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SHORT COMMUNICATION

Drug-Metabolizing Enzymes in Pharyngeal Mucosa and in Oropharyngeal Cancer Tissue

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ABSTRACT. Cytochrome P4501A1 (CYP1A1) and the UDP-glucuronosyltransferase isoform UGT1A6 were studied in pharyngeal mucosa and squamous cancer tissue obtained from 27 male subjects (10 healthy nonsmoking volunteers, 10 smokers, and 7 smokers with pharyngeal cancer). CYP1A activity (7-ethoxyresorufin *O*-deethylase) was significantly induced in smokers as compared to nonsmokers (2.3 ± 1.1 and 0.8 ± 0.4 pmol · min^{-1} · mg $protein^{-1}$, respectively). Immunoblot analysis demonstrated enhanced CYP1A1 protein in smokers. UGT activity towards 4-methylumbelliferone and 1-naphthol was also detectable in oropharyngeal mucosa. RT-PCR (reverse transcriptase-polymerase chain reaction) analysis indicated that UGT activity was at least in part due to the expression of UGT1A6. In cancer tissue, CYP1A activity was decreased in comparison with surrounding healthy mucosa (1.2 ± 0.9 in tumor tissue vs. 2.2 ± 0.7 pmol · min^{-1} · mg $protein^{-1}$, respectively), whereas means and medians of UGT activity were unchanged. The results suggest that phase I and II drug-metabolizing enzymes are detectable in oropharyngeal mucosa and that CYP1A activity is inducible by constituents of cigarette smoke. BIOCHEM PHARMACOL **54**;10:1159–1162, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. drug-metabolizing enzymes; CYP1A1; UDP-glucuronosyltransferase UGT1A6; smoking; head and neck cancer

Cigarette smoking and alcohol consumption have been identified as main etiological factors in the development of pharyngeal cancer [1–4]. Polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene are found in cigarette smoke and account in part for its carcinogenic effects [5, 6]. A group of drug-metabolizing enzymes is adaptively induced by PAH through the action of the aryl hydrocarbon receptor [7], including CYP1A1, which is responsible for the bioactivation of PAH and the UGT isoform UGT1A6 responsible for conjugation of planar phenolic metabolites including mono- and diphenols of PAH [8]. Characteristic changes in enzyme activities have also been detected in preneoplastic and tumor tissue, which may in part be responsible for the toxin-resistance phenotype [9]. In this phenotype, CYP1A activity is decreased and conjugating enzymes are not affected or increased.

Previously, Phase I and II drug-metabolizing enzymes and their responses to smoking and drug treatment have been studied in duodenal biopsies [10]. In the present study, these enzymes have been investigated in oropharyngeal mucosa and in corresponding squamous head and neck cancer tissues in an attempt to further our knowledge as to the regulation of these enzymes in human tissues.

MATERIALS AND METHODS

Subjects

Three groups of male subjects participated in the study: 10 smokers (smoking >20 cigarettes/day), 10 nonsmokers, and 7 tumor patients (smoking >20 cigarettes/day). The smokers, nonsmokers and tumor patients were 20 to 40 years old. No drugs and no additional diseases were known, and the patients' alcohol consumption was moderate (<40 g/day). With smokers and nonsmokers biopsies were taken during tonsillectomy or nasal sinus surgery. Histological examination of the tumors revealed squamous cell carcinoma of the oro- and hypopharynx (staging >63, >T2). After extensive information and written consent, all the patients were treated by surgery due to ear-nose-throat (ENT) indications.

Preparation of Tissue Homogenates and Microsomes

All samples were immediately frozen in dry ice, then transferred to Eppendorf tubes and stored for 1 to 4 weeks at −80°. After thawing biopsy samples were homogenized in 0.25 M sucrose buffered with 10 mM Tris-HCl, pH 7.4, and sonicated for 20 sec. For the preparation of microsomal fractions, homogenates were centrifuged at 15,000 × *g* for

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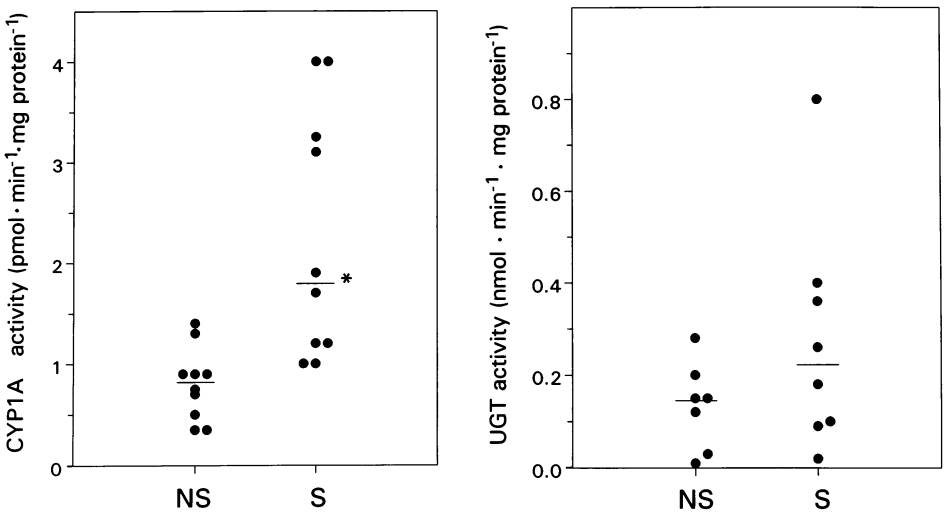

FIG. 1. CYP1A and UDP-glucuronosyltransferase (UGT) activities in pharyngeal mucosa of nonsmokers (NS) and smokers (S). Enzyme activities were determined in tissue homogenates. Medians are indicated by horizontal lines. *P < 0.05.

15 min. Subsequently, the supernatant was sedimented at 100,000 × *g* for 1 hr. Microsomal pellets were resuspended in buffered sucrose and stored at −80°. Care was taken that all preparations were carried out at 4°. Protein was measured according to Lowry et al. [11].

Enzyme Assays

CYP1A[‖] activity was determined fluorimetrically as EROD (7-ethoxyresorufin-*O*-deethylase) activity, as described [10]. Tissue homogenates (*ca.* 0.6 mg protein) were used for the EROD assay. UGT activity towards 4-methylumbelliferone [10] and 1-naphthol [12] was measured according to published procedures. UGT activity was tested in the activated state by addition of 0.05 mg Brij 58/mg protein to the tissue homogenate. Activation was *ca.* 2-fold.

Immunoblotting of CYP1A1

Microsomal protein (40 μg) was submitted to SDS-PAGE (10% polyacrylamide) and the separated proteins were transferred to Immobilon P membranes (Millipore). Nonspecific binding sites were blocked with PBS containing Tween 20 (0.05%) and milk powder (5%) for 1 hr. The membrane was then washed with PBS/Tween and incubated overnight with rat polyclonal antibodies against CYP1A1 [13] at a 1:1000 dilution, as described previously [10]. As standard, 1 μg yeast microsomal protein of cloned expressed CYP1A1 was used [14].

RT-PCR of UGT1A6 from Pharyngeal Tissue

Total RNA of the tissue samples was prepared from biopsies by the guanidinium thiocyanate method of Chomczynski and Sacchi [15]. Total RNA (1 μg/9 μL) was heated at 65° for 5 min and cooled on ice. Reverse transcription was performed exactly as described [16].

Statistics

Data are given as mean (±SD) and as medians. Comparison of data were made by Wilcoxon, Mann and Whitney rank test, $p < 0.05$ being considered statistically significant.

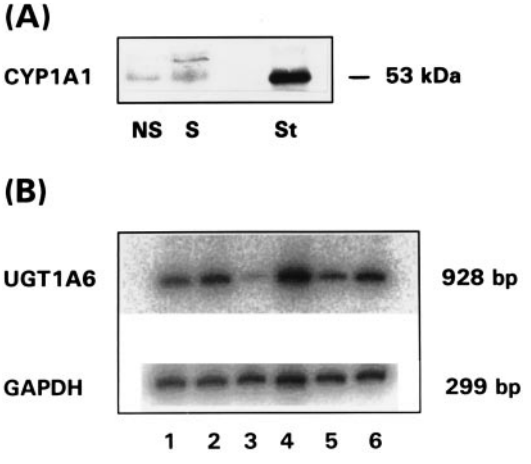

FIG. 2. Immunoblot analysis of CYP1A1 in pharyngeal microsomes (A) and RT-PCR analysis of UGT1A6 mRNA in pharyngeal tissues (B). Pharyngeal microsomes were obtained from pooled tissues of 3 nonsmokers (NS) and 3 smokers (S). As standard (St), 1 μg yeast microsomal protein of cloned expressed CYP1A1 was used (14). The band of high molecular weight is unknown. In B, pharyngeal tissues were obtained from a tumor and normal tissue of the same patient (1 and 2, respectively), from areas with leucoplakia of 3 patients (3, 4 and 5) and from normal pharyngeal mucosa of a nonsmoker (6). Total RNA (0.2 μg) was used for RT-PCR (16). With the same samples, RT-PCR for GAPDH (glyceraldehyde-phosphate dehydrogenase) was carried out to control equal loading.

[‖] *Abbreviations:* CYP1A1, cytochrome P4501A1; EROD, 7-ethoxyresorufin *O*-deethylase; PAH, polycyclic aromatic hydrocarbons; RT-PCR, reversed transcriptase-polymerase chain reaction; UGT, UDP-glucuronosyltransferase (EC 2.4.1.17).

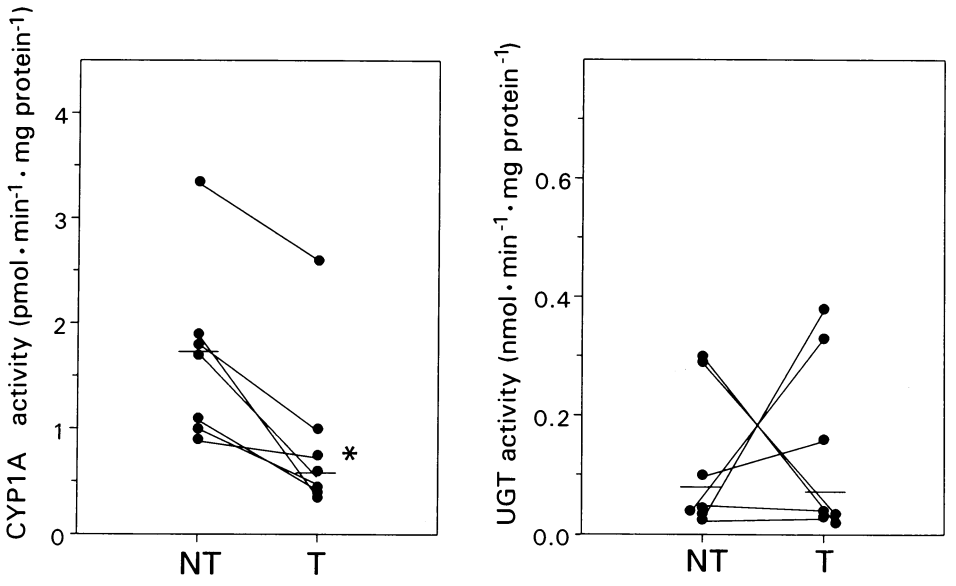

FIG. 3. CYP1A and UDP-glucuronosyltransferase (UGT) activity in paired pharyngeal biopsies from tumor tissue (T) and from surrounding nontumor tissue (NT). Enzyme activities were determined in tissue homogenates. Medians are indicated by horizontal lines. *$P < 0.05$.

RESULTS AND DISCUSSION

CYP1A activity was detectable in all pharyngeal tissue samples examined (Fig. 1). It was significantly increased in smokers despite large interindividual variations. As shown by immunoblot analysis, enhanced enzyme activity in smokers was due to increased CYP1A1 protein (Fig. 2A). Densitometry of pooled samples revealed that CYP1A1 was moderately induced *ca.* 1.5-fold (this trend was supported in individual samples from 3 smokers and 3 nonsmokers despite large variability; not shown). UGT activity was also detectable (Fig. 1) and tended to be higher in smokers although it did not reach statistical significance. UGT activities towards 4-methylumbelliferone and 1-naphthol (nmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1}) were similar in 4 samples (nonsmokers and smokers: 0.14 $\pm$ 0.10 and 0.42 $\pm$ 0.33 for 4-methylumbelliferone; 0.11 $\pm$ 0.05 and 0.32 $\pm$ 0.21 for 1-naphthol). In cancer patients, paired biopsies from the tumor and adjacent mucosal tissue were studied (Fig. 3). CYP1A activity was significantly decreased in tumor tissue (1.2 $\pm$ 0.9 vs. 2.2 $\pm$ 0.7 pmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1}), whereas means and medians of UGT activity were unchanged.

More than 10 UGT isoforms have been detected in human tissues and divided into 2 families (phenol/bilirubin UGTs and steroid UGTs) based on evolutionary divergence [17]. Characterization of family 1 members indicated that they are encoded by a large UGT1 locus consisting of multiple nested first exons encoding isoform-specific sequences which are followed by an identical sequence present in all family 1 members (encoded by exons 2 to 5; 18). Human UGT1A6 is the product of the sixth unique exon. To be able to measure UGT1A6 expression in small tissue samples, a special RT-PCR method has been developed [16]. This method uses intron-overlapping forward and reverse primers from exons 1 and 2 and a 'hot-start' modification. Utilizing this method, UGT1A6 expression was measured for the first time in pharyngeal mucosa, tumor tissue and areas with leukoplakia (considered to be a preneoplastic lesion; Fig. 2B). With the exception of sample 3 (in which there was evidence for partial mRNA degradation, based on additional low molecular weight bands; not shown), no marked difference (<2-fold) in UGT1A6 expression was found in normal mucosa or in leukoplakial and tumorous tissue, substantiating UGT activity data.

UGT1A6 is at least partially responsible for the conjugation of planar phenols and arylamines, including metabolites of the carcinogens 2-naphthylamine and benzo(a)-pyrene present in cigarette smoke [19]. Hence, the presence of this isoform in pharyngeal mucosa may play an important protective role. Identification of UGT1A6 in pharyngeal mucosa is in line with accumulating evidence for its expression in many human tissues [16]. Regulation of this isoform is complex and tissue-specific. In some tissues, low basal expression and inducibility by PAH-type inducers has been found, e.g. in human hepatocyte cultures and in the colon carcinoma cell line Caco-2. In contrast, in other cell lines (lung carcinoma A549 cells) high constitutive expression and no inducibility by PAH-type inducers was observed [16].

In oro- and hypopharyngeal tumors CYP1A activity was decreased, whereas means and medians of UGT activity and UGT1A6 expression appeared to be unchanged. These changes resemble the toxin-resistance phenotype found in preneoplastic lesions in experimental animals [12].

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