Oxidative damage and mechanisms of antioxidant defense in the red blood cells of longhorn sculpin (*Myoxocephalus octodecemspinosus*)

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Due to their physiological role as oxygen transporters, red blood cells (RBCs) are continuously exposed to reactive oxygen species. Using the RBCs from longhorn sculpin our aim was to describe the natural levels of oxidative damage and antioxidant defense mechanisms in the nucleated RBCs of a teleost fish. The data acts as a point of departure for future work investigating the role of oxidative stress in the process of aging of nucleated RBCs in teleost fishes.

Under natural conditions, red blood cells (RBCs) are continuously exposed to reactive oxygen species (ROS) as a consequence of their role as oxygen transporters[2,3]. Unlike their mammalian counterparts, teleost RBCs retain a nucleus throughout their lifespan. These RBCs also have fully functioning mitochondria and ribosomes that support continual synthesis of proteins. As such, these nucleated RBCs have an additional source of ROS due to their high rates of aerobic metabolism. The free radical theory treats aging as the result of cumulative oxidative damage to biomolecules such as proteins, lipids and nucleic acids. The current work provides baseline levels of oxidative damage and antioxidant defense mechanisms in the nucleated RBCs of fish under control conditions. Future studies will build on this knowledge by investigating the relationship between RBC age and levels of oxidative damage/antioxidant defense mechanisms in order to determine if the free radical theory is the cause of aging in nucleated RBCs of fish and their subsequent targeted removal from circulation.

Baseline levels of oxidative damage and antioxidant defense mechanisms were measured in RBCs from six longhorn sculpin (*Myoxocephalus octodecemspinosus*) held under ambient conditions. Blood was drawn from the caudal vessel in sculpin anaesthetized with clove oil (80 mg/L) and separated into separate vials for each biochemical analysis. Aliquots of whole blood were taken from each vial for analysis of hemoglobin via Drabkin method[1]. Remaining blood samples were centrifuged to separate plasma and packed RBCs. RBCs were lysed with dH_20. Levels of lipid peroxidation were analyzed in the hemolysate solution via the thiobarbituric acid reactive species (TBARS) commercial kit assay (Cayman chemicals). Remaining vials of hemolysate were centrifuged to isolate the cytosolic contents for analysis of glutathione peroxidase (GPx)[5] activity, total glutathione (GSH) and oxidized glutathione (GSSG) levels[6]. All values of oxidative damage and antioxidant defense in the RBCs were normalized per gram of hemoglobin.

Table 1. Levels of lipid peroxidation, total glutathione (GSH), oxidized glutathione (GSSG), glutathione redox status (GSH:GSSG) and activity of glutathione peroxidase (GPx) in the RBCs of longhorn sculpin (*Myoxocephalus octodecemspinosus*). Data presented as mean ± SE (n=6).

Oxidative Damage	Antioxidant Defense Mechanisms			
Lipid Peroxidation (ηmol MDA/g Hb)	GPx (μmol NADH/min/g Hb)	GSH (μmol/g Hb)	GSSG (μmol/g Hb)	GSH:GSSG (redox status)
450 ± 5.0	13.1 ± 3.1	3.8 ± 0.9	0.68 ± 0.2	6.8 ± 1.5

Levels of lipid peroxidation in the sculpin are comparable to published values for RBCs from both freshwater and marine teleost species that ranged from 717-1435 ηmol MDA/g Hb[7]. Lipid peroxidation has been shown to be highly variable in teleost fish and to undergo significant seasonal variation. Glutathione peroxidase is a major antioxidant enzyme in cells that catalyzes the reduction of hydroperoxides (including H_2O_2) that can be extremely damaging to lipid membranes. The RBCs of sculpins have elevated levels of GPx compared to the enucleated RBCs of humans and the nucleated RBCs of chickens (5.7 ± 1.5 and 5.2 ± 1.0 μmol NADH/min/g Hb)[4], which suggests sculpin may have a greater potential to protect against lipid peroxidation than other vertebrates. Glutathione is the major endogenous antioxidant produced by cells. Cells with high

levels of the reduced form of glutathione (GSH) have a high antioxidant defense potential. In future studies, any increase in the concentration of oxidized glutathione (GSSG) or a reduction in the glutathione redox status (GSH:GSSG) would be indicative of elevated exposure to free radicals, either due to environmental stress or decreased functioning of the antioxidant defense mechanisms.

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