4 研究内容

In vitro screening of natural and dietary ligands as potential modulators against multiple avian AHR isoforms

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[**Aim**]

The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that plays an important role in assessing the toxicity of environmental pollutants, such as dioxin-like compounds (DLCs). While early studies of AHR focused on its role in regulating the expression of drug-metabolizing enzymes and mediating the toxicity of DLCs, recent reports suggest that natural ligands, including indole-3-carbinol (I3C), 3,3'-diindolymethane (DIM), resveratrol, daidzein, and quercetin, also play a role in various in vivo regulations. These natural ligands have been found to have health benefits or risks due to their antioxidant, growth inhibitory, estrogenic, or antiestrogenic properties. Indoles such as I3C and DIM have been shown to have immunomodulatory properties by balancing the activity of regulatory T cells (Treg) and T helper 17 cells (Th17). While most studies of AHR in avian species have focused on DLCs, few studies have examined the transcriptional response of natural ligands. In this study, we examined the in vitro response of chicken and cormorant AHR isoforms to natural ligands. This study will help expand the use of natural ligands as AHR modulators.

[**Procedure**]

In this study, an in vitro reporter gene assay was performed. The cells, COS-7 cells (5×10^4 cells/well) or LMH cells (6×10^4 cells/well), were seeded on 24-well plates. A total of 300 ng of plasmid DNA was transfected into the cells. Expression vectors containing ckAHR1, ckAHR2, ccAHR1, ckARNT1, ccARNT1 were previously constructed by inserting the respective full-length cDNA into the pcDNA3.1 Zeo (+) plasmid. For COS-7 cells, 3 ng of AHR isoform, 20 ng of CYP1A5, 50 ng of ARNT, 226.8 ng of pcDNA3.1 Zeo (+) and 0.2 ng of CMV Renilla were used. For the LMH cells, 20 ng of ckCYP1A5 and 0.2 ng of CMV Renilla were used. The ligands used in the transfected cells are FICZ, I3C, DIM, quercetin, resveratrol, baicalin, genistein and daidzein. DMSO was used as solvent control. As for the sample obtained by cell lysis, the firefly luciferase activity was measured using the dual luciferase reporter assay system, and the overall transcriptional

activity of AHR was measured with the relative luciferase unit (RLU) value. Prism 5 was used to calculate the 50% effective concentration (EC_{50}) for AHR transcriptional activity. The Dunnett's test was used for statistical processing to assess significance.

[**Results and Discussion**]

The sensitivity of chicken AHR1 and AHR2 (ckAHR1, ckAHR2) and LMH cells to FICZ and natural ligands (I3C, daidzein, baicalin, etc.) was investigated by measuring transcriptional activity and calculating EC_{50} values. FICZ elicited similar EC_{50} values of 0.041 nM, 0.028 nM, and 0.049 nM for ckAHR1, ckAHR2, and LMH cells, respectively. Comparison of EC_{50} values for ckAHR1 and ckAHR2 revealed that I3C and daidzein were more sensitive in ckAHR1, whereas DIM and quercetin were more sensitive in ckAHR2. Both ckAHR1 and ckAHR2 showed similar EC_{50} values of 6.5 uM and 4.7 uM, respectively, in the case of baicalin. The EC_{50} values of natural ligands in LMH cells were comparable to those of ckAHR1 compared to ckAHR2. This may be due to the higher endogenous expression level of ckAHR1 in LMH cells compared to ckAHR2. Cormorant AHR1 (ccAHR1) had a similar EC_{50} for FICZ to ckAHR1, suggesting similar sensitivity across species and AHR isoforms. In a comparison between chickens and cormorants, ckAHR1 was slightly more sensitive than ccAHR1 for all natural ligands except resveratrol, which showed no significant response in both chickens and cormorants compared to controls. These results suggest that transcriptional activity by native ligands in avian AHR is species- and ligand-dependent.

[**Perspectives in future**]

This study clarified transcriptional responses of eight natural ligands in chickens and cormorants with multiple AHR isoforms. Avian AHR isoforms exhibited transcriptional activation ability for most natural ligands, but reactivity was species-specific, with chicken AHR1 being slightly more sensitive than cormorant AHR1. The sensitivity to natural ligands differed between isoforms of AHR1 and AHR2, suggesting species- and ligand-specific regulation of these responses.