Ah RECEPTOR CHARACTERIZATION IN HUMAN PLACENTA SAMPLES

Allan B. Okey[1], David K. Manchester[2], M. Mark Feeley[3], Donald L. Grant[3] and Andrew Gilman[3].

1. Dept. of Pharmacology, University of Toronto, Toronto, Canada. M5S 1A8.
2. The Children's Hospital Kempe Research Centre, University of Colorado School of Medicine, Denver, Colorado. 80218.
3. Health Protection Branch, Tunney's Pasture, Food Directorate (MMF,DLG), Environmental Health Directorate (AG), Ottawa, Ont. Canada. K1A 0L2.

INTRODUCTION

Since the discovery in the mid-1970's of a high affinity, saturable cytosolic protein binding site in rodent liver for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), numerous lines of evidence have been drawn supporting the hypothesis that this Ah (aryl hydrocarbon) receptor is involved in the co-ordinate expression of various TCDD-induced biological and toxicological responses (enzyme induction, receptor modulation, dermal toxicity, immunotoxicity, teratogenicity, tumour promotion) observed in experimental animals[1].
With the observation of certain similar biological responses in humans (enzyme induction, receptor effects, chloracne) accidentally exposed to TCDD and other "dioxin-like" compounds[2], increasing research emphasis has focused on both the identification of a similar receptor in humans and its functionality.

The current study was designed to investigate the variation in Ah receptor binding characteristics (Bmax and Kd) in a relatively large, diverse human sample and if any possible association exists between adverse pregnancy outcomes and Ah receptor properties, including AHH activity.

Methods

Human placenta samples were collected between late 1992 and early 1993 from The Children's Hospital in Denver, Colorado (DKM) and Toronto General Hospital/Mt. Sinai Hospital in Toronto, Ontario (ABO). Cytosolic fractions were prepared from each placenta immediately after delivery as previously described[3] and stored in liquid nitrogen until Ah receptor quantification (ABO). The Ah receptor extraction buffer contained 20 mM molybdate as this has previously been reported to be required for Ah receptor stabilization during extraction for both Ah "nonresponsive" rodent and human samples[4].

Briefly, aliquots of placenta cytosolic fractions were incubated at 0-4 °C with 0.5-40.0 nM (^{3}H)-TCDD (saturation binding) for 2 hours. At the end of the incubation period, specific binding in the 9S region was determined by velocity sedimentation on sucrose density gradients. Concentration of specific binding sites (Bmax) and receptor binding affinity (Kd) for (^{3}H)-TCDD were determined by Woolf plot analysis, as previously described[5]. Aryl hydrocarbon hydroxylase (AHH) activity was determined in placenta microsomal samples and expressed as pmole 3-hydroxybenzo(a)pyrene generated per minute per mg protein (see[6]).

Results

Detectable levels of Ah receptor were found in all placental samples analyzed (Table 1). As the placental tissue used in this study is genetically of fetal origin (syncytial trophoblast tissue), it may be reasonable to hypothesize that functional Ah receptor could be present in other fetal tissues at a late pre-natal developmental stage.

TABLE 1

PLACENTAL CYTOSOLIC Ah RECEPTOR BINDING

TORONTO (n=37)			DENVER (n=58)	
Kd (nM)	Bmax (fmol)*		Kd (nM)	Bmax (fmol)*
6.0	161.9	mean	9.4	55.2
2.5	62.2	SD	7.5	42.9
0.4	10.2	SEM	0.9	5.6
6.9	178.2	upper 95% CI	11.4	66.5
2.2-14.8	29.7-347.7	range	0.26-38.8	4.9 -200.0

* per mg cytosolic protein

When the Ah receptor results from the Denver placenta samples were subdivided into 4 groups based on pregnancy outcome (normal full-term (1), premature delivery (2), intrauterine growth retardation (IUGR) +/- prematurity (3) and pregnancies associated with one or more fetal structural anomaly (4)), there appeared to be a tendency for higher receptor concentrations (Bmax) in groups 3 and 4 vs. 1 and 2 (Fig. 1). However, due to the small sample size and large standard deviation, none of the differences were statistically significant. Ah receptor binding affinity (Kd) values were also not significantly associated within any one particular pregnancy outcome group (results not shown).
When placental AHH activity was grouped according to smoking history, a significant increase ($p<0.001$) was found, as expected, in the samples from smokers (10.06 pmole/min./mg protein) compared to the samples from nonsmokers (0.31 pmole/ min./mg protein) (Fig. 2).

Fig. 1

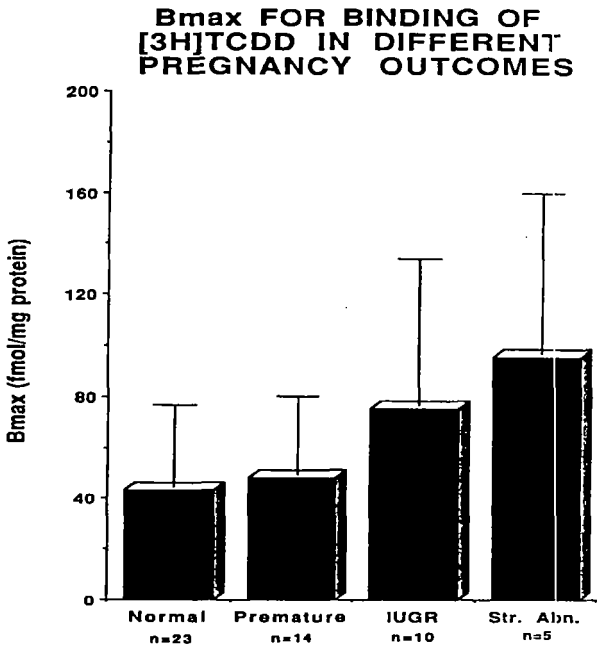
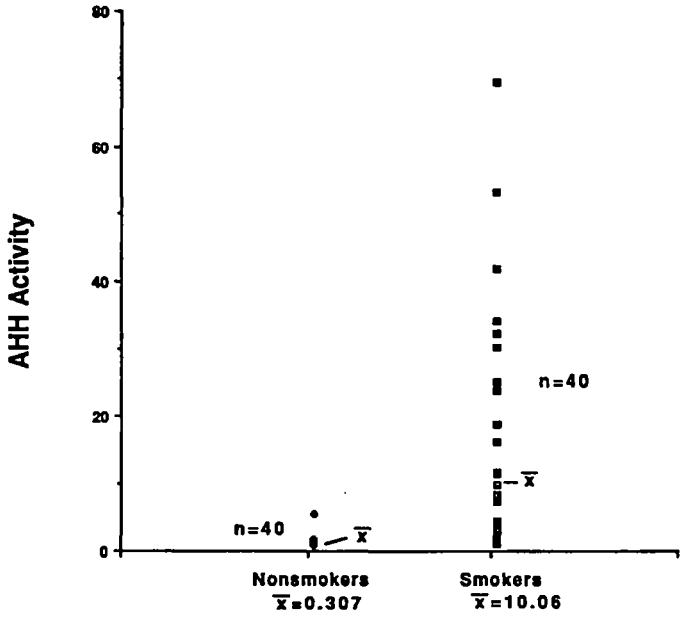

Bmax FOR BINDING OF
[3H]TCDD IN DIFFERENT
PREGNANCY OUTCOMES
Bmax (fmol/mg protein)
200
160
120
80
40
0
Normal
n=23
Premature
n=14
IUGR
n=10
Str. Abn.
n=5

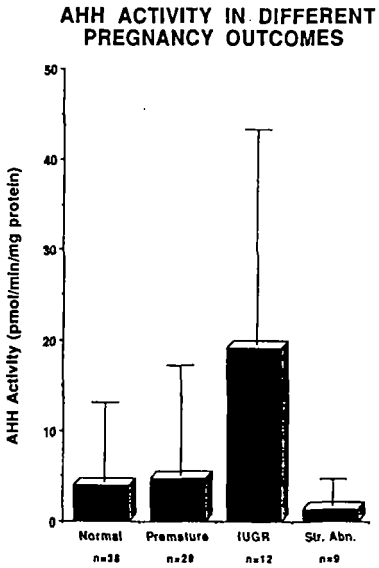
Fig. 2

AHH ACTIVITY* IN PLACENTAL MICROSOMES FROM SMOKERS AND NONSMOKERS



* pmole 3-OH B(a)P/min/mg protein

Fig. 3



Although placenta samples from pregnancy outcome group 3 (IUGR) tended to be positively associated with higher AHH activity (Fig. 3), no statistical association was observed between AHH activity and Ah receptor Bmax or Kd values in the entire experimental population or in the smoking vs. nonsmoking subsets.

The significantly higher receptor Bmax values found in the Toronto samples was probably reflective of the sample collection procedures and receptor lability. Caesarean delivery was favoured for sample collection in Toronto while the majority of Denver samples were from vaginal births.

Discussions

Although previously, Ah receptor-mediated enzyme activity (AHH) had been detected in a variety of fetal tissues[7], initial attempts to identify an Ah receptor-like binding protein from placenta were unsuccessful[8]. However, by employing experimental techniques previously used in the isolation of cytosolic steroid receptors, detectable quantities of Ah receptor have been found in human placenta and a wide variety of other human tissues and cell lines[4].

Several possible conclusions can be made from this study.

1) detectable levels of Ah receptor were found in all placenta samples.

2) large interindividual variations were found to exist in placental Ah receptor binding properties (up to 10-fold for the possibly critical Kd values).

3) the affinity with which (^{3}H)-TCDD binds to the placental Ah receptor is on average lower than that observed with Ah "responsive" rodent species. The Kd values from both the Denver and Toronto samples averaged from 6.0-9.4 nM compared to Kd values for hepatic Ah receptor of 1 nM from TCDD-sensitive rodents (i.e., C57BL/6J mice) and 16 nM from "nonresponsive" strains (i.e., DBA/2J mice).

4) while the conditions required for placenta Ah receptor isolation and the average Kd values found support the hypothesis that humans may be less responsive to TCDD and related dioxin-like compounds, it remains to be determined whether this regulatory gene product could be a discerning factor in human risk characterization.

This research was supported by Health Canada's Great Lakes Health Effect Program.

References

1. Birnbaum, L.S. (1994). Evidence for the role of the Ah receptor in response to dioxin. Prog. Clin. Biol. Res., 387, 139-154.

2. Lucier, G.W. (1991). Humans are a sensitive species to some of the biochemical effects of structural analogs of dioxin. Environ. Tox. Chem., 10, 727-735.

3. Manchester, D.K., Gordon, S.K., Golas, C.L., Roberts, E.A. and Okey, A.B. (1987). Ah receptor in human placenta: stabilization by molybdate and characterization of binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 3-methylcholanthrene and benzo(a)pyrene. Cancer Res., 47, 4861-4868.

4. OKey, A.B., Riddick, D.S. and Harper, P.A. (1994). The Ah receptor: mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin(TCDD) and related compounds. Toxicol. Lett., 70, 1-22.

5. Okey, A.B., Vella, L.M. and Harper, P.A. (1989). Detection and characterization of a low affinity form of cytosolic Ah receptor in livers of mice nonresponsive to induction of cytochrome P_1-450 by 3-methylcholanthrene. Mol. Pharmacol., 35, 823-830.

6. Manchester, D.K., Parker, N.B. and Bowman, C.M. (1984). Maternal smoking increases xenobiotic metabolism in placenta but not umbilical vein endothelium. Pediatr. Res., 18, 1071-1075.

7. Rifkind, A.B., Tseng, L., Hirsch, M.B. and Lauersen, N.H. (1978). Aryl hydrocarbon hydroxylase activity and microsomal cytochrome content of human fetal tissues. Cancer Res., 38, 1572-1577.

8. Wong, T.K., Sloop, T. and Lucire, G.W. (1986). Nondetectable concentrations of human placental Ah receptors are asociated with potent induction of microsomal benzo(a)pyrene hydroxylase in individuals exposed to polychlorinated biphenyls, quaterphenyls, and dibenzofurans. Tox. Appl. Pharmacol., 85, 60-68.