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Relationship between brain and liver oxidative state and maximum lifespan potential of different fish species

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ABSTRACT

The aim of the present study is to determine whether a relationship exists between the maximum lifespan potential (MLSP) of different fish species (*Squalius pyrenaicus*, *Cyprinus carpio*, *Carassius auratus*, *Luciobarbus sclateri* and *Oncorhynchus mykiss*) and the oxidative state of brain and liver. For this purpose, the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione reductase (GR), glutathione *S*-transferase (GST) and DT-diaphorase (NAD(P)H:quinone oxidoreductase1) were measured together with the lipid peroxidation degree (malondialdehyde, MDA) in liver and brain tissues of the collected species. Only the brain tissue manifested a higher activity of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and glutathione *S*-transferase in addition to a negative correlation between the values of MDA and MLSP among the different fish species. Hypotheses are proposed from the analysis of the obtained results that open new areas of research and converge on the importance of the theory of free radicals in the processes that condition the maximum life expectancy of an animal species.

1. Introduction

As noted by Barja (2004, 2005) in his reviews, all animal species age, particularly those, such as humans, that cease growing once they reach the adult stage. The origin of the ageing process is internal (genetic), which explains its progression despite a lack of exposure of the animal to any source of environmental damage. This endogenous character also explains why different animal species age at very different rates despite living in an identical environment. External factors may modify the ageing process but cannot be its intrinsic cause.

The theory of ageing by free radicals (Harman, 1956; Hulbert et al., 2007) of mitochondrial origin is the most accepted theory to explain the characteristics of ageing, as mitochondria are an endogenous source of free radicals, and all cells of vital organs produce reactive oxygen species (ROS) continuously in their mitochondria.

Much of the animal longevity studies initially performed focused on antioxidant factors. One of the first hypotheses stated that ageing could be due to decreased levels of antioxidants as the individual ages. This idea was discarded as it was observed that variations in cellular antioxidant levels depending on the age and maximum lifespan potential (MLSP) do not follow a uniform pattern (Tolmasoff et al., 1980; Ono and Okada, 1984; Sohal and Brunk, 1992; Mockett et al., 1999; Huang et al., 2000).

Several studies to date have found that ROS production by mitochondria isolated from post-mitotic tissues is lower in long-lived animal species than in short-lived species (Ku et al., 1993; Barja, 2004). This finding could explain the negative correlation between antioxidants and maximum longevity that has been found in many cases: long-lived animals have low levels of antioxidants simply because their intensity of ROS production per unit time is low.

In addition to mitochondrial ROS production, a second parameter negatively related to maximum longevity is the degree of unsaturation of fatty acids present in the cell membranes of vital organs (Couture and Hulbert, 1995; Hulbert, 2005, 2008).

Another key factor concerns the increase in maximum longevity induced by caloric restriction. Several studies have shown that the mechanism of action of caloric restriction on the maximum longevity includes a decrease in mitochondrial ROS production and subsequent damage to mitochondrial DNA (López- Torres et al., 2002; Judge et al., 2004; Ramsey et al., 2004). It is remarkable that mitochondrial ROS production and oxidative damage to mitochondrial DNA are lower both in long-lived animals and in those with restricted feeding. This finding suggests that the decrease in the intensity of the generation of reactive oxygen species by mitochondria is a highly conserved evolutionary mechanism that is utilised to slow down the ageing of an animal and to increase the maximum longevity of a species (Barja, 2005).

It has been shown in a variety of species and tissues that aging process involves the oxidative damage to DNA, protein and lipid (Richter

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et al, 1988; Barja, 2000; Barja and Herrero;, 2000; Gemma et al, 2007). Despite all these studies, some authors continue to view the role of oxidative stress as a determinant of longevity as an open question (Buffenstein et al., 2008; Montgomery et al., 2011).

In previous studies on the oxidative state of the brain and other tissues from the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and the sturgeon *Acipenser naccarii* Bonaparte, 1836, it has been demonstrated that the potential of most of the major antioxidant enzymes of the sturgeon brain is significantly greater than that of the rainbow trout, contrary to what occurs in gills, plasma, red blood cells, skin, liver or heart (Trenzado et al., 2006; Furné et al., 2008; unpublished results), and significantly lower values of lipid peroxidation have been found in sturgeon brain than in trout brain. Because sturgeon is longer-lived than trout, the existence of a relationship between the maximum lifespan of an animal species and its capacity for antioxidant protection in the brain has been postulated (unpublished results).

The aim of the present study is to determine whether a relationship exists between the MLSP of different fish species [*Squalius pyrenaicus* (Günther, 1868); *Cyprinus carpio* Linnaeus, 1758; *Carassius auratus* (Linnaeus, 1758); *Luciobarbus sclateri* (Günther, 1868); and *Oncorhynchus mykiss* (Walbaum, 1792)] and the oxidative state of the brain. Besides studying brain tissue, liver tissue has been also studied to see if the behaviour of both tissues is similar or not. For this purpose, the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione *S*-transferase (GST) and DT-diaphorase (DTD) has been measured and the degree of lipid peroxidation (concentration of malondialdehyde, MDA) has been determined.

2. Materials and methods

2.1. Studied animals

The study was carried out with five fish species with different MLSP (Kottelat and Freyhof, 2007): the Iberian chub (*Squalius pyrenaicus*), 7 years; the common carp (*Cyprinus carpio*), 50 years; the goldfish (*Carassius auratus*), 20 years; the Andalusian barbel (*Luciobarbus sclateri*), 18 years, and the rainbow trout (*Oncorhynchus mykiss*), 10 years. Individuals were collected from the wild in the Granada province: the Iberian chubs from Guadalfeo river (30S 462988 4082280, 310 m a.s.l.), the common carp and Andalusian barbel from Cubillas river (30S 438733 4125431, 620 m a.s.l.), and the rainbow trout from Riofrío river (30S 392960 4113033, 490 m a.s.l.). The goldfish were collected from an artificial pond in Alomartes (30S 418260 4125034, 770 m a.s.l.). All samples were taken during March and April 2011.

The physico–chemical variables of the environment (pH, temperature, oxygen content and conductivity) were recorded with a VWR SymPHony SP90M5 multiparametric probe (Table 1).

Fishes were collected by mean of electric fishing during the middle of the day. For the electric fishing, portable equipment was employed. Eight to ten individuals per species were collected and quickly slaughtered. Afterwards, weight and total length were recorded. Scale samples were also taken to determine the age of individuals following the method of Gulland (1966). Posteriorly individuals were introduced in liquid nitrogen and stored at −80 °C until tissue extraction.

2.2. Treatment of samples

For the laboratory treatment of the samples, brain and liver were extracted and homogenized in ice-cold buffer (100 mM Tris–HCl, 0.1 mM EDTA and 0.1% triton X-100 (v/v), pH 7.8) at a ratio of 1:9 (w/v). Homogenates were centrifuged at 30,000 *g* for 30 min and the supernatant was collected and frozen at −80 °C.

All enzymatic assays were carried out at 25 ± 0.5 °C using a Power Wave$_x$ microplate scanning spectrophotometer (Bio-Tek Instruments, USA) in duplicate in 96-well microplates (UVStar®, Greiner Bio-One, Germany). The enzymatic reactions were started by the addition of the tissue extract, except for SOD where xanthine oxidase was used. The specific assay conditions were as follows: catalase (CAT; EC 1.11.1.6) activity was determined by measuring the decrease of H_2O_2 concentration at 240 nm according to Aebi (1984). The reaction mixture contained 50 mm potassium phosphate buffer (pH 7.0) and freshly prepared 10.6 mM H_2O_2. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured spectrophotochemically by the ferricytochrome c method using xanthine/xanthine oxidase as the source of superoxide radicals (McCord and Fridovich, 1969). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine, 0.013 mM cytochrome c and 0.024 IU mL^{-1} xanthine oxidase. One unit of activity was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome c reduction rate measured at 550 nm (McCord and Fridovich, 1969). Glutathione peroxidase (GPX; EC 1.11.1.9) activity was measured following the method of Flohé and Günzler (1984). A freshly prepared glutathione reductase solution (2.4 U mL^{-1} in 0.1 M potassium phosphate buffer, pH 7.0) was added to a 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM sodium azide, 0.15 mM NADPH and 0.15 mM cumene hidroperoxide. After the addition of 1 mM GSH (reduced glutathione), the NADPH-consumption rate was monitored at 340 nm. Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as described by Calberg and Mannervik (1975), with some modifications, by measuring the oxidation of NADPH at 340 nm. The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.63 mM NADPH, and 0.15 mM GSSG. Glutathione *S*-transferase (GST; EC 2.5.1.18) activity was determined by the method of Habig et al. (1974) adapted to microplate. The reaction mixture consisted of 0.1 M phosphate buffer (pH 6.5), 1.2 mM GSH and 1.23 mM solution of 1-chloro-2,4-dinitrobenzene in ethanol were prepared just before the assay. GST activity was monitored at 340 nm by the formation of glutathione-CDNB-conjugate. DT-diaphorase (DTD; NAD(P)H:Quinone oxidoreductase1; EC 1.6.99.2) activity was measured as described by Sturve et al.(2005). The reaction mixture contained 50 mM Tris-HCl (pH 7.3), 50 μM DCPIP (2,6-dichlorophenol indophenol) and 0.5 mM NADH. Control reaction was measured with the addition of purified water instead of sample extract. DTD activity was defined as the difference between sample and control DCPIP reduction; glucose-6-phosphate dehydrogenase (G6PDH; 1.1.1.49) activity was determined by measuring the reduction of NADP + at 340 nm. The assay mixture consisted of 50 mM imidazole–HCl buffer (pH 7.4), 5 mM $MgCl_2$, 2 mM NADP+, and 1 mM glucose-6 phosphate.

Except for SOD, for which the units have already been mentioned, one unit of activity is defined as the amount of enzyme required to transform 1 μmol of substrate/min under the above mentioned assay conditions. To estimate the enzymatic specific activity, soluble protein of the extracts was determined by BCA (bicinchoninic acid) protein assay reagent (Thermo Scientific, Pierce Biotechnology, Rockford, IL, USA) using bovine serum albumin as the standard.

Lipid-peroxidation levels were determined according to Buege and Aust (1978), based on malondialdehyde (MDA) levels generated as product of lipids peroxides degradation. In the presence of thiobarbituric acid, MDA reacts producing a colored thiobarbituric-acid-reacting substance

Table 1
Water characteristics of the different sampling sites.

Species	Sampling site	Temperature (° C)	Conductivity (μS/cm^2)	Dissolved oxygen (mg/L)	pH
S. pyrenaicus	Río Guadalfeo	7.7	198.1	9.15	8.25
O. mykiss	Riofrío-Arroyo salado	13.7	631	8.25	8.42
L. sclateri	Río Cubillas	13.5	476	8.33	8.53
C. carpio					
C. auratus	Pond in Alomartes	17.8	487	8.57	6.84