DNA Adduct Formation in the Human Nasal Mucosa as a Biomarker of Exposure to Environmental Mutagens and Carcinogens

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Human exposure to chemical compounds, often termed xenobiotics, has been linked to a number of enhanced incidences of various neoplasias. A majority of these enter the human body through inhalation. Most xenobiotics are metabolized in the body to more hydrophilic metabolites before excretion in the urine and bile. During this process, promutagens and procarcinogens could be activated and could interact with proteins as well as DNA to form adducts. DNA adducts formed by chemical carcinogens can, therefore, be used as biomarkers of exposure and other host factors. This study shows that DNA adduct analysis can be performed on cells from human nasal mucosa. Using the nasal lavage procedure performed on 20 healthy volunteers, 5 × 106 to 5 × 108 cells were obtained from which 5 to 40 µg DNA was isolated. DNA adducts were analyzed by the 32P-postlabeling assay. The DNA adduct levels ranged between 1.4 and 6 adducts/106 nucleotides. In addition to its simplicity, the nasal lavage procedure is an inexpensive, noninvasive procedure that requires no anesthetics or specific equipment. Moreover, the cells obtained are the first to come in contact with air pollutants. DNA adduct analysis from human nose mucosa cells could therefore be used to develop a technique suitable for the assessment of exposure to chemical carcinogens through inhalation. — Environ Health Perspect 104(Suppl 3):471–473 (1996)

Key words: human exposure, biomarkers, nasal lavage, DNA adducts, 32P-postlabeling

Introduction

The exponential increase in the production and use of chemicals has had a profound impact on the environment and created unforeseen hazards to man’s well-being. Many of those compounds, termed xenobiotics, are not water soluble and thus must be metabolized in the body to more polar metabolites (biotransformation) prior to excretion in the urine and bile. Usually this

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Abbreviations used: Triton X, polyethylene glycol p-isocyanophenyl ether; Tris-HCl, tris(hydroxymethyl)aminoethane-hydrochloride acid.

series of events is considered to be a mechanism invented by nature to detoxify deleterious compounds; however, in some cases during biotransformation, reactive intermediates can be produced and can either uncouple integrated biochemical processes or combine with cellular constituents such as DNA, RNA, and protein. It is now evident that carcinogenesis, mutagenesis, and cellular necrosis are often attributable to the action of such reactive intermediates (1–5). DNA adduct formation is therefore thought to represent an early and critical step in the process of chemical carcinogenesis; thus, it is reasonable to assume that human populations with chronically elevated DNA adduct levels may be at higher risk of developing cancer (6–8). DNA adducts formed in human cells by chemical carcinogens can be used as biomarkers and dosimeters of exposure.

Because humans are predominantly nose breathers and the ventilation rates through the nasal passages can exceed 10,000 liters per day, the cells lining the nasal passages are the first to come in contact with air pollutants. These cells also constitute the first line of defense against various xenobiotics. There is therefore every reason to believe that inhaled airborne pollutants might provide adverse effects on the nose mucosa cells. The cells in human nasal cavity could, however, be an excellent material for the development of biomarkers related to airborne carcinogens. The main goal of this study has been to determine if samples provided by the nasal lavage procedure contain sufficient amount of DNA for assessing and quantifying DNA adducts.

Materials and Methods

Subjects

Nasal mucosa cells were collected from 20 healthy volunteers from our institute. All the subjects had a normal-appearing nasal mucosa; subjects with ongoing symptoms of nasal diseases or ongoing drug treatment were excluded from this study.

Cell and Nucleus Isolation

Nasal mucosal cells were isolated by the nasal lavage procedure as described previously (9). Briefly, nasal lavage was performed with 10 ml sterile isotonic saline solution (0.9%), 5 ml in each nasal cavity, using a needleless syringe while the subject gently flexed his head backward and did not breathe or swallow. The saline was held in the nasopharyngeal region by palatal pressure for 10 sec; the subject then forcibly expelled the nasal lavage fluid containing mucosal cells into a collection vessel. The lavage fluid from each subject (8 ml) was rapidly transferred into Falcon tubes to which 0.8 ml acetylcystein (200 mg/ml, a mucus digestant purchased from Kabi Pharmacia, Stockholm, Sweden) was added and incubated at room temperature for 30 min with a shaking frequency of 100 cycles/min. After this incubation, the volume of lavage fluid was adjusted to 10 ml and a 0.5 ml aliquot was taken out for the total cell counting (monitored in a hemocytometer). The remaining sample (9.5 ml), which was used for DNA isolation, was centrifuged at 2,000 × g for 10 min. The resulting cell pellets were suspended in 5 ml of a medium composed of 5% Triton X (polyethylene glycol
p-isoctylphenyl ether), 75 mM NaCl, and 10 mM Tris- HCl (pH 7.4). To obtain the cell nucleus, the cell suspensions were shaken vigorously for 10 min at room temperature and then centrifuged at 2,000 x g for 10 min. The nuclear pellet was resuspended in 200 µl buffer containing 75 mM NaCl and 10 mM Tris-HCl (pH 7.4) and stored at -20°C until isolation of DNA.

Purification of DNA and $^{32}$P-postlabeling Analysis of Adducts

DNA was isolated from nuclei using a standard procedure involving enzymatic digestion of RNA and protein by treatment with pancreatic ribonuclease and proteinase K followed by extraction with phenol and chloroform:isoamyl alcohol (24:1) as described earlier (10). Isolated DNA was quantitated spectrophotometrically by measuring the absorption at 260 nm and assuming 20 OD/mg DNA. The 260/280 ratios for DNA samples ranged from 1.5 to 1.6. DNA (1-5 µg) was digested into normal deoxynucleosides and adducts by nuclease P1-mediated procedure (11). Digested DNA was labeled with 7 µCi of γ-$^{32}$P-ATP (3,000 Ci/mm), and the adducts were analyzed by multidirectional thin-layer chromatography as described earlier (12,13).

Results

As shown in Table 1, $2 \times 10^6$ cells were obtained from each nasal lavage from which DNA was isolated. The analyses were performed on 20 healthy volunteers from 25 to 55 years of age. The levels of adducts detected in this study ranged between 1.4 and 6.0 adducts/10$^8$ nucleotides with a mean of 3 adducts/10$^8$ nucleotides. Since the purity of the isolated DNA was relatively low (260/280 ratios for DNA samples; range 1.5-1.6), it is indicated that the adduct levels are underestimated.

Adduct patterns from three individuals are shown in Figure 1. The adduct spots seem to vary among individuals, which may be related to the distribution of the cells collected, smoking habit, etc. So far, the material is too small to point out the variables that affect the quantity and pattern of adducts of the nasal cells.

Discussion

Because it is the first site of air entry, the nasal cavity is a primary target for effects from environmental contaminants (14). To protect the lower airways from airborne chemical toxicants, the cells lining the nasal passages are furnished with a very effective defense system. Thus, the human nasal mucosa contains high levels of xenobiotic-metabolizing enzymes, e.g., cytochrome P450 isozymes, epoxide hydrolase, glutathione S-transferase, and DT-diaphorase (15,16). High levels of xenobiotic-metabolizing enzymes have also been detected in other mammalian species such as rat, mouse, rabbit, hamster, monkey, etc. (17-20). These high levels of xenobiotic-metabolizing enzymes are probably necessary to rapidly metabolize and remove the inhalated xenobiotics; however, promutagens and procarcinogens could be activated by this nasal biotransformation and interact with proteins as well as DNA, forming DNA adducts.

During the last decade, extremely sensitive methods for assessing and quantifying DNA adducts have been developed (6,7). Because of its high sensitivity with certain adducts (1 adduct/10$^{10}$ nucleotides), the low amount of DNA needed for each assay (1-5 µg DNA), and its applicability to almost all hydrophobic DNA adducts, the $^{32}$P-postlabeling assay has emerged as a suitable method for specimens from humans exposed to environmental mixtures of carcinogens and mutagens (6,7).

This study was designed to investigate if the cells obtained from the human nasal mucosa contain a sufficient amount of DNA for adduct analysis. The results presented here demonstrate that, from each nasal lavage procedure, 5 to 40 µg DNA can be isolated. It is therefore suggested that nasal cells obtained by this procedure provide a sufficient amount of DNA for DNA adduct analysis on a routine basis. In addition, the nasal lavage procedure is a simple, inexpensive, and noninvasive procedure that requires no anesthetics or special equipment and provides human tissue, which is the first target of and the first line of defense against airborne chemical carcinogens. By combining the sensitivity of the $^{32}$P-postlabeling assay with the specificity of the nasal mucosa to the airborne chemical exposures, the DNA adduct analysis from human nasal mucosa cells represents an ideal technique in the assessment of exposure to airborne chemical carcinogens. Further work however is needed in order to evaluate the above technique in comparison with results obtained from other tissues from the same individuals, e.g., isolated lymphocytes.
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