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Comparative study of enzymatic antioxidants in muscle of elasmobranch and teleost fishes

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ABSTRACT

Exercise may cause an imbalance between pro-oxidants and antioxidants. In skeletal muscle, oxygen flow can increase considerably during vigorous exercise. The antioxidant system in athletes contributes to neutralize the concomitant rise in reactive oxygen species (ROS) production. The objective of this study was to compare the antioxidant system in muscle of three species of elasmobranchs and teleosts, considering differences in swimming capacity among species within each group and evolutionary differences between the two groups. Muscle samples were collected from elasmobranchs (*Isurus oxyrinchus*, *Prionace glauca*, *Mustelus henlei*) and teleosts (*Totoaba macdonaldi*, *Kajikia audax* and *Coryphaena hippurus*) in the coast of the Baja California peninsula, Mexico. The enzymatic activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) was determined by spectrophotometry. The activity of the antioxidant enzymes CAT, GPx and GST was higher in elasmobranchs, as a group, than in teleosts. In fish species with high swimming capacities, *P. glauca*, *K. audax* and *C. hippurus*, antioxidant enzyme activity was higher in comparison with species with lower swimming capacities, *M. henlei* and *T. macdonaldi*. It is possible that antioxidant enzymes, particularly SOD, GPx and GST, contribute to avoidance of oxidative damage in teleost and elasmobranch species with higher swimming capacities. The antioxidant enzyme activities in fish appear to depend mainly on their swimming capacity and life style rather than the evolutionary group (elasmobranchs, teleosts).

1. Introduction

Among both teleosts and elasmobranchs, there are some species that are slow swimmers, others move extremely quickly, while others can reach high speed but are able to maintain it only for a short period of time (Bennet, 1991; Ji et al., 1998; Kieffer, 2000; Ji, 2008). Exercise can cause alterations in the intracellular homeostasis, specifically an imbalance between pro-oxidants and antioxidants (Finaud et al., 2006). The increase in metabolic rate results in increased production of reactive oxygen species (ROS), mainly superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), in the mitochondrial membranes (Wilhelm-Filho et al., 1993; Ji, 1999; Wilhelm-Filho, 2007). This increased production of ROS may result in oxidative damage to lipids, proteins and genetic material (Ji et al., 1998; Aguiló et al., 2005; Tauler et al., 2005; Finaud et al., 2006; Ji, 2008; Fernández et al., 2009).

In skeletal muscle, oxygen flow can increase several-fold during vigorous exercise (Ji, 1995), leading to increased ROS production (Ferreira and Reid, 2008). The antioxidant system contributes to neutralize ROS and avoid or repair the consequent oxidative damage. The antioxidant defense system includes enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST), as well as other molecules, such as vitamin E, vitamin C and glutathione (GSH) (Wilhelm-Filho et al., 1993; Ji, 1995; Janssens et al., 2000; Fang et al., 2002; Hermes-Lima and Zenteno-Savín, 2002; Aguiló et al., 2005; Martínez-Álvarez et al., 2005; Trenzado et al., 2006; Grim et al., 2010). Despite the evolutionary differences between elasmobranchs and bony fishes, many of the species studied to date have the same molecules as part of their antioxidant defenses (Leveelahti et al., 2014). It has been suggested that in early elasmobranch species, such as *Squalus acanthias*, the antioxidant system is mainly composed of non-enzymatic antioxidants (Rudneva, 1997). Differences in the enzymatic antioxidant system found between elasmobranchs and teleosts in liver, heart and blood were ascribed to swimming activity, being higher in the most active species (Wilhelm-Filho and Boveris, 1993; Wilhelm-Filho et al., 1993). Among sharks, ROS production and the antioxidant enzyme activity in muscle was reported to be higher in those species capable of vigorous exercise as compared to passive swimmers (López-Cruz et al., 2010).

Therefore, the objective of this study was to compare the enzymatic antioxidant system in muscle of three species of elasmobranchs and three species of teleosts, taking into account the differences in

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swimming capacity among species within each group, as well as the evolutionary differences between the two groups. The species included in the elasmobranch group were mako shark (*Isurus oxyrinchus*), blue shark (*Prionace glauca*) and brown smooth hound (*Mustelus henlei*). Among the teleost group, the species analyzed were striped marlin (*Kajikia audax*), common dolphinfish (*Coryphaena hippurus*) and totoaba (*Totoaba macdonaldi*). For the objectives of this study, those fish species capable of developing swimming speeds ~70 km h^{-1} and that are at the top levels of the food chain having a more oceanic distribution, *i.e.*, *I. oxyrinchus* and *P. glauca*, among the elasmobranchs, and *K. audax* and *C. hippurus*, among teleosts, were considered to have the highest swimming capacities.

2. Materials and methods

2.1. Sample collection

Samples were collected from fish caught by commercial fishermen, sports fishing and during scientific sampling trips from March 2011 to June 2012. White muscle samples (5 g approximately) were collected from the caudal area of six fish species, three elasmobranchs, *I. oxyrinchus* (n = 20), *P. glauca* (n = 30) and *M. henlei* (n = 15), and three teleosts, *K. audax* (n = 30), *C. hippurus* (n = 27) and *T. macdonaldi* (n = 30) in Baja California Sur, Mexico. Samples from *I. oxyrinchus*, *P. glauca* and *M. henlei* were collected at Punta Lobos and San Lazaro fishing camps, samples of *K. audax* were collected in Cabo San Lucas and samples of *C. hippurus* in Ensenada de Muertos and El Sargento fishing camps; samples of *T. macdonaldi* were collected in the upper Gulf of California. All tissue samples were stored in the dark and frozen at −80 °C until processing.

2.2. Sample analyses

2.2.1. Activity of antioxidant enzymes

Prior to the determination of the antioxidant enzyme activities, samples (100 mg) were homogenized in cold potassium phosphate buffer (50 mM, pH 7.5) with EDTA (60 mM) and centrifuged at 906 ×*g* for 15 min at 4 °C. The supernatant was recovered and used immediately. For all enzyme activity assays, the methods described by Vázquez-Medina et al. (2006) and López-Cruz et al. (2010), based on the original methodologies, were followed, using a spectrophotometer (Jenway 6305, Princeton, NJ, USA). Briefly, total SOD activity was measured by the method of Suzuki (2000). The enzymatic activity is expressed in units of SOD mg^{-1} protein. One unit of SOD is defined as the amount of enzyme required to inhibit 50% of the maximum reaction of $O_2^{\bullet-}$ with nitroblue tetrazolium (NBT). Catalase activity was determined using the methodology proposed by Aebi (1984). The activity of this enzyme is expressed in units of CAT mg^{-1} protein. One unit of CAT is defined as the amount of enzyme required to reduce 1 μmol of H_2O_2 per minute. Glutathione peroxidase activity was measured by the method proposed by Folhé and Günzler (1984). The enzymatic activity is expressed in units of GPx mg^{-1} protein. One unit of GPx is defined as the amount of enzyme required to oxidize 1 mol of GSH to oxidized glutathione (GSSG) per min. Glutathione reductase activity was determined by the method of Goldberg and Spooner (1987). The activity of this enzyme is expressed in units of GR mg^{-1} of protein. One unit of GR is defined as the amount of enzyme required to reduce 1 mol of GSSG to GSH per min. The activity of GST was determined by the methodology proposed by Habig and Jakoby (1981). The results are expressed in units of GST mg^{-1} of protein. One unit of GST is defined as the amount of enzyme that catalyzes the conjugation of 1 mol of 1-chloro-2,4-dinitrobenzene (CDNB) per min.

2.2.2. Total protein quantification

To standardize the data, the amount of soluble protein in each sample was determined using the method described by Bradford (1976), as described by Vélez-Alavez et al. (2013), utilizing a commercial kit (Bio-Rad Laboratories, Hercules, CA, USA) adapted to microplate. The results are expressed in mg of protein mL^{-1}.

2.3. Statistical analyses

Given the distribution of the data and because the assumptions of normality and homoscedasticity were not met, non-parametric statistical analyses were performed. Mann–Whitney (independent samples) U tests were used to determine differences in enzyme activity between the fish groups (elasmobranchs *vs.* teleosts) and between pairs of species, one elasmobranch *vs.* one teleost species with similar swimming capacities (*I. oxyrinchus vs. K. audax*, *P. glauca vs. C. hippurus*, and *M. henlei vs. T. macdonaldi*). Kruskal–Wallis tests were performed to assess possible differences between species within each group (Durán et al., 2003). In the cases where enzymatic activity was below the detection limit of the methodology, the simple substitution method, which consists in replacement of the value for one-half the specific detection limit (Helsel, 1990), was used. All analyses were performed using Statistica v.8. (StatSoft, Inc., 2007).

3. Results

The results of the antioxidant enzyme activities in the six fish (elasmobranch and teleost) species analyzed are summarized in Figs. 1 and 2. No differences in SOD activity were detected between elasmobranchs and teleosts (Fig. 1-I). Within the group of elasmobranchs, SOD activity was significantly lower in *I. oxyrinchus* than in *P. glauca* ($p = 0.01$) (Fig. 1-IA); while among teleosts the activity of SOD was lower in *T. macdonaldi* than in *C. hippurus* ($p = 0.003$) and *K. audax* ($p = 0.002$) (Fig. 1-IB). When comparing between pairs of species with similar swimming capacities, SOD activity was lower in *I. oxyrinchus* than in *K. audax* ($p = 0.02$), both species with the highest swimming capacity within their group, and was higher in *M. henlei* than in *T. macdonaldi* ($p = 0.01$), both species with the lowest swimming capacity within their group (Fig. 1-I).

Catalase activity was below the detection limit (1.0 U mg^{-1} protein) in 80% of the samples from *I. oxyrinchus*, 72% from *P. glauca*, 67% from *M. henlei*, 34% from *K. audax*, 37% from *C. hippurus* and 67% from *T. macdonaldi*. Regardless, CAT activity was 82% higher in teleosts than in elasmobranchs as a group ($p = 0.001$) (Fig. 1-II). There were no significant differences in CAT activity among species within the group of elasmobranchs (Fig. 1-IIA) nor within teleosts (Fig. 1-IIB). Catalase activity was lower in *I. oxyrinchus* than in *K. audax* ($p = 0.0002$), the species with greatest swimming capacity in their respective group (Fig. 1-II).

Glutathione peroxidase activity was 68% higher in the group of elasmobranch fishes than in teleosts ($p = 0.0001$) (Fig. 2-I). Within the group of elasmobranchs, GPx activity was significantly higher in *P. glauca* than in *M. henlei* ($p = 0.004$) (Fig. 2-IA); whereas within the group of teleosts the activity of GPx was lower in *C. hippurus* than in *K. audax* ($p = 0.0001$) and in *T. macdonaldi* ($p = 0.01$) (Fig. 2-IB). When comparing between pairs of species with similar swimming capacities, GPx activity was higher in *P. glauca* than in *C. hippurus* ($p = 0.0001$), species with intermediate swimming capacity within their group (Fig. 2-I).

There were no significant differences in GR activity between groups (elasmobranchs *vs.* teleosts) (Fig. 2-II). There were no significant differences in GR activity between species among elasmobranchs (Fig. 2-IIA). Among teleosts, GR activity was lower in *C. hippurus* than in *K. audax* ($p = 0.02$) and *T. macdonaldi* ($p = 0.001$) (Fig. 2-IIB). When comparing between pairs of species with similar swimming capacities, GR activity was higher in *P. glauca* than in *C. hippurus* ($p = 0.0006$), species with intermediate swimming capacity within their group (Fig. 2-II).

Glutathione S-transferase activity was 40% higher in elasmobranchs than in teleosts, as a group ($p = 0.006$) (Fig. 2-III). Within the group of elasmobranchs, no significant differences between species were found