Relationship between Cadmium, Lead Concentration and Aryl Hydrocarbon Hydroxylase (AHH) Activity in Placenta of Cigarette Smoking Pregnant Women

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Abstract

The aim of this study was to investigate the interrelationship between cadmium, lead and Aryl Hydrocarbon Hydoxylase (AHH) activities in placenta of smoking and non-smoking women. The study was carried out on 90 smokers, 70 passive smokers (exposed to cigarette smoke) and 30 non smokers as control group. Results showed that, AHH activity increased with increasing numbers of cigarettes smoked per day. In this context, AHH activity values in placental homogenate obtained from the women who smoked 25 cigarettes per day were 8.8 and 4.1 times greater than the non-smokers, and the women smoking 5 cigarettes per day during pregnancy respectively. The statistical test of results was carried out utilizing paired and unpaired t-tests and Pearson correlation test was used for correlation analysis. Furthermore, Women who passively exposed to cigarette smoking had significantly ($p < 0.01$) higher AHH values 2.2 times than the controls. Similarly, the placental cadmium and lead levels increased considerably in smoking women compared with the control group. This study showed that there was a clear increase in placental Cd and Pb levels with increasing number of cigarette smoked per day. The results showed significant positive correlations between placental Cd – AHH activity ($r = 0.866$; $p < 0.01$) and Pb – AHH activity ($r = 0.890$; $p < 0.01$) in smokers. In addition, it has been shown that there was a significant positive correlation between placental Cd – AHH activity ($r = 0.818$; $p < 0.01$) and Pb – AHH activity ($r = 0.767$; $p < 0.01$) in pregnant women passively exposed to cigarette smoke.

***Keywords*:** AHH, Lead, Cadmium, Cigarette smoking, Placenta

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a large class of environmental contaminants in the atmosphere, soil, waterways, oceans, and food chain. Epidemiological studies indicate that the environment is a significant determinant in the incidence of human cancer [1]. A causal agent in human cancer is the inhalation of cigarette smoke, resulting in a high incidence of lung cancer among smokers. Many PAHs in cigarette smoke are powerful carcinogens in experimental animals and likely cause lung cancer in humans. The PAHs may also contribute to the etiology of cancer at organ sites other than lung [1]. After ingestion, absorption, and transport, the initial biological receptors for the metabolism of the PAHs are the microsomal mixed-function oxidases (MFOs) containing cytochrome P450 [2]. Benzo[a]pyrene (BP), a common by-product of tobacco burning, was shown to be a potent carcinogen in experimental animals. Many drugs and environmental chemicals metabolized in the placenta enter fetal circulation [3]. Aryl Hydrocarbon Hydroxylase (AHH) is present in placental microsomes and is induced to high levels in microsomes from women who smoked during pregnancy. Cigarette smoking was also shown to induce or reduce some other placental enzyme activities [3].

The placenta serves as the point of contact between maternal and fetal circulation. It functions as the means by which all necessary nutrients are delivered to the fetus, as well as a barrier to prevent passage of toxic substances, including metals. If the latter is accomplished by binding of the metals to the placenta, it may interfere with placental function, in particular the transport of essential trace elements required for fetal growth and development [4, 5]. Cadmium, for example, is known to accumulate in the placenta [6, 7]. Studies employing Cd-109 (a radioactive isotope) have shown that cadmium cross the placenta and may accumulate in the fetus [8]. In a study by Lagerkvist et al. (*1992*), the cadmium levels in the umbilical cord blood were reported to be about 70% of those in the mothers. The placental passage of lead is well documented [10]. Boadi et al. [11] examined the effects of cadmium as $CdCl_2$ on some placental enzyme activities after explants had been incubated with the salt for 6 or 24 hr. The results indicated that, for both incubation periods, Cd at low doses had a stimulatory effect on AHH. This effect was dose and time dependent. The activities of AHH showed a biphasic response with increases at the lower dose levels and decreases with higher ones [11].

MATERIALS AND METHODS

Chemicals and Reagents

Potassium chloride, Na_2CO_3, NaOH, sodium + potassium tartarate, $CuSO_4$, Tris HCl, $MgCl_2$, $CaCl_2$, sucrose, acetone, hexane were obtained from Merck, Darmsttadt, Germany.

BSA was purchased from Serva Feinbiochemica Heidelberg, New York. Dithiothreitol was obtained from Aldrich Chemical Company, NADPH and Folin's phenol reagent were purchased from Merck, Darmstadt, Germany. 3-OHBP and B[a] P were obtained from Midwest Research (Kansas City, MO USA).

Sample Preparation

Normal-appearing segments of placenta were obtained from 190 women delivering at the Government Hospital, Soykan Hospital and East Clinic from Malatya and Zekai Tahir Burak Cebeci Maternity-home (Hospital) from Ankara. The placentas were stored frozen (-70°C) until assayed. All the mothers participating in this study completed a questionnaire with regard to age, ethnic origin, employment, smoking habits, pregnant women passively exposed to cigarette smoke, diet and drug use. The data recorded included baby birth weight and length, number of previous pregnancies and medication given during pregnancy and labor.

Each placenta was thawed overnight at 4°C and then minced after removal of the membranes, umbilical cord and blood. A 5-10 g portion of the minced homogenate was removed and a sub-portion was homogenized for 2-3 minutes in ice cold 0.25 M sucrose-0.05 M Tris-chloride buffer (pH 7.7) using a PCV type homogenizer and was frozen (-70°C) and stored until assayed for AHH activity. Protein content was determined by the method of Lowry et al [12].

Aryl Hydrocarbon Hydroxylase Assay

AAH assay was a minor modification of that of Daudel et al [13]. The reaction mixture in a total volume of 1.0 ml, contained 920 µl of 0.05 M Tris-HCl buffer, pH 7.7, 10 µl of 5.4 mmol NADPH in 1% $NaHCO_3$, 10 µl 7.5 mmol $MgCl_2$, 50 µl of placental homogenate (containing 1-2 mg protein / ml) and 10 µl of 10 mmol BP in methanol (added just prior to incubation). The mixture was incubated, at 37° C for 30 min in air with gentle shaking. The reaction was stopped by the addition of 4 ml acetone-hexane (1:3, v/v) and samples were read against blanks to which acetone-hexane was added before the addition placental homogenate. Tubes were vortexed and the upper organic phase was transferred to 1 ml 1 M NaOH and vortexed for 15 sec. The fluorescence change in the alkaline phase was recorded with a F-4010 Model Hitachi Fluorescence Spectrophotometer of 3- hydroxybenzo[a]pyrene in samples was determined using with the following settings: slit width 10 nm; excitation, 387 nm; emission 504 nm. Standard curve was constructed with known concentrations of 3-hydroxybenzo[a]pyrene. The Fluorescence Spectrophotometer was calibrated each time with rhodamine B. The quantity of BP derivatives formed was calculated by comparing the net fluorescence (sample minus blank) of the final alkaline extract with a standard plot of 3-OHBP concentration versus fluorescence. AHH activity is expressed as picomoles product formed per milligram protein per minute. Product refers to the alkali-extractable metabolites of BP measured spectropfluorometrically, relative to 3-OHBP.

Elemental Analysis

Duplicate samples of (3-5 g) wet placental tissue were homogenized for 2-3 min in ice cold 0.01 M 10 ml Tris-HCl (pH 7.7) buffer. These homogenates were digested in 3.0 ml concentrated HNO_3, 1.5 ml concentrated H_2SO_4 and 0.5 ml 30 % H_2O_2 mixed over a hot plate under a reflux cap, until the samples were colorless. Digested placental samples were diluted to 10 ml with double distilled deionized water (ddH_2O) produced by a Milli-Q system (Millipore Corporation, Bedford, USA) and stored at 4 °C until analysis. All glassware was soaked in 2 M nitric acid for at least three days, washed three times with ddH_2O. Pb and Cd levels were analyzed with using hanging mercury drop electrode EG&G PARC Model 303A instrument. Aliquots (500 µl) of the digested sample were added into a cell containing 9.5 ml 0.1 M citric acid-NH_3 (pH 3.0) buffer. This solution was deoxygenated for 15 min and maintained under nitrogen during the experiment. Cadmium and lead determinations were performed in the same solution the hanging mercury drop electrode method. The same digestion procedure was used for bovine liver reference standard (Standard Reference Material – Bovine Liver 1577b from National Institute of Standards & Technology, USA) at about 0.5 g. After every series of 10 samples, standard reference material was run and analyzed for elements. The limit of detection was calculated to be 0.28 ng / g tissue for Cd and 2.34 ng / g tissue for Pb. Data on smoking habits were validated by analysis of urine cotinine in placental sample (Urine Nicometer®, Serex, Inc., Maywood, NJ, USA; 16).

STATISTICS

Statistical calculations were performed using SPSS 10.0 analysis software. Statistically paired and unpaired t-tests were used and Pearson correlation was used for bivariate comparisons.

RESULTS

In this study we determined the effect of cigarette smoking on placental AHH activity, cadmium and lead levels. The placental homogenates from 30 non-smokers, 90 cigarette smokers and 70 women who were exposed to cigarette smoking were assayed

Table 1. Content of cadmium, lead and AHH enzyme activity in placental samples of smoking, passive smoking and non-smoking women.

Group	Cig.	n	Cd	A	Pb	A	AHH	A
Control	0	30	0.29 ± 0.11	-	2.79 ± 0.78	-	0.70 ± 0.04	-
Passive	5	20	1.39 ± 1.64	4.8	11.77 ± 2.17	4.2	0.83 ± 0.07	1.19
Smoker	5	20	4.54 ± 1.05	15.6	42. 23 ± 3.71	15.1	1.49 ± 0.28	2.13
Passive	10	18	2.60 ± 0.79	9.0	15.87 ± 0 .16	5.7	1.08 ± 0.08	1.50
Smoker	10	20	6.97 ± 1.19	24.0	74.25 ± 12.32	26.6	2.21 ± 0.32	3.16
Passive	15	17	3.95 ± 0.66	13.6	25.80 ± 6.87	9.3	1.25 ± 0.03	1.79
Smoker	15	20	11. 69 ± 0.74	40.3	139.15 ± 6.76	49.8	2.61 ± 0.06	3.73
Passive	20	15	4.12 ± 0.78	14.2	29.76 ± 0.89	10.7	1.55 ± 0.22	2.21
Smoker	20	20	18.48 ± 2.95	63.7	209.06 ± 5.65	74.9	3.12 ± 0.35	4.46
Smoker	25	10	23.06 ± 3.03	79.5	258.86 ± 2.44	92.7	6.17 ± 0.45	8.81

for AHH activity. In general there was a correlation between increased AHH activity and number of cigarettes smoked per day. AHH activity profiler are summarized in Table 1 and in Figure 1 placental homogenate obtained from the women who smoked 25 cigarettes per day had 8.8 times higher AHH than non-smokers.

Cig., number of cigarettes smoked or exposed (for passive smokers) per day; n, number of samples (placenta) from different women; A, the rate of increase in heavy metal concentration and AHH activity in cigarette smokers or passive smokers compared to controls. Cd and Pb values are given as ng g^{-1} tissue and AHH activity as pmol 3-OHBP pmol min^{-1} mg^{-1} $protein^{-1}$. All data points are the average of 3 independent experiments with ± STDEVs ($n_{\sigma-1}$).

This figure was about 4.0 times higher than the women who passively exposed to cigarette smoke daily during their pregnancies. These values were significant ($p<0.01$), when compared with control group. The results clearly showed that cigarette smoking markedly increased AHH activity. Average AHH activity in non-smoker (control) placental homogenate was 0.70 ± 0.04 pmole min^{-1} mg^{-1} $protein^{-1}$ and was 1.55 ± 0.22 pmole min^{-1} mg^{-1} $protein^{-1}$ in women who exposed to 20 cigarettes per day. This corresponds to a 2.2 fold higher activity of AHH in later group than the former. Furthermore, these values were significantly ($p<0.01$) higher than the control group.

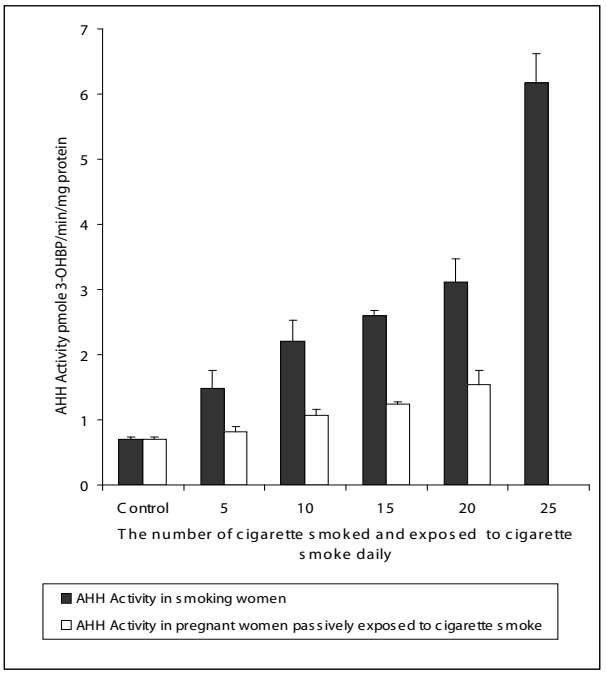

Figure 1. The distribution of AHH activity in placental homogenates from control, passive smoking and smoking women.

The elemental analysis showed that cadmium and lead levels increased with the number of cigarettes smoked per day (Table 1 and Figure 2). As it is apparent from Table 1 and Figure 2 placental cadmium levels in women smoking 25 cigarettes and women smoking five cigarettes per day were 79.5 and 15.6 times higher than the controls, in the same respect placental lead levels also showed 92.7 and 15.1 times higher values.

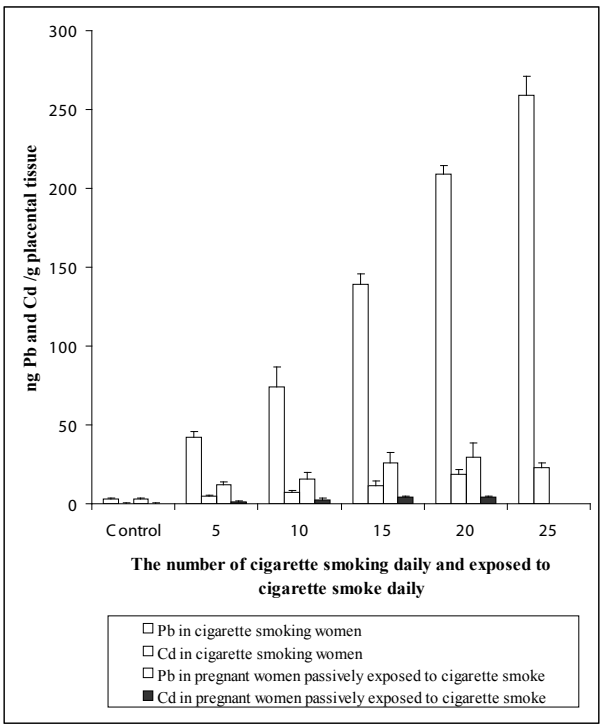

Figure 2. The distribution of cadmium and lead levels in placental homogenates control, passive smoking and smoking women.

As shown in Table 1 and Figure 2, placental cadmium levels in passive smokers who were exposed to 20 and 5 cigarettes per day were 14.2 times and 4.8 times higher than control respectively. Lead levels, also, showed 10.7 and 4.2 times were higher than the controls, in that respect. The results showed significant positive correlations between placental Cd – AHH activity

($r = 0.866$; $p < 0.01$) and Pb – AHH activity ($r = 0.890$; $p < 0.01$) in smoking women. Furthermore, there was a significant positive correlation between placental Cd – AHH activity ($r = 0.818$; $p < 0.01$) and Pb – AHH activity ($r = 0.767$; $p < 0.01$) in smoking women passively exposed to cigarette smoke (Table 1 and Figure 1 and 2).

DISCUSSION

The aim of this study was to investigate the interrelationship between cadmium, lead and AHH activities in placenta of smoking and non-smoking women. We have shown that AHH activity values in placental homogenate obtained from the women who smoked 20 cigarettes per day were 4.5 times greater than non-smokers, and 2.0 times higher than the women exposed to cigarette smoking during their pregnancies. Furthermore, an increase in AHH activity appears to be correlated with increasing numbers of cigarettes smoked per day. The AHH results are generally in agreement with other related studies [14, 15]. The values were significantly different ($p<0.01$) compared with controls. In a related study, the levels of AHH activity in placenta of cigarette smokers was determined to be 45.7 and 6.4 times higher than in non-smokers and passive smokers, respectively [16]. In this respect, another study showed that AHH activity in placenta

from smoking women was 2 to 25 fold greater than the AHH in placentas from non-smokers [3]. These results clearly show that cigarette smoking markedly increases AHH activity [14, 15]. Here we have showed that AHH activity in non-smoker placental homogenate was 0.70 pmole min^{-1} mg^{-1} $protein^{-1}$ and was 1.55 pmole min^{-1} mg^{-1} $protein^{-1}$ in women who exposed to 20 cigarettes per day, figures that correspond to 2.21 times higher AHH activity in the placenta of smoking women than that of the controls. Results clearly demonstrate that there is a relationship between number of cigarettes smoked per day and the placental AHH activity. The toxic effects of PAHs in tissues such as placenta have been demonstrated to be due to their metabolites, epoxides, which interact with DNA [17]. In this context, it has been found that there was a positive linear correlation between DNA adducts and AHH activity. The number of adducts were 5 times higher in smokers compared with nonsmokers [1]. Ours and the results of others show that the placental homogenates have higher AHH activity in smokers and these rates were highly significant ($p<0.01$) compared to non-smokers. Furthermore, results of this study demonstrate that there is a certain increase in placental cadmium and lead levels with increasing number of cigarette smoking per day. These increases were significant ($p<0.01$) compared to non-smokers. Our results are in accordance with the others showing that placental Cd concentrations were found in higher levels in smokers than nonsmokers [18]. In an animal experimental test, the lead administered at 250 to 2000 ppm concentrations between 6th and 14th day of gestation of pregnant rats the lead uptake by placenta was higher than that of controls [19]. Similar results in motor activity and neurochemical alterations in rats exposed to Pb and Cd were reported [20]. The metal bound to metallothionein (MT) was mainly Zn and insignificant amounts of Cu. The MT concentration in placenta did not increase in the Cd injected mouse, but the ratio of Cd/Zn in MT increased proportionally at doses up to 2 mg/Kg [21]. Both Cd and MT contents in the placenta of the Cd injected pregnant rats had higher levels than those of the control and there was a significant increase over the gestational period [22], showing the possibility of their transport through placenta. Infant cadmium levels were about 70% of maternal levels in most pairs. Serum cadmium was significantly higher in mothers and babies passively exposed to tobacco smoke [23]. Maternal exposure to Cd seems to increase during early delivery, which leads to a lower birth weight. Also, the Cd is transferred in part to the next generation through breast milk [24]. Human studies indicate that exposure to lead is associated with decreased sperm quality while modest, if any, effects on conventional reproductive endocrine profile [25] is observable.

Our results showed significant positive correlations between placental Cd–AHH activity ($r=0.866$; $p<0.01$) and Pb–AHH activity ($r = 0.890$; $p < 0.01$) in smoking women. Lead concentration was shown to be a determinative factor for normal pregnancy. Falcon et al (2003) showed that the proportion of abnormal pregnancy outcome in the group of placentas with lead concentrations above 120 ng /g was 40.6 versus 8.8% in placentas below this concentration. When pregnant guinea pig was exposed to cadmium chloride inhalation, the cadmium level increased in several tissues of mother and fetus. It has been suggested that low-level inhalation of Cd may pass through the guinea pig placenta and accumulate in fetal brain, liver, and heart [27]. The amount of MT transported by milk to the mammary gland is smaller in smokers than in non-smokers, which may prove to be advantageous to an infant because of the higher toxicity of the Cd-MT complex than of inorganic Cd salts [28]. There was an observed 780 g difference between the average weight of the infants between 20 cigarette smoker group and the control group. Similarly, there was a 3.81 cm decrease in the average infant length in 20 cigarette group compared to non smoker group. Furthermore, there was a 548 g weight reduction and a 1.77 cm length reduction in 20 cigarette exposed group relative to the non-smoker group [29]. We have shown that there is a significant positive correlation between placental Cd–AHH activity ($r = 0.818$; $p < 0.01$) and Pb–AHH activity ($r = 0.767$; $p < 0.01$) in pregnant women passively exposed to cigarette smoke. Our results are in good agreement with the previous report of Boadi et al. [11] who treated the placenta tissue with $CdCl_2$ and then they measured aryl hydrocarbon hydroxylase (AHH), quinine reductase and catecholamine-O-methyltransferase (COMT) enzyme activities. Their results indicated that, Cd at low doses had a stimulatory effect on aryl hydrocarbon hydroxylase (AHH). Activity of AHH showed a biphasic response; increase at the lower Cd dose levels, but in contrast, AHH activity decrease with higher dose of Cd [11]. In a study carried out at a metallurgical industrial and heavy traffic density area, it was shown that levels of placental Pb and Cd were considerably higher than those at less polluted areas [30]. Finally a study showed that cultured mouse thymocytes exposed to 10 μM Cd, were killed by apoptosis rather than necrosis [31]. It has been shown that, high level of expression of CYP4A11 in liver is significantly correlated with high Cd level in liver [32].

CONCLUSIONS

The results here showed that cigarette smoking cause accumulation of some heavy metals and result in higher levels of AHH enzyme activity in placentas of pregnant women. The elevation of toxic metals in placental tissue may have abnormal effects on developing fetus.

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