Sex Differences in Lung CYP1A1 Expression and DNA Adduct Levels among Lung Cancer Patients

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Abstract

Several epidemiological studies have indicated that female tobacco smokers may be at higher risk of lung cancer than males. In a study of lung cancer cases, we have found that female smokers had a significantly higher level of aromatic/hydrophobic DNA adducts in their non-tumor lung tissue compared with male smokers. Females had significantly higher levels of adducts/pack-year (females 0.95 ± 0.82 adducts/pack-year and males 0.46 ± 0.46, P = 0.0004) and adducts/cigarette/day (females 1.48 ± 1.29 and males 0.89 ± 0.74, P = 0.015). By quantitative reverse transcription-PCR, it was found that female smokers exhibited a significantly higher expression level of lung CYP1A1 mRNA (15.39 ± 9.47 adducts/10⁶ nucleotides, n = 29) than male smokers (12.08 ± 8.14, n = 93; P = 0.047). By quantitative reverse transcription-PCR, it was found that female smokers exhibited a significantly higher expression level of lung CYP1A1 mRNA (494 ± 334 CYP1A1 mRNA/10⁶ glyceraldehyde-3-phosphate dehydrogenase mRNA, n = 15) compared with males (210 ± 208, n = 12; P = 0.016). Furthermore, for both sexes combined, a significant correlation between CYP1A1 expression and DNA adduct level was found (r = 0.50, P = 0.009). In conclusion, the observed sex difference in aromatic/hydrophobic DNA adduct levels may at least in part be explained by different levels of CYP1A1 expression.

Introduction

Lung cancer is the most frequent cancer in the world today, and the epidemic of this disease is still ongoing. Globally, carcinomas of the lung are the most common cause of cancer death, and cigarette smoking is widely accepted as the major risk factor for the incidence of lung cancer. More attention is necessary on the issue of smoking and women because several epidemiological studies indicate that for a given number of cigarettes smoked, females may be at higher risk of lung cancer compared with males (2–4). This sex difference may be due to a higher susceptibility to tobacco carcinogens among females.

PAHs and nitroso compounds are considered to be carcinogens of major importance in tobacco smoke. The human CYP1A1 gene codes for a central enzyme in the metabolic activation of PAHs, leading to the formation of highly reactive diol-epoxides that may bind to DNA. Smoking-related DNA adducts in lung tissue may be a molecular exposure marker. DNA adduct levels have been shown to correlate with the level of CYP1A1 activity in smokers, which again correlates with the level of CYP1A1 mRNA. A good correlation between the carcinogenic potency and DNA adduct formation has been observed experimentally for several carcinogens, including PAHs (for review, see Ref. 9). In humans, some studies have indicated that the level of hydrophobic DNA adducts may be a risk factor for the development of lung cancer (5, 6, 10). In addition, among lung cancer patients, high levels of DNA adducts have been related to an early onset of the disease (11).

CYP1A1 expression is induced by PAHs and halogenated aromatic hydrocarbons. In human lung tissue, CYP1A1 expression is inducible by cigarette smoking (8). Both a high degree of interindividual variability in CYP1A1 inducibility, and a correlation between level of inducibility in human lymphocytes and risk of lung cancer have been demonstrated in smokers (12). It seems that induction of CYP1A1 expression is controlled primarily at the level of transcription.

We have reported previously (11, 13) a higher level of lung hydrophobic DNA adducts and a higher frequency of G:C→T:A transversions in the p53 gene in the lung tumors of female lung cancer patients, which lends support to the hypothesis that females are at increased risk of developing smoke-induced lung cancer. To clarify this sex dependence of PAH-induced DNA adducts further, we have determined the levels of hydrophobic DNA adducts in non-tumor lung tissue of lung cancer patients in the present, extended study. Furthermore, we have used quantitative competitive RT-PCR to study expression of the CYP1A1 gene in the lung tissue of currently smoking patients.

Materials and Methods

Study Population. Samples of normal lung tissue adjacent to tumor tissue were resected at the time of surgery of 159 previously untreated lung cancer patients. The tissue was snap-frozen and stored at −80°C. Demographic patient data on sex, age, and smoking history (see Table 1) were obtained by questionnaires as described previously (11). Hydrophobic DNA adduct levels were measured in 29 female and 93 male smokers as well as 13 female and 24 male nonsmokers (never-smokers and ex-smokers of 2 or more years of abstinence). CYP1A1 gene expression was studied in a subset consisting of 15 female and 12 male current smokers: mean age ± SD was 55 ± 13 years (females) and 60 ± 11 years (males; nonsignificant); smoking history was 12.2 ± 5.2 cigarettes/day (females) and 15.8 ± 7.1 cigarettes/day (males; nonsignificant); and pack-years was 20 ± 9 (females) and 30 ± 16 (males; P = 0.04).

Quantitative RT-PCR. Gene expression was determined using quantitative competitive RT-PCR modified from Celi et al. (14) and Willey et al. (15). In brief, CYP1A1 and GAPDH cDNA (native) was ampliﬁed in the presence of known amounts of synthetic CTS derived from the respective cDNA sequences. The CTS give rise to PCR products that are identical to the native cDNA sequence apart from a deletion and will be amplified by the native primers. In each case, cDNA was titrated against the corresponding CT to determine the amount needed to result in equal levels of PCR products.

Total RNA was extracted from frozen non-tumor lung tissue by the TRIzol reagent (Life Technologies, Inc., Rockville, MD) following the supplier’s protocol. mRNA was reversibly transcribed by aid of the 1 Strand cDNA Synthesis kit for RT-PCR (1st Strand cDNA Synthesis Kit for RT-PCR [AMV], Boehringer Mannheim, Mannheim, Germany), using oligo-pdT₁₅ primers. Primer sequences for quantitative competitive PCR CYP1A1 were: (a) CYP1A1 native upper 5’-CAT CCC CCA CAG AAC AAG-3’; (b) CYP1A1 native lower 5’-AGA GCA GGC ATG CT CAT CT GGT-3’; and (c) CYP1A1 CT lower 5’-AGA GCA GGC ATG CT CAT CT GGT TCT CAC CGA.
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TAC ACT TCC GCT-3' (15). These primers give rise to PCR-products of 355 bp (native) and 248 bp (CT). GAPDH primer sequences were: (a) GAPDH native upper 5'-ACA GTC CAT GCC ATC ACT GCC-3'; (b) GAPDH native lower 5'-GCC TGC TTC ACC TTC TTG; and (c) GAPDH CT upper 5'-ACA GTC CAT GCC ATC ACT GCC ATC CCT GCC TCT ACT GGC-3'. Primer products for GAPDH were 266 bp (native) and 200 bp (CT). The cDNA sequence for GAPDH was from the GenBank, and primer sequences were chosen using the Oligo software (National Biosciences, Plymouth, MN). For the PCR reactions, the DynaZyme II Thermostable recombinant DNA polymerase from Finnzymes Oy (Espoo, Finland) was used together with the supplier’s buffer conditions. Because of differences in mRNA levels of several orders of magnitude, CYP1A1 and GAPDH were amplified in separate reactions for 30 and 20 cycles, respectively, of 94°C for 20 s, 58°C for 30 s, and 72°C for 60 s. PCR products were electrophoresed on 6.7% polyacrylamide gels (29:1), visualized by the SYBR Green I nucleic acid gel stain (FMC BioProducts, Rockland, ME), and photographed with a CCD camera. Levels of PCR products were measured by densitometric analysis of gel images using the public domain NIH Image program.

**Results**

In a pilot study, we have previously shown that female lung cancer patients have higher levels of DNA adducts in their normal lung tissue than males, when adjusted for smoking dose (11). In the present study, the levels of hydrophobic/aromatic DNA adducts were measured in lung tissue from 159 lung cancer patients by 32P postlabeling analysis with the nuclease P1 modification. As shown in Table 1, the levels of DNA adducts were highly significantly increased among both smoking females and smoking males compared with nonsmokers (P = 3 × 10⁻⁷ and P = 4 × 10⁻⁸, respectively). Significantly higher levels of DNA adducts were found among female smokers compared with male smokers (P = 0.047) although the latter had smoked more, verifying our previously published data (11). When expressing DNA adduct levels in relation to smoking dose, we found that levels of adducts/pack-year (females, 0.95 ± 0.82, and males, 0.46 ± 0.46) and adducts/cigarette/day (females, 1.48 ± 1.29, and males, 0.89 ± 0.74) were significantly higher among females (P = 0.0004 and P = 0.015, respectively).

The expression of the CYP1A1 gene was determined in normal lung tissue from a subset consisting of currently smoking lung cancer patients. CYP1A1 expression was normalized to the expression of GAPDH by quantitative competitive RT-PCR as described in “Materials and Methods” (Fig. 1). Large interindividual variations were observed with a total range of 21–112 CYP1A1 mRNA/10⁶ GAPDH mRNA. When grouped by sex, a significantly higher mean CYP1A1 expression was found for females than for males (P = 0.016; Fig. 2).

Linear regression analysis revealed that the hydrophobic DNA adduct levels were significantly related to the level of CYP1A1 expression (with the exclusion of one outlier), r = 0.50, P = 0.009 (Fig. 3). The excluded data point from the linear regression analysis was...
found to be an outlier in a normal probability plot, as well as having a twice as high residual value as the data point with the second largest value. No correlation was found between CYP1A1 expression and either number of cigarettes smoked per day or age of the patients.

Discussion

The formation of hydrophobic/aromatic DNA adducts in the lung is induced by PAH present in tobacco smoke. The metabolism of PAH is complex, including an activation reaction (Phase I) that involves members of the cytochrome P450 monoxygenases and a detoxifying conjugation reaction via the various Phase II enzymes. Several previous studies have shown significantly higher DNA adduct levels in smokers compared with nonsmokers (5, 6, 10). Among smokers, our data showed that PAH-DNA adduct levels were inversely related to age, years of smoking, or pack-years. Years of smoking was the most significant single smoking-related parameter explaining the variation in adduct levels. Thus, it may be interpreted that some individuals are predisposed to high adduct levels without regard to the daily smoking dose. These individuals will statistically have an earlier onset of lung cancer than individuals with low adduct levels. In our study, females had significantly higher PAH-adduct levels than males and an earlier onset of the disease (lower mean age at the time of diagnosis). This indicates that females are more susceptible to PAH exposure than males, which confirms our previous results (11).

AHH has been found to correlate with CYP1A1 mRNA (Ref. 8 and unpublished data). In human lung tissue both CYP1A1 mRNA and AHH activity are induced by PAHs present in tobacco smoke (8, 17). To evaluate the role of AHH activity in the observed sex difference in smoking-induced DNA adducts, CYP1A1 gene expression was measured in currently smoking lung cancer patients. We found that females had significantly higher levels of CYP1A1 expression in their nontumor lung tissue than males. Because it has been reported that CYP1A1 gene expression is reduced as early as 2 weeks after smoking cessation (8), only patients who were smoking up to the time of surgery were included in the CYP1A1 expression study.

We have used a highly reliable quantitative competitive RT-PCR method to study CYP1A1 expression, relating the CYP1A1 gene transcript to the expression of the GAPDH gene. Both cDNAs were amplified in the presence of CTs that were identical to the particular cDNA sequence apart from a deletion. Titration of the cDNA against known amounts of the respective template allowed accurate quantification of the expression. Because of a high degree of sequence homology between the genes for CYP1A1 and CYP1A2, the PCR primers for CYP1A1 cDNA amplification will also amplify CYP1A2 cDNA, which results in PCR products that are indistinguishable upon gel electrophoresis. However, CYP1A2 has been reported not to be expressed in human lung (18). In addition, similar to the results reported in the present study, a significantly higher expression level of pulmonary CYP1A1 among smoking female lung cancer patients was found by using semiquantitative RT-PCR and primers that were specific for CYP1A1 only (19).

Hydrophobic DNA adduct levels were found to be significantly related to the level of CYP1A1 expression, although a large interindividual variation was observed for both DNA adducts and CYP1A1 expression. In agreement with our data, a significant correlation has been demonstrated between AHH activity and DNA adducts in nontumor lung tissue of smoking lung cancer patients (20). Considering the rapid turnover of CYP1A1 mRNA, and a half-life of 1.7 years for DNA adducts in bronchial tissue from ex-smokers (6, 8), we found a correlation coefficient (r) of 0.50, which means that 25% of the variation in the level of DNA adducts could be explained by the variation in CYP1A1 expression. Clearly, the level of DNA adducts is influenced by factors other than CYP1A1, such as exposure to PAH, Phase II metabolism, DNA repair processes, and cell turnover.

Several, although not all, epidemiological studies indicate that females may be at greater risk of developing lung cancer than males (2–4). In addition to higher levels of smoking-induced CYP1A1 gene expression and higher levels of DNA adducts among female lung cancer patients, women seem to have higher frequencies of G/C—>T:A base substitution mutations in the p53 gene (11, 13, 21). Together, these data provide evidence for sex differences in lung cancer susceptibility at the genetic and biochemical level. The mechanism(s) is unknown, but the hypothesis of a possible role of hormones has been put forward (22). In vitro studies have suggested complex interactions between the ER and the aryl hydrocarbon receptor pathways (23). It is possible that circulating female steroid hormones(s) may interact with receptors present in the lung and modulate the expression of PAH metabolizing enzymes. Sex-steroid-receptor expression has been found to be more frequent in lung tumors from female than from male patients (24). Expression of ER-α, as detected by immunocytochemistry, is infrequent in normal lung. Little is known about the expression of the recently cloned ER-β in adult human lung, but this receptor subtype has been demonstrated to be frequently expressed in rat lung tissue as well as in fetal human lung (25, 26).

References

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