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Oxidative status in nestlings of three small passerine species exposed to metal pollution

Miia J. Rainio [a,*], Mirella Kanerva [b], Juha-Pekka Salminen [c], Mikko Nikinmaa [b], Tapio Eeva [a]

[a] Department of Biology, Section of Ecology, University of Turku, FI-20014 Turku, Finland
[b] Department of Biology, Division of Genetics and Physiology, University of Turku, FI-20014 Turku, Finland
[c] Laboratory of Organic Chemistry and Chemical Biology, 20014 University of Turku, FI-20014 Turku, Finland

HIGHLIGHTS

- Species-specific oxidative status was studied in birds exposed to metal pollution.
- Species showed interspecific variation in their antioxidant enzyme activities.
- Oxidative status was only weakly related to fecal metal exposure.

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ABSTRACT

Antioxidant defense has an important role in the protection of organisms against oxidative stress caused by reactive oxygen species (ROS). Many metals are capable of generating ROS and inducing oxidative damage, and may therefore lead to changes in oxidative regulation. We studied species-specific variation in the oxidative status of great tit (*Parus major*), blue tit (*Cyanistes caeruleus*) and pied flycatcher (*Ficedula hypoleuca*) nestlings in a vicinity of a non-ferrous smelter. Non-enzymatic (glutathione [tGSH], GSH:GSSG ratio, and carotenoids) and enzymatic (glutathione peroxidase [GP], glutathione-S-transferase [GST], superoxide dismutase [SOD], and catalase [CAT]) antioxidants were evaluated to determine the effects of metal exposure on the oxidative status of the birds. We found strong evidence of interspecific variation in CAT and SOD activities, whereas less variation was observed in parameters related to glutathione metabolism. Oxidative state (in terms of tGSH and GSH:GSSG) did not vary between species, suggesting that different species may employ different antioxidant pathways to achieve the same oxidative state. Oxidative status was only weakly related to metal exposure, and these associations were further obscured by species-specific environmental effects. Our results indicate that effects on oxidative status observed in one species cannot be generalized to other ones. Future work should attempt to incorporate species-specific biology and environmental context into assessments of contaminant impacts on oxidative regulation of passerine birds.

1. Introduction

Metals appear naturally in the environment, and several of them participate in important metabolic and signaling pathways (Valko et al., 2005). However, at high concentrations these metals can be harmful to organisms, possibly owing to metal-induced oxidative stress caused by increased production of reactive oxygen species (ROS) (Halliwell and Cross, 1994; Halliwell and Gutteridge, 2007). As with metals, ROS play an important role in cell signaling and regulation of redox status (Jackson, 2005; Thannickal and Fanburg, 2000), but may pose hazards to the organisms when produced in excess (e.g. via oxidation of DNA, proteins, and lipids) (Bae et al., 2009; Beckman and Ames, 1998; Harman, 1956).

Metals can be found in different forms in the environment, their toxicity being related to their oxidative state and reactivity with other compounds (Scheuhammer, 1987; Valko et al., 2005; Walker, 1995). Metals can be divided either redox-active or -inactive metals according to their function. Redox-active metals (e.g. copper and iron) catalyze Fenton reactions, which generate reactive hydroxyl radicals and are commonly associated with membranous fractions such as mitochondria, microsomes and peroxisomes, whereas redox-inactive metals (e.g. lead, nickel and cadmium) deplete major cellular antioxidants, such as glutathione. Thus, metals are able to either increase ROS production or reduce antioxidant defense (Ercal et al., 2001; Stohs and Bagchi, 1995; Valko et al., 2005).

Antioxidants are molecules that protect against oxidative damage by reducing ROS (or preventing their formation), thereby rendering them unable to cause further damage to the cell. Antioxidants may be taken up directly from the environment (as in the case of carotenoids), or they may be generated internally by a series of enzymatic and

* Corresponding author. Tel.: +358 2 333 6050; fax: +358 2 333 6550.
E-mail addresses: miikoi@utu.fi (M.J. Rainio), mmkane@utu.fi (M. Kanerva), j-p.salminen@utu.fi (J.-P. Salminen), miknik@utu.fi (M. Nikinmaa), teeva@utu.fi (T. Eeva).

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non-enzymatic reactions (Sies, 1993). Glutathione is an antioxidant that has been found in almost all organisms examined to date (Andrews, 2000; Pinto et al., 2003). It functions by binding metals at sulfhydryl groups, thereby preventing them from creating ROS (Andrews, 2000; Pinto et al., 2003). Each glutathione molecule cycles between reduced (GSH) and oxidized (GSSG) states, with the overall ratio of GSH:GSSG serving as an indicator of current oxidative onslaught. Cycling between GSH and GSSG is catalyzed by a number of enzymes, including glutathione peroxidase (GP) and glutathione-S-transferase (GST) (Halliwell and Gutteridge, 2007). In normal cells, we expect to see a relatively high GSH:GSSG ratio, and low activity of associated enzymes. In contrast, a low GSH:GSSG ratio is indicative of oxidative stress (Halliwell and Gutteridge, 2007; Hoffman, 2002; Isaksson et al., 2005; Stephensen et al., 2002). In the event that metal concentrations overwhelm the protective capacity of glutathione, cells also have a number of mechanisms through which ROS can be deactivated directly. Two enzymes, superoxide dismutase (SOD) and catalase (CAT), are among these regulatory antioxidants (Ercal et al., 2001; Pinto et al., 2003). In this paper, the term oxidative state is used to refer to oxidative state in terms of tGSH and the ratio of GSH:GSSG, and the term oxidative status is used to cover both enzyme activities (GP, GST, SOD and CAT) and the antioxidant GSH and the GSH:GSSG ratio.

In this paper, we report on antioxidant (tGSH, GSH:GSSG ratio, and carotenoids) levels and antioxidant enzyme (GP, GST, SOD, and CAT) activities in nestlings of three passerine species, great tits (*Parus major*), blue tits (*Cyanistes caeruleus*), and pied flycatchers (*Ficedula hypoleuca*), living near a metal smelter in Harjavalta, Finland. Previous studies have reported reductions in survival, reproductive success, genetic integrity, plumage brightness, carotenoid levels, and food availability associated with living on metal-contaminated areas (Berglund et al., 2010; Dauwe et al., 2006; Eeva et al., 2006; Eeva and Lehikoinen, 1996; Eeva et al., 2003, 1998; Geens et al., 2010), but little is known about management of oxidative stress (Bel'skii and Stepanova, 1995; Berglund et al., 2007; Geens et al., 2009; Koivula et al., 2011). Studies that have sought to address this question have yielded conflicting results, with some researchers reporting increased oxidative stress at polluted sites, and others reporting no apparent association (Berglund et al., 2007; Isaksson et al., 2005, 2009; Koivula et al., 2011). Part of the reason for this discrepancy may be that different species regulate antioxidant metabolism in different ways.

The goals of our study were two-fold: First, we sought to generate baseline data on regulation of antioxidants in three avian species while accounting for variation due to environment and phylogeny. Second, we attempted to determine whether oxidative regulation of nestlings reared on contaminated areas deviated from these baselines as a result of increased exposure to metals. The use of several species in the same study set-up enables us to standardize many sources of variation, such as habitat quality, breeding season, tissue used in the analyses and measurement techniques. Fecal samples, which have been proven to be good indicators of metal pollution, especially when studying food chain contamination (Berglund et al., 2011; Dauwe et al., 2000, 2004; Eeva and Lehikoinen, 1996), were used to measure actual metal exposure. In insectivorous birds the transfer of metals via the food chain has been considered as a major source of metal contamination (Berglund et al., 2009). Plasma carotenoids were also examined, because carotenoids are small-molecule antioxidants and their levels have been shown to vary in relation to environmental pollution due to pollution-related changes in food webs (Eeva et al., 2008, 2009b; Geens et al., 2009).

2. Materials and methods

2.1. Study area and study species

The study was conducted in summer 2008 in the vicinity of a metal smelter complex in the town of Harjavalta (61°20′ N, 22°10′ E), one of the most metal polluted areas in Finland. The main pollutants in the smelter area are nickel (Ni) and copper (Cu), but arsenic (As), zinc (Zn), lead (Pb) and sulfuric oxides are also emitted in appreciable amounts (Kiikkilä, 2003). Studies of wild birds have been ongoing in this area for the past 21 years, and several effects of pollution exposure have been reported in past until today (Eeva et al., 2009a; Eeva and Lehikoinen, 1996, 1998; Koivula et al., 2011), in spite of fact that pollution levels have declined considerably since the 1990s (Kozlov et al., 2009; Kubin et al., 2000).

We used thirteen study sites, each with 40–50 nest boxes at a distance of 0.6–11.2 km from the smelter, in three main directions (SW, SE and NW) (Fig. 1). Seven sites were in the polluted area (zone 1; <2.5 km from the smelter) and six in the unpolluted area (zone 2; >2.5 km from the smelter), where the pollution levels are close to the background levels (Eeva et al., 2008). We used similar wooden nest boxes for each species that were hanged 2 m above the ground level onto the trees, at the range of 35–40 m from each other. A description of the nest boxes is given in Lambrechts et al. (2010). The habitat type was barren Scots pine (*Pinus sylvestris*) dominated forest in all our study areas to avoid habitat-related variation between the study areas. All three study species are abundant at our sites and breed in the nest boxes.

Altogether 243 nestlings of the great tits (n = 93), blue tits (n = 47) and pied flycatchers (n = 103) were included in this study. Approximately half of the nestlings were born in polluted area and half in unpolluted areas (great tit; 47 in zone 1 and 46 in zone 2, blue tit; 31 in zone 1 and 16 in zone 2, pied flycatcher; 52 in zone 1and 51 in zone 2). We visited nest boxes daily throughout the breeding season in order to collect information on clutch size, hatching success, and fledging success. The study was performed under the licenses of the Animal Care & Use Committee of Turku University (ESLH-2008-02274/Ym-23) and Regional Environment Centre (LOS-2008-L-224-254).

2.2. Sampling methods

2.2.1. Sampling in the field

All nestlings were uniquely ringed at either 6 (pied flycatchers) or 7 (great tits and blue tits) days of age. At 9 (pied flycatchers) or 11 (great and blue tits) days old, we randomly selected two nestlings per brood (excluding runts) on which to perform measurements and collect blood samples. The difference between the times of sample collection was due to difference in the nestling development rate between pied flycatcher and the Parid species, pied flycatchers developing faster than Parids. The blood samples were taken by brachial venipuncture using 75 μl heparinized capillary tubes, and centrifuged immediately for 5 min at 4000 rpm to separate the plasma from red blood cells. Plasma and red blood cells were then immediately placed in liquid nitrogen until permanent storage at −80 °C.

2.2.2. Carotenoid analyses

We determined plasma concentrations of lutein, zeaxanthin, and β-carotene using high performance liquid chromatography (HPLC). Briefly, a known volume of plasma (10–35 μl) was extracted 3× with 100% acetone. The solvent was evaporated from the combined extract under vacuum and the residue was dissolved into a small volume of 80% acetone. The carotenoid composition of the extracts (lutein, zeaxanthin, and β-carotene) was analyzed with HPLC at 450 nm using a Merck Purospher STAR RP-18 (55 × 2mm, i.d., 3 μm) column (Darmstadt, Germany). β-carotene was quantified as β-carotene and other carotenes as lutein equivalents.

2.2.3. Fecal metal analyses

As for blood samples, we analyzed fecal metal concentrations of two nestlings per brood. Following collection, fecal sacs were pooled by brood and dried at 50 °C for 72 h. Thereafter, we combined 0.15–0.20 g of fecal material with 2 ml of Supra-pure HNO_3 and 0.5 ml of H_2O_2 were added to the samples in Teflon bombs for digestion with a microwave system