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Note

Carboxylesterase activities in chondrichthyans of the western Mediterranean Sea

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ABSTRACT

Sharks, rays, skates and chimaeras play an important role as predators in the ecosystems. These species could serve as potential sentinels for the presence of xenobiotics in marine ecosystems. In this study, liver carboxylesterase (CbE) activity was determined for 20 species of chondrichthyans. Carboxylesterase activity, a known esterase involved in the metabolism of pesticides, lipids and certain drugs, was measured using two substrates: 4-nitrophenyl acetate (pNPA) and α-naphthyl acetate (αNA). Rajiformes (rays and skates) showed higher CbE activity than sharks, and the order Chimaeriformes showed the lowest values. *In vitro* sensitivity to CbE inhibitors was assessed in the shark *Scyliorhinus canicula*. The substrate αNA was found to be the most adequate as it displayed the highest activity and was more sensitive to the organophosphate dichlorvos in the liver (IC50 = 2.37 μM) and plasma (IC50 = 0.051 μM). The high interspecific variation of CbE activity and its sensitivity to inhibition by fenofibrate suggest that this enzyme could contribute to species-specific differences in drug detoxification.

A wide range of pollutants identified as chemicals of environmental concern are present in aquatic environments, impacting natural ecosystems, human resources and health (Halpern et al., 2008). Nowadays, multiresidual analytical methodologies allow for the direct analysis of a broad range of pollutants; however, often the methodology is complex, expensive and does not consider the effect of the chemical mixtures on the aquatic organisms. Thus, biomarkers based on measurements of particular enzymes present in living organisms have been used as indicators of exposure to certain xenobiotics in the environment (Alves et al., 2015, 2016; Narvaez et al., 2015). The ability of carboxylesterases (CbEs) to respond to the presence of pollutants like pyrethroids, organophosphates and carbamates, makes them an adequate biomarker of exposure (to the pollutants) and susceptibility (of the organisms to be affected by pollutants). This way, CbEs are playing an important role in the protection of other enzymes like acetylcholinesterase and take part in the metabolism of lipids (Wheelock et al., 2008). CbEs are a group of enzymes of the family α,β-serine hydrolases, which act as a detoxifying mechanism due to their capacity to hydrolyze esters, including a wide range of xenobiotics such as pesticides, PPCPs (Pharmaceuticals and Personal Care Products), as well as endogenous compounds (Masson and Lockridge, 2010; Sogorb and Vilanova, 2002). Moreover, CbEs are found in most of animal tissues, having small variations (isoforms) but conserving the same function. This makes them interesting to study as they could be used as biomarkers of pollution in many species (Narvaez et al., 2015; Solé et al., 2010).

Marine organisms occupying high trophic levels in the ecosystems are particularly susceptible to the bioaccumulation and biomagnification of some pollutants, and are important sentinels for the presence of xenobiotics in the marine domain (Alves et al., 2016; Gilbert et al., 2015; Koenig et al., 2013). This is the case for chondrichthyans (sharks, rays, skates and chimaeras), important predators in the marine ecosystems occupying high trophic positions within the food webs (Baum and Worm, 2009; Heupel et al., 2014). However, although some studies have proposed chondrichthyans as bioindicators of pollution (Alves et al., 2015, 2016; Torres et al., 2016; Torres et al., 2014), to our knowledge few studies have analyzed CbE activity levels in these marine predators (Scott et al., 1976; Solé et al., 2010).

Here, CbE activities in the liver of 20 species of chondrichthyans (1 chimaera, 11 sharks and 8 rays/skates) from the western Mediterranean Sea, a highly productive marine area notably im-

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Table 1
Reported mean and standard deviation of carboxylesterase activity (in nmol · min^{-1} mg $ptrotein^{-1}$) measured in the liver of 20 chondrichthyan species from the western Mediterranean Sea. The threatened category in the Mediterranean based on IUCN evaluations (Dulvy et al., 2016) is also indicated.

Order/species	IUCN category	N	CbE(pNPA)	CbE(αNA)
Chimaeriformes				
Chimaera monstrosa	Near threatened	16	15.80 ± 6.63	10.37 ± 3.97
Rajiformes				
Dipturus oxyrinchus	Near threatened	2	13.56 ± 0.72	34.15 ± 1.95
Leucoraja naevus	Near threatened	3	15.85 ± 8.01	17.52 ± 4.24
Raja clavata	Near threatened	6	14.88 ± 3.80	47.52 ± 13.65
Raja montagui	Least concern	8	18.24 ± 21.45	56.43 ± 66.35
Raja asterias	Near threatened	16	34.05 ± 9.96	126.82 ± 47.31
Raja polystigma	Least concern	11	22.98 ± 5.02	44.50 ± 19.56
Myliobatiformes				
Gymnura altavela	Critically endangered	1	6.17	6.51
Mobula mobular	Endangered	1	71.95	52.92
Carcharhiniformes				
Galeorhinus galeus	Vulnerable	1	24.28	215.41
Galeus melastomus	Least concern	18	10.99 ± 1.38	30.36 ± 6.43
Prionace glauca	Critically endangered	4	11.67 ± 3.58	22.87 ± 6.07
Scyliorhinus canicula	Least concern	18	13.04 ± 7.53	69.54 ± 64.69
Hexanchiformes				
Hexanchus griseus	Least concern	8	15.97 ± 8.99	23.93 ± 14.80
Squaliformes				
Centroscymnus coelolepis	Least concern	16	16.73 ± 3.26	47.29 ± 11.32
Centrophorus granulosus	Critically endangered	16	26.54 ± 7.02	62.73 ± 18.70
Etmopterus spinax	Least concern	14	13.05 ± 2.46	17.51 ± 5.14
Oxynotus centrina	Critically endangered	3	6.22 ± 0.58	8.63 ± 4.00
Somniosus rostratus	Data deficient	5	22.72 ± 8.08	58.21 ± 25.96
Squalus acanthias	Endangered	4	18.54 ± 4.24	50.36 ± 9.51

pacted by human activities (Coll et al., 2012; Navarro et al., 2016), were analyzed (Table 1). Specifically, a portion of liver (0.2 g) was taken from 171 individuals of 20 species of chondrichthyans opportunistically fished as bycatch in commercial trawlers in the western Mediterranean Sea between 2011 and 2014 (Fig. 1; Barría et al., 2015). The populations of the species included in the present study are considered to be at different risk levels by the International Union of the Conservation of Nature (IUCN) (Table 1; Dulvy et al., 2016). Despite their state of conservation and their importance in the ecosystems, as either top predators, mesopredators or in some cases keystone species, knowledge of this group is very scarce, especially in terms of pollution and their potential as sentinel species for Mediterranean marine pollution.

CbE activity was measured in all of the 20 species and an *in vitro* characterization was carried out with four individuals of *Scyliorhinus canicula*, an abundant demersal small-sized shark frequently present as bycatch in the Mediterranean fisheries. The CbE activity analyses were conducted on a portion of liver of each individual of the 20 species. CbEs activity was also measured in plasma of *S. canicula* (only species with availability of plasma samples; Valls et al., 2016). Two substrates were used (aNA and pNPA), following the procedures described in Solé et al. (2012). For the species comparisons, αNA was assayed at 250 μM and pNPA at 1 mM final concentrations. For the four extra samples of *S. canicula* characterization, six concentrations of the substrates αNA (0.03–1 mM) and six of pNPA (0.3–10 mM) were assayed to determine kinetic constants (Vmax and Km). Also for this 4 samples, the organophosphate dichlorvos and the drug fenofibrate were used for *in vitro* inhibition studies using the S10 fraction of the liver and plasma.

The results revealed a high variability of CbE activity measured in the liver between species and orders (based on ANOVA tests). The values obtained represent the first measures of this enzyme activity in 18 out of the 20 studied species. CbEs activity also showed differences depending on the substrate assayed. The activity with the substrate αNA was usually higher than with pNPA in the liver of most chondrichthyans except *Mobula mobular* (Order Myliobatiformes) and *Chimaera monstrosa* (Order Chimaeriformes). These differences are probably due to the non-specific nature of the substrates for the different CbE forms. The affinity to each substrate varies among species and organs because the CbE isoforms are differently expressed (Al-Ghais et al., 2000). αNA usually responded more specifically to OP pesticides in *S. canicula* and other fish species (Nigam et al., 2014; Solé et al., 2012).

CbE showed values between 4.27 and 95.91 and 1.12 and 547.07 nmol min^{-1} mg $protein^{-1}$ for the substrates pNPA and αNA, respectively. This broad variability indicates high interspecific differences in the CbE activity, for CbE (pNPA) (ANOVA tests; $F_{16,165} = 2.93$, $p < 0.05$; Table 1 and Fig. 2a), with differences between *Raja asterias* and *Galeus melastomus*, *Centroscymnus coelolepis*, *S. canicula, Etmopterus spinax* and *C. monstrosa*. Similarly, CbE (αNA) values also differed ($F_{16,165} = 2.57$, $p < 0.05$, Table 1 and Fig. 2), with differences between *R. asterias* and *Hexanchus griseus*, *G. melastomus*, *E. spinax* and *C. monstrosa*. The skate *R. asterias* was the only species that differed from the others showing high activity values, especially with the substrate αNA (Table 1; Fig. 2). As for variability at the order level, Rajiformes showed the highest activity differing from the Carcharhiniformes with the substrate pNPA ($F_{5,169} = 4.31$, $p < 0.05$; Fig. 2b) and from the Chimaeriformes with the substrate αNA ($F_{5,169} = 2.47$, $p < 0.05$; Fig. 2b) (Fig. 2b).

Kinetic parameters of Vmax, Km and catalytic efficiency (Vmax/Km) confirmed that αNA was a better substrate for measuring CbE as it displayed the highest activity and more sensitivity to the OP dichlorvos in the liver and more significantly in the plasma of *S. canicula* (Table 2). The antihyperlipidemic drug fenofibrate also inhibited CbE activity in *S. canicula,* as evidenced in several other aquatic species (Solé and Sanchez-Hernandez, 2015). The same range of concentrations of this drug as for dichlorvos were assayed *in vitro* (0.02–320 μM), however this drug only interacted with CbE (αNA) in the liver (IC50 = 39.21 μM). In plasma, the maximum inhibition reached at the highest dose assayed was 35% using the same substrate and 26% using pNPA in liver. This difference in the sensitivity suggests different isoform compositions in the plasma and liver. Moreover, the fact that BChE activity was negligible in this species while CbE activity in plasma was significant, suggests that CbE could display a protective role normally attributed to plasmatic BChE in other fish species (Sturm et al., 2000).

In comparison with other species of fish, most of the elasmobranchs presented lower CbE activity and a high intraspecific variation as previously described in Solé et al. (2010). However, our results are not directly comparable with this study because the substrates employed are different and the affinity of CbEs is substrate dependent. None-