Advances in Brief

Metabolic Phenotypes of Retinoic Acid and the Risk of Lung Cancer


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Abstract

The metabolic activity of cytochrome P-450 enzymes has been associated with an increased risk of developing lung cancer. We found previously that all-trans retinoic acid is catabolized by these oxidative enzymes, and that an inhibitor of this system discriminated between two populations of lung cancer patients. We examined the association between this metabolic phenotype and the risk of lung cancer in 85 subjects. The area under the plasma concentration × time curve (AUC) was calculated after a single oral dose of all-trans retinoic acid (45 mg/m²). The mean AUC for patients who had either squamous or large cell carcinomas was significantly lower than that of patients with adenocarcinomas (P = 0.0001) or control subjects (P = 0.01). Individuals with an AUC < 250 ng·h/ml had a greater likelihood of having squamous or large cell carcinoma (odds ratio = 5.93). This study suggests that the “rapid” catabolism of all-trans retinoic acid is linked to an increased risk of squamous or large cell cancers of the lung.

Introduction

The incidence of lung cancer continues to increase. In the United States, this disease is itself responsible for the overall increase in death from all cancers, and world-wide deaths from lung cancer are expected to exceed 2 million by the end of the decade. Approximately 85–90% of lung cancer is attributable to smoking; however, less than 20% of individuals who smoke actually develop the disease. Thus, the identification of potential differences in susceptibility to this disease, especially among smokers, may be critical to the development of successful prevention strategies.

Heritable differences in the metabolic ability to catabolize various environmental or xenobiotic agents have been linked to the pathogenesis of lung cancer. In particular, epidemiological studies have suggested an association between an increased susceptibility to lung cancer and the oxidative activity of certain cytochrome P-450 enzymes. Whereas most studies have focused on metabolic activation of specific carcinogens, other reports have noted an association with the metabolism of apparently unrelated compounds (such as the antihypertensive drug dapsone) that are catabolized by specific enzyme isozymes.

Recently, we observed marked intersubject differences in the pharmacokinetics of a natural retinoid, all-trans retinoic acid, in patients with non-small cell lung cancer (1). The oral administration of all-trans retinoic acid to these patients distinguished a cohort of subjects who appeared to have an inherent capacity to metabolize this retinoid at a markedly accelerated rate. This high catabolic activity could be attenuated by a single oral dose of ketoconazole, a known inhibitor of several cytochrome P-450 oxidases. Because all-trans retinoic acid is an essential factor for normal growth and differentiation of tracheobronchial epithelium, we suggested that an association might exist between this “rapid” catabolic phenotype for all-trans retinoic acid and the development of lung cancer (1). We tested this hypothesis by conducting a hospital-based case-control study.

Materials and Methods

Study Population. Patients were identified who had pathologically confirmed non-small cell lung cancer and who had not received chemotherapy or radiation therapy. The pathological material was reviewed by a reference pathologist (D. S. K.). Study controls were selected from two populations of subjects, neither of which had any history of cancer. The first group comprised individuals with chronic obstructive pulmonary disease who met one of the following criteria: a 10-20 pack-year history of cigarette smoking; forced expiratory volume at 1.0 s ≤75% of the predicted normal volume; or a forced expiratory volume at 1.0 s forced vital capacity ratio ≤75% of the predicted normal ratio. The second group (control subjects) comprised healthy individuals with no history of major medical illness. All subjects were required to have a Karnofsky performance status ≥70, total serum bilirubin ≤2.0 mg/dl (≤33.9 mmol/liter), serum aspartate aminotransferase ≤54 units/liter (≤54 units/mmol), and serum creatinine ≤2.0 mg/dl (≤177 mmol/liter). First-degree relatives, pregnant or lactating women, and subjects with unstable angina or congestive heart failure were excluded. General anesthesia was not permitted within 3 days of study entry. Subjects taking drugs known to affect cytochrome P-450 enzymes were also excluded. These agents included phenobarbital, phenytoin, primidone, carbamazepine, glutethimide, antipyrine, rifampin, griseofulvin, cimetidine, phenylbutazone, sulfisoxazole, sulfasalazine, clarithromycin, azithromycin, chloramphenicol, ketoconazole, isoniazid, propoxyphene, and disulfiram. Signed informed consent was obtained, and the study was reviewed and approved in advance by this Center's Institutional Review Board.

Data Collection. A structured interview of approximately 45 min was conducted to collect data on demographic and occupational characteristics; family history of cancer; and recent and remote use of tobacco, alcohol, vitamins, diet, and medications. Items in the medication history included information regarding dose, schedule, duration of therapy, and date and time of last dose.

Pharmacology Study Design. On the study day, subjects were instructed to take nothing by mouth after midnight. All nonessential medications were withheld on that morning, and subjects then ingested all-trans retinoic acid (45 mg/m²) as a single oral dose. The drug, formulated as soft gelatin capsules (Vesanoid®; Hoffmann-La Roche, Inc., Nutley, NJ), was taken immediately after ingestion of 240 ml of a liquid formula with defined lipid content (Ensure®, 8.8 g fat/240 ml). Heparinized blood samples were collected immediately prior to drug ingestion and then 1, 2, 3, 4, and 6 h thereafter. Plasma was separated by centrifugation and frozen at −20°C until assayed. All samples were protected from direct light and were transported in amber-colored bags to prevent degradation.

Assay Methods and Pharmacokinetic Calculations. Plasma concentrations of all-trans retinoic acid were measured by reverse phase HPLC using the modified method of Eckhoff and Nau (2). Briefly, an equal volume of isopropanol was added to the plasma sample and then centrifuged to remove

Received 2/6/96; accepted 4/25/96.

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1 Supported by CA-57645 from the National Cancer Institute, Department of Health and Human Services. J. R. R. and V. A. M. are recipients of the American Cancer Society Clinical Oncology Career Development Award.

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3 The abbreviations used are: HPLC, high performance liquid chromatography; AUC, area under the plasma concentration × time curve; RAR, retinoic acid receptor.
The protein precipitate. A 200-μl aliquot of the supernatant was injected using a SP8775 autosampler (Spectra-Physics, Fremont, CA) onto a Spherisorb ODS-2 5-μm column (4.6 x 250 mm) (Phase Separation, Norwalk, CT). The sample was eluted using a gradient that ranged from 70% solvent A to 30% solvent B to 100% solvent B over 60 min with a flow rate of 1 ml/min using a SP8800 gradient pump (Spectra-Physics) [solvent A, 50% methanol and 50% 40 mM ammonium acetate, (pH 7.8); solvent B, 100% methanol]. All-trans retinoic acid was detected at 354 nm by a SP8450 UV spectrophotometer (Spectra-Physics) at a retention time of approximately 34 min (Fig. 1). Standard curves for all-trans retinoic acid were linear over a range from 10 to 1000 ng/ml with a squared correlation coefficient of 0.95 to 0.99. The AUC over the 6 h after the single oral dose of all-trans retinoic acid was calculated according to a trapezoidal method.

Statistical Methods. The difference of the mean values of all-trans retinoic acid AUCs between controls and each case group was compared by t test. The association between lung cancer risk and all-trans retinoic acid AUC and other risk factors (e.g., pack-years of cigarette smoking, occupational exposure, and other risk factors) was measured by the odds ratio and its 95% confidence interval. The odds ratio for all-trans retinoic acid AUC is the odds in favor of a rapid catabolizer (defined as subjects having an AUC of <250 ng-h/ml) developing lung cancer relative to the control group. Smoking exposure was classified by pack-years of cigarette smoking in three categories: nonsmokers; subjects who smoked from 1 to 49 pack-years; and subjects who smoked 50 or more pack-years. The odds ratios for each of the smoking groups were estimated by comparing them to the nonsmoking group. Possible dose-response relationships were evaluated by the Mantel-Haenszel trend test. A multiple unconditional logistic-regression method was used to estimate the adjusted odds ratio after controlling for potentially confounding variables such as age. For this purpose, the FREQ, TTEST, and LOGISTIC procedures in SAS computer software were used (SAS/STAT version 6; SAS Institute Inc., Cary, NC).

Results

Subject Characteristics. Ninety-four subjects were enrolled into this urban hospital-based case-control study. Nine individuals were excluded from the analysis: three lung cancer patients and one control subject failed to completely ingest the all-trans retinoic acid; three lung cancer patients had a history of another cancer; and two patients failed to completely ingest the all-trans retinoic acid. The plot demonstrates all-trans retinoic acid, with retention time 34 min (1) and retinol, with retention time 48 min (2).

79% of the lung cancer cases were 50 years of age compared to 43% of the control subjects (P = 0.003).

AUCs for Cases and Control Subjects. The mean AUC values for the cases and controls are presented in Table 1. The mean plasma AUC for the 42 control subjects was 371 ± 47 ng-h/ml (mean ± SE). This value was similar to the mean AUC for all lung cancer cases (372 ± 46 ng-h/ml) (P = 0.994). However, the mean plasma AUC for the lung cancer patients with a pathological diagnosis of squamous or large cell carcinoma of the lung (162 ± 26 ng-h/ml) was significantly lower than that of the control subjects (P = 0.0108). Moreover, this value was also significantly lower than the value for lung cancer patients with adenocarcinomas (571 ± 62 ng-h/ml; P = 0.0001). We also observed that patients with adenocarcinomas had significantly higher AUCs (571 ± 62 ng-h/ml) than that of the control subjects (P = 0.0251). A frequency histogram of the distribution of all-trans retinoic acid AUCs for the 85 study subjects is depicted in Fig. 2. The histogram shows that the control subjects were distributed bimodally around an all-trans retinoic acid AUC of approximately 250 ng-h/ml. The histogram also depicts a dominant distribution of all-trans retinoic acid AUCs <250 ng-h/ml for the cases with squamous or large cell carcinomas (15 of 21). These 15 cases exhibited the rapid catalytic behavior for exogenously administered all-trans retinoic acid.

In contrast, only 2 (of 22) adenocarcinoma cases had AUCs <250 ng-h/ml; in fact, 14 of these cases had values >500 ng-h/ml. The highest all-trans retinoic acid AUCs were recorded in nonsmoking women with adenocarcinoma of the lung.

Univariate Analyses. In view of the differences in AUC distribution for cases with adenocarcinoma, and squamous or large cell histologies, these groups were analyzed independently. The crude odds ratio for individuals with AUCs < 250 ng-h/ml was 2.27 (95% confidence interval, 0.74–6.99) for the squamous cell or large cell lung cancer histologies. In comparison, the crude odds ratio for the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>AUC of all-trans retinoic acid for study subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
</tr>
<tr>
<td>Controls</td>
<td>42</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>43</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>22</td>
</tr>
<tr>
<td>Large/squamous cell</td>
<td>21</td>
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</table>

* Comparison of mean all-trans retinoic acid AUC for case groups of lung cancer, adenocarcinoma, and large cell/squamous cell carcinomas with the mean AUC of all-trans retinoic acid for the control group. A comparison of mean all-trans retinoic acid AUC for case groups of lung cancer (adenocarcinoma versus large cell/squamous cell carcinomas; P = 0.0001).
Multivariate Analysis. After controlling for age, lifetime cigarette smoking, and occupational exposure to lung carcinogens, the estimated adjusted odds ratio for the rapid all-trans retinoic acid catabolic phenotype was 5.93 (95% confidence interval, 1.30–27.15) for squamous or large cell carcinomas of the lung compared to the control subjects. The estimated adjusted odds ratio for the rapid phenotype was 0.12 (95% confidence interval, 0.02–0.65) for the cases of adenocarcinoma when compared to the control subjects (Table 2).

Discussion

Although almost 90% of lung cancer patients are current or past cigarette smokers, a relatively low proportion of smokers (10–20%) will actually develop lung cancer. Therefore, cancer prevention studies are constrained by requirements for large numbers of subjects and prolonged periods of observation due to the relatively low annual incidence of new lung cancers that occur even in heavy smokers. This constraint has prompted efforts to define additional factors that will identify individuals at particularly high risk (and therefore most likely to benefit from specific interventions).

In previous studies, factors such as inheritance, diet, environmental exposure, metabolic phenotypes (3), and genetic alterations have been explored. The relationship of various heritable traits, especially differences in the metabolism of potential carcinogens, have been examined. Polycyclic aromatic hydrocarbons found in tobacco smoke were thought to be activated by CYP1A1 isozyme of the cytochrome P-450 family. However, case-control comparisons of lymphocyte aromatic hydrocarbon hydroxylase activity in lung cancer have been conflicting and inconclusive. Similarly conflicting results have been reported with antipyrine. The antihypertensive agent debrisoquine is hydroxylated by a P-450 isozyme, CYP2D6. Although studies have found an association between "extensive" metabolism of debrisoquine and an increased risk of lung cancer (4), several others have not confirmed this association (5).

More than 70 years ago, Wolbach and Howe (6) reported the first studies that described the importance of retinoids in the normal development of mucociliary epithelium. More recently, dietary deficiencies of retinol (vitamin A) have been associated with precancerous changes in tracheobronchial epithelium, and supplementation with retinoids protected these tissues from carcinogen-induced squamous metaplasia (7). Several epidemiological studies have demonstrated an inverse relationship between carotenoid intake and the risk or incidence of lung cancer (8). These studies have also revealed differences in the histological subtypes of lung cancer related to carcinogen exposure, dietary intake of carotenoids, and serum retinol levels. For example, smoking is the dominant factor for squamous cell carcinoma of the aerodigestive tract (9), whereas adenocarcinoma of the lung

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### Table 2 Multivariate analysis of the AUC, smoking, and occupational exposure

<table>
<thead>
<tr>
<th>Variables</th>
<th>All lung cancer cases versus controls</th>
<th>Adenocarcinoma versus controls</th>
<th>Large cell and squamous cell versus controls</th>
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<tr>
<td></td>
<td>Odds ratio 95% confidence interval</td>
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<td>AUC (ng h/ml)</td>
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<td>&lt;250</td>
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<td>1.03–1.13</td>
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<td>1.02–1.18</td>
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Fig. 2. Distribution of all-trans retinoic acid AUCs for 21 lung cancer cases with squamous or large cell histologies (blue), 22 lung cancer cases with adenocarcinoma (green), and 42 control subjects (red). 

Adenocarcinoma cases with AUCs <250 ng/h/ml versus control subjects was 0.09 (95% confidence interval, 0.02–0.44). We then assessed the individual contribution of specific factors to this association including age, race, education, lifetime pack-years of cigarette smoking, history of occupational exposure to lung carcinogens, recent smoking status, laboratory tests of hepatic and renal function, family history of cancer, and use of multivitamins. Lifetime cigarette smoking of ≥50 pack-years and occupational exposure to lung carcinogens produced odds ratios of 2.36 (95% confidence interval, 0.76–7.34) and 1.69 (95% confidence interval, 0.66–4.30), respectively, for the lung cancer cases when compared to the control subjects. Cigarette smoking was associated with large cell and squamous cell carcinomas of the lung. The odds ratio for these histological subtypes of lung cancer was 2.89 (95% confidence interval, 0.53–15.91) for those who smoked less than 50 pack-years and 6.50 (95% confidence interval, 1.18–35.80) for those who smoked 50 or more pack-years (trend test; P = 0.02). However, no association was observed between lifetime smoking status, laboratory tests of hepatic and renal function, family history of cancer, and occupational exposure to lung carcinogens, the estimated adjusted odds ratio for the rapid phenotype was 5.93 (95% confidence interval, 1.30–27.15) for large cell and squamous cell carcinomas of the lung compared to the control subjects (Table 2).
occurs most frequently in nonsmoking women (10). As yet, however, there is no evidence that dietary supplementation with carotenoids can alter this risk (11).

This case-control study links the constitutive rapid catabolism of all-trans retinoic acid to an increased risk of developing squamous or large cell lung cancer. Previously, we had observed marked intersubject variability in the pharmacokinetics of all-trans retinoic acid after ingestion of single oral doses (1). We also found that administration of ketoconazole (a broad-spectrum inhibitor of cytochrome P-450 3A4 oxidases) prior to dosing with all-trans retinoic acid could distinguish two populations of patients with lung cancer. One group exhibited a constitutively high rate of clearance (the rapid catabolic phenotype) that was attenuated by the administration of ketoconazole; the other group showed a slower rate of clearance (the "normal" catabolic phenotype), similar to patients with acute promyelocytic leukemia whom our group had studied previously (Ref. 12; Fig. 3). Initial clearance rates in the latter group were not affected by ketoconazole; however, continuous dosing with the retinoid resulted in induced catabolism of the drug, and this inducible type of accelerated clearance was also attenuated by both ketoconazole and liarozole (1, 13). We also found that these two populations of lung cancer patients differed in their endogenous plasma concentrations of natural retinoids. Patients who exhibited the rapid catabolic phenotype had significantly lower endogenous plasma levels of all-trans retinoic acid compared to those with a normal catabolic phenotype (1). Thus, these preliminary findings suggested that the rapid phenotype was associated with constitutively higher activity of a cytochrome P450-dependent catabolic pathway that resulted in significantly lower levels of endogenous retinoids.

The oxidative catabolism of all-trans retinoic acid to 4-hydroxy- and 4-oxo-all-trans retinoic acid (the major oxidative metabolite reported in adult patients) is mediated by cytochrome P450 3A4 oxidases. If the rapid catabolic phenotype observed in this study is mediated by a single, constitutively expressed cytochrome P450 enzyme, its identification may lead to the description of a characteristic genotypic polymorphism associated with this trait. Using in vitro cell microsomes enriched genetically with specific P-450 isozymes, Muindi and Young (14) showed that CYP3A4 had the greatest oxidative activity for all-trans retinoic acid. This isoform is the most abundantly expressed P-450 oxidase in human liver, and it demonstrates marked heterogeneity in interindividual activity. Ketoconazole, the agent we used to differentiate the two populations of lung cancer patients, is a potent inhibitor of CYP3A4 (15), and human CYP3A4 has been shown in vitro to be highly inducible in liver and small bowel (16).

The mechanisms by which retinoids regulate cellular growth and differentiation have not been elucidated fully. These compounds are known to mediate transcription of target genes by activation of specific nuclear receptors. Three RARs have been identified. The expression of one receptor, RAR-β, is inducible by all-trans retinoic acid in normal human tracheobronchial epithelial cells (17). Decreased RAR-β expression is a consistent abnormality in cancers of the aerodigestive tract (18), and this phenomenon has also recently been associated with the progression from normal epithelium to invasive squamous head and neck cancer, a tobacco-related disease (19, 20). Conversely, transfection of RAR-β into squamous cell tumors reduces both tumorigenicity and growth (21). Conceivably, the failure of RAR-β to up regulate could allow these cells to escape normal growth controls and progress to neoplasia after carcinogenic exposure. If all-trans retinoic acid is critical to normal RAR-β expression, then the rapid catabolic phenotype we identified may reflect accelerated oxidative activity that as a consequence markedly decreases effective intranuclear concentrations of retinoic acid. This feature, even in the presence of adequate dietary retinoid intake, could result in diminished RAR-β expression and increased susceptibility to carcinogenesis. That predisposition, coupled with chronic cigarette smoke exposure, may be central to the development of the squamous and large cell subtypes of non-small cell lung cancer.

Retinoid supplements have proven most effective in the treatment and prevention of smoking-related premalignant or squamous cell cancers of the lung and head and neck (22, 23). Conceivably, characterization of the retinoic acid metabolic phenotype may identify subjects at highest risk for lung cancer, thus selecting those individuals who would be most likely to benefit from the specific use of retinoids for chemoprevention.

References
11. Heinonen, O. P., and Albanes, D. The effect of vitamin E and β carotene on the


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