Brief Communication

Metabolism of Dimethylnitrosamine and 1,2-Dimethylhydrazine in Cultured Human Bronchi

Curtis C. Harris,1 Herman Autrup, Gary D. Stoner, Elizabeth M. McDowell,2 Benjamin F. Trump,2 and Paul Schafer3

Human Tissue Studies Section, Experimental Pathology Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, 20014 [C. C. H., H. A. G., D. S. J.]; Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland 21201 [E. M. M., B. F. T.]; and Department of Surgery, Washington Veterans Administration Hospital, Washington, D. C. 20420 [P. W. S.]

SUMMARY

The metabolic activation of several chemical classes of procarcinogens is being studied in cultured human bronchi. Previous studies have shown that carcinogenic polynuclear aromatic hydrocarbons are metabolically activated by the bronchial epithelium. In the study reported here, dimethylnitrosamine and 1,2-dimethylhydrazine were also found to bind to both cellular DNA and protein. Bronchial DNA was methylated in both the O-6 and N-7 positions of guanine. In addition to polynuclear aromatic hydrocarbons, an aliphatic nitrosamine and a methylhydrazine can now be added to the list of xenobiotic chemical carcinogens metabolized by human bronchus.

INTRODUCTION

DMN4 and 1,2-DMH are both procarcinogens, they require enzymatic activation into electrophilic metabolites that bind readily to cellular macromolecules covalently (23, 24). Intracellular binding of chemicals to DNA is presumptive evidence, but not proof, that they are carcinogens in the target cells responsible for their metabolic activation. Therefore, we have chosen to measure the binding of DMN and of 1,2-DMH to DNA as an initial assay of their metabolic activation in cultured human bronchial mucosa. Previous studies (12-15) have shown that several carcinogenic polynuclear aromatic hydrocarbons bind to DNA in cultured human bronchus.

MATERIALS AND METHODS

Bronchial specimens were obtained either at surgery (Patients 109, a 70-year-old male with adenocarcinoma of the lung; 110A, a 60-year-old male with squamous cell carcinoma of the lung; and 112, a 53-year-old male with adenocarcinoma) or at "immediate autopsy" (31) (Patients 110, a 70-year-old male with adenocarcinoma of the lung; and 1110A, a 60-year-old male with squamous cell carcinoma) or at "immediate autopsy" (31) (Patients 110, a 70-year-old male with adenocarcinoma of the lung; and 112, a 53-year-old male with adenocarcinoma) or at "immediate autopsy" (31) (Patients 110, a 70-year-old male with adenocarcinoma of the lung; and 112, a 53-year-old male with adenocarcinoma). The specimens were immediately immersed in L-15 medium (Grant Island Biological Co., Grand Island, N. Y.) at 4° until cultured as 1- x 1-cm explants by methods previously described (2, 14, 30). Six to 8 explants per experimental variable were cultured for 7 days in a chemically defined medium [CMRL 1066 (Grand Island Biological Co.) supplemented with (per ml): 0.1 µg hydrocortisone (The Upjohn Co., Kalamazoo, Mich.), 0.1 µg β-retinyl acetate (Hoffman-LaRoche, Nutley, N. J.), 1.0 µg bovine insulin (Eli Lilly, Indianapolis, Ind.), 100 units Penicillin G, 100 g streptomycin, and 0.25 µg amphotericin B] and in an atmosphere of 50% O2, 45% N2, and 5% CO2. The explants were then cultured in L-15 medium in an atmosphere of 50% O2 and 50% N2 and were exposed for 24 hr to either [14C]DMN [27.6 or 276 µM; 35 mCi/m mole; prepared by National Cancer Institute Contract N01-CP-55677 and purified in one of our laboratories by C. C. Harris by the method of den Engelse et al. (4)] or 1,2-[14C]DMH (1.29 mm; 3.78 mCi/m mole; New England Nuclear, Boston, Mass.). [14C]DMN and 1,2-[14C]DMH were each dissolved directly in L-15 medium. The radiopurity of [14C]DMN and 1,2-[14C]DMH was greater than 98% by radioscan of thin-layer chromatograms. After the bronchial mucosa was scraped from the supporting stroma, mucosa from 5 explants were pooled, and DNA was then isolated by phenol extraction and by CsCl gradients as previously described (15).

After hydrolysis of DNA with 0.1 M HCl at 37° for 20 hr, bases were isolated by liquid-column chromatography (Sephadex G-10; Pharmacia Fine Chemicals, Piscataway, N. J.; 50 mM phosphate buffer, pH 7.0) (1). Markers for N7, O6, methylnitrosamine, and N2-methyladenine (0.3 mg each) were added to the hydrolyzed DNA; the elution was monitored at 254 nm, and 4-ml fractions were collected. The fractions associated with each peak of absorbance were pooled and evaporated to a final volume of 4 ml, and the radioactivity was measured by liquid scintillation methods using an internal standard for calculating counting efficiency.

Protein was precipitated by adding 3 volumes of ice-cold ethanol to the phenol fraction which was kept at 4° for 48 hr. The precipitate was washed twice with acetone and then repeatedly extracted with ether until the level of radioactivity in the supernatant was negligible. The proteins were then redissolved in 0.1 N NaOH, and the concentration was determined by the method of Hartree (16). One explant from

---

1 To whom requests for reprints should be addressed, at Building 37, Room 3A07, National Cancer Institute, NIH, Bethesda, Md. 20014.
2 Supported in part by Contract NO1 CP 43237.
3 Supported in part by Interagency Agreement Y01 CP 60204.
4 The abbreviations used are: DMN, dimethylnitrosamine; 1,2-DMH, 1,2-dimethylhydrazine.
5 Received November 23, 1976; accepted April 29, 1977.
each variable was fixed in 3% glutaraldehyde for monitoring cell viability by high-resolution light microscopy (14, 30). At the doses of 1,2-DMH and DMN selected, no cytotoxicity was observed.

RESULTS AND DISCUSSION

We are developing model systems for studying carcinogenesis in human tissues (12). One objective of these studies is to determine whether the metabolic pathway(s) of different chemical classes of precarcinogens is the same in the human target epithelium as that known to occur in the target cells of the animal models. If similar pathways are found, then an extrapolation of carcinogenesis data obtained in the experimental animal is more likely to be valid than if different pathways of metabolic activation are observed. Studies of the metabolic activation of precarcinogens in human epithelium are also important because, unlike most experimental investigations using inbred animals, the human population is genetically heterogeneous. Genetic factors are largely responsible for the marked variation among people in the metabolism of drugs (32), which raises the possibility that differences in the ratio of carcinogen activation to detoxification may be responsible for the varying individual susceptibility to environmental chemical carcinogens. We have recently found a 75-fold interindividual variation in binding levels of benzo(a)pyrene to DNA in cultured human bronchi (13).

DMN contaminates the environment from several sources. It has been detected in tobacco smoke (22), smoked fish (7), and the atmosphere of certain cities (10), and is formed in vivo from ingested nitrites and secondary amines under the acidic conditions found in the stomach (27). Although DMN is carcinogenic in a large number of different species, including the rat, mouse, hamster, rabbit, mink, trout, and guinea pig (see review, Ref. 19), carcinogenicity data in man are as yet unavailable. However, DMN is hepatotoxic in humans (11), and both human liver and lung have been shown to metabolize DMN in vitro. Montesano and Magee (25) reported that slices of human liver can metabolize [14C]DMN into both [14C]CO2 and intermediates that alkylate bases in nucleic acids forming, primarily, 7-methylguanine. Slices of human lung metabolize [14C]DMN, as measured by production of [14C]CO2, at a rate significantly higher than that for rat lung (3). We have found that [14C]DMN binds to both DNA and protein of cultured human bronchi (Table 1). Binding to DNA was dependent on dose of DMN in a nonlinear manner. The relative binding of DMN to DNA and protein varied greatly among the 3 patients studied. These findings suggest that the bronchial epithelium has the capacity to metabolize [14C]DMN, a precarcinogen, to reactive intermediates.

The organ specificity of N-nitrosamines varies among species. For example, DMN causes primarily hepatic carcinomas in Syrian Golden hamsters (29) and in rats (20), but lung tumors are also found in mice (28). The route of administration of DMN can also alter the site of tumor induction. When DMN is given by inhalation instead of p.o., carcinomas of the nasal cavity instead of the liver are found in rats (6). The organ specificity of N-nitrosamines in humans has yet to be demonstrated.

<table>
<thead>
<tr>
<th>Patient</th>
<th>DMN (µM)</th>
<th>DNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>27.6</td>
<td>308</td>
<td>990</td>
</tr>
<tr>
<td>110A</td>
<td>27.6</td>
<td>700</td>
<td>3,334</td>
</tr>
<tr>
<td>112</td>
<td>27.6</td>
<td>10,380</td>
<td>2,792</td>
</tr>
<tr>
<td></td>
<td>276.0</td>
<td>668</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>276.0</td>
<td>786</td>
<td>320</td>
</tr>
</tbody>
</table>

* pmoles of DMN bound per 10 mg of either DNA or protein.

Table 1

Binding of DMN to DNA and to protein in cultured human bronchus

Bronchial explants were cultured for 7 days in a chemically defined medium before addition of [14C]DMN for 24 hr.

In contrast to DMN, 1,2-DMH is not a common environmental contaminant. At the present time, it is not synthesized commercially in large quantities, although 1,2-DMH has been tested experimentally as a rocket fuel. 1,2-DMH has been studied extensively in experimental animals; it causes colonic carcinomas in rodents (5, 33), and this organotropism has been successfully utilized in the development of animal models of human colonic carcinoma. Recent biochemical studies (8, 9) have identified both azothane and methylazoxymethanol as metabolites of 1,2-DMH. Although the metabolism of 1,2-DMH in human tissues has not been previously investigated, the bronchial mucosa can activate 1,2-DMH into forms that bind to cellular macromolecules (Table 2). Binding to protein exceeds that to DNA in the 4 cases studied. In this small number of cases, the interindividual variation is approximately 10-fold for binding to DNA and 3-fold for binding to protein. The intra-individual variation in binding levels of carcinogens to DNA due to experimental methodology is small (mean coefficient of variation, 0.1) (Ref. 14; H. Atrup and C. Harris, unpublished results). No correlation was evident between the binding levels to both protein and DNA among the individuals tested. Cultured human colon can also activate 1,2-DMH and DMN into metabolites that bind to DNA, but the binding levels are lower than those in the human bronchus (1).

Both 1,2-[14C]DMN and [14C]DMN methylate DNA at both the O-6 and N-7 positions of guanine as measured by liquid-column chromatography (Table 3). The ratio of O6-methylguanine to N7-methylguanine found after a 24-hr exposure in vitro to either DMN or 1,2-DMH was high, i.e., greater than 1, when compared to most reported observations in experimental animals (17, 26). Recent investigations (21) have reported evidence for excision of N7-methylguanine and for persistence of O6-methylguanine in DNA of hamster tissues with increasing time after in vivo exposure of DMN. The kinetics of both alkylation of guanine and excision of alkylguanine in human bronchial DNA have not as yet been investigated. However, our preliminary finding of O6 methylation of DNA in cultured human bronchi is intriguing, because a positive association has been found in experimental animals between the capability of agents to form O6-methylguanine in DNA of target tissues and their carcinogenic potency in those tissues (17). Loveless (18) has noted that O6 methylation of guanine in DNA yields a miscoding base during replication of DNA; this conclusion is consistent with the hypothesis that somatic mutation may be an important event in chemical carcinogenesis.
Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>DNA (dpm)</th>
<th>Protein (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>109</td>
<td>7,328</td>
<td>20,674</td>
</tr>
<tr>
<td>110</td>
<td>1,501</td>
<td>18,503</td>
</tr>
<tr>
<td>110A</td>
<td>14,598</td>
<td>20,798</td>
</tr>
<tr>
<td>112</td>
<td>3,619</td>
<td>6,006</td>
</tr>
</tbody>
</table>

Binding of 1,2-DMH to DNA and to protein in cultured human bronchus

Bronchial explants were cultured in a chemically defined medium for 7 days before 1,2-[14C]DMH was added for 24 hr.

*pmoles of 1,2-DMH per 10 mg of either DNA or protein.

Table 3

<table>
<thead>
<tr>
<th>Base</th>
<th>1,2-DMH (Experiment 1)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N°-methylguanine</td>
<td>1027* (51)*</td>
<td>192 (21)</td>
<td>570 (25)</td>
</tr>
<tr>
<td>N°-methyladenine</td>
<td>314 (16)</td>
<td>153 (17)</td>
<td>348 (15)</td>
</tr>
<tr>
<td>N°-methyladenine</td>
<td>313 (14)</td>
<td>108 (12)</td>
<td>313 (14)</td>
</tr>
<tr>
<td>Guanine + adenine</td>
<td>467 (23)</td>
<td>128 (14)</td>
<td>561 (25)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>205 (10)</td>
<td>337 (36)</td>
<td>494 (22)</td>
</tr>
</tbody>
</table>

DMN

Binding of 1,2-DMH to DNA and to protein in cultured human bronchus

Bronchial explants were cultured in a chemically defined medium for 7 days before 1,2-[14C]DMH was added for 24 hr.

<table>
<thead>
<tr>
<th>Base</th>
<th>1,2-DMH (Experiment 1)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>O°-methylguanine</td>
<td>1027* (51)*</td>
<td>192 (21)</td>
<td>570 (25)</td>
</tr>
<tr>
<td>N°-methylguanine</td>
<td>314 (16)</td>
<td>153 (17)</td>
<td>348 (15)</td>
</tr>
<tr>
<td>N°-methyladenine</td>
<td>313 (14)</td>
<td>108 (12)</td>
<td>313 (14)</td>
</tr>
<tr>
<td>Guanine + adenine</td>
<td>467 (23)</td>
<td>128 (14)</td>
<td>561 (25)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>205 (10)</td>
<td>337 (36)</td>
<td>494 (22)</td>
</tr>
</tbody>
</table>


REFERENCES

7. Fiola, E. S., Kulakos, C., Bobotas, G., and Weisburger, J. H. Detection of N°-methylguanine/V7-methylguanineW3-methyladenineGuanine Binding of 1,2-DMH to DNA and to protein in cultured human bronchus

Bronchial explants were cultured in a chemically defined medium for 7 days before 1,2-[14C]DMH was added for 24 hr.

*pmoles of 1,2-DMH per 10 mg of either DNA or protein.


dpm.

Numbers in parentheses, percentage of the total number of dpm added to the column.

ND, none detectable.

On April 20, 2017. © 1977 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from
Metabolism of DimethylNitrosamine and 1,2-Dimethylhydrazine in Cultured Human Bronchi

Curtis C. Harris, Herman Autrup, Gary D. Stoner, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/37/7_Part_1/2309

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.