4-OH-Tamoxifen is not an agonist of the frog aryl hydrocarbon receptor

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Abstract

The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor found in all vertebrates. The AHR mediates toxicity of a wide range of xenobiotics, including such planar halogenated aromatic hydrocarbons as the classical agonist 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). 4-hydroxy-tamoxifen (4-OH-TAM) is an active metabolite of the selective estrogen receptor (SERM) tamoxifen. SERMS have been used to treat and prevent breast cancer connected with abnormal estrogen receptor (ER) signaling by estradiol in ERα positive breast cancers. Studies have shown that 4-OH-TAM regulates the activity of the AHR in MCF7 breast cancer cells. Along with other genes, *CYP1A1* mRNAs were induced. This project sought to use low affinity AHRs from *Xenopus laevis* to test the hypothesis that 4-OH-TAM is a classical agonist of the AHR. Like TCDD, we expect 4-OH-TAM to induce frog CYP*1A6* expression with a potency much lower than in mouse cells.

CYP1A mRNA induction by 4-OH-TAM was tested in dose-response studies in the XLK-WG frog cell line, and compared against induction in the Hepa1c1c7 mouse hepatoma line. CYP*1A6* mRNA was not induced by 4-OH-TAM in the frog cells. Consistent with data from other groups, 4-OH-TAM was a weak inducer of CYP*1A1* in the mouse cells. At 5mM, it induced CYP*1A1* ~6-fold over control, compared with >400-fold for 100 nM TCDD. This suggests that like many other compounds which induce CYP*1A1* in mice, 4-OH-TAM is not an AHR agonist in *X. laevis*.

Aryl hydrocarbon receptor

•The AHR is a ligand activated transcription factor which induces monooxygenase expression (*CYP1A* enzymes) in response to various exogenous and endogenous compounds.

•The AHR has a role in the detoxification of xenobiotics such as the environmental contaminant TCDD.

•AHR agonists, such as TCDD, upregulate *CYP1A* expression.

•The AHR has a relatively promiscuous ligand-binding pocket [1]. Multiple metabolites of tamoxifen have been shown to act as AHR ligands in MC7 breast cancer cells [2].

•The AHR in *X. laevis* binds the canonical agonist TCDD with a much lower affinity than observed in other model systems.

•The *X. laevis* AHR is relatively more responsive to some ligands, including the endogenous compound formylindolo[3,2-*b*] carbazole (FICZ) [4].

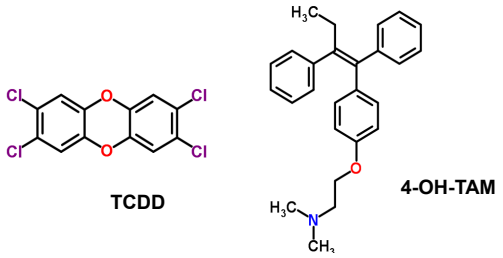


Figure 1. Aryl hydrocarbon agonist 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and selective estrogen receptor 4-hydroxy tamoxifen (4-OH-TAM).

4-hydroxy tamoxifen

• 4-OH-TAM (4OHT) is an active metabolite of the selective estrogen receptor (SERM) tamoxifen (TAM).

• SERMS have been used to treat and prevent breast cancer connected with abnormal estrogen receptor (ER) signaling by estradiol in ERα positive breast cancers [2].

• In breast tissue, SERMS compete with estrogen at the ER binding site. Thus, SERMS function by starving tumor cells of the estrogen necessary for their survival and proliferation [3].

• The TAM metabolite 4OHT can act as both an agonist and antagonist depending on its location in differing cell types.

• Recently, it has been shown that multiple metabolites of TAM, including 4OHT, affect AHR signaling independently of ER [2].

Objective

•To determine if *CYP1A6* mRNA's are induced by 4-OH-TAM in XLK-WG cells.

•To observe CYP1A mRNA induction by 4-OH-TAM in dose-dependent studies in XLK-WG and Hepa1c1c7 cells.

CYP1A mRNA induction

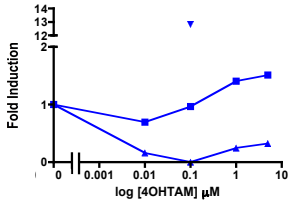


Figure 2. CYP1A1 mRNA induction by 4-OH-TAM in XLK-WG cells

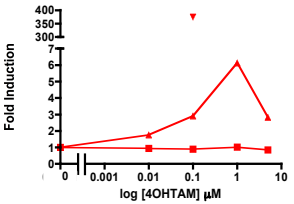


Figure 3. CYP1A6 mRNA induction by 4-OH-TAM in Hepa1c1c7 cells

Figures 2-3. XLK-WG were exposed to serial dilutions of 4-OH-TAM to examine the dose-dependency of mRNA induction. Mouse Hepa1c1c7 cells were used as a positive control so that the responsiveness of both *X. laevis* and mouse cells to 4-OH-TAM could be compared. Induced *CYP1A* mRNA were quantified with real-time PCR.

Figure 4. Comparison of highest fold CYP1A mRNA induction by 4-OH-TAM in XLK-WG and Hepa1c1c7 cells

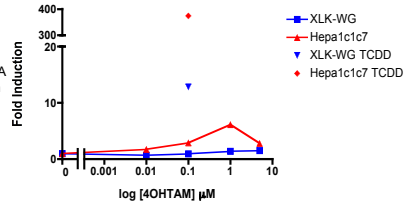
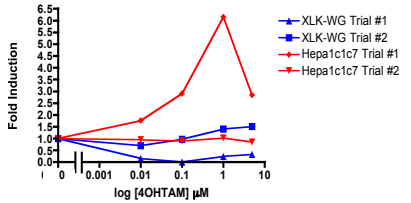


Figure 5. Comparison of CYP1A mRNA induction for all trials by 4-OH-TAM in XLK-WG and Hepa1c1c7 cells



Figures 4-5. Comparisons of CYP1A mRNA induction by 4-OH-TAM in XLK-WG and Hepa1c1c7 cells.

Conclusion

▪4-OH-TAM is a weak inducer of *CYP1A1* in mouse cells, inducing only ~6-fold over control at 5mM. C*YP1A1* induction by TCDD was >400-fold over control at 100 nM TCDD.

▪ 4-OH-TAM is not an AHR agonist in frog cells.

▪ At certain concentrations, 4-OH-TAM may actually inhibit CYP1A mRNA induction compared to control in frog cells.

References

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