b], Polona Jamnik [c], Milenko Roš [a]

[a] National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia
[b] University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia
[c] University of Ljubljana, Biotechnical Faculty, Food Science and Technology Department, Chair of Biotechnology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Received 15 April 2007; received in revised form 20 July 2007; accepted 24 July 2007
Available online 1 August 2007

Abstract

Biochemical biomarkers are a popular measure of toxic effects on organisms due to their assumed fast response, and are usually assessed after acute exposure of the organism to the stressor. However, increasing interest in the use of biochemical biomarkers in environmental pollution monitoring calls for more laboratory long-term studies of contaminants' effects on biochemical endpoints. In this study, four biochemical biomarkers (protein content, activity of cholinesterase (ChE), catalase (CAT) and glutathione *S*-transferase (GST), were correlated with standardised reproductive and survival endpoints of water fleas (*Daphnia magna*) after chronic exposure to Cr (VI) and Cd. No effect on the reproduction and survival was noticed up to the highest tested concentration of Cr (VI) (52.5 μg/L), while the protein content, and the ChE and CAT activity decreased, and GST activity increased. Cd affected reproduction of daphnids above 0.656 μg/L, but the protein content and ChE activity were changed at 0.328 μg/L and 0.082 μg/L of Cd, respectively. Biochemical biomarkers in some cases proved to be equally or more sensitive than reproduction and mortality. We recommend more frequent use of a battery of biochemical biomarkers in combination with other higher-level biomarkers also in chronic studies and not only in the acute ones.

Keywords: Cadmium (Cd); Catalase; Cholinesterase; Chromium (Cr (VI)); Chronic toxicity test; *Daphnia magna*; Glutathione *S*-transferase

1. Introduction

The utility of biochemical approaches in environmental pollution monitoring and characterization of effect/exposure to stressor for the use in environmental risk assessment is based on the assumption that low concentrations of a toxicant will cause biochemical responses within individual organisms before these effects are observed at higher levels of biological organization (Sarkar et al., 2006). Such biochemical responses are considered to be rapidly responding endpoints (Adams, 2002), and thus most biochemical biomarkers in the laboratory studies are assessed after acute exposure to chemicals. In the water flea *Daphnia magna* Straus, a number of biochemical biomarkers have been studied after acute exposure (Day and Scott, 1990; Guilhermino et al., 1998; Sturm and Hansen, 1999; Diamantino et al., 2000; De Coen and Jassen, 2003; Printes and Callaghan, 2003; Barata et al., 2005; Jemec et al., 2007a), but they have rarely been assessed after 21 d exposure (Jemec et al., 2007b). The need for longer-term laboratory studies using biochemical biomarkers which may serve as reference points with which to develop biomarkers of chronic exposure situations, usually faced by organisms in the field, has been proposed by Handy et al. (2003).

Among the most frequently analyzed biochemical biomarkers in toxicity studies are the enzymes cholinesterase, glutathione *S*-transferase and catalase. Cholinesterase (ChE) hydrolyzes the neurotransmitters such as acetylcholine at the nerve synapse. In the absence of such hydrolysis, neurotransmitter accumulates and as a consequence, prolonged electrical activity at nerve endings occurs. Inhibition of ChE activity is usually regarded as an indicator of organophosphorus and carbamate exposure, but metals can also influence this enzyme (Ishaaya, 2001). Glutathione *S*-transferase (GST) belongs to a family of detoxification enzymes, and catalyses the conjugation of glutathione with xenobiotics including organophosphorus pesticides (Booth and O'Halloran, 2001), and cytotoxic aldehydes produced during lipid peroxidation (Halliwell and

* Corresponding author. Tel.: +386 14760245; fax: +386 14760300.
E-mail address: anita.jemec@ki.si (A. Jemec).

1532-0456/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2007.07.006

Gutteridge, 1999). Catalase (CAT) decomposes hydrogen peroxide formed extensively during oxidative stress (Halliwell and Gutteridge, 1999). In chemically stressed animals, increase or decrease of CAT and GST activities is possible, depending on the type of the chemical, time and dose of exposure. Protein content in *D. magna* has previously been used as a biomarker of chronic chemical exposure (Knowles and McKee, 1987, Bodar et al., 1988a), and reflects the entire physiological state of the organism (Printes and Callaghan, 2003).

In our previous work (Jemec et al., 2007a) acute (48 h) effects of Cr (VI) and Cd on the activities of ChE and GST in *D. magna* were studied. Since, except for the induction of ChE at lower concentrations of Cd, no significant changes of selected enzyme activities were detected, it was to conclude that the applicability of these biomarkers in routine acute toxicity tests is limited. In the present chronic (21 d) study the effects of Cr (VI) and Cd on biochemical, reproductive, and survival endpoints in *D. magna* were assessed. These two metals were chosen because they are common pollutants in a variety of aquatic environments, and Cr (VI) is used as a reference chemical in standard acute *D. magna* test (ISO 6341:1996). It is well established, that their toxic action is mediated through the induction of oxidative stress (Stohs and Bagchi, 1995; Halliwell and Gutteridge, 1999). Additionally, Cd has been shown to directly inhibit GST activity (Dierickx, 1982). For both metals, the inhibition of ChE activity was expected (Guilhermino et al., 1998). We compare the sensitivities of biochemical biomarkers to higher-level chronic (reproduction, survival) and acute (immobility) endpoints and discuss the use of such biomarkers in chronic toxicity studies.

2. Materials and methods

2.1. Chemicals

The following chemicals were purchased from Sigma (Germany): dibasic and monobasic potassium phosphate, hydrogen peroxide (30%), 1-chloro-2,4-dinitrobenzene, L-glutathione (reduced form), 5,5-dithiobis-2-nitrobenzoic acid, sodium hydrogen carbonate, acetylthiocholine iodide, and ethylenediaminetetraacetic acid. BCA Protein Assay Reagents A and B, cadmium chloride, and potassium dichromate were purchased from Pierce (U.S.A.). All chemicals were of the highest commercially available grade, typically 99% or higher.

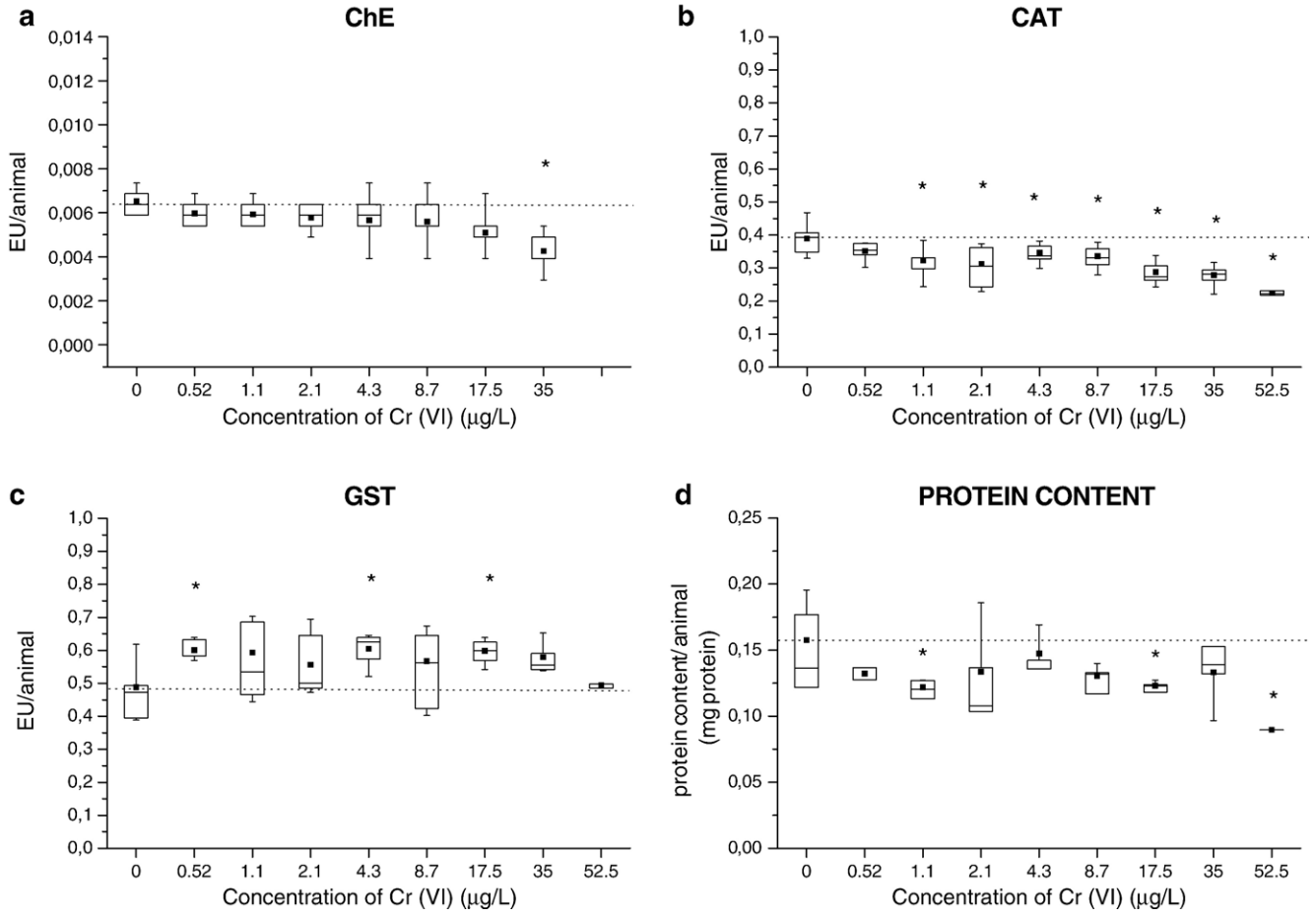

Fig. 1. ChE (a), CAT (b), GST (c) activities and protein content (d) in *D. magna* exposed to Cr (VI). Symbols on the box plot represent maximum and minimum value (whiskers: ⊥), mean value (■), and significant changes compared to control (*) (ANOVA, $P<0.05$). The dashed line represents the mean value of the control.