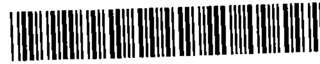
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DNA REPAIR CAPACITY IN THE RAT RESPIRATORY TRACT

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Abstract — *A product of alkylating agents and DNA, O^6-methylguanine, can mispair with thymine, resulting in initiation of a carcinogenic tissue response. O^6-alkylguanine-DNA alkyltransferase (AGT) is an acceptor protein responsible for repairing O^6-methylguanine. The purpose of our experiments was to characterize AGT activity in vitro in tissue and cell extracts of the respiratory tract, a target tissue for inhaled alkylating agents. Removal of [^{3}H]methyl from O^6-methylguanine was measured by high-pressure liquid chromatography after incubation of tissue and cell extracts with the [^{3}H]DNA. With the exception of tracheal and bronchial extracts, all tissues and cells analyzed contained AGT activity, which increased in proportion to the amount of protein added to reaction flasks. AGT activity in tracheal and bronchial extracts was only detected at the highest protein concentration used (1.5 mg protein/mL) and ranged from 10-15 fmole/mg protein. AGT activity in the respiratory tract was highest in the lung and a region of the nasal tissue (i.e., ethmoturbinates) and ranged from 45-75 fmole/mg protein. These data suggest that methylated DNA in specific regions of the rat respiratory tract should be readily repaired, albeit to different extents.*

Some of the products formed by reaction of mutagenic and carcinogenic alkylating agents with DNA are O^6-alkylguanine, 7-alkylguanine, and 3-alkyladenine. One of the products of methylation with dimethylnitrosamine is O^6-methylguanine. Goth and Rajewsky[1] hypothesized that the inability of a cell to remove O^6-alkylguanine (i.e., persistence of O^6-alkylguanine in organs) and repair the DNA damage correlates with tissue susceptibility to alkylating carcinogens. O^6-alkylguanine, which is produced to a large extent by N-nitroso carcinogens, can mispair with thymine.

The protein referred to as O^6-alkylguanine-DNA alkyltransferase (AGT) removes the alkyl group from O^6-alkylguanine and transfers it to a cysteine acceptor site on the protein. The bulk of evidence indicates that AGT is the major mechanism for removal of alkylated guanines, specifically methylated and ethylated guanines in DNA. While there have been several reports on the presence of O^6-methylguanine in tissues and cells of animals exposed to alkylating carcinogens, there are very little data available on the AGT activity in tissues and cells of the respiratory tract. It is particularly important to determine which tissues and cells of the respiratory tract have low AGT activity, since these cells may be target cells for airborne alkylating agents. The purpose of our studies was to characterize AGT activity in vitro in tissue and cell extracts of the respiratory tract. Our results indicate that AGT activity is not uniformly distributed along the respiratory tract. Higher levels of activity were seen in a region of the nasal tissue (i.e., ethmoturbinates) and in the lung than in the trachea and bronchi. AGT activity in the ethmoturbinates, lung, and alveolar type II cells is similar to the AGT activity measured in the liver.

METHODS

All rats were raised at this Institute and were killed by CO_2 asphyxiation. Immediately after death, the thoracic cavity of the rat was opened, and the trachea and lungs were removed intact. Concomitantly, the head was removed from the carcass and the nasal cavity was split from the nares to the nasopharynx, exposing the right and left lateral and septal mucosal surfaces. Anatomically defined regions throughout the respiratory tract were sampled. These included two regions of the lateral wall of the left and right nasal airway (maxilloturbinates and ethmoturbinates), the trachea, mainstem bronchi, and peripheral lung (i.e., alveolar tissue). In addition, a sample of liver was removed.

Primary type II alveolar cells were obtained from male F344/N rats (9-12 wk) sacrificed by CO_2 asphyxiation. Cells were obtained by the method of Johnson *et al.*[2] The cell preparation consisted of type II alveolar cells (85%) and alveolar macrophages and other cells (15%) as determined by electron microscopy. Tissue and cell extracts of the type II alveolar cells were obtained, as previously described.[3]

Assay of AGT activity in tissue and cell extracts was performed according to previously published methods.[4] AGT activity was determined by incubation of a reaction mixture containing: cell or tissue extracts (0-1.5 mg protein/mL), 1.3 mg calf thymus DNA, [^{3}H]DNA (containing 0.12 pmole of O^6-methylguanine; ~ 36,000 dpm), 50 mM Tris-HCl, 5 mM dithiothreitol, and 0.1 mM EDTA. The final volume of the reaction mixture was 1 mL. Control reaction flasks contained boiled cell or tissue extracts and the reaction contents described above. Reaction mixtures were incubated for 0-30 min in a heated (37°C), shaking water bath. Following incubation, reactions were stopped by addition of 2N percholoric acid to bring the reaction mix to a concentration of 0.25 M perchloric acid. The precipitate was collected by centrifugation (16,000 rpm; 30 min) and the DNA was hydrolyzed in 0.1 N HCl at 70°C for 30 min.

Hydrolyzed DNA was analyzed by high-performance liquid chromatography (HPLC) using two Whatman Partisil 10-SCX columns linked in series. The bases were eluted isocratically at room temperature with 75 mM ammonium formate (pH 2.5-3.0) containing 20% methanol. The flow rate was 1.5 mL/min. Absorbance was monitored at 254 nm (V4, Isco Instruments, Lincoln, NE). Fractions of eluate were collected at 30-sec intervals; the amount of radioactivity in each fraction was determined by liquid scintillation spectroscopy. The retention times for guanine, adenine, 7-methylguanine, and O^6-methylguanine were 8.8, 13.8, 14.8, and 19.1 min, respectively. AGT activity was calculated by subtraction of the O^6-methylguanine (O^6-MG)/7-methylguanine (7-MG) ratio in test reactions from the O^6-methylguanine/7-methylguanine ratio in control reactions.

RESULTS

Removal of O^6-methylguanine as a function of the amount of protein added to incubation flasks was investigated in respiratory tract tissues and cells. The incubation times used for these experiments were based on preliminary studies. Incubation times used for the maxilloturbinates, ethmoturbinates, trachea, bronchi, lung, type II alveolar cells, and liver were 10, 10, 30, 30, 20, 30, and 30 min, respectively. Figure 1 shows the data from these experiments (except trachea and bronchi). AGT activity increased proportionally to the amount of protein added to reaction flasks. AGT activity was detected at all protein concentrations except in the maxilloturbinates, the trachea and the bronchi. For the maxilloturbinates, no AGT activity was detected using 0.5 mg protein/mL, and AGT activity in tracheal and bronchial extracts was only detected at 1.5 mg protein/mL.

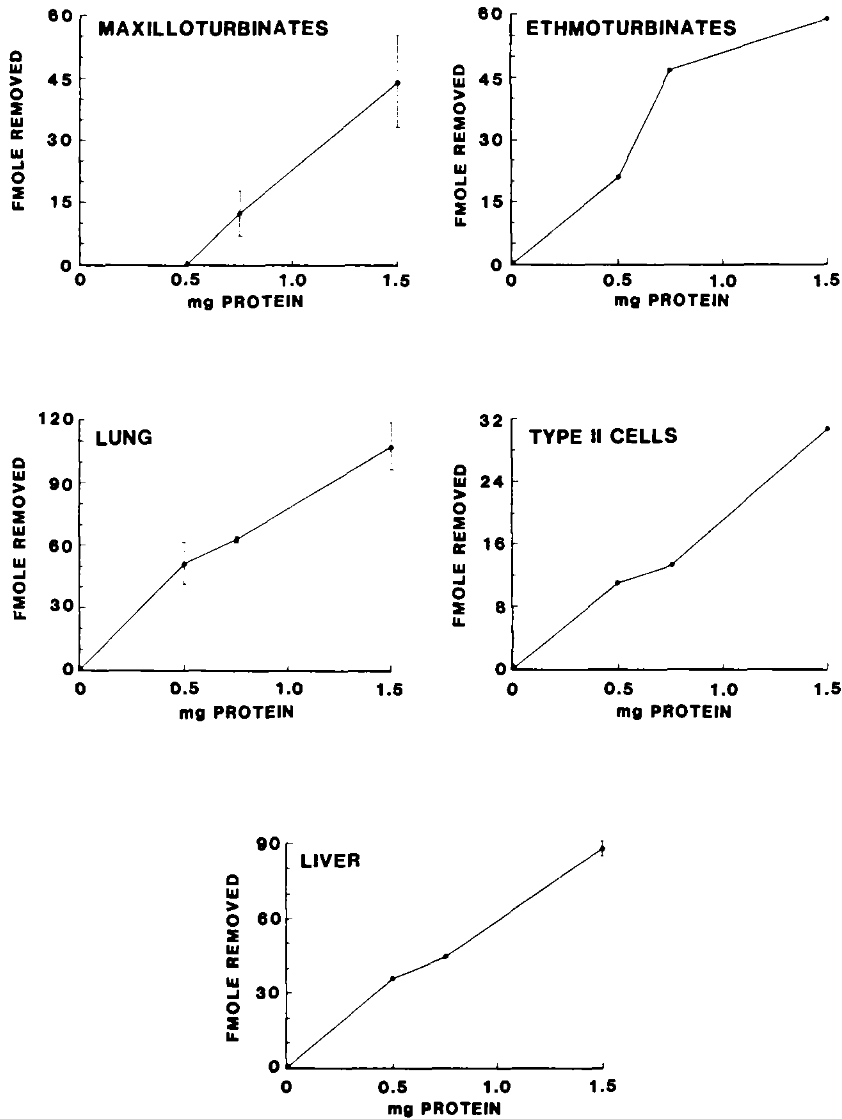

Figure 1. Removal of O^6-methylguanine as a function of the amount of protein extract added to incubation flasks. Incubation times used for the maxilloturbinates, ethmoturbinates, lung, type II alveolar cells, and liver were 10, 10, 20, 30, and 30 min, respectively. Values represent the mean ± SE for two experiments. AGT activity in trachea and bronchi was detected at only 1.5 mg protein/mL and, therefore, no protein-response curves are shown for these tissues.

Figure 2 compares the AGT activity in tissues and cells of the respiratory tract. Values shown in this graph were obtained by calculating the slope of the protein-response curves (except the trachea and bronchi) (Fig. 1) and are expressed as fmole O^6-methylguanine removed/mg protein. AGT activity in the respiratory tract was nonuniformly distributed. The highest activity was detected in the lung (~ 75 fmole/mg protein) and a region of the nasal tissue (i.e., ethmoturbinates; ~ 45 fmole/mg protein). The lowest activity was measured in tissues of the major conducting airways, the trachea and bronchi, and in alveolar type II cells and ranged from about 10-20 fmole/mg protein. AGT activity in the maxilloturbinates was about 50% less than the AGT activity measured in the ethmoturbinates. Liver AGT activity reported here (~ 60 fmole/mg protein) was similar to AGT activity in livers of F344 and Sprague Dawley male rats (70-90 fmole/mg protein) reported elsewhere.

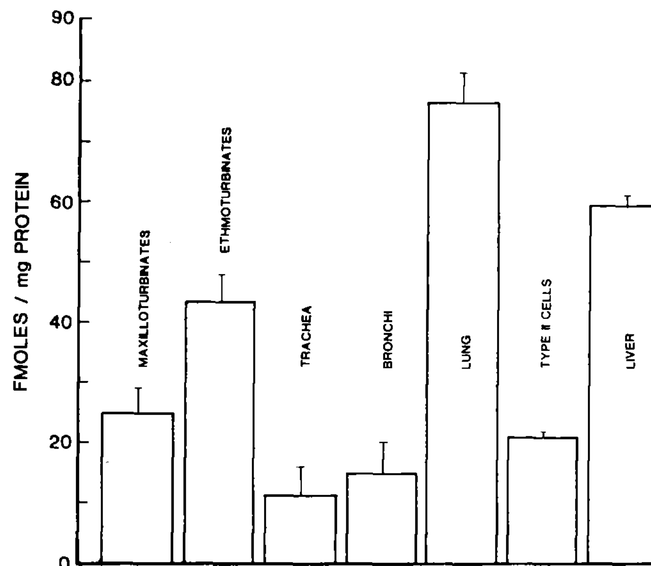

Figure 2. Comparison of AGT activity in tissues and cells of the respiratory tract and the liver. Rates of AGT activity were obtained by determining the slopes of the protein-response curves (see Fig. 3). Values represent the mean ± SE. For trachea and bronchi, AGT activity was seen at only 1.5 mg protein/mL and the data presented represent AGT activity at this protein concentration.

DISCUSSION

Analysis of the data presented in this paper on *in vitro* AGT activities in various regions of the respiratory tract leads to the conclusion that AGT activity is distributed nonuniformly throughout the respiratory tract. The highest AGT activity was detected in the lung and the lowest activity was seen in the trachea and bronchi which contained about 20% of the activity measured in the lung. It is possible that the lower AGT activities in the trachea and bronchi may be due to the higher structural connective tissue component of these tissues compared to other regions of the respiratory tract sampled. The structural connective tissue component would not be expected to contain AGT activity, but would contribute to the total protein content removed from these tissues. We were able to measure AGT activity in a specific lung cell type, the type II alveolar cell, and found activity to be similar to that measured in the maxilloturbinates. It is possible, however, that AGT activity in the isolated cells was due to cells other than the type II alveolar cells (e.g., pulmonary alveolar macrophages).

Our studies indicate that different regions of the respiratory tract can effectively repair methylated DNA with lung > ethmoturbinates > maxilloturbinates ≅ alveolar type II cells > trachea ≅ bronchi. These data confirm and extend the observations of Belinsky *et al.*[5] who reported on AGT activity *in vitro* and demonstrated that repair of O^6-methylguanine occurs in lungs and nasal tissue from rats treated with the tobacco-specific carcinogen, 4-(N-methyl-N-nitrosamin)-1-(3-pyridyl)-1-butanone (NNK). Our experiments provide additional information on AGT activity in various regions of the respiratory tract. Additional studies are necessary to characterize AGT activity in other cells of the rat respiratory tract (e.g., Clara cells, tracheal epithelial cells). While we do not yet have enough data to allow prediction of target tissue/cell specificity following exposure to alkylating agents, the data do suggest that methylated DNA in the respiratory tract should be readily repaired, albeit to differing extents.

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