AROMATIC AMINES AS ARYL HYDROCARBON (Ah) RECEPTOR AGONISTS AND PARTIAL ANTAGONISTS

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Abstract:

The competitive Ah receptor binding affinites of 1-amino 3,7,8-trichlorodibenzo-*p*-dioxin (1-amino 3,7,8-TrCDD), 2-amino 3,7,8-trichlorodibenzo-*p*-dioxin (2-amino 3,7,8-TrCDD), 2-amino 7,8-dibromodibenzo-*p*-dioxin (2-amino 7,8-DBDD), 1-amino 3,6,8-trichlorodibenzofuran (1-amino 3,6,8-TrCDF) and 3,3'-dichlorobenzidine-dihydrochloride (3,3'-DCbenzidine-2HCl) were determined using hepatic cytosol from Long Evans rats and [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as the radioligand. The temperature-dependent transformation of the rat cytosolic Ah receptor was also determined by gel mobility shift assays using a consensus ^{32}P-labeled dioxin responsive element (DRE). All of the aromatic amines exhibited binding affinity to the Ah receptor however, 2-amino 7,8-DBDD, 1-amino 3,6,8-TrCDF and 3,3'-DCbenzidine-2HCl were 2-, 10-, and 10-fold less active than TCDD. In coincubation studies, these aromatic amines inhibit TCDD-induced transformation of the cytosolic Ah receptor. In addition, the Ah receptor antagonist acivity of these ligands was determined from their concentration-dependent inhibition of TCDD-induced ethoxyresorufin O-deethylase (EROD) activity in rat hepatoma H4II E cells. Cotreatment of cells with 1 nM TCDD plus 1 μM of 2-amino 7,8-DBDD and 1-amino 3,6,8-TrCDF resulted in a 10 and 76% decrease in TCDD-induced EROD activity.

Introduction:

The aryl hydrocarbon (Ah) receptor binds with high affinity to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene (MC) and structurally related aromatic and halogenated aromatic hydrocarbons[1-5]. The molecular mechanisms of Ah receptor mediated responses have been derived from studies on the molecular biology of MC- and TCDD-induced CYP1A1 gene expression[6-9]. TCDD initially binds to the cytosolic Ah receptor (AhR), which is associated with heat shock protein 90 (hsp 90) and possibly other factors; the liganded complex undergoes transformation to a lower molecular weight heterodimer which exhibits increased DNA binding affinity; this complex translocates into the nuclear fraction of the target cell, binds to specific genomic sequences in the 5'-flanking region (dioxin responsive elements, DREs) of the CYP1A1 gene and acts as a nuclear ligand-responsive transcriptional factor (LTF)[10]. This paper

focuses on structure activity relationships of substituted aromatic amines as Ah receptor ligands.

Materials and Methods:

Chemicals All of the substituted aromatic amines were synthesized in this laboratory with the exception of 3,3'-dichlorobenzidine-dihydrochloride which was purchased from Pfatz and Bauer Inc., Waterbury, CT.

Treatment of cells Rat hepatoma H4II E cells were grown as a continuous cell line in α-MEM medium supplemented with 2.2 mg/ml tissue culture grade sodium bicarbonate, $NaHCO_3$, 5% fetal calf serum, 10 ml/l antibiotic/antimycotic solution. Cells were grown in 150 cm^2 tissue culture flasks and incubated in a humidified mixture of 5% CO_2 and 95 % air under atmospheric pressure.

Preparation of Hepatic Cytosol Male Long-Evans rats (21 days old, ≈100g) were obtained from Harlan Laboratories (Houston, TX). and hepatic cytosol was prepared according to the described procedure[11].

Hydroxylapatite (HAP) Assay The IC_{50} values for competitive receptor binding affinities were determined using freshly prepared rat hepatic cytosol (2 mg protein/ml) and the HAP procedure essentially as described[11]. Different concentrations of the ligands were used to determine displacement curves; the IC_{50} values were determined from a probit plot of the percentage of [^{3}H]-TCDD bound versus log concentrations of the ligands.

Gel Mobility Shift Assay DNA binding was measured using a gel mobility shift assay with a complementary pair of oligonucleotides containing the sequence 5'-GATCTGGCTCTTCTCACGCAACTCCG-3' as described[12].

Ethoxyresorufin O-deethylase (EROD) Induction Assay EROD activity was determined fluorometrically as described[13].

Statistics and Controls The statistical differences between different treatment groups were determined by ANOVA and the data were expressed as means ± standard errors.

Results and Discussion:

The ligands selected for this study were a series of five substituted aromatic amines. The competitve displacment binding affinities of the congeners using [^{3}H]2,3,7,8TCDD as the radioligand were determined using Long Evans rat hepatic cytosol and the HAP binding assay procedure . Logit analysis of the competitve binding data gave IC_{50} values of 1.4±0.3, 4.3±1.5, 33.3±8.3, 45.8±16.0, and 100.0±27.0 nM for 1-amino 3,7,8-TrCDD, 2-amino 3,7,8-TrCDD, 2-amino 7,8-DBDD, 1-amino 3,6,8-TrCDF, and 3,3'-DCbenzidine-2HCl, respectively. The congeners with three lateral chlorine substituents exhibited the highest binding affinty to the Ah receptor.

The ligand concentration-dependent transformation of the rat cytosolic Ah receptor was also determined using the gel mobility shift assay procedure (Fig. 1). There was a concentration-dependent increase in the formation of the transformed receptor complex: the highest levels were observed for the congeners with three lateral chlorine substituents. The lowest levels of transformation relative to 10 nM TCDD were observed for 1-amino 3,6,8-TrCDF, 2-amino 7,8-DBDD, and 3,3'-DCbenzidine-2HCl and these congeners were futher investigated for their effect as inhibitors of the TCDD-induced transformation as determined by the gel mobility shift assay. The intensity of the retarded DRE-complex obtained after incubation with 5 nM TCDD alone was arbitrarily

set at 100% and the relative band intensities in the different treatment groups were compared to this value. Cotreatment of the cytosol with 5 nM TCDD plus 5 μM of these congeners resulted in a decrease in the intensity of the TCDD-induced retarded band that was similar to these observed for 5 μM of these ligands alone (Table I).

In parallel studies, the effects of the aromatic amines as inhibitors of TCDD-induced CYP1A1 gene expression in rat hepatoma H4II E cells were also investigated. The results in Table II summarize the interactive effects of different concentrations of aromatic amines on the induction of EROD activity by 1 nM TCDD in rat hepatoma H4II E cells. The results in Table II show that those congeners with three lateral chlorine substituents induced EROD activity. Cotreatment of the cells with 1 nM TCDD plus 1 μM 2-amino 7,8-DBDD and 1-amino 3 6,8-TrCDF resulted in a decrease in TCDD-induced EROD activity however, treatment of cells with 3,3'-DCbenzidine-2HCl in the presence or absence of TCDD failed to induce or inhibit EROD activity. These results demonstrate that aromatic amines exhibit Ah receptor agonist activity and at least one of the compounds, 1-amino 3,6,8-TrCDF, acts as a partial Ah receptor antagonist.
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Table I. Effects of Aromatic Amines on TCDD-Induced Transformation.

Treatment	DRE Binding % of response to 5 nM TCDD (100%)
TCDD + 5 μM 2-amino 7,8-DBDD	58.6±6.6*
5 μM 2-amino 7,8-DBDD	58.7±3.0*
TCDD + 5 μM 1-amino 3,6,8-TrCDF	16.4±5.8*
5 μM 1-amino 3,6,8-TrCDF	6.5±3.9*
TCDD + 5 μM 3,3'-DCbenzidine-2HCl	48.7±7.7*
5 μM 3,3'-DCbenzidine-2HCl	9.7±5.6*

*Statistically lower ($p<0.05$) than cytosol transformed with 5 nM TCDD alone.

Table II. Effect of Aromatic Amines on 1 nM TCDD-Induced EROD Activity.

Treatment	EROD Activity % of response to 1 nM TCDD (100%)
TCDD + 1 μM 2-amino 7,8-DBDD	89.5±3.3
1 μM 2-amino 7,8-DBDD	54.5±0.7*
TCDD + 1μM 1-amino 3,6,8-TrCDF	23.8±0.5*
1 μM 1-amino 3,6,8-TrCDF	0.0±0.0*
TCDD + 1 μM 3,3'-DCbenzidine-2HCl	102.7±4.7
1 μM 3,3'-DCbenzidine-2HCl	0.0±0.0*
1 μM 1-amino 3,7,8-TrCDD	104.4±1.7
1 μM 2-amino 3,7,8-TrCDD	103.6±4.9

*Statistically lower ($p<0.05$) than cells treated with 1 nM TCDD alone.

References:
1. Poland, A., Glover, E., and Kende, A. S. (1976) *J. Biol. Chem.* **251**, 4936-4946.
2. Okey, A. B., Bondy, G. P., Mason, M. E., Kahl, G. F., Eisen, H. J., Guenthner, T.M., and Nebert, D.W. (1979) *J. Biol. Chem.* **254**, 11636-11648.
3. Farrell, K., Safe, L., and Safe, S. (1987) *Arch. Biochem. Biophys.* **259**, 185-195.
4. Hannah, R. R., Nebert, D. W., and Eisen, H. J. (1981) *J. Biol. Chem.* **256**, 4584-4590.
5. Poellinger, L., Lund, J., Gillner, M., Hansson, L.-A., and Gustafsson, J.-A. (1983) *J. Biol. Chem.* **258**, 13535-13542.12.6.
6. Safe, S. H. (1988) *ISI Atlas of Science: Pharmacology* **2**, 78-83.
7. Poland, A., and Knutson, J. C. (1982) *Annu. Rev. Pharmacol. Toxicol.* **22**, 517-554.
8. Whitlock, J. P. Jr. (1986) *Annu. Rev. Pharmacol. Toxicol.* **26**, 333-369.
9. Whitlock, J. P. Jr. (1987) *Pharmacol. Rev.* **39**, 147-161.
10. Evans, R. M. (1988) *Science* **240**, 889-895.
11. Bandiera, S., Safe, S., and Okey, A. B.,(1982) *Chem. -Biol. Interact.* **39**, 259-277.
12. Dension, M. S., and Deal, R. M. (1990) *Mol. Cell. Endocrinol.* **69**, 51-55.
13. Pohl, R. J., and Fouts, J. R. (1980) *Anal. Biochem.* **107**, 150-155.

Figure 1. Concentration-dependent formation of the transformed cytosolic Ah receptor complex as determined by gel shift assays