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The Effect of Cigarette Smoke on Aryl-hydrocarbon Hydroxylase (AHH) Activity of the Human Kidney

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Abstract—*AHH activity was measured in kidney cortex biopsies obtained during surgery from patients with hydronephrosis and from unaffected portions of kidneys with renal cell carcinomas. AHH activity was slightly, but not significantly, higher in the group of non-smoking cancer patients when compared to non-smoking hydronephrotic subjects. Smoking induced a significant increase of AHH activity in the hydronephrotic kidneys but not in cancer kidneys, probably due to the higher baseline values and scatter in the cancer group. Pooling cancer and non-cancer kidneys, smokers had a higher AHH activity than non-smokers, this increase being dose-related.*

INTRODUCTION

MICROSOMAL mono-oxygenases detoxify foreign chemicals and activate procarcinogens to their ultimate reactive metabolites [1]. Aryl-hydrocarbon hydroxylase (AHH) catalyzes the oxidation of benzo(a)pyrene, producing polar derivatives that are considered to be proximate carcinogens [2]. A considerable amount of work, therefore, has been dedicated to the study of the variations of AHH activity in cancer patients, with the aim of identifying metabolic differences in humans that might explain the individual susceptibility to the carcinogenic action of benzo(a)pyrene and other polycyclic hydrocarbons.

Although many studies of AHH activity of human lymphocytes in cancer patients and in controls have ended with contradictory conclusions [3–8], some evidence was produced indicating a higher activity of AHH in cancer patients when AHH levels of lymphocytes and macrophages were measured [9]. The purpose of this study was to determine the activity of human kidney AHH and to study its variation as a function of disease and smoking habits, in order to ascertain whether variations of AHH activity might have a role in the induction of kidney cancer.

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MATERIALS AND METHODS

Collection of samples

Kidney fragments were obtained during nephrectomy on subjects undergoing surgery for kidney cancer or hydronephrosis. All cancers were clear-cell carcinomas, affecting only a portion of the kidney. Fragments of about 1.5 g were obtained from zones of the cortex macroscopically and microscopically unaffected by the tumor. Hydronephrotic kidneys were obtained from patients with congenital UPJ stenosis or with calculotic stenosis. All subjects were on a standard hospital diet and under no drug therapy. Age, sex, smoking habits and current clinical parameters were recorded for all patients.

Handling of samples

Kidney samples (1–1.5 g), kept in ice immediately after removal, were transferred to a deep-freezer (−80°C) and thawed within a week for the analysis of AHH activity. The samples were homogenized with a Teflon–glass homogenizer (1:3 w:v in 0.154 M KCl) and centrifuged for 15 min at 9000 ***g*** to obtain the S9 supernatants that were used for the determination of AHH activity.

AHH activity determination

AHH activity was determined fluorometrically according to Nebert and Gelboin [10], using 3-OH-benzo(a)pyrene as a standard.

Proteins were determined with the method of Lowry *et al.* [11].

RESULTS

Table 1 shows the individual values of AHH activity in kidneys from patients with hydronephrosis. The values of enzymatic activity were rather homogeneous, only two cases being in the higher range. A non-smoking subject operated on for a nephrectomy after kidney trauma had a value of AHH activity of 4.4 pmol/mg/10 min, in the range of this group of patients. Smoking increased AHH activity about 3-fold (Table 1) and this difference was highly significant ($F = 13.1$, $P < 0.005$, one-way analysis of variance).

Table 2 shows that some but not all of the values of AHH activity in kidneys of non-smoking cancer patients were elevated. The mean was also higher in the cancer group (12 vs 6.87 pmol/mg/10 min). However, due to the scatter of individual values, this difference was not statistically significant ($F = 3.4, P > 0.05$). Table 2 also shows that smoking only slightly increased the mean AHH activity in this group of patients ($F = 0.59$, $P > 0.05$).

By pooling cancer and non-cancer cases, non-smokers had a mean value of AHH activity of 9.8 ± 7.8 and smokers had a value of 16.3 ± 6.8 ($F = 6.6$, $P < 0.05$).

Figure 2 shows that the increase of AHH activity is roughly correlated to the amount of cigarettes smoked ($r = 0.64$, $P < 0.05$).

DISCUSSION

The data just reported show that although some individual values of AHH activity in the kidneys of non-smoking cancer patients were in a higher range when compared to hydronephrotic ones, most of the data actually overlapped. The data also show that smoking increased AHH activity when cancer and non-cancer cases were considered as a single group. Kidney AHH activity seems, therefore, to be influenced by smoking, as demonstrated for human macrophages [12] and placentas [13]. The values of AHH activity that we found were lower than those reported in human kidneys by Prough *et al.* [14]. These authors, however, measured the total metabolic transformation of benzo(a)pyrene and not the 3-OH-benzo(a)pyrene, as in the fluorescent assay. Moreover, they measured the activity of 78,000 $g \times 60$ min microsomal pellets, and these two factors may explain the higher reported activity.

The relationship between AHH activity and tumor susceptibility is a complicated one. Higher AHH levels have been reported to be associated

Table 1. *Individual values of kidney AHH activity in hydronephrotic kidneys in both smokers and non-smokers*

Non-smokers	Sex	Diagnosis	Age (yr)	AHH activity (pmol/mg/10 min)
G.D.A.	F	hydronephrosis	63	13.0
S.B.	F	calculosis	73	2.4
B.L.	F	hydronephrosis	64	4.4
M.B.	F	hydronephrosis	23	7.7
I.B.	F	calculosis	52	2.5
I.S.	F	hydronephrosis	24	5.5
B.B.	F	hydronephrosis	18	18.8
L.B.	M	hydronephrosis	25	7.1
G.D.S.	M	calculosis	57	3.0
A.C.	M	calculosis	55	3.2
A.C.	M	hydronephrosis	49	5.5
F.A.	M	hydronephrosis	76	8.0
E.P.	M	calculosis	60	8.3
μ			49.1	6.87
$\pm$ S.D.			19.8	4.7
Smokers	**No. of cigarettes**			
B.C.	5	M	hydronephrosis	41	11.6
S.C.	15	M	hydronephrosis	67	11.9
A.R.	20	M	calculosis	58	34.4
A.D.	10	M	calculosis	49	12.5
G.C.	10	M	hydronephrosis	52	15.4
M.B.	12	M	hydronephrosis	61	22.8
μ				54.6	18.1
$\pm$ S.D.				9.3	9.0

Table 2. Individual values of AHH activity of kidneys with carcinomas in both smokers and non-smokers

Non-smokers		Sex	Diagnosis	Age (yr)	AHH activity (pmol/mg/10 min)
G.S.		M	kidney carcinoma	73	4.8
A.A.		M	,,	57	6.1
G.B.		M	,,	23	40.0
G.T.		M	,,	47	12.7
F.V.		M	,,	67	14.0
E.C.		M	,,	61	4.8
G.C.		M	,,	59	22.6
E.P.		M	,,	59	6.2
E.F.		M	,,	76	9.3
G.R.		M	,,	73	10.7
P.T.		M	,,	65	9.0
G.T.		M	,,	65	11.3
A.B.		F	,,	53	7.3
G.C.		F	,,	69	5.3
R.O.		F	,,	21	5.8
L.R.		F	,,	34	26.0
M.S.		F	,,	71	7.8
M.B.		F	,,	58	11.8
μ				57.3	12.0
± S.D.				16.3	9.1
Smokers	No. of cigarettes				
M.C.D.G.	10	M	kidney carcinoma	52	15.4
M.C.	15	M	,,	62	13.5
L.M.	10	M	,,	67	13.3
B.T.	8	M	,,	67	14.0
P.B.	14	M	,,	68	23.8
A.C.	11	M	,,	34	8.6
B.C.	15	M	,,	59	14.8
μ				58.4	14.8
± S.D.				12.0	4.5

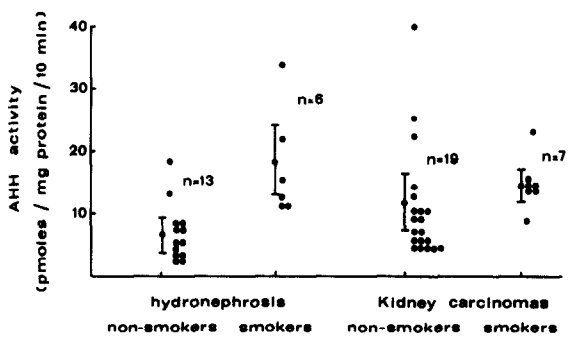

Fig. 1. Levels of AHH activity in hydronephrotic kidneys (smokers and non-smokers) and in unaffected parts from kidneys with carcinomas (smokers and non-smokers); means ± S.D. and the individual values are reported.

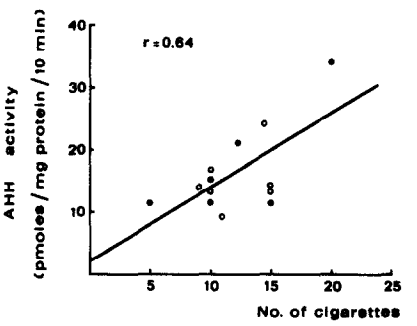

Fig. 2. Correlation between levels of AHH activity and number of cigarettes smoked (● hydronephrotic kidneys; ○ kidneys with cancer).

with increased cancer incidence in mice treated either with methylcholanthrene or benzo(a)pyrene [15,16]. Moreover, the formation of benzo(a) pyrene7-8-diol, which is catalyzed by AHH, has been shown to be correlated with the level of binding of benzo(a)pyrene to human DNA [17].

In our study the effect of smoking and not the presence of kidney cancer seems to have an effect on the levels of AHH activity. This observation indicates that an increased capability of activating procarcinogens is unlikely to be a major cofactor in the induction of kidney cancer.

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