Mutation Research 702 (2010) 24–31

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Genotoxicity in herring gulls (*Larus argentatus*) in Sweden and Iceland

Halldora Skarphedinsdottir[a,*], Gunnar Thor Hallgrimsson[b], Tomas Hansson[a], Per-Åke Hägerroth[a], Birgitta Liewenborg[a], Ulla Tjärnlund[a], Gun Åkerman[a], Janina Baršienė[c], Lennart Balk[a]

[a] Department of Applied Environmental Science, Unit of Environmental Toxicology and Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden
[b] Institute of Biology, University of Iceland, IS-101 Reykjavík, Iceland
[c] Institute of Ecology, Vilnius University, Akademijos 2, LT-08412 Vilnius 21, Lithuania

ARTICLE INFO

Article history:
Received 2 February 2010
Received in revised form 16 June 2010
Accepted 23 June 2010
Available online 17 July 2010

Keywords:
DNA adducts
Micronuclei
^{32}P-postlabelling
Larus argentatus
Pulli
Baltic Sea
Sweden
Iceland

ABSTRACT

Adult and young herring gulls (*Larus argentatus*) in Sweden and Iceland were investigated with respect to DNA adducts, analysed with the nuclease-P1 version of the ^{32}P-postlabelling method, and micronucleated erythrocytes. Three important aims were: (1) to estimate the degree of exposure to genotoxic environmental pollutants in the Baltic Sea area and Iceland, (2) to evaluate the utility of the investigated biomarkers in birds, and (3) to investigate if there was any relationship between genotoxic effects and thiamine deficiency. The results demonstrate that both Swedish and Icelandic herring gulls are exposed to genotoxic pollution. Urban specimens have higher levels of DNA adducts than rural specimens, but background exposure to genotoxic environmental pollutants, such as PAHs, is also significant. In the herring gull the general level of DNA adducts in the liver seems to be higher than in fish. DNA adducts were most abundant in the liver, followed by the kidney, intestinal mucosa, and whole blood, in decreasing order. The frequency of micronucleated erythrocytes was probably slightly elevated in all the investigated sites, reflecting a significant background exposure. The level of DNA adducts was unrelated to the frequency of micronucleated erythrocytes, and both these variables were unrelated to symptoms of thiamine deficiency. The investigation confirmed the utility of DNA adducts, and probably also micronucleated erythrocytes, as biomarkers of genotoxicity in birds.

1. Introduction

During the last decades, many bird species breeding in Sweden and the Baltic Sea area have suffered from conspicuous population declines [1,2] and some of them are even endangered over their entire range, *e.g.* the Fennoscandian subspecies of the lesser black-backed gull (*Larus fuscus fuscus*) [3]. In a recent study, paralysis and mass mortality in the herring gull (*Larus argentatus*) and several other bird species in the Baltic Sea area were explained by thiamine deficiency, which was also suggested as a possible cause for the observed population declines [4]. In the present study, genotoxicity was measured in the same specimens that were examined with respect to thiamine deficiency [4]. Hence, one important aim was to investigate if there was any relationship between genotoxic effects and thiamine deficiency.

So far, birds have received very little attention in studies of environmental genotoxicity. Previous work on genotoxicity in herring gulls included the effect of industrial pollution on mutation rates quantified by multi-locus DNA fingerprinting [5,6] and on DNA damage measured as strand length (median molecular length of DNA) [7]. In the present study, genotoxicity was measured as DNA adducts and frequency of micronucleated erythrocytes. Two important aims were: (1) to estimate the degree of exposure to genotoxic environmental pollutants in the Baltic Sea area and Iceland, and (2) to evaluate the utility of these biomarkers in birds. To corroborate our results we investigated the relationship between the biomarker responses and the actual pollution level of the surroundings. The Baltic Sea has historically received large inputs of pollutants and is currently considered to be one of the most polluted seas in the world [8] with eutrophication and pollution affecting its entire ecosystem [9].

Genotoxic substances produce chemical and/or physical modifications to the DNA, and damaged DNA may lead to a range of consequences. Several studies have demonstrated relationships between DNA damage and reduced fitness, such as gene and protein dysfunction, tumour initiation, growth impairment, embryonic malformations, reduced fecundity, and negative effects on longevity [10–15]. Polycyclic aromatic hydrocarbons (PAHs) are a major class of genotoxic pollutants commonly present in polluted areas. The genotoxic effects of PAHs are well known, and especially the formation of DNA adducts [*e.g.* 16–18]. Adducts are formed when reactive electrophilic metabolites of chemicals bind

* Corresponding author. Tel.: +46 8 674 7270; fax: +46 8 674 7638.
E-mail address: halldora@itm.su.se (H. Skarphedinsdottir).

1383-5718/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrgentox.2010.07.002

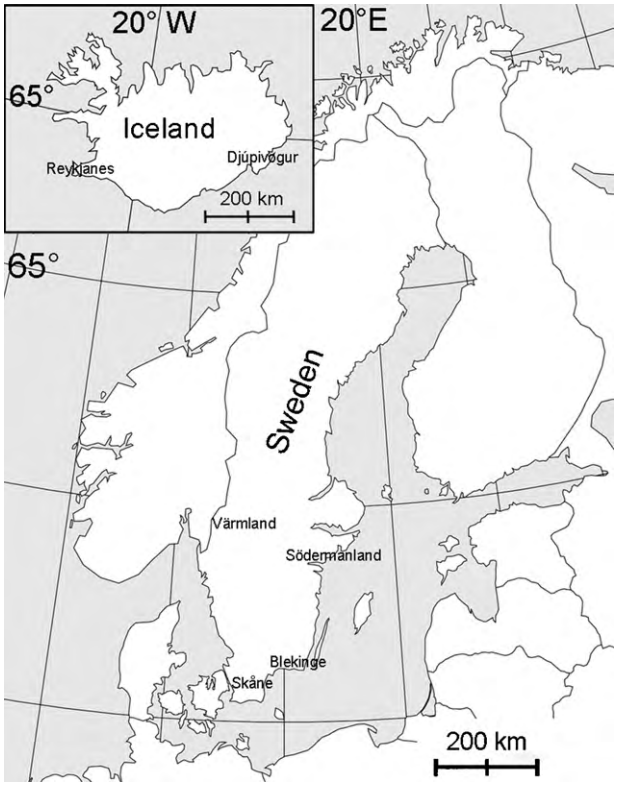

Fig. 1. The investigated regions. Adult herring gulls (*Larus argentatus*) were collected in the County of Södermanland, the County of Blekinge, and the County of Skåne in Sweden and on the Reykjanes peninsula and at Djúpivogur on Iceland. Herring gull pulli were collected in the County of Värmland and the County of Södermanland in Sweden and on the Reykjanes peninsula on Iceland. The County of Skåne was the only urban site (Malmö harbour). The other sites were rural.

covalently to the DNA. Such reactive electrophilic metabolites also readily attack protein, RNA, and other cell constituents, thereby increasing the risk for a wide range of dysfunctions. Consequently, DNA adducts may be used both as a measure of the direct genotoxic effect, and as an indicator of a wider range of toxic exposure [10,19]. DNA adducts have been widely used as a biomarker in fish [*e.g.* 18,20,21] and mussels [*e.g.* 22,23], and are considered to be one of the best biomarkers of PAH exposure.

The formation of micronuclei is another genotoxic effect. Micronuclei are small DNA-containing bodies outside the cell nucleus, formed by chromosome breakage, centromere or spindle dysfunction, or defective cytokinesis (cell division) [24]. They may be generated by aneugens, which damage the spindle, and/or by clastogens, which induce chromosomal breaks [25]. The frequency of micronucleated erythrocytes has been used as a biomarker for more than 25 years [26].

2. Methods

2.1. Bird material and sampling

Adult herring gulls (*L. argentatus*) were collected in three regions in Sweden and two regions in Iceland (Fig. 1) from mid-May to mid-August 2004. The Swedish regions were: the County of Skåne (Malmö harbour), an urban site with high anthropogenic pollution load (Table 1), and the Counties of Blekinge and Södermanland, which are both rural sites with comparatively lower anthropogenic pollution load (Table 1). Herring gull pulli were collected in the County of Värmland and the County of Södermanland, which are both rural sites. The Icelandic regions were the Reykjanes peninsula (Reykjanes and Miðnesheiði) and Djúpivogur, which are two rural sites without known anthropogenic pollution sources. The two Icelandic regions were equivalent with respect to DNA adducts and micronucleated erythrocytes in the adult herring gulls, and were therefore pooled and used as a single control for the Baltic Sea area. Icelandic pulli were collected only on the Reykjanes peninsula (not Djúpivogur). The adult herring gulls were caught with cannon-nets (Iceland) or fyke or hand nets (Sweden), whereas the pulli were collected by hand or with a hand-net. The birds were transported to the laboratory, where they were examined and sampling was performed. The occurrence of paralytic symptoms was noted. Sacrifice was performed by cervical dislocation. The condition was estimated by visual inspection on a four-grade scale based on the amount of fat on inner organs: 0 = emaciated (completely devoid of body fat), 1 = poor, 2 = intermediate, and 3 = good. Liver-body index (LBI) was obtained as 100 times the liver weight divided by the body weight. The LBI values fell into two distinct groups (without overlap). The group with LBI $\geq$ 2.5% were considered to suffer from hepatomegaly. The exact age of the full-grown birds was not determined. The weight range of the pulli was 100–670 g and, accordingly, all of them had started to eat food provided by the parents. Collection of birds in the field for invasive sampling was performed in compliance with permits issued by the Swedish Environmental Protection Agency and the Icelandic Ministry for the Environment.

2.2. Sample preparation

Before sacrifice, blood was sampled directly from the heart with a 10-mL syringe. Blood smears for determination of the frequency of micronucleated erythrocytes were prepared with the wedge procedure described by Vives Corrons et al. [29]. The blood smears were air-dried, fixed for 10 min in 99.5% methanol, air-dried again, and kept in the dark until staining and counting of micronucleated erythrocytes. Tissue samples for DNA-adduct analysis were taken from the liver, kidney, intestinal mucosa, and whole blood. The samples were placed in cryotubes, snap-frozen in liquid nitrogen and kept in a −80 °C freezer until analysis.

2.3. Chemicals

Giemsa stain (11700), micrococcal endonuclease (N-3755), nuclease P1 (7160), RNase A (R-4642), spermidin (S-2626), spleen phosphodiesterase (P-9041), standard DNA (salmon sperm, D-1626), and Trizma® base (T1503) were obtained from Sigma–Aldrich Sweden AB (Stockholm, Sweden). α-Amylase (102814), phenol (1814303), proteinase K (1000144), RNase T1 (109193), and T_4-polynucleotide kinase (3′-phosphatase free, 838292) were obtained from Roche Diagnostics Scandinavia AB (Bromma, Sweden). Radio-labelled ATP ([γ-^{32}P]ATP) with specific activity 3000 Ci/mmol (110 TBq/mmol) was obtained from GE Healthcare Bio-Sciences (Uppsala, Sweden). The benzo[*a*]pyrene standard adduct, 7*R*,8*S*,9*S*-trihydroxy,10*R*-(N^2-deoxyguanosyl-3′-phosphate)-7,8,9,10-tetrahydro-benzo[*a*]-pyrene (B[*a*]PDE-dG-3′p), was obtained from Midwest Research Institute (Kansas City, MO, USA). Cellulose (MN-301) was obtained from Machery-Nagel (Düren, Germany). Vinyl strips (PVC foil, 0.2-mm thickness), used for the groundwork of the polyethyleneimine cellulose sheets were obtained from Andrén & Söner (Stockholm, Sweden). Ultima Gold™ scintillation fluid (6013329) was obtained from CiAB (Sollentuna, Sweden). All other chemicals were of analytical purity and were obtained from common commercial sources.

2.4. Analysis of DNA adducts

Our experience of other vertebrates is that DNA-adduct levels are higher in the liver than in other organs. Therefore, in order to avoid values below the detection limit in the kidney, intestinal mucosa and whole blood, a stepwise procedure was used. DNA adducts were first measured in the liver of 25 adult herring gulls. Then a sub-sample, consisting of specimens with high levels of liver DNA adducts, was selected for measurement of DNA adducts in the kidney, intestinal mucosa, and whole blood. The Swedish part of the sub-sample consisted of the specimens with ranks 1–3, 7, and 12 (three highest plus two among the 12 highest with respect to liver DNA adducts), whereas the Icelandic part of the sub-sample consisted of the specimens with ranks 1–6 (six highest with respect to liver DNA adducts).

The tissue samples were semi-thawed and the DNA was extracted and purified according to Dunn et al. [16], and Reichert and French [30] with slight modifications according to Ericson and Balk [31]. DNA adducts were analysed with the nuclease-P1 version of the ^{32}P-postlabelling method [32] with some modifications described below. Purified DNA was hydrolysed to 3′-nucleoside monophosphates by incubation of 12.5-μL aliquots at 37 °C for 4 h with micrococcal endonuclease (24 mU/μg DNA) and spleen phosphodiesterase (3.2 mU/μg DNA) in a solution of 0.1 mM $CaCl_2$ and 10 mM succinate buffer, pH 6.0. DNA adducts were enriched by the nuclease-P1 method: normal nucleotides were hydrolysed to nucleosides by addition of 0.41 μg nuclease P1 per μg DNA and incubation of the sample at 37 °C for 30 min. After adjustment of the pH to approximately 7.8 by the addition of 1 μl of 0.5 M Tris–HCl, the mixture was evaporated to dryness in a SpeedVac® centrifuge (Savant Instrument Inc. Farmingdale, NY, USA). The DNA adducts were radio-labelled by incubation at 37 °C for 30 min with a mixture containing 8.3 U of T_4-polynucleotide kinase, 3.1 MBq [γ-^{32}P]-ATP (110 TBq/mmol), 10 mM $MgCl_2$, 10 mM dithiothreitol, 3.5 mM spermidine, and 50 mM Tris–HCl, pH 7.6.

The radio-labelled DNA adducts were separated by means of multidirectional thin-layer chromatography (TLC) on polyethyleneimine cellulose thin-layer sheets prepared according to Reichert and French [30] with a Desaga spreader (Desaga, Heidelberg, Germany). Chromatography solvents used for the separation were essentially the same as those described by Reddy and Randerath [32]: