Molecular Epidemiology Linking Exposure and Disease: A Case Study on Polycyclic Aromatic Hydrocarbons (PAHs)

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Environmental Sources of Polycyclic Aromatic Hydrocarbons (PAHs)

- Incomplete burning of wood, coal, oil and gas, or other organic substances like tobacco or charbroiled meat (Chuang, J. C., et al. 1991, Lewtas, J. 1994)

- In urban ambient air from traffic, particularly diesel trucks and buses, heating fuels (IARC, 1983)

- Among the 189 hazardous air pollutants covered under the Clean Air Act
Fossil Fuel-Related Outdoor Air Pollution and PAHs
Sources of Indoor Air PAH Pollution

Active/Passive Smoking

Indoor Cooking
## Milestones in the research on toxicology of PAHs

<table>
<thead>
<tr>
<th>Year</th>
<th>Milestone</th>
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<tbody>
<tr>
<td>1775</td>
<td>Observation of scrotal cancer in chimney sweeps from occupational exposure to soot (Pott, 1775)</td>
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<tr>
<td>1929-</td>
<td>Dibenz[a,h]anthracene (synthetic) and benzo[a]pyrene (isolated from coal-tar pitch) shown to be carcinogenic in treated animals (Cook et al., 1932; Kennaway, 1955)</td>
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<tr>
<td>1930</td>
<td></td>
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<tr>
<td>1950</td>
<td>The first proposed metabolic activation of PAHs by epoxidation (Boyland, 1950)</td>
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<tr>
<td>1974</td>
<td>Identification of the vicinal bay-region diol epoxide, a powerful genotoxic metabolite from benzo[a]pyrene (Sims et al., 1974)</td>
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<tr>
<td>1982</td>
<td>Detection of PAH-DNA adducts in humans (Perera et al. 1982)</td>
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<tr>
<td>The last decades</td>
<td>Reproductive and developmental toxicity (Mattison &amp; Thorgeirsson, 1979); epigenetic effects (Wilson and Jones, 1983, Wojciechowski and Meehan, 1984)</td>
</tr>
</tbody>
</table>
Accurate Repair

Cancer. Other?
Epigenotoxicity

Benzo[a]pyrene

Hypermethylation → Metabolites → Hypomethylation

Transcriptional inactivation, and loss of protein expression

Genomic instability, exemplified by misalignments, DNA breakage, deletions and duplications during DNA replication

Mutational gene inactivation

Deamination of methylated cytosine in CpG dinucleotides

Inactivation of tumor suppressor genes such as p53 and LDL receptor
Exposure

PAHs in tobacco smoke and air pollution

Internal Dose

PAH metabolites in urine

Biologically effective dose

PAH-DNA or -protein adducts

Preclinical effect

P53 mutation, chromosomal aberrations, altered gene/protein expression

Clinical Disease

Markers of Susceptibility

Genetic (CYP, GST, XRCC1 etc.);
Nutritional (antioxidant levels);
Immunologic factors

Molecular epidemiology has provided evidence of causality for specific environmental PAH exposures

- Tobacco smoke/PAHs and lung cancer
- Ambient air pollution and lung cancer
Benzo[a]pyrene-DNA Adducts in Lung Cancer

A pilot project in molecular cancer epidemiology: determination of benzo[a]pyrene–DNA adducts in animal and human tissues by immunoassays

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1 Division of Environmental Sciences, School of Public Health, Columbia University College of Physicians and Surgeons, New York, NY 10032; 2 Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bethesda, MD 20005; and 3 Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York, NY 10032.

(Received on 6 July 1982; accepted on 6 October 1982)

A highly sensitive immunoassay utilizing antisera specific for benzo[a]pyrene covalently bound to DNA has been employed to probe for adducts in the DNA of animal and human tissues and peripheral blood mononuclear cells. By enzyme-linked immunoassay (ELISA), a dose-related increase in levels of benzo[a]pyrene–DNA adducts was observed in DNA from lung tissue of mice and rabbits injected i.p. with benzo[a]pyrene. Quantitation with ELISA was confirmed by p.i. injection of [3H]benzo[a]pyrene and determination of adduct levels in DNA by radiolabeling. Thus, the ELISA assay was determined to be quantitative for benzo[a]pyrene–DNA adducts in vivo, and the lower limits of detectability established at 0.08–0.10 fmol/μg DNA. At this level of sensitivity no significant differences were observed between DNA from peripheral blood mononuclear cells of dogs on smoke inhalation machines and controls. In an attempt to probe for benzo[a]pyrene–DNA adducts in subjects resulting from chronic environmental exposure, lung tissue, lung tumor and blood samples were obtained from patients hospitalized for lung cancer and other disease conditions. A detailed history of exposure to environmental sources of benzo[a]pyrene and to factors known to influence polycyclic aromatic hydrocarbon metabolism was attempted for 15 patients. DNA was extracted from the lung tissue of 12 patients and blood cells of several individuals and assayed by ELISA; 5 patients appeared to have low but measurable levels of benzo[a]pyrene–DNA adducts as determined by ELISA. All of these patients were in the lung cancer group. However, the number of subjects was too small to draw conclusions relating exposure history to occurrence of hydrocarbon–DNA adducts. These preliminary results should encourage future studies on the utilization of immunoassays for carcinogen–DNA adducts as a potential method in epidemiological studies attempting to relate biologically-effective dose of carcinogen to human cancer risk.

Introductions

In the epidemiology of human carcinogenesis serious difficulties are apparent in estimating dose from exposure data and predicting the metabolic fate of a chemical carcinogen in exposed subjects. New methods of quantifying the biologically effective dose of a carcinogen are required. The amount of activated carcinogen directly interacting with critical cellular targets can be defined as the biologically effective dose and is presumed to be directly involved in the carcinogenic process (1,2). Quantitation of carcinogen–DNA adducts by immunoassays (3) may provide a useful indication of biologically-effective dose since there is evidence that covalent binding to DNA is a critical early event in the process of tumorigenesis (4). In general the carcinogenic potency of a number of polycyclic aromatic hydrocarbons (PAH) correlates with their ability to form specific covalent adducts with DNA (4–7).

The technology recently developed in our laboratory permits implementation of a pilot project to probe for benzo[a]pyrene–DNA (BP–DNA) adducts in lung tissue and peripheral blood cells obtained from patients. In addition these patients provided a detailed history of exposure to sources of PAH. BP is a significant and pervasive airborne pollutant arising from widespread sources, including incomplete combustion of fossil fuels and cigarette smoking, and is a ubiquitous contaminant in food and water (8–10). An increasing environmental concern is the metabolism of BP to activated intermediates and form the DNA adducts in question (9–18). Since this particular assay could be viewed as a prototype indicator of biologically effective dose for DNA-binding carcinogens, we designed a pilot project to apply the assay to human samples, after first determining the lower limits of detectability in lung tissue and blood from animals exposed to BP. It was our intention to probe for BP–DNA adducts in human lung tissue and peripheral blood mononuclear cells to examine the possibility that lung cancer patients might have measurably higher levels of BP adducts in these tissues. Finally, an attempt was made to develop a comprehensive history of past exposure to PAH and/or other factors which might modify binding and to correlate this data with that obtained from the immunoassays.
## Elevated PAH/Aromatic DNA Adducts ($^{32}$P) in WBC as Predictors of Lung Cancer (PHS)*

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current</td>
<td>2.98 (1.05-8.42)</td>
<td>0.04</td>
</tr>
<tr>
<td>Former/Never smokers</td>
<td>1.00 (0.46-2.18)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*89 cases and 173 controls high/low adducts stratifying on the median

[Tang, Phillips, Stampfer...Perera, Cancer Res 2001 61:6708]
Correlation Between Adducts in WBC (surrogate) and Lung (target) Tissue of Cases

<table>
<thead>
<tr>
<th>Method</th>
<th>Tissue</th>
<th>r, p, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>WBC: Ltumor</td>
<td>0.34, p=0.05</td>
</tr>
<tr>
<td>(n=34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32 P</td>
<td>MNC: L Nontumor</td>
<td>0.77, p=0.001 (n=31)</td>
</tr>
</tbody>
</table>

1 Tang, Santella, Blackwood...Perera CEPB 1995 4:341
2 Wiencke, Thurston, Kelsey... Christiani JNCI 1999 91: 614
Lung Cancer and Long-Term Exposure to Fine Particulate Air Pollution

Table 2. Adjusted Mortality Relative Risk (RR) Associated With a 10-µg/m³ Change in Fine Particles Measuring Less Than 2.5 µm in Diameter

<table>
<thead>
<tr>
<th>Cause of Mortality</th>
<th>1979-1983</th>
<th>1999-2000</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause</td>
<td>1.04(1.01-1.08)</td>
<td>1.06(1.02-1.10)</td>
<td>1.06(1.02-1.11)</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td>1.06(1.02-1.10)</td>
<td>1.08(1.02-1.14)</td>
<td>1.09(1.03-1.16)</td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>1.08(1.01-1.16)</td>
<td>1.13(1.04-1.22)</td>
<td>1.14(1.04-1.23)</td>
</tr>
<tr>
<td>All other cause</td>
<td>1.01(0.97-1.05)</td>
<td>1.01(0.97-1.06)</td>
<td>1.01(0.95-1.06)</td>
</tr>
</tbody>
</table>

*...controlling for age, sex, race, smoking, education, marital status, body mass, alcohol consumption, occupational exposure, and diet

[Pope et al. JAMA 287:1132. 2002]
Molecular epidemiology has identified groups susceptible to or “at greater risk” from PAHs

- Fetus and child
- Individuals with genetic polymorphisms
Parallel/International Studies of *In Utero* PAH Exposures and Childhood Disease

- **Poland**
  - Growth & Development
  - Asthma
  - Cancer Risk
  - Cohort N=550M, 550C

- **New York City**
  - Growth & Development
  - Asthma
  - Cancer Risk
  - Cohort 730M, 730N
  - Community-Based Intervention

- **China**
  - Growth & Development
  - Asthma
  - Cancer Risk
  - Serial Birth Samples 300M, 300N
  - Energy Policy

**WTC Study**
320 mothers, 320 newborns
International Cohort Studies
The Need for Prevention

- 9,500 U.S. children new cases each year; incidence rate: 14.5/100,000 Leukemia incidence in children <15 years old has increased in the past 20 years U.S.

- 12.5% of U.S. children have been diagnosed with asthma; >20% in Harlem, NYC

- 17% of U.S. children have one or more developmental disability

- In other countries, patterns are similar
Mechanisms in Fetal/Child Susceptibility

- Differential exposure
- Greater absorption and retention of toxics
- Decreased efficiency in detoxification/repair
- Higher rate of cell proliferation
- Time for cancer to develop
Mechanisms By Which *In Utero* Exposure to PAHs Can Affect Disease Risk

- Induction of metabolic enzymes
- Binding to placental growth factor receptors
- DNA damage activating apoptosis, mutation
- Effects on immune system
- Estrogenic/hormonal effects
- Alteration of gene methylation and gene and protein expression
- Interactions with genetic, nutritional factors