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Arylhydrocarbonhydroxylase Inducibility and Smoking Habits in Patients with Laryngeal Carcinomas

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There is considerable evidence that the inducible enzyme aryl hydrocarbon hydroxylase (AHH) plays an important role in the activation of polycyclic aromatic hydrocarbons (PAH) to ultimate carcinogens. In man, a genetic heterogeneity of AHH inducibility has been demonstrated, and correlated to susceptibility to bronchogenic carcinomas following exposure to PAH. We assessed AHH inducibility in a control group of 102 healthy Swedish citizens and in 41 patients with laryngeal carcinomas. Frequencies of the three phenotypes of high, intermediate and low AHH inducibility in our control group; 8.8%, 42.2% and 49%, respectively, did not differ significantly from frequencies found in a white US population. In the laryngeal carcinoma group, there was a statistically highly significant overrepresentation of patients with high AHH inducibility, 36.6%, whereas 43.9% had an intermediate and 19.5% a low level. Most of the patients were heavy smokers. These findings add further support to the concept that susceptibility to PAH-induced carcinomas is associated with high levels of inducible AHH activity.

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The excess incidence of laryngeal carcinomas among heavy smokers has been established in numerous studies and epidemiological surveys (1, 2), as well as the predisposition of smokers to cancers of the mouth, the lungs and the bladder. These and recent data published by the American Cancer Society (3) show that the cancer risks increase statistically with the amount of inhaled tobacco smoke. However, genetic differences in susceptibility to smoke carcinogens have been demonstrated between individual smokers in clinical materials (4).

A considerable amount of evidence has now accumulated in the literature that the polycyclic aromatic hydrocarbons (PAH), regarded as the main carcinogens of tobacco smoke, are metabolically activated to electrophils, or ultimate carcinogens, in the cells (5). Only a few of the chemical carcinogens normally occur as electrophils, and the activation of PAH is mediated by a component enzyme system of the microsomal mixed-function oxidase complex. The component system, commonly referred to as aryl hydrocarbon hydroxylase (AHH), hydroxylates PAH to epoxides (6–8) and these microsomal metabolites of PAH form covalent bonds with DNA, RNA and certain proteins (9, 10). It has even been postulated that the epoxide-induced DNA-alteration might represent the biochemical basis of carcinogenesis (11, 12).

This would also explain the observation that every cocarcinogen tested to date has inhibited DNA repair replication in normal human lymphocytes. Enhancement of tumori-

genesis by cocarcinogens would thus be actuated by the depression of normal enzymatic DNA repair activity. AHH is inducible by various agents such as drugs, steroids, insecticides and PAH (13–15). More than 200 compounds have been shown to influence the activities of the microsomal mixed-function oxidase. These compounds have been divided into two main groups, of which the phenobarbital group enhances the formation of cytochrome P-450 and the enzyme activities towards a variety of substrates. The PAH group enhances the enzyme activities towards a limited number of substrates and stimulates the formation of cytochrome P-448 (13, 16).

AHH inducibility has been demonstrated in various tissues in a number of laboratory animals, e.g. the mouse, where inducibility is believed to be controlled by a single autosomal locus (17).

In man, AHH inducibility has been assayed in leukocytes, especially lymphocytes, lung preparations, foreskin tissue cultures and pulmonary alveolar macrophages. Fibroblasts from different embryonic organs have been shown to metabolize PAH to the same extent, while variations in the metabolism of PAH in fibroblasts from different embryos have been demonstrated. This genetic heterogeneity of AHH inducibility in man has been extensively studed by Kellerman et al. (18) who suggested a single autosomal locus of genetic control. Two co-dominant alleles, resulting in three phenotypes of high, intermediate and low inducibility, were found in frequencies of approximately 0.3 and 0.7.

The association of AHH activity and PAH-induced tumours has been much debated. Earlier results stemming from experiments on laboratory animals have been mostly inconclusive and even to some extent contradictory (19). However, more recently an association between AHH activity and susceptibility to 3-methylcholanthrene-induced subcutaneous carcinomas in mice has been demonstrated (20). Presence of the microsomal enzyme inhibitor 7,8-benzoflavone has been shown to increase the mean latent period for skin tumours induced by chemical carcinogens, and in some cases completely suppress malignant cell transformation (21, 22). Finally, it has been demonstrated that chemical carcinogens highly mutagenic in cells with mixed-function oxidases are of very weak mutagenicity in cells without this enzyme system, e.g. Chinese hamster V79 cells (23).

In a human material of lung cancers, Kellerman et al. showed a striking overrepresentation of patients with high AHH inducibility (24). In a review of the more recent epidemiological and clinical data, Gelboin also concluded that AHH may be involved in smoking-related cancers of the respiratory tract and oral cavity (25), but that more information is required. We report here the results of AHH assays in a laryngeal carcinoma material, together with some relevant clinical findings.

MATERIALS AND METHODS

Clinical material

The material stems from a total of 64 males and 1 female with invasive laryngeal carcinomas diagnosed at the Department of Oto-rhino-laryngology in Malmö, in 1962–75. All patients subsequently underwent surgery and/or radiotheraphy, and histopathological examination verified spinocellullar laryngeal carcinomas in all but one (male) case who had a primary epiglottal malignancy. Of the remaining 64 cases, 54 (all male) were alive in December 1975, and 53 were still living in Malmö. 10 patients were over 80 years of age. For practical reasons, only the 43 patients below 80 years of age were invited to the investigation. 2 of them did not respond to repeated invitations, thus 42 patients left venous blood samples for AHH assessment at the Department of Preventive Medicine in Malmö. A detailed smoking history was obtained in all but one of these cases.

Control group

The control group was composed of 102 healthy hospital personnel who voluntarily offered blood samples to have their AHH inducibility assayed. 42 were smokers, 19 of whom were heavy smokers with a daily tobaco consumption exceeding 20 cigarettes.

None of the hospital staff in the control group had malignant diseases at the time of the examinations or had undergone treatment for malignant neoplasms previously.

Age and smoking habits of the carcinoma patients did not in detail match those of the healthy control individuals, who on average were younger, with a correspondingly lower total tobacco consumption.

Analytical methods

Some 8–10 ml of venous blood was withdrawn aseptically into a heparinized tube. The tubes were sent by special delivery to the Department of Tumour Cytogenetics in Lund, where they were kept at +4°C overnight. The following day lymphocyte suspensions were prepared by a onestep centrifugal technique using Lymphoprep®, according to Bøyum and Thorsby & Bratlie (26, 27). Lymphocyte yields were calculated by the standard trypan blue dye exclusion procedure.

The lymphocytes were then cultured in Parker 199 medium complemented with 15% fetal bovine serum, glutamine, antibiotics and phytohemagglutinin, a modification of the method described by Busbee et al. Following incubation at 37°C for 72 hours, the lymphoblasts were divided in two aliquots, and 3 µl 1.5 mM 3-methylcholanthrene in acetone were added to one of these and allowed to take effect for an additional 24 hours. The AHH inducibility was then assayed by a modification of the methods of Kellerman et al. (18) and Nebert & Gelboin (25), and expressed as fold induction in comparison with the non-induced lymphoblast aliquot from the same patient. The fold induction was classified according to Kellerman et al. (18) into high (>3.6), intermediate (2.6–3.5) or low (<2.5) level.

RESULTS

In the control material, the frequencies of high, intermediate and low induction were 8.8%, 42.2% and 49%, respectively.

In the larynx cancer group, the same frequencies were 36.6%, 43.9% and 19.5% (Table I).

The expected frequencies were computed from the phenotype distribution in the control group, which did not differ significantly from data obtained by Kellerman et al. (18, 24) or from preliminary data from Sweden (28).

Table I. *Observed and expected frequencies (number of cases) of high, intermediate and low AHH induction levels in the larynx cancer material*

The statistical significance of the differences in the distributions was calculated by the χ^2-test with two degrees of freedom. Expected frequencies were calculated from the AHH distribution in the control material

	High AHH inducibility *n*	Intermediate AHH inducibility *n*	Low AHH inducibility *n*	Statistical significance
Observed	15	18	8	$p<0.0001$
Expected	3.6	17.3	20.1	

The observed number of carcinoma patients with high AHH inducibility compared with the expected number of such patients reveals a statistically highly significant ($P<0.001$ by χ^2-test) overrepresentation.

There was also an underrepresentation of low AHH inducibility (Table I). This underrepresentation was likewise statistically highly significant ($p<0.001$ by χ^2-test) when compared with the expected numbers in Table I.

Of the 41 carcinoma patients, only 2 were nonsmokers, one with intermediate and one with low AHH inducibility. Both of the nonsmokers belonged to the group of 11 patients with carcinomas classified as small (T_1).

The majority of the patients were heavy smokers and these were more heavily represented in the group with high AHH inducibility, as shown in Table II. The table also shows that the age of the patients was lowest in this group and highest in the low AHH induction group.

Occupational histories recorded from all patients revealed no obvious correlation to

Table II. *Age at diagnosis of laryngeal carcinoma, smoking habits, and clinicopathological characteristics of the laryngeal carcinomas in the high, intermediate and low AHH induction groups*

1 cigarette is equivalent with 1 g of tobacco; >20 cigarettes a day were considered as heavy smoking. The table gives the mean and standard deviation of the tobacco consumption in the whole subgroups. The size of the larynx cancer is expressed according to the TNM classification as T_1 (small), T_2 (intermediate) and T_3 (large)

	High AHH inducibility	Intermediate AHH inducibility	Low AHH inducibility
Number of patients	15	18	8
Age at diagnosis of larynx cancer (mean and range; in years)	53.5 (43–76)	60.7 (43–70)	65.4 (55–75)
Number of smokers[a]	15	17	7[b]
Cigarettes only	8	8	1
Pipe only	2	5	2
Cigarettes/cigars and pipe	5	4	3
Duration of smoking[a] (mean and range; in years)	36.5 (24–56)	39.2 ((0)–10–57)	39.4 ((0)–42–50)
Average daily consumption[a] (mean and range; in grammes)	16.7 (10–30)	13.1 ((0)–5–25)	7.7 ((0)–4–10)
Approximate total consumption[a] (mean and range; in kilogrammes)	213.6 (109.5–319.4)	209.3 ((0)–18.3–428.9)	130.8 ((0)–61.3–182.5)
Site of larynx cancer (right/left/medial; number)	9/6/0	4/13/1	5/2/1
Size of larynx cancer ($T_1/T_2/T_3$)	3/8/4	6/8/4	2/4/2
Differentiation of larynx cancer (high or intermediate/low; number)	14/1	18/0	7/1
Metastases (number of cases)	2	0	2
Recurrences (number of cases)	1	0	2
Family history of larynx cancer (number of cases)	1	1	1

[a] Smoking habits until diagnosis of laryngeal carcinoma.
[b] Based upon 7 complete smoking histories.

occupational health hazards, such as exposure to carcinogens, and no significant differences between the various AHH groups.

Gross clinicopathological features of the carcinomas, as summarized in Table II, were also similar.

DISCUSSION

Because of the restricted number of patients, no definite conclusions could be drawn, but the results of our study add further support to the concept that microsomal mixed-funtion oxidases play an important role in activating proximate chemical carcinogens to ultimate carcinogens, i.e. epoxides (25).

Individual differences in epoxide metabolism might also be of some importance in PAH-induced carcinogenesis, although previous studies have reported that AHH inducibility and epoxide hydrase inducibility are under the same genetic control and might even constitute a coupled mono-oxygenase–hydrase system (29, 30).

All patients in the high-inducibility group were smokers, most of them heavy smokers with a daily tobacco consumption in the range of 20 grammes. One of the patients in this group had 14 years earlier undergone surgical treatment for a pulmonary carcinoma. Examinations of the other 40 patients revealed no symptoms of malignancies of the respiratory tract below the larynx.

Hypothesizing that the incidence of laryngeal carcinomas in comparison with the incidence of bronchogenic carcinomas might be approximately proportionate to the epithelial surfaces exposed to PAH in larynx and bronchi, we are also aware of the importance of inhaling habits, smoke turbulences in the laryngeal cavity, etc. However, we have not been able to obtain conclusive data of these factors, since their evaluation is especially difficult in retrospective materials.

The theoretical considerations of Kellerman et al., that subjects with high AHH activity would require less exposure to PAH for tumour development, could not be confirmed in our material. Rather, the tobacco consumption was greater in the high than in the intermediate and low AHH inducibility groups (Table II). On the other hand, the age (and smoking duration) tended to be lower. The limited size of the material precludes close scrutiny of these observations. With due respect to possible still unknown factors in PAH-induced carcinogenesis, we nevertheless feel justified in concluding that the findings underline the association between laryngeal carcinomas and excessive smoking, and that the statistically highly significant over- and underrepresentation of carcinoma patients in the high- and low- inducibility groups, respectively, may be of some value in identifying individuals at high risk.

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A complete list of references can be obtained on request from the authors.