Ah Receptor Mediating Induction of Aryl Hydrocarbon Hydroxylase: Detection in Human Lung by Binding of 2,3,7,8-[^3]H]Tetrachlorodibenzo-p-dioxin

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ABSTRACT

In laboratory animals and in mouse hepatoma cells in culture the Ah receptor previously has been shown to mediate induction of aryl hydrocarbon hydroxylase (cytochrome P-450) by 3-methylcholanthrene, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. We examined human lung cytosols to determine whether the Ah receptor was present in human tissues. Cytosol was prepared from grossly normal lung tissue obtained at pulmonary lobectomy for presumed lung cancer from 53 consecutive adult patients including 32 males (42–77 years old) and 21 females (18–81 years old). Ah receptor in the cytosols was identified and quantitated by specific binding of [3H]TCDD after separation by ultracentrifugation on sucrose gradients. Specific binding of [3H]TCDD to a component which met the criteria for Ah receptor was detected in 10 of the 53 specimens. As previously established in tissues from laboratory animals, the specific [3H]TCDD-binding component sedimented at 9S. Binding of [3H]TCDD to the 9S component was competitively inhibited by incubation in the presence of 2,3,7,8-tetrachlorodibenzofuran, dibenz(a,h)anthracene, and nonradioactive TCDD, all known to be potent agonists for Ah-receptor-mediated induction of aryl hydrocarbon hydroxylase. Specific Ah receptor also was detected in some specimens by direct binding of [3H]-3-methylcholanthrene. The human population studied exhibited striking heterogeneity in Ah receptor concentrations. Only 10 of the 53 individuals studied had detectable Ah receptor. In specimens with detectable specific binding, the mean concentration of binding sites was 6.9 ± 1.2 (SE) fmol/mg cytosolic protein. These concentrations are approximately 10–30% of the concentrations of Ah receptor found in lung cytosols from laboratory animals. Our experiments indicate that the Ah receptor can be detected in lung cytosol from some humans and suggest that the regulatory mechanism mediating human cytochrome P-450 induction may be similar to that in the murine model. Aryl hydrocarbon hydroxylase, the major enzyme induced under control of the Ah receptor, plays an important role in the metabolism of several carcinogenic compounds, including polycyclic aromatic hydrocarbons such as benzo(a)pyrene. It is possible that differences in the Ah receptor content within the human population may be genetically based and that variation at the Ah receptor locus may be an important determinant of individual susceptibility to certain chemically induced cancers.

INTRODUCTION

The Ah (aromatic hydrocarbon) receptor has been extensively studied in tissues of laboratory animals. The receptor is a soluble intracellular protein which binds specific halogenated aromatic compounds such as TCDD and certain polycyclic aromatic hydrocarbons such as MC (4–6), BP (6, 7), and DB(a,h)A (6).

TCDD acts as a tumor promoter in rodent skin (8) and liver (9) and it appears that tumor promotion by TCDD is mediated by the Ah receptor (8). The most thoroughly characterized response mediated by the Ah receptor is induction of AHH (cytochrome P-450). Ligands for the Ah receptor including TCDD, BP, MC, and DB(a,h)A are potent agonists for AHH induction in many tissues and cells of laboratory animals and humans (10). The mechanism of AHH induction appears to involve binding of the Ah receptor-ligand complex to specific gene regions (10–12), thereby stimulating synthesis of cytochrome P-450 mRNA (11) which subsequently is translated into cytochrome P-450 apoprotein.

AHH is a major enzyme system implicated in the metabolic activation of several compounds, especially polycyclic aromatic hydrocarbons, into ultimate carcinogenic forms such as diol-epoxides (13, 14). In studies with inbred mice it has been established that susceptibility to several types of polycyclic aromatic hydrocarbon-induced tumors is strongly associated with Ah receptor-mediated inducibility of AHH (15). It also has been suggested that genetic differences in AHH inducibility in humans might be important in determining susceptibility to lung cancer (14, 16). Little is known about the occurrence of Ah receptor in human cells or tissues or about the role of the receptor in induction of human cytochrome P-450. The purpose of our study was first to determine whether or not the Ah receptor could be detected in human lung and, if so, to determine in what proportion of the human population the Ah receptor was present.

MATERIALS AND METHODS

Chemicals. [3H]TCDD (ring-labeled; 50 Ci/mmole) and nonradioactive TCDD were purchased from KOR Isotopes (Cambridge, MA); [3H]MC (generally labeled; 37 Ci/mmole, 95% chemical purity) was from Amersham Corp. (Oakville, Ontario, Canada). [3H]TCDD was greater than 95% pure, as determined in our laboratory by high performance liquid chromatography on a C18 µ-Bondapak reverse phase column (Waters Associates, Milford, MA) eluted with 25% water in acetonitrile (17). Nonradioactive MC was purchased from Eastman Organic Chemicals (Rochester, NY); nonradioactive DB(a,h)A, BP, dextran, dithiothreitol, and phenobarbital were from Sigma Chemical Co. (St. Louis, MO). Nonradioactive TCDBF was a generous gift from Dr. S. Safe. 4-(2-Hydroxyethyl)-1-piperazinethanesulfonic acid was from the Calbiochem-Behring Corp. (La Jolla, CA). Sucrose (SDG grade) was from Beckman Instruments (Toronto, Ontario, Canada); and dimethyl sulfoxide, glycerol, charcoal (Norit A), and EDTA were from the Fisher Scientific Co. (Toronto, Ontario, Canada).

Tissue Specimens. Male C57BL/6N mice (6 months old) were obtained from Charles River Breeding Laboratory of Canada (Montreal, Quebec, Canada). Human lung tissue specimens were from 53 consecutive patients undergoing pulmonary lobectomy for presumed lung cancer. These patients included 32 males, 42 to 77 years old, and 21 females, 18 to 81 years old. Pathological diagnoses in these patients included carcinoma of the lung (48 patients), metastatic adenocarcinoma of the colon (one patient), carcinoid tumor (3 patients), and no pathological diagnosis (one patient). A piece of nontumorous lung,
The concentration of specific Ah receptor sites in mouse lung was assayed for Ah receptor in order to provide a basis for comparison with human lung samples. As shown in Fig. 1, mouse lung cytosol exhibits a large peak of specific [3H]TCDD in the absence of an excess of nonradioactive TCDD. After treatment with charcoal-dextran, samples were analyzed by velocity sedimentation on sucrose gradients as described in "Materials and Methods." The concentration of specific Ah receptor binding sites detected was 49 fmol/mg cytosol protein.

**RESULTS**

**Sucrose Gradient Centrifugation.** Lung cytosol from C57BL/6N mice was assayed for Ah receptor in order to provide a basis for comparison with human lung samples. As shown in Fig. 1, mouse lung cytosol exhibits a large peak of specific [3H]TCDD in the absence of an excess of nonradioactive TCDD. After treatment with charcoal-dextran, samples were analyzed by velocity sedimentation on sucrose gradients as described in "Materials and Methods." The concentration of specific Ah receptor binding sites detected was 49 fmol/mg cytosol protein.

**Specific binding of [3H]TCDD to Ah receptor in cytosol from lungs of C57BL/6N mice.** Cytosol (4.5 mg protein/ml) from lungs of two 6-month-old mice was incubated with 10 nM [3H]TCDD in the absence or presence of a 100-fold molar excess of nonradioactive TCDBF. After treatment with charcoal-dextran, samples were analyzed by velocity sedimentation on sucrose gradients as described in "Materials and Methods." The concentration of specific Ah receptor binding sites detected was 49 fmol/mg cytosol protein.

**Fig. 1.** Specific binding of [3H]TCDD to Ah receptor in cytosol from lungs of C57BL/6N mice. Cytosol (4.5 mg protein/ml) from lungs of two 6-month-old mice was incubated with 10 nM [3H]TCDD in the absence or presence of a 100-fold molar excess of nonradioactive TCDBF. After treatment with charcoal-dextran, samples were analyzed by velocity sedimentation on sucrose gradients as described in "Materials and Methods." The concentration of specific Ah receptor binding sites detected was 49 fmol/mg cytosol protein.

**Fig. 2.** Specificity of [3H]TCDD binding in human lung cytosol as demonstrated with 2,3,7,8-tetrachlorodibenzofuran and dibenzo(a,A)anthracene as competitors. Top, aliquots of lung cytosol (7.8 mg protein/ml) were incubated with 10 nM [3H]TCDD in the absence or presence of a 100-fold molar excess of nonradioactive TCDBF. After treatment with charcoal-dextran, samples were analyzed by velocity sedimentation on sucrose gradients as described in "Materials and Methods." The concentration of specific binding sites (fmol/mg protein) detected was 6.7. Bottom, aliquots of cytosol from the same lung (6.1 mg protein/ml) were incubated with 10 nM [3H]TCDD in the absence or presence of a 100-fold molar excess of nonradioactive DB(a,h)A. Analysis was as in the previous sample. The concentration of specific binding sites detected was 6.7 fmol/mg protein.

**Fig. 3.** Specificity of [3H]TCDD binding in human lung cytosol as demonstrated with 2,3,7,8-tetrachlorodibenzofuran and dibenzo(a,A)anthracene as competitors. Top, aliquots of lung cytosol (7.8 mg protein/ml) were incubated with 10 nM [3H]TCDD in the absence or presence of a 100-fold molar excess of nonradioactive TCDBF. After treatment with charcoal-dextran, samples were analyzed by velocity sedimentation on sucrose gradients as described in "Materials and Methods." The concentration of specific binding sites (fmol/mg protein) detected was 6.7. Bottom, aliquots of cytosol from the same lung (6.1 mg protein/ml) were incubated with 10 nM [3H]TCDD in the absence or presence of a 100-fold molar excess of nonradioactive DB(a,h)A. Analysis was as in the previous sample. The concentration of specific binding sites detected was 6.7 fmol/mg protein.
Most binding experiments with the human lung specimens were conducted with [3H]TCDD as the radioligand. However, in some specimens we also tested [3H]MC, a compound which has been shown to be an effective Ah receptor ligand in tissues from laboratory animals. Fig. 3 illustrates that specific binding can be detected in the 9S region with [3H]MC as the radioligand; binding of [3H]MC in the 9S region is inhibited by a 100-fold molar excess of DB(a,h)A (Fig. 2) and by TCDBF (data not shown).

Fig. 4 illustrates detection of a very low concentration of Ah receptor in human lung cytosol. Aliquots of lung cytosol (3.9 mg protein/ml) were incubated with 10 nM [3H]MC in the absence or presence of a 100-fold molar excess of nonradioactive DB(a,h)A. After treatment with charcoal-dextran, samples were analyzed by velocity sedimentation on sucrose gradients as described in "Materials and Methods." The concentration of specific binding sites detected was 28.0 fmol/mg protein.

DISCUSSION

These experiments establish that human lung contains a binding component with the same sedimentation properties and chemical specificity as the Ah receptor, which previously was discovered and characterized in tissues from laboratory animals (2, 3). Little previous work has been done to determine whether human tissues contain Ah receptor. Preliminary reports have indicated that Ah receptor could be detected in lymphocytes (20) and placenta (21) from a few individuals. Ah receptor also has been reported in human squamous cell carcinoma lines (22), but was not detectable in MCF-7 human breast carcinoma cells (23).

One reason for the paucity of information on Ah receptor in the human population is the relatively low concentration of receptor sites in human tissues or cells. The mean receptor concentration, 6.9 fmol/mg cytosolic protein, detected in our experiments on human lungs is much lower than the concentration of Ah receptor in lungs from laboratory animals. In lungs from adult Sprague-Dawley rats, Hartley guinea pigs, and C57BL/6 mice, cytosolic Ah receptor concentrations have been reported to range from 20 to 80 fmol/mg protein (24–26). Thus the maximum concentration of Ah receptor detected in human lung is only about 10–30% of that detected in lungs from common laboratory animals.

It is probable that the assays we conducted underestimate the prevalence and concentration of Ah receptor in human lung. It is inherently difficult to procure and process human tissues immediately after resection. The Ah receptor is known to be susceptible to thermal degradation and to proteolysis (3, 27). It is also possible that Ah receptor concentrations are abnormal in patients with lung carcinomas because of the disease itself. We assayed portions of the lung which were distant from the primary tumor and free of disease by macroscopic inspection. No animal studies have yet been conducted, however, to deter-
mine whether the presence of tumors in the same organ or in distant organs alters Ah receptor concentrations. It also should be emphasized that radioligand-binding methods presently available for determination of Ah receptor concentrations can detect only those receptor sites which are not already occupied by a ligand. In animal studies it has been shown that in vivo treatment with chemicals which induce AhH temporarily reduces the concentration of detectable cytosolic Ah receptor sites (3). The apparent “disappearance” of receptor from cytosol probably is due to occupancy of the receptor sites by the injected chemical inducer and subsequent binding of the ligand-receptor complex to chromatin. In humans who smoke or who are otherwise exposed to AhH-inducing chemicals, a certain fraction of Ah receptor sites may be occupied and thus are not detectable when incubated with radioligands such as [3H]-TCDD. Reliable “exchange” assays, which would permit measurement of “occupied” as well as “free” sites, have not yet been developed for the Ah receptor.

Despite these technical limitations, we believe that our data clearly establish the existence of Ah receptor in human lung and that the data indicate considerable heterogeneity in the human population in regard to concentrations of Ah receptor. Confirmation of the presence of Ah receptor in human tissues along with data from other laboratories (22, 23, 28) strongly suggests that the mechanism which regulates induction of cytochrome P-450 (AhH) is fundamentally the same in humans as in laboratory animals.

AhH activity in human tissues and cells has been extensively studied over the past several years. AhH activity has been demonstrated in a number of human tissues, including lung (29, 30), liver (31, 32), skin (33, 34), peripheral lymphocytes (16, 35), and mammary tumors (36). AhH activity is induced in placenta by smoking (35) and in cultured lymphocytes by treatment with chemicals such as benz(a)anthracene (16). Recently complementary DNA for human cytochrome P-450 (which mediates AhH activity) was sequenced and the deduced protein sequence was shown to be highly homologous to mouse cytochrome P-450 (28). The human cytochrome P-450 gene has been localized to chromosome 15 (37). The Ah locus previously associated with chromosome 2 may represent a regulatory gene. This further emphasizes that regulation of AhH activity in humans may occur by mechanisms similar to those previously characterized in laboratory animals. Variability in the inducibility of enzyme activities associated with human cytochrome P-450 may be due to differences in the regulatory gene (i.e., Ah receptor gene) rather than in the structural gene for human cytochrome P-450 (38).

Human populations exhibit wide variation in AhH activity (39). Considerable effort has been expended in attempts to determine whether phenotypic differences in AhH inducibility within the human population are related to susceptibility to lung cancer or other forms of cancer. There is overwhelming evidence that human lung cancer is strongly associated with smoking and that smoke contains many chemical substituents which are substrates for AhH (40). Kouri et al. (16) reported a positive correlation between high AhH inducibility in cultures of human lymphocytes and the occurrence of primary lung cancer, whereas no association was found between AhH inducibility and the occurrence of leukemias or solid tumors in children (41). Although there are many difficulties in design, execution, and interpretation of such studies (14), human AH inducibility potentially is of great importance in understanding individual differences in susceptibility to certain chemically induced tumors.

In mice, genetic differences in AhH inducibility are primarily determined by the Ah receptor phenotype (10). Mice with an apparent high-affinity form of Ah receptor (e.g., C57BL/6N) exhibit a high degree of AhH inducibility when treated with nonhalogenated compounds such as MC, benz(a)anthracene, or BP. Mice with a low-affinity (possibly mutated) form of the Ah receptor do not exhibit significant AhH induction when treated with the same chemicals. Phenotypic variation in the molecular forms and concentrations of Ah receptor in mice leads to significant differences in their responses to toxic and carcinogenic chemicals (42). Our data indicate considerable heterogeneity in AhH receptor concentrations in the human population. Whether the forms of AhH receptor (high affinity versus low affinity) in human tissues are genetically variable, and whether the concentration of receptor sites is a critical determinant of susceptibility to carcinogens remain to be determined.

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