CHARACTERIZATION OF CYTOCHROME P450 IN THE ANTARCTIC KEY SENTINEL SPECIES *TREMATOMUS BERNACCHII*

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Introduction

The constant increase of global anthropogenic pressure is considered an important issue for Antarctic ecosystems. Local contamination can arise from the development of scientific bases, while persistent organic pollutants (POPs) are transported from industrialized regions (1). Other chemicals, such as trace metals, are natural components of the Antarctic marine environment, but their naturally elevated concentrations can influence biological responses and sensitivity of the organisms. To obtain basic ecotoxicological knowledge regarding the sensitivity and responsiveness of classical biomarkers, laboratory exposures still represent a fundamental approach for Antarctic organisms. In the present study, the endemic rock cod, *Trematomus bernacchii*, was selected as a biological model to investigate the natural variability and responsiveness of cytochrome P450 (CYP1A) toward aromatic xenobiotics and the potential influence of cadmium in modulating the biotransformation pathway. *T. bernacchii* is a key species in the Antarctic environment, widely distributed, a benthic organism commonly found within the first 200 meters of depth and well characterized for its ecology and reproductive biology (2). Organisms were exposed, in single dose or in combination, to cadmium and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), chemicals of special interest for Terra Nova Bay potentially reflecting inputs from natural, local or remote origin. Cadmium is an important element for Antarctic marine species from Terra Nova Bay, due to the elevated natural levels caused by upwelling phenomena, while 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was selected as a model for ubiquitous POPs released around the world and transported to polar regions by air masses, global distillation and fractionated processes.

Materials and methods

Specimens of *T. bernacchii* were sampled in Tethys Bay, close to the Italian Scientific Station, from November 2001 to January 2002 to characterize the seasonal variability of cytochrome P4501A. For laboratory exposures, organisms were acclimatized in aquaria and intraperitoneally injected with cadmium (Cd, 2.5 µg/g) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 1 ng/g), dosed alone or in combination. After one week, livers were rapidly dissected out, frozen in liquid nitrogen and stored at -80°C until analyses. Activity of cytochrome P4501A (EROD) was assayed spectrofluorimetrically using an adaptation of the method of Peters and Livingstone (3). Incubation of samples was carried out at 25°C and fluorimetric analyses (535/585 nm) were quantified by reference to resorufin standards (0.01-1µM). The CYP1A protein levels were confirmed by Western blot analyses using anti-rat CYP1A1 polyclonal antibodies (2). Concentration of cadmium was analyzed by atomic absorption spectrophotometry with electrothermal atomization (SpectrAA 300 Zeeman; Varian), after digestion under pressure with nitric acid and hydrogen peroxide. The subcellular distribution of metal also was analyzed in enriched fractions obtained using the method described by Fattorini and Regoli (4) with slight modifications. The same parameters were analyzed also in a temperate species, the European eel *Anguilla anguilla*, exposed to the same chemicals.

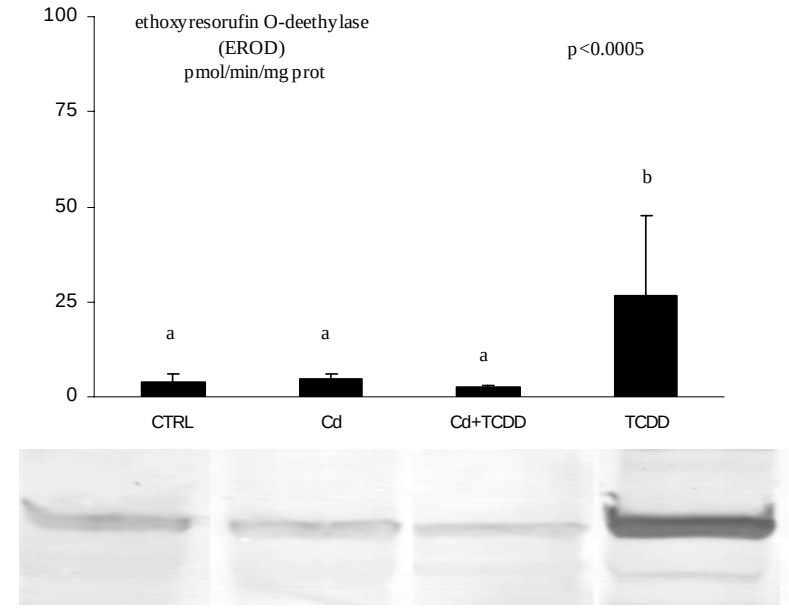
Results and discussions

T. bernacchii did not exhibit significant differences in EROD activity between male and female specimens, and values, ranging between 2.5 and 6 pmol/min/mg prot, were almost constant in various sampling periods. These results suggest that cytochrome P450 was not markedly influenced by both biological (reproductive cycle) and ecological factors (i.e. phytoplanctonic blooms and food availability).

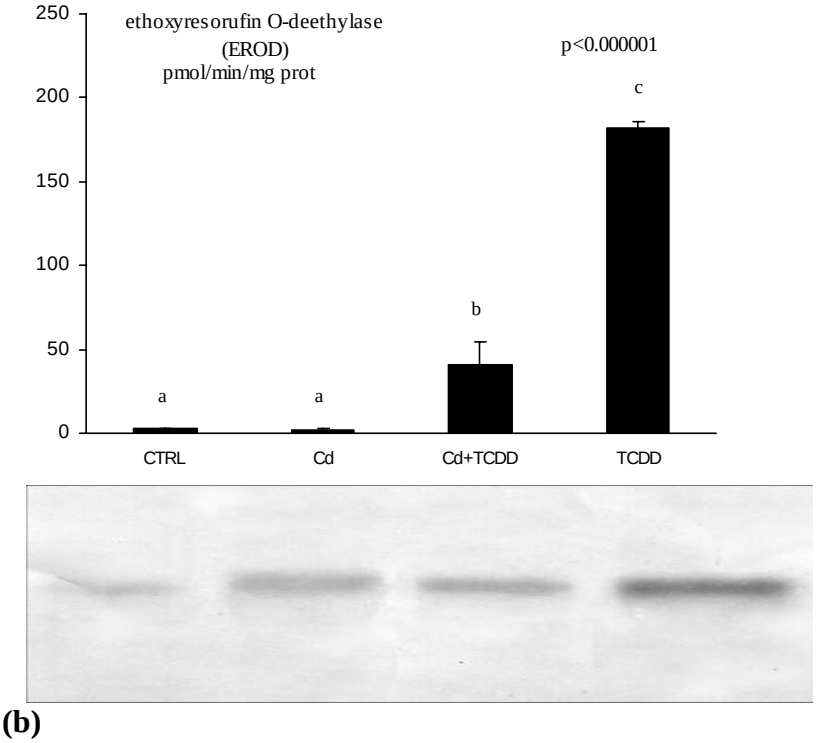
The results from laboratory exposures highlighted similar responses in *T. bernacchii* and *A. anguilla* despite the inducibility rate of cytochrome P450 in the Antarctic fish was much lower than in the temperate species (6 fold compared to 70 fold increase after TCDD exposure). The marked induction of EROD activity by dioxin was greatly reduced in both the species on concomitant treatment with cadmium (Fig.1a-b).

To characterize at which level cadmium prevents elevated EROD activity in co-exposed organisms, western immunoblots analyses were carried out. Levels of CYP1A hepatic protein were low in organisms exposed to both toxicants together, while a strong enhancement of CYP1A protein levels was induced by TCDD alone. Different transcriptional or post translational mechanisms might be considered for explaining the Cd-TCDD interaction, including the induction of Heme-oxygenase 1 (HO-1) by cadmium and the consequent low availability of heme group for CYP1A proteins, the interaction of cadmium with –SH groups and the enhanced formation of reactive oxygen species (2).

Other reciprocal interactions between metabolism of trace metals and aromatic xenobiotics were observed on cadmium bioaccumulation. Natural hepatic concentrations were approximately 10 fold higher in *T.bernacchii* than in eels (24 vs 2 ppm), confirming an elevated bioavailability of this element in organisms from Terra Nova Bay (5).



(a)

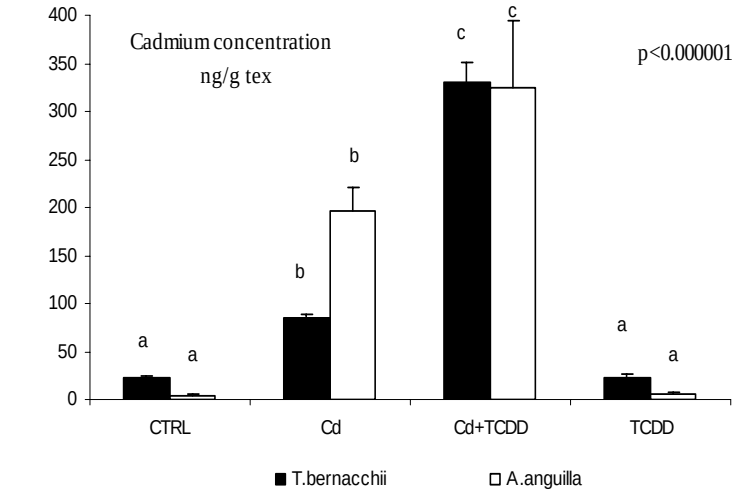


(b)

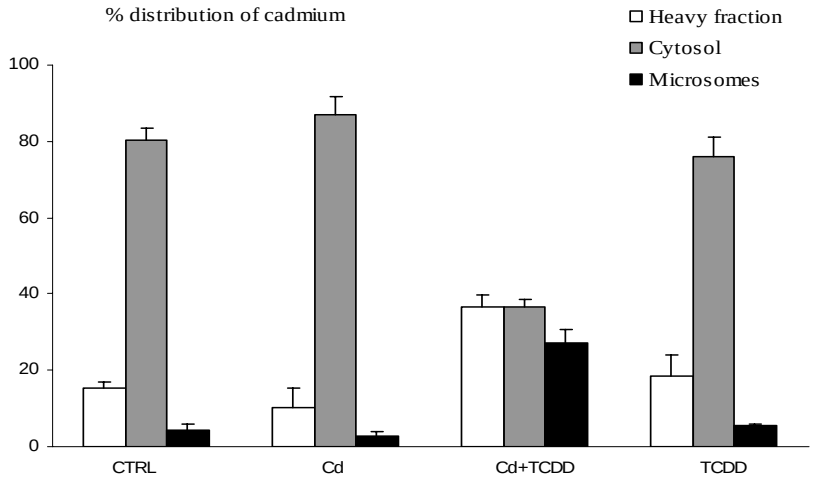
Figure 1. Enzymatic activity of ethoxyresorufin *O*-deethylase (EROD) and levels of CYP1A1 proteins in liver of *Trematomus bernacchii* (a) and of *Anguilla anguilla* (b) under various experimental conditions. Enzymatic results are given as mean values ± standard deviations (n = 10). The *p* values are reported for significant variations and different letters indicate significant differences between groups of means (post hoc comparison).

Laboratory exposure to cadmium caused a significant increase of metal concentrations which were particularly enhanced during co-treatment with TCDD (Fig.2a).

Analysis of subcellular distribution of cadmium revealed that, while the metal is generally sequestered in the cytosol, a significant contribution of microsomal fraction becomes evident during co-exposures (Fig.2b); in this respect, the proliferation of endoplasmatic reticulum caused by dioxin, would increase the storage of Cd within microsomal vescicles due to the atomic similarity with calcium (6).



(a)



(b)

Figure 2. Concentrations of cadmium in liver of *Trematomus bernacchii* and *Anguilla anguilla* under various experimental conditions (a); percentage distribution of cadmium in subcellular fractions of *T.bernacchii* (b). Results are expressed as mean values ± standard deviations (n = 10). The *p* values are reported for significant variations and different letters indicate significant differences between groups of means (post hoc comparison).

In conclusions the present results emphasized the relatively low sensibility of CYP1A response in Antarctic fish compared to temperate organisms and the marked effect of cadmium in reducing biotransformation capability. The elevated natural levels of cadmium in organisms from Terra Nova Bay could thus influence the basal response of this system with important implications for biological effects of aromatic xenobiotics in key sentinel species.

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