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Ah RECEPTOR MEDIATING INDUCTION OF CYTOCHROME P450IA1 IN A NOVEL CONTINUOUS HUMAN LIVER CELL LINE (Mz-Hep-1)

DETECTION BY BINDING WITH [^{3}H]2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN AND RELATIONSHIP TO THE ACTIVITY OF ARYL HYDROCARBON HYDROXYLASE*

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Abstract—The Ah receptor regulates induction of cytochrome P450IA1 and mediates certain toxicities of polyhalogenated aromatics such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). It has been characterized previously in continuous cell lines, notably the mouse hepatoma line Hepa 1, the human squamous cell carcinoma line A431, and the human liver cell line Hep G2. The present work extends our knowledge of the Ah receptor in continuous human liver cell lines. Ah receptor can be detected in Mz-Hep-1, a hepatitis B virus-negative cell line derived from a Thorotrast-induced hepatocellular carcinoma. The mean concentration of Ah receptor in Mz-Hep-1 cells was 341 ± 22 fmol/mg cytosol protein (mean ± SEM, nine separate determinations). This is equivalent to ~30,000 sites per cell. The concentration of Ah receptor in Mz-Hep-1 cells is similar to that in Hepa 1 cells and approximately three times higher than that in Hep G2 cells. The Mz-Hep-1 Ah receptor sedimented in continuous sucrose gradients at ~9 *S*. Specificity of binding by [^{3}H]TCDD was demonstrated by competitive binding of non-radiolabeled 2,3,7,8-tetrachlorodibenzofuran, 3-methylcholanthrene (MC), and dibenz[*a*,*h*]anthracene in 50-fold molar excess. Phenobarbital, which is not a substrate for P450IA1, did not compete with [^{3}H]TCDD for binding to Mz-Hep-1 Ah receptor. Dexamethasone and estradiol also did not compete with [^{3}H]TCDD for binding, suggesting non-identity of Ah receptor with glucocorticoid or estrogen receptor. In separate experiments, glucocorticoid receptor was identified in Mz-Hep-1 cells. By Scatchard plot analysis, the apparent equilibrium dissociation constant (K_d) for binding of [^{3}H]TCDD to Mz-Hep-1 Ah receptor was estimated to be 4.4 nM, compared to 0.8 nM in Hepa 1 cells. By Woolf plot analysis the K_d was 5.4 nM, compared to 1.2 nM in Hepa 1 cells. The [^{3}H]TCDD·Ah receptor complex extracted from nuclei of Mz-Hep-1 cells incubated with [^{3}H]TCDD in culture at 37° sedimented at ~6 *S* under conditions of high ionic strength. Aryl hydrocarbon hydroxylase (AHH) activity was detectable in Mz-Hep-1 cells after pretreatment with inducing chemicals. Mz-Hep-1 cells have the highest concentrations of Ah receptor in any continuous human liver cell line thus far investigated. The Mz-Hep-1 Ah receptor is similar physicochemically to that described in murine systems. AHH activity is inducible in Mz-Hep-1 cells.

The Ah receptor is a soluble intracellular protein which regulates the induction of cytochrome P450IA1§ and appears to mediate some toxicities of polyhalogenated aromatic compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [2–6]. It has been characterized in detail in hepatic cytosol from various species including the C57BL/6 mouse and the Sprague–Dawley rat [7, 8]. It has also been detected in high concentrations in the mouse hepatoma line, Hepa 1 [9–11]. These and other studies have indicated the mechanism by which the Ah receptor regulates induction of cytochrome P450IA1. The inducer, a polycyclic aromatic hydrocarbon (PAH), which is typically a substrate for aryl hydrocarbon hydroxylase (AHH), or a polyhalogenated aromatic hydrocarbon, binds to the Ah receptor in the cytoplasm. After a temperature-dependent transformation, the receptor·ligand complex binds to specific regulatory sites near the 5′-end of the cytochrome P450IA1 gene [12]. Subsequently, mRNA for cytochrome P450IA1 apoprotein is produced [13]. It is not yet entirely clear whether Ah receptor prior to ligand binding is predominantly in the cytosol or in the nucleus [14–16]. However, transformation of the Ah receptor·ligand complex to a high nuclear affinity-activated form and its subsequent binding to DNA are critical features of Ah receptor function.

In general, little is known about the regulation of human cytochromes P450. The regulation of human

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§ The term cytochrome P450IA1 [1] denotes the same cytochrome previously known as cytochrome P_1-450 in the mouse, P-450$_c$ in the rat, and "form 6" in the rabbit.

P450IA1 has been established in the greatest detail. Recent studies have indicated that regulation of human P450IA1 is similar to that of murine P450IA1 [17, 18]. Additionally, Ah receptor has been identified in human lung, placenta, and thymus [19–21] and in various continuous human cell lines [22–24]. Studies of Ah receptor in human liver are comparatively few in number. We have identified Ah receptor in low concentrations in 17/26 human liver specimens.* Moreover, to define the relationship between Ah receptor function and enzyme induction in the human liver, human liver cell culture systems are needed. Ah receptor has been characterized recently in the human liver cell line Hep G2 in detail [25, 26]. Ah receptor has also been studied in the continuous human liver cell line Hep 3B [25], but this line has the disadvantage of carrying hepatitis B virus, which may affect its pharmacological functions [27]. Ah receptor is detectable in low concentrations in the line SK-Hep-1 [28] but this cell line is comparatively dedifferentiated.

The continuous human liver cell line Mz-Hep-1 was produced from a Thorotrast-induced hepatocellular carcinoma [29]. It secretes certain proteins, including complement components (C'2, C'3, C'4), haptoglobin and retinol-binding protein, into culture medium. It is negative for α-fetoprotein production. Although its hepatocellular functions are less fully characterized than those of Hep G2, it appears to be well-differentiated. There is no evidence of hepatitis B virus infection, such as production of hepatitis B surface antigen. Drug metabolism has not yet been characterized in Mz-Hep-1 cells. The aim of these studies was to determine whether Ah receptor was present in cytosol from Mz-Hep-1 cells and, if so, to investigate induction of AHH in these cells. The mouse hepatoma line Hepa 1 was used as a standard of comparison [9–11].

MATERIALS AND METHODS

Chemicals. [^{3}H]TCDD (32 Ci/mmol) and nonradioactive 2,3,7,8-tetrachlorodibenzofuran (TCDF) were the gifts of Dr. S. Safe (Texas A & M University). These extremely toxic substances require handling with special precautions as described by Poland and Glover [30]. The radiochemical purity of [^{3}H]TCDD when analyzed in our laboratory by high-performance liquid chromatography as previously described [8] was >95%. [^{3}H]3-Methylcholanthrene (MC) (generally-labeled, 37 Ci/mmol, 95% chemical purity) was from the Amersham Corp. (Oakville, Ontario, Canada). [^{3}H]MC was repurified by thin-layer chromatography before use. [^{3}H]Triamcinolone acetonide (33 Ci/mmol) was obtained from DuPont Canada-NEN Research Products (Lachine, Quebec, Canada). Nonradioactive MC and benz[*a*]anthracene (BA) were purchased from Eastman Organic Chemicals (Rochester, NY); nonradioactive dibenz[*a*,*h*]anthracene (DB[*a*,*h*]A), dextran, dithiothreitol, sodium molybdate, and bovine serum albumin (BSA) were from the Sigma Chemical Co. (St. Louis, MO). Benzo[*a*]pyrene (BP) was from the Aldrich Chemical Co. (Milwaukee, WI), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) from the Calbiochem-Behring Corp. (La Jolla, CA), and sucrose (SDG grade) from Beckman Instruments (Toronto, Ontario, Canada). Dimethyl sulfoxide, glycerol, charcoal (Norit A), EDTA, KCl, KH_2PO_4, $NaHPO_4$, sodium citrate and glucose were from the Fisher Scientific Co. (Toronto, Ontario, Canada).

Buffers. The standard buffer used for these procedures was HEDGM [25 mM HEPES; 1.5 mM EDTA; 1 mM dithiothreitol; 10% (v/v) glycerol; 20 mM sodium molybdate, at pH 7.6]. For preparation of cell cytosol, cells were homogenized in half the total estimated volume of buffer prepared as HEDM buffer [25 mM HEPES; 1.5 mM EDTA; 1 mM dithiothreitol; 20 mM sodium molybdate at pH 7.6]; after homogenization the second half volume of buffer "HEDM2G" [25 mM HEPES; 1.5 mM EDTA; 1 mM dithiothreitol; 20 mM sodium molybdate; 20% (v/v) glycerol, at pH 7.6] was added. This procedure was used to avoid possible interference with homogenization by the glycerol.

HEDGMK buffer ("high salt" buffer) refers to HEDGM buffer containing 0.5 M KCl used for preparation of the nuclear extract.

PBS^- (phosphate-buffered saline without Ca^{2+} or Mg^{2+}) was used for washing cells to remove medium.

Cell lines and culture medium. Cell monolayers of the continuous human liver cell line Mz-Hep-1 were routinely maintained in Eagle's α-minimum essential medium (α-MEM) with 10% fetal bovine serum (FBS) added at 37° in a humidified atmosphere with 5% CO_2/95% room air. Doubling time was 27 hr. Cell cultures were subcultured every 6–8 days at 1:5 split ratios.

The cell line Hepa 1c1c9, the gift of Dr. M. Dufresne, University of Windsor, Windsor, Ontario, Canada, was maintained similarly. Under these conditions the cell doubling time was approximately 48 hr. This cell line will be referred to as "Hepa 1" in the rest of this paper.

Cell culture materials. Trypsin (Canlab, Toronto, Ontario, Canada) was prepared as a 1% solution in citrate saline plus glucose. All plastic ware for cell culture was from Falcon (Becton Dickinson, Oxnard, CA).

Preparation of cytosol. Forty to fifty 100-cm^2 plates were seeded with 1–2 × 10^6 cells in α-MEM with 10% FBS and grown to confluence. For cytosol preparation, the plates were washed once with ice-cold PBS^-; cells were removed from culture plates by exposure to trypsin for 7–8 min and pooled in a container on ice. Pooled cells were washed twice with ice-cold PBS^- and then suspended in HEDM buffer and homogenized with a glass homogenizer (60 strokes). The volume of buffer was adjusted to the cell number so that the protein concentration in the resulting supernatant would be 4–8 mg/mL. Extent of cell breakage was assessed microscopically; cell breakage was acceptable if >90%. The homogenate was centrifuged at 11,000 *g* for 4 min and the resulting supernatant fraction was centrifuged at 105,000 *g* for 1 hr. Cytosol was removed carefully with a Pasteur pipette to avoid disturbing any surface lipid layer. Protein concentrations were determined

* Roberts EA *et al.*, manuscript in preparation.

by the method of Bradford [31] using BioRad reagents (BioRad Laboratories, Missisauga, Ontario, Canada). So far as possible, all procedures in the preparation and analysis of cytosol samples were performed at 0–4°.

"Nuclear extract" was prepared by suspending the pellet resulting after the "11,000 *g*" step of cytosol preparation in HEDGMK buffer and incubating it for 1 hr. The suspension was then centrifuged at 105,000 *g* for 1 hr, and the supernatant was used for further analysis.

Cytosol incubation. Samples for sucrose gradient analysis were prepared by incubating 500 μL of cytosol with 10 nM [^{3}H]TCDD for 1 hr at 0–4°. In some experiments cytosols were incubated with [^{3}H]MC. Radioligands were added to cytosol in 10 μL dimethyl sulfoxide/mL cytosol; dimethyl sulfoxide was also used as the solvent for nonradioactive competitor chemicals. To check specificity and saturability of binding, each sample of cytosol was incubated with the [^{3}H]TCDD plus solvent only and in a parallel incubation with [^{3}H]TCDD plus a 50-fold molar excess of nonradioactive competitor. The competitors used were TCDF, MC, and DB[*a*,*h*]A, all of which have been shown previously to be potent agonists for the Ah receptor. After incubation, the dextran-charcoal adsorption step to remove unbound and loosely bound radioligands (previously a routine feature of this assay) was omitted, as preliminary experiments had shown that this did not improve detection of Ah receptor in these cells.

Sucrose gradient analysis. Aliquots (300 μL) of cytosol samples were layered onto linear sucrose gradient prepared in HEDGM buffer in 5.1-mL polyallomer tubes (Beckman Instruments). [^{14}C]Formaldehyde-labeled BSA (4.4 *S*) was added to each gradient as an internal sedimentation marker. This marker protein was labeled with [^{14}C]formaldehyde as previously described [8]. Gradients were centrifuged for 2 hr in a vertical-tube rotor (Beckman VTi65, $G_{average}$ = 372,000) [32]. After centrifugation, 25 fractions (200 μL each) were collected from each gradient using an ISCO model 640 gradient fractionator (Instrumentation Specialities Co., Lincoln, NE). Radioactivity in each fraction was determined by liquid scintillation counting and corrected for counting efficiency.

Detection of Ah receptor·ligand complex in nuclear extract. We modified the method of Okey *et al.* [9] in order to detect nuclear binding of the Ah receptor·ligand complex in cells in suspension, as opposed to monolayer culture. Briefly, near-confluent cultures were trypsinized. The trypsinized cells were resuspended at a concentration of 6×10^6 cells/mL in medium containing [^{3}H]TCDD (with and without non-radiolabeled competitor) and incubated at 37° for 2 hr. After incubation cells were washed and cytosol and nuclear extract were prepared as described above. The cytosol and nuclear extract were placed directly on sucrose gradients, ultracentrifuged, fractionated and counted for radioactivity. An additional aliquot of cytosol was incubated with [^{3}H]TCDD to assess the presence of Ah receptor not bound by whole cell incubation with [^{3}H]TCDD.

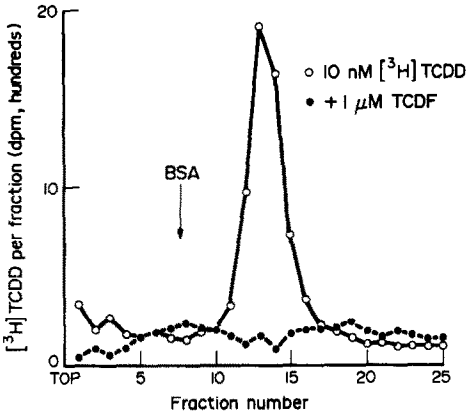

Fig. 1. Specific binding of [^{3}H]TCDD to Ah receptor in cytosol from mouse hepatoma line Hepa 1. Cytosol (2.8 mg protein/mL) from Hepa 1 was incubated with 10 nM [^{3}H]TCDD in the absence or presence of a 100-fold molar excess of nonradioactive TCDF. Samples were analyzed by velocity sedimentation on sucrose gradients as described in Materials and Methods. The arrow marked "BSA" indicates the sedimentation position of [^{14}C]formaldehyde-labeled bovine serum albumin (4.4 *S*), added as an internal standard in each gradient.

Measurement of enzyme activities. Aryl hydrocarbon hydroxylase (AHH) activity was measured by the method of Nebert and Gelboin [33] modified for cells in culture, with and without preincubation (×24 hr) with 13 μM BA or MC and over a range of concentrations (0.8 to 26 μM for BA; 0.65 to 52 μM for MC).

RESULTS

Detection of Ah receptor in Hepa 1 cytosol. Ah receptor is readily detectable in the continuous cell line Hepa 1 derived from a mouse hepatoma. Specific assay conditions for Hepa 1 cytosol were: cytosol protein 2–10 mg/mL, 10 nM [^{3}H]TCDD, 100-fold molar excess of non-radioactive competitor, 1-hr incubation with radiolabeled ligand, incubation with 2 mg dextran-charcoal/mg cytosol protein, and preparation of cytosol with HEDGM buffer. Figure 1 shows typical findings with Hepa 1 cytosol incubated with [^{3}H]TCDD and analyzed by velocity centrifugation on a continuous sucrose gradient. There is a large peak of specific [^{3}H]TCDD binding at ~9 *S*, eliminated by simultaneous incubation with 100-fold molar excess of nonradioactive TCDF. The concentration of specific Ah receptor binding sites in this Hepa 1 cytosol sample was 422 fmol/mg cytosol protein.

Analytical considerations for detection of Ah receptor in Mz-Hep-1 cytosol. Our previous experience with detecting Ah receptor in cytosol prepared from the human liver cell lines Hep G2 and SK-Hep-1 has shown that assay conditions have to be optimized for each individual cell line. For Mz-Hep-1 these conditions were: cytosol protein 4–8 mg/mL,

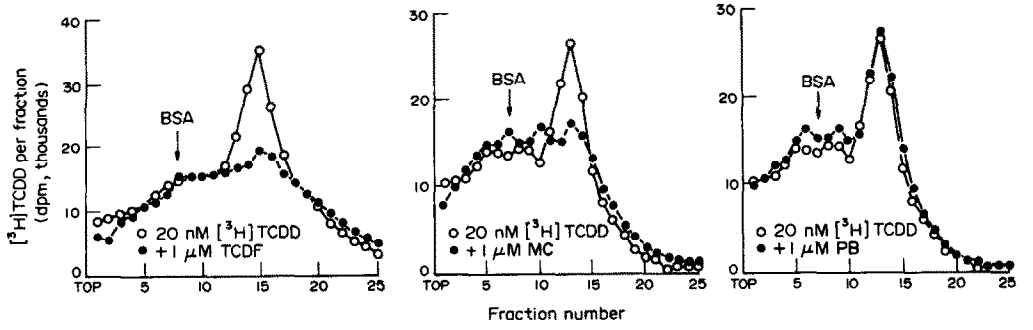

Fig. 2. Specificity of [^{3}H]TCDD binding in Mz-Hep-1 cytosol as demonstrated with 2,3,7,8-tetrachlorodibenzofuran, 3-methylcholanthrene and phenobarbital as competitors. (Left panel) Aliquots of Mz-Hep-1 cytosol (8.0 mg protein/mL) were incubated with 20 nM [^{3}H]TCDD in the absence or presence of a 50-fold molar excess of nonradioactive TCDF. Samples were analyzed by velocity sedimentation on sucrose gradients as described in Materials and Methods. The concentration of specific binding sites detected was 250 fmol/mg cytosol protein. (Center panel) Aliquots of Mz-Hep-1 cytosol (6.0 mg protein/mL) were incubated with 20 nM [^{3}H]TCDD in the absence or presence of a 50-fold molar excess of nonradioactive MC. The concentration of specific binding sites detected was 113 fmol/mg protein. (Right panel) Aliquots of the same cytosol as in the center panel were incubated with 20 nM [^{3}H]TCDD in the absence or presence of a 50-fold molar excess of non-radioactive PB.

20 nM [^{3}H]TCDD, 50-fold molar excess of non-radioactive competitor, 1-hr incubation with radiolabeled ligand, and no incubation with dextran-charcoal. Specific binding of [^{3}H]TCDD to a component meeting the criteria for Ah receptor, sedimenting at ~9 *S*, could then be demonstrated (Fig. 2). Moreover, preparation of cytosol with 20 mM sodium molybdate added to homogenizing buffers enhanced detection of Ah receptor in Mz-Hep-1 cytosol (data not shown).

Using these conditions, we assayed nine different samples of cytosol prepared from Mz-Hep-1 cells at near-confluence. In each case the tritiated ligand was [^{3}H]TCDD and the non-radiolabeled competitor was TCDF. The mean concentration (±SEM) of Ah receptor was 341 ± 22 fmol/mg cytosol protein. Thus, these cells are similar to Hepa 1 cells with respect to concentration of Ah receptor. This is equivalent to approximately 30,000 sites per cell, compared to approximately 35,000 sites per cell in Hepa 1.

Chemical specificity of Ah receptor binding in Mz-Hep-1 cytosol. Specific binding of [^{3}H]TCDD to Ah receptor was further investigated by demonstrating competitive inhibition by TCDF, MC (Fig. 2), and DB[*a*,*h*]A (data not shown) in 50-fold molar excess. Binding of [^{3}H]TCDD to Ah receptor was not inhibited by PB in 50-fold molar excess (Fig. 2). Specific binding by [^{3}H]MC, competitively inhibited by TCDF, could be demonstrated (Fig. 3).

Non-identity of this receptor with steroid hormone receptors was investigated in two ways. Neither dexamethasone nor estradiol competitively inhibited binding of [^{3}H]TCDD to Mz-Hep-1 Ah receptor. Glucocorticoid receptor was identified specifically by incubation with [^{3}H]triamcinolone acetonide with 100-fold molar excess of dexamethasone as a competitive inhibitor of binding (data not shown). The concentration of specific glucocorticoid receptor binding sites was 151 fmol/mg protein.

Tertiary structure of Mz-Hep-1 Ah receptor.

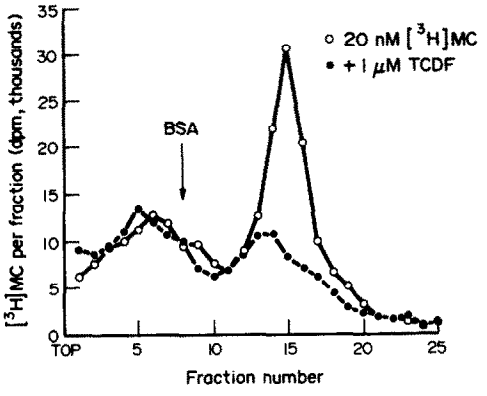

Fig. 3. Sucrose gradient profile illustrating specific binding of [^{3}H]MC in Mz-Hep-1 cytosol. Aliquots of Mz-Hep-1 cytosol (6.4 mg protein/mL) were incubated with 20 nM [^{3}H]MC in the absence or presence of 50-fold molar excess of nonradioactive TCDF. Samples were analyzed by velocity sedimentation on sucrose gradients as described in Materials and Methods. The concentration of specific binding sites detected was 504 fmol/mg protein.

Incubation of Mz-Hep-1 cytosol at 50° for 5 min prior to incubation with [^{3}H]TCDD destroyed specific binding. In a "high-salt" sucrose gradient, the Mz-Hep-1 Ah receptor was detectable only in reduced concentrations with one binding peak at ~9 *S* (data not shown).

Apparent affinity of binding [^{3}H]*TCDD to Ah receptor.* The apparent affinity with which [^{3}H]TCDD binds to this receptor was assessed by Scatchard plot and by Woolf plot [34] analysis. The range of [^{3}H]TCDD concentrations used was 0.8–30 nM. These studies were performed on three separate preparations of Mz-Hep-1 cytosol. The K_d was found to be 4.4 ± 1.1 (SEM) nM by Scatchard plot analysis and 5.4 ± 0.6 (SEM) nM by Woolf plot analysis

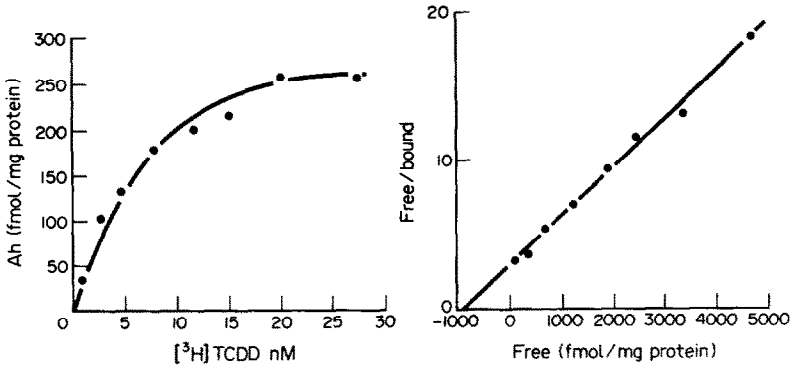

Fig. 4. Saturation and Woolf plot analysis of specific binding of [^{3}H]TCDD to Ah receptor in Mz-Hep-1 cytosol. Aliquots of cytosol (5.0 mg protein/mL) were incubated with [^{3}H]TCDD in concentrations ranging from 0.8 to 30 nM, and specific binding was measured by velocity sedimentation on sucrose gradients. B_{max} was 257 fmol/mg cytosol protein and the dissociation constant (K_d) was 5 nM, both calculated by least squares linear regression.

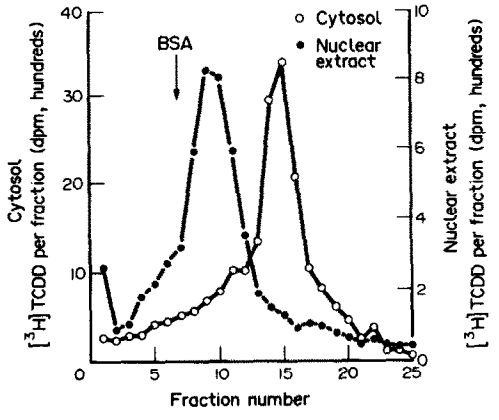

Fig. 5. Nuclear [^{3}H]TCDD·Ah receptor complex in Mz-Hep-1-cells. Mz-Hep-1 cells were incubated in suspension with 5 nM [^{3}H]TCDD at 37° for 2 hr; cytosol and nuclear extract were then prepared as indicated in Materials and Methods. Each was analyzed by velocity sedimentation on sucrose gradients without prior treatment with dextran-charcoal. Specific binding of [^{3}H]TCDD in the cytosol was 59 fmol/mg cytosol protein with a sedimentation coefficient of ~9 *S*. Specific binding in the nuclear extract was 103 fmol/mg protein with a sedimentation coefficient of ~6 *S*.

(Fig. 4). For Hepa 1 cytosol, Scatchard plot analysis showed a K_d of 0.8 nM and Woolf plot analysis showed a K_d of 1.2 nM (data not shown).

Detection of Ah receptor·ligand complex in nuclear extract. In mouse Hepa 1 cytosol, the Ah receptor·ligand complex has been demonstrated previously to undergo temperature-dependent change in mass and shape followed by binding to specific chromosomal elements in the nucleus. As shown in Fig. 5, after incubation of whole Mz-Hep-1 cells with [^{3}H]TCDD at 37°, Ah receptor·ligand complexes were detected in both cytosol and nuclear extract. Those in the nuclear extract migrated at 6.7 *S*, consistent with transformation prior to binding to nuclear sites.

Table 1. Induction of aryl hydrocarbon hydroxylase (AHH) by benz[*a*]anthracene (BA) in Mz-Hep-1 cells

Concentration of BA (μM)	AHH activity (pmol/min/mg protein)
26	12.8
13	5.29
8.7	4.46
6.5	0
3.2	0
0.8	0
0	0

Samples were assayed in duplicate; intersample variation was ≤ 10%. Controls including (1) DMSO (vehicle for BP) only and (2) zero incubation time were comparable.

AHH induction in Mz-Hep-1 cells. Induction of AHH activity could be demonstrated in Mz-Hep-1 cells with either BA or MC as inducer. AHH activity was assayed in the basal non-induced state and after 24-hr incubation in medium containing BA (Table 1). AHH activity was not detectable without prior induction. The mean maximum activity after induction with BA was 12.8 pmol/min/mg protein. For comparison, in Hepa 1 cells the mean maximum AHH activity was 59 pmol/min/mg protein after induction with BA; there was no basal activity detected. After induction with MC the mean maximum AHH activity in Hepa 1 cells was 59.4 pmol/min/mg protein, with no basal activity detected. Although AHH activity was induced after incubation with MC, Mz-Hep-1 cells appeared exquisitely sensitive to the toxic effects of MC. The mean maximum activity after incubation with MC was 1.76 ± 0.21 pmol/min/mg protein (mean ± SEM, four determinations). After treatment with concentrations of MC ≥ 13 μM, AHH activities were consistently lower than maximum (data not shown). Cell death, likely due to accumulation of toxic metabolites, was the probable cause of reduced activity at these higher concentrations of MC.

DISCUSSION

The continuous human liver cell line Mz-Hep-1 is derived from a drug-induced neoplasm and appears well-differentiated; however, its retained pharmacological functions have not been characterized extensively. The studies presented here have shown that AHH activity can be induced in these cells and moreover that Mz-Hep-1 cells express the Ah receptor, which has been shown to mediate induction of cytochrome P450IA1 in other systems. Physicochemically, the Mz-Hep-1 Ah receptor is similar to Ah receptor in mouse liver and in other human tissues studied. In continuous sucrose gradients it sediments at ~9 *S*. Binding by TCDD or MC is competitively inhibited by PAHs. Compounds such as PB, dexamethasone and estradiol, do not bind to the Ah receptor. The estimated number of binding sites per cell (30,000) is similar to the number of binding sites per Hepa 1 cell.

The affinity for binding (estimated K_d of ~5 nM) is lower than that of mouse hepatoma Hepa 1 Ah receptor but somewhat higher than that estimated for human placental Ah receptor [20]. This apparent binding affinity was similar whether computed by the Woolf plot or the more usual Scatchard plot method. The Woolf plot may be more appropriate for these data since measured, not deduced, parameters are used. When velocity sedimentation data are used for estimating the binding affinity of TCDD for the Ah receptor, binding affinity appears to vary with total protein concentration in the cytosol sample [35]. Therefore, to compare these measurements of apparent binding affinity in different tissues, we have used data obtained at similar cytosol protein concentrations.

To characterize the Mz-Hep-1 Ah receptor more completely, we investigated binding of the receptor · ligand complex in the nucleus. After ligand binding, the Ah receptor · ligand complex undergoes transformation before binding to sites in the nucleus. In Mz-Hep-1 cells incubated with [^{3}H]TCDD at 37°, Ah receptor · ligand complexes were detected in the nuclear extract. The nuclear Ah receptor · ligand complex had a smaller sedimentation coefficient consistent with transformation having taken place.

AHH activity could be induced in Mz-Hep-1 cells. There was no detectable AHH activity in these cells prior to treatment with inducing chemicals. MC was a more potent inducer than BA, but it was also more toxic. High concentrations of MC appeared to result in cell death.

Ah receptor in Mz-Hep-1 cells and Ah receptor in Hep G2 cells, another well-differentiated continuous human liver cell line in which we [26] and others [25, 36] have studied regulation of cytochrome P450IA1 extensively, are similar. We found the following differences: the estimated number of binding sites per cell was twice as high in Mz-Hep-1 as in Hep G2 (~30,000 sites per cell vs ~14,000 per cell in Hep G2) and the mean concentration of Ah receptor detected in cytosol was also higher in Mz-Hep-1 cytosol (340 vs 110 fmol/mg cytosol protein in Hep G2) when each is assayed under ideal conditions. Mz-Hep-1 Ah receptor was only partly denatured in high salt buffer compared to complete loss of Hep G2 Ah receptor in high salt buffer. Ah receptor from either line was denatured by heat.

The apparent K_d for binding of TCDD to Mz-Hep-1 Ah receptor was slightly lower than that of the Hep G2 Ah receptor (whose K_d is ~9 nM), but this difference may not be biologically important. AHH activity can be induced in both lines. Maximum induced AHH activities were found to be lower in Mz-Hep-1 than in Hep G2. Other factors besides the Ah receptor itself may influence AHH induction. There may be differences between these two cell lines with respect to other metabolic pathways contributing to apparent AHH activity when BP is used as substrate. Alternatively, there may be differences in the specific regulatory sites near the 5′-end of the cytochrome P450IA1 gene [17]. Moreover post-transcriptional events may differ in these two lines.

The "4 *S* protein" is another binding protein for PAHs, which is apparently distinct from the Ah receptor, and which has been described in the mouse and rat as a possible mediator of induction of cytochrome P450IA1 [37–39]. However, it has not been identified in human tissues [20, 23, 26]. The 4 *S* protein was also not detectable in Mz-Hep-1 cells.

Mz-Hep-1 cells express glucocorticoid receptor which was detected in Mz-Hep-1 cytosol by specific binding of [^{3}H]triamcinolone acetonide. Whether other steroid receptors are expressed in these cells remains uncertain. Glucocorticoids have been shown to enhance induction of P450IA1 in fetal and adult rat hepatocytes in primary cell culture, and glucocorticoid regulatory elements have been identified in intron I of the rat P450IA1 gene [40, 41]. The glucocorticoid receptor appears to be necessary for this potentiation of P450IA1 induction in rat liver cells.

These studies indicate that another continuous human liver cell line besides Hep G2 expresses Ah receptor. Mz-Hep-1 cells have high concentrations of Ah receptor and retain inducibility of cytochrome P450IA1. Mz-Hep-1 appears to be an excellent liver cell culture system for studying Ah receptor and P450IA1. The data presented for Mz-Hep-1 provide further support for the hypothesis that *human hepatic* cytochrome P450IA1 is regulated by a mechanism similar to that delineated in mouse liver and in the mouse hepatoma line Hepa 1. Comparisons between Hep G2 and Mz-Hep-1 may help to elucidate details of this regulatory mechanism in the human liver.

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