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Diethyldithiocarbamate injection induces transient oxidative stress in goldfish tissues

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Abstract

The effects of intraperitoneal injection of diethyldithiocarbamate (DDC) on free radical processes were examined in brain, liver and kidney of goldfish (*Carassius auratus*). Levels of oxidatively modified lipids and proteins as well as the activities of antioxidant and associated enzymes were measured. Intraperitoneal injection of DDC at a concentration of 0.01 mg/g wet mass decreased SOD activities by about 30–50% after 48 and 168 h compared to corresponding sham-injected values. This treatment resulted in transient oxidative stress. Lipid peroxide content increased after DDC injection at all time points in the kidney, after 48 h in the liver and was elevated in most experimental groups in the brain. Thiobarbituric-acid reactive substances (end products of lipid peroxidation) rose within the first 48 h after injection, but returned to initial levels after 168 h. Two other indices of oxidative stress were also transiently modified: protein carbonyl levels in the brain and kidney increased 24 h post-injection, and the low-molecular mass thiol content was reduced over the same period in all tissues examined. Activities of catalase, glutathione peroxidase, glutathione-*S*-transferase, glutathione reductase, and glucose-6-phosphate dehydrogenase showed differential responses to DDC treatment that rebounded by 168 h post-injection. Glutathione peroxidase activities were reduced by 60, 45 and 65% in the brain, liver and kidney, respectively, after 24 h but rebounded thereafter. After 48 h post-injection with DDC significant decreases were also seen in liver and kidney catalase, GST activities in all three tissues, and kidney GR and G6PDH activities. In some cases, catalase, GST, GR and G6PDH activities transiently increased after 24 h. It was concluded that DDC injection depleted SOD and simultaneously stimulated lipid peroxidation, but did not require compensatory enhancement of other enzymatic defenses. Different actions of the superoxide anion in cellular metabolism and possible consequences of the impairment of superoxide dismutase are discussed.

Keywords: Goldfish; Superoxide dismutase; Diethyldithiocarbamate; Oxidative stress; Superoxide anion

1. Introduction

The goldfish *Carassius auratus* is highly tolerant of multiple environmental stresses [1]. This species endures both long-term anoxia [2–5] and hyperoxia [6] as well as a wide range of low and high temperatures [7–10]. All these stresses are accompanied by an environmen-

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tally induced elevation of reactive oxygen species (ROS) in tissues, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) [11,12]. The steady-state level of ROS is the balance between production and decomposition, and an imbalance in these processes in favor of the former has been termed "oxidative stress" [11–13]. Oxidative stress can disturb and damage many cellular processes, sometimes leading to cell death.

Organisms subjected to environmental stress often need to deal with at least three problems – energy provision, accumulation of metabolic end products, and emerging oxidative stress. The first two aspects of biochemical adaptation have been well studied in many species with respect to low oxygen stress. The responses to these problems include high levels of fermentable substrates, mainly glycogen, that are used for anaerobic energy production [3], and high activities of glycolytic enzymes [14,15]. The problem of end product build-up is tackled in one of several ways; in goldfish, the lactic acid produced by all organs is converted by skeletal muscle into ethanol and carbon dioxide which are then excreted into the environment via the gills [2–4,16]. The third problem, oxidative stress development, has not received enough attention and just a few years ago it was shown that goldfish possess relatively high levels of antioxidants which may be altered according to the needs of the organism. Elevation of several oxidative stress markers, particularly oxidized proteins and lipids, has been described in tissues of goldfish exposed to anoxia and recovery [5], hyperoxia [6], and heat shock [9,10]. To help clarify the involvement of specific mechanisms of antioxidant defense in the protection of goldfish tissues against ROS, we inhibited the antioxidant enzyme, catalase, using aminotriazole and found that this procedure resulted in oxidative stress in brain [17], liver and kidney [18].

The present study was designed to assess the metabolic consequences of inhibition of another key enzyme of antioxidant defense, the copper and zinc containing superoxide dismutase (Cu,Zn-SOD). To investigate this, we inhibited SOD *in vivo* using diethyldithiocarbamate (DDC). DDC inhibits Cu,Zn-SOD both *in vitro* [19] and *in vivo* [20–22] by extracting the copper ion from the enzyme active center. However, DDC may also affect other enzymes like xanthine oxidase [23] by the same mechanism. Here we evaluated the consequences of DDC treatment on the activities of other antioxidant enzymes as well as on the levels of oxidatively modified proteins and lipids in goldfish tissues. We hypothesized that SOD inhibition would result, firstly, in oxidative stress development in goldfish tissues and then, secondly, in compensatory responses by other antioxidant enzymes. We compared organs with highly intensive oxidative metabolism, namely brain, liver and kidney; the latter two organs have major involvement in the catabolism of xenobiotics, whereas brain, that is the most protected organ in animals is also highly oxygen dependent and consumes high amount of oxygen.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), 1-chloro-2,4-dinitrobenzene (CDNB), cumene hydroperoxide, diethyldithiocarbamate (DDC), 2,4-dinitrophenylhydrazine (DNPH), 5,5′-dithio-*bis*(2-nitrobenzoic acid) (DTNB), ethylenediamine-tetraacetic acid (EDTA), glucose-6-phosphate (G6P), reduced glutathione (GSH), oxidized glutathione (GSSG), $NADP^+$, NADPH, phenylmethylsulfonyl fluoride (PMSF), thiobarbituric acid (TBA), sodium azide, xylenol orange, and yeast glutathione reductase (GR) were purchased from Sigma–Aldrich Chemical Co. (USA). *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), quercetin and Tris–HCl were from Reanal (Hungary), and guanidine–HCl was from Fluca. All other reagents were of analytical grade.

2.2. Animals and experimental design

Goldfish (*C. auratus* L.) of both sexes weighing 25–53 g were purchased at a local fish farm (Ivano-Frankivsk district, Ukraine). They were kept in dechlorinated tap water and fed with standard fish food. Temperature was maintained at $20 \pm 1\,^{\circ}C$ with a natural light–dark cycle with light from about 8:00 to 17:00. Goldfish were acclimated to these conditions for at least a month before experimentation.

In preliminary studies 10 specimens per group were injected intraperitoneally with DDC diluted in physiological saline (0.9%, w/v NaCl) at final concentrations of 0.01, 0.025 or 0.05 mg/gram wet body mass (gwm). The volume of injected solution was 0.3% of body mass. Concentrations of 0.025 and 0.05 mg DDC/gwm had lethal effects, and a sublethal concentration of 0.01 mg/gwm was chosen for further experiments. Two experimental groups (5–7 animals per group) were set up: (a) sham-injected animals that received only 0.9% NaCl, and (b) DDC-treated fish that were injected with 0.01 mg/gwm DDC solution. Control fish were not treated. After 24, 48 or 168 h fish injected with DDC or NaCl solutions were sampled and brain, liver and kidney tissues were