VARIATION IN CYTOCHROME P4501A mRNA INDUCIBILITY AMONG INDIVIDUAL CHICKENS AND HERRING GULLS

Jessica A. Head[1], Vance L. Trudeau[1], Sean W. Kennedy[1,2]

[1]CAREG, Department of Biology, University of Ottawa, Ottawa, ON
[2]Environment Canada, Canadian Wildlife Service, National Wildlife Research Centre, Ottawa, ON

Introduction

Dioxin-like compounds are a class of environmental contaminants which includes dioxins, dibenzofurans and polychlorinated biphenyls (PCBs). Elevated concentrations of dioxin-like compounds in the Great Lakes have been associated with adverse effects and population declines in herring gulls (*Larus argentatus*) and other colonial fish-eating birds[1]. Compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) bind to the aryl hydrocarbon receptor (AHR) with high affinity and alter transcription rates of a number of genes, most notably cytochrome P4501A (CYP1A) isoforms.

The clearly described dose-response relationship between dioxin exposure and CYP1A induction makes the AHR pathway useful for assessing exposure to dioxin-like environmental contaminants. Activity of the CYP1A4 enzyme in chickens can be measured with the ethoxyresorufin-*O*-deethylase (EROD) assay, and is frequently used as a biomarker for dioxin exposure. The two known chicken isoforms, CYP1A4 and CYP1A5, are both strongly induced by TCDD[2]. Two analogous isoforms have been cloned in herring gull (AY233271, AY220876), but it is not known which catalyzes the EROD reaction or if both are induced by dioxin-like compounds.

Species differ in sensitivity to TCDD in terms of both EROD activity and toxicological effects. Chickens, the most sensitive birds studied to date, are upwards of 30 times more sensitive to EROD induction by TCDD than herring gulls. This observation is relevant in terms of risk assessment because the EROD response is predictive of *in vivo* toxicity; species of birds with low EROD EC_{50}s tend to be more sensitive to the toxicological effects of dioxin-like compounds *in ovo*[3]. Interindividual variation in sensitivity to dioxin-like compounds has also been described within a species. For example, sensitivity to EROD induction in herring gulls, ring-billed gulls, chickens, terns and cormorants was found to range widely between individuals[4].

In this study we characterized induction of CYP1A mRNA expression in TCDD treated embryo hepatocyte cultures from individual herring gulls and chickens. The main objectives of this work were to characterize the range of interindividual variation in the CYP1A response, and to assess whether this bioassay can be used to identify individuals and species that are resistant to the effects of dioxin-like compounds.

Materials and Methods

Eggs and Incubation: Fertilized chicken (*Gallus gallus*) eggs (several breeds) were incubated at 37.5°C, 60% relative humidity for 19 days (one day pre-hatch). Fertilized herring gull (*Larus argentatus*) eggs were collected from Chantry Island, Lake Huron, or Middle Sister Island, Lake Erie in April 2002, and incubated at 37.5°C, 60% relative humidity for 26 days (one day pre-hatch). Since each egg was collected from a different nest, none of the embryos shared the same mother.

Cell culture: Individual avian hepatocyte cultures were prepared as previously described[5]. After 24 hours of incubation, medium was removed and replaced with medium dosed with TCDD or solvent control (DMSO). TCDD doses were chosen to elicit a maximal response in each species. Cells were exposed for 24 hours, medium was removed, and the plates were frozen at -80°C.

RNA Isolation and cDNA synthesis: Total RNA was isolated from cells with the Qiagen RNeasy 96 kit and on-column DNAse treatment according to manufacturer's instructions (Qiagen, Mississauga, ON, Canada). A 50% ethanol solution was used for RNA extraction because this produced higher yields than the recommended 70% ethanol solution. RNA was reverse transcribed to cDNA with SuperSCRIPT™ II and random primers according to manufacturer's instructions (Invitrogen, Burlington, ON, Canada).

Quantitative RT-PCR: Gene expression was measured using a quantitative RT-PCR method developed for this study. Primer and probe sets were designed based on GenBank sequences for chicken CYP1A4 (X99453), CYP1A5 (X99454), and beta-actin (L08165) and herring gull beta-actin (AY045724). Primers and probes for herring gull CYP1A5 were designed based on a cloned fragment. Herring gull CYP1A4 (AY233271), and CYP1A5 (AY220876) have since be identified by a different group. Multiplex reactions were run for chicken or herring gull sequences with beta-actin as endogenous control.

Results and Discussion

Variation is common within natural populations, and is a necessary precondition for natural selection to occur. In this paper we describe interindividual variation in TCDD induced expression of CYP1A isoforms in herring gulls and chickens. Previous studies found a large degree of interindividual variation in the EROD inducing potency of TCDD in these two species[4]. Our research substantiates the finding of interindividual variation in an AHR-mediated response with the observation of a large degree of variation at the mRNA level (Figure 1).

Concentrations of CYP1A mRNA were measured in TCDD treated and untreated hepatocyte cultures from 16 chickens and 16 herring gulls (Figure 1). Expression in treated cells over basal expression (fold induction), was calculated for each individual. The degree of variation between individuals for a given response was calculated as the coefficient of variation (CV = standard deviation/mean). The CVs for induction of chicken CYP1A4 and CYP1A5 were 1.0 and 0.9 respectively and the pattern of expression between individuals was similar for the two isoforms. These similarities were expected since these genes are activated through the same biochemical pathway. The CV for induction of herring gull CYP1A5 was 0.6. The lower CV suggests that there is less variation in Great Lakes herring gulls than in chickens tested for this response.

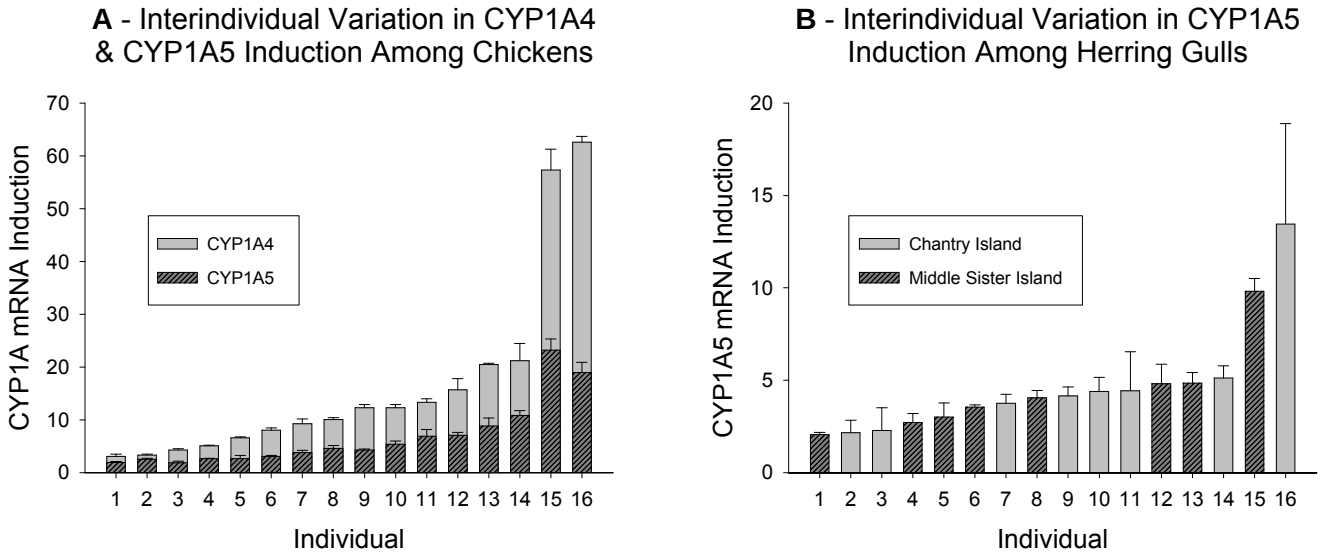

Figure 1: Interindividual variation in CYP1A mRNA induction in 3nM TCDD treated chicken embryo hepatocytes (A), or 100 nM TCDD treated herring gull embryo hepatocytes (B). Bars represent CYP1A mRNA expression over basal levels for each individual. Data are based on 3 replicate analyses of the same sample, and error bars represent the standard deviation of these 3 readings. TCDD doses were chosen to elicit a maximal response in each species. The coefficients of variation (CVs) for these data are as follows: chicken CYP1A4 (1.0), chicken CYP1A5 (0.9), herring gull CYP1A5 (0.6). There was no observed effect of gender on CYP1A inducibility in chickens or in herring gulls.

Herring gull eggs were collected from a relatively clean site (Chantry Island) or a contaminated site (Middle Sister Island) in the Great Lakes. Each egg was collected from a different nest. A large degree of variation in CYP1A5 inducibility was observed among individuals at both sites. There was no significant difference between sites. This may indicate that genetic differences among individuals are a more important contributor to variation in the CYP1A response than environmental factors associated with collection site (such as contaminant load). Genetic factors would not be expected to contribute to differential sensitivity between the sites, since gulls from isolated colonies in the Great Lakes form one interbreeding population[6]. It is possible that genetic factors contribute to differential sensitivity between herring gulls at a population level. We plan to test this hypothesis by comparing CYP1A inducibility in gulls from the Great Lakes, and gulls from a genetically distinct population with a history of lower contaminant levels.

The main purpose of this study was to characterize interindividual variation in a molecular response to TCDD, and to determine if these data could be used as a means of identifying sensitive and resistant individuals. In both species tested, we succeeded in identifying individuals that appeared to be particularly sensitive to CYP1A induction in cell culture. At doses known to elicit a maximal response in each species, CYP1A5 fold induction in herring gull hepatocytes was comparable to CYP1A5 fold induction in chicken hepatocytes. This was somewhat surprising since EROD data demonstrates that chickens are dramatically more sensitive than herring gulls in terms of both maximal response and EC_{50}[3]. Because expression of herring gull CYP1A isoforms has not been previously described, it is difficult to explain this observation. One possibility is that herring gull CYP1A isoforms are different from chicken isoforms in terms of either expression patterns, or EROD activity. In chickens, CYP1A4 catalyzes the EROD reaction and is induced to a higher level than CYP1A5. It is not known if these properties also apply to the herring gull isoforms. We are currently developing assays to measure CYP1A4 mRNA induction in herring gull embryo hepatocytes in order to clarify this point.

Conclusions

Significant interindividual variation in CYP1A mRNA induction by TCDD was observed in both chicken and herring gull embryo hepatocyte cultures. Certain individuals of both species appeared to be particularly sensitive to induction. No differences in average response were found between herring gull eggs collected from a contaminated and a clean site in the Great Lakes.

References

1. Gilbertson M., Kubiak T., Ludwig J., Fox G. (1991) J Toxicol Environ Health. 33, 455
2. Mahajan S.S., and Rifkind A.B. (1999) Toxicol Appl Pharmacol. 155, 96
3. Kennedy S.W., Lorenzen A., Jones S.P., Hahn M.E., Stegeman J.J. (1996) Toxicol Appl Pharmacol. 141, 214
4. Sanderson J.T., Kennedy S.W., and Giesy J.P. (1998) Environ Toxicol Chem. 17(10), 2006
5. Kennedy S.W., Jones S.P., and Elliott J.E. (2003) Ecotoxicology. 12, 163
6. Yauk C.L., Quinn J.S. (1999) J Great Lakes Res. 25(4), 856