GENOTOXICITY OF ENVIRONMENTAL AGENTS IN HUMAN MAMMARY EPITHELIAL CELLS: A TRIGGER FOR HUMAN BREAST CANCER

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INTRODUCTION

Breast cancer is the most common cancer in American women, resulting in a mortality second only to the mortality caused by cigarette-induced lung cancer (American Cancer Society, 1990). Further, the incidence of breast cancer is increasing at a steady rate (American Cancer Society, 1990). Despite its prevalence, the etiology of human breast cancer is poorly understood. While many environmental variables such as diet may modulate the promotion and/or progression of breast cancer, the only environmental agent that has been proven to induce breast cancer in women is ionizing radiation (Howe, 1984, Tokunaga, et al, 1984). While no clear epidemiological link between environmental chemicals and human breast cancer has been established, it has been suggested that passive smoking may increase that risk of breast cancer (Horton, 1988). The ability of environmental xenobiotics such as polycyclic aromatic hydrocarbons (PAH) to induce mammary cancer in rodents is, however, well documented (Dao, 1969, Huggins, 1979). Furthermore, ubiquitous environmental pollutants such as PAH can be stored in human breast fat (Obana, et al, 1981), suggesting that human breast tissue might be exposed to potential carcinogens. Thus, it is important to determine if the human breast can metabolically activate carcinogens and if it may be a target for neoplastic transformation by environmental agents. The question of the relevance of rodent studies to human carcinogenesis is of major concern. One approach to this question is to develop in vitro models for key biological activities of environmental chemicals using human cells.

(12) was modified for human mammary epithelial cells (HMEC) (Eldridge, et al., 1992). Normal HMEC were derived from residual surgical material from reduction mammoplasties of five healthy women 19 to 22 years of age (Cases B402, B499, B708, 3014, and 3085) following procedures for the isolation and cultivation of HMEC (20, 21).

Since species and tissue specificities are observed in chemical carcinogenesis (Langenbach, 1983), it is important to establish predictive tests in target cells. The measurement of chemically induced DNA repair as UDS in normal HMEC provides a qualitative indication of potential risk rather than quantitative risk assessment.

ROLE OF ENVIRONMENTAL FACTORS IN TRIGGERING BREAST CANCER

In view of the increasing incidence of human breast cancer and the possible role of environmental factors, it is important to identify environmental agents that are genotoxic in HMEC. Since carcinogens often exhibit species- and tissue-specific differences in factors such as biotransformation, the most relevant mechanistic information that is useful for risk assessment may be derived from test systems that utilize the actual human tissue at risk. Toward this end, we developed an assay for measuring DNA repair in secondary cultures of HMEC. This assay provides a qualitative indication of potential genotoxic activity in these cells.

A wide variety of agents were examined to determine their ability to induce UDS in HMEC (Table 1). The results reflect the metabolic activation and excision-repair capabilities of the HMEC. Positive responses were observed with chemicals requiring metabolic activation: benzo(a)pyrene (BP), aflatoxin B₁ (AFB₁), 1,6-dinitropyrene (1,6-DNP) and 2-acetylaminofluorene (2-AAF). BP is a ubiquitous environmental agent of a chemical class known to induce mammary tumors in rat (Dao, 1969, Huggins, 1979). AFB₁ is a naturally occurring fungal metabolite that has been detected in human breast milk (Lamplugh, et al, 1988). 1,6-DNP is a mutagenic environmental pollutant found in diesel exhaust, effluents from coal-fired power plants, and cigarette smoke (Mermelstein, et al, 1981, Rosenkranz, 1982). It is a polycyclic aromatic nitro compound and potent inducer of DNA repair in rat and human hepatocytes (Butterworth, et al, 1983). 2-AAF, a mouse liver and bladder carcinogen, induced a positive response in the HMEC UDS assay. Thus, the positive response in HMEC for all of these agents may suggest a potential health hazard from exposure to these compounds.

Tobacco smoke condensate (TSC) produced a weak but positive response in secondary cultures of HMEC (Table 1). TSC contains numerous components and is genotoxic in a variety of test systems (DeMarini, 1983). Nicotine and its metabolite cotinine are detected in breast milk of mothers who smoke (Luck, et al, 1985, Woodward, et al, 1986). Epidemiology data do not support a causal relationship between smoking and breast cancer (Baron, 1984). However, these studies have considered only smokers and non-smokers. When age at start of smoking was examined, a 30% increase in relative risk was found for women who started smoking prior to age 17 years (Brinton, et al, 1986). These results are consistent with radiation-induced breast cancer, in which the greatest risk is among women under 20 years of
age at the time of exposure (Committee on the Biological Effect of Ionizing Radiation, 1990). Furthermore, exposure to passive smoke is suggested to be major risk factor for breast cancer (Horton, 1988). Taken together, these data suggest an additional potential health risk to women from cigarette smoke, particularly when exposure to tobacco smoke occurs during childhood.

Comparisons of the DNA repair response in HMEC to two PAH revealed BP to be a much stronger inducer of UDS than an equimolar concentration of DMBA (Table 1). These data correlate with in vitro mutagenicity and DNA binding levels in HMEC (Gould, et al, 1986, Moore, et al, 1986, Moore, et al, 1987). When in vitro mutagenicity and DNA binding levels of BP and DMBA were compared in rat mammary epithelial cells, a pattern opposite to that of HMEC was obtained which correlates with the carcinogenic activity of DMBA in the rat mammary gland (Gould, et al, 1986, Moore, et al, 1986, Moore, et al, 1987). DMBA is a more effective mammary carcinogen than BP in rats (Meselson, 1977). When sister chromatid exchange induced by BP and DMBA was compared in human and rat mammary epithelial cells, results confirmed the notion that BP has a greater genotoxic effect than DMBA in HMEC (Mane, et al, 1990). The existence of DNA adducts in HMEC in situ has been demonstrated; human and rat mammary epithelial cells form different DNA adducts when exposed to BP in vitro (Seidman, et al, 1988). The differential UDS response observed with BP and DMBA in HMEC, together with the previous studies mentioned above, suggest that caution be applied when extrapolating rodent data to humans in the absence of mechanistic information and that the potential involvement of BP in human breast cancer should be considered.

CONCLUSIONS

Despite an increasing incidence of human breast cancer, its etiology remains unknown. Since some environmental chemicals are stored in human breast fat and are rodent mammary carcinogens, determining the carcinogenic potential of environmental agent in this key target tissue is important. Genotoxicity assays are used to identify potential carcinogens based on their ability to alter the DNA. One widely used genotoxicity assay is the measurement of DNA repair as a means to assess the ability of a test agent to reach and alter the DNA. Since species and tissue specifications are observed in chemical carcinogenesis, it is important to establish predictive tests in target cells. Therefore, the UDS assay was developed to measure genotoxic activity of environmental agents in normal HMEC. A wide variety of agents were examined to determine their ability to induce UDS in early passage cultures of normal HMEC derived from reduction mammaplasties. Of the chemicals demonstrated to be genotoxic in HMEC, some are known to cause cancer in rodents or to be contaminants in human breast tissue. The observation of genotoxic activity in the HMEC UDS assay suggests that mammary cells might be target for carcinogenic activity of some environmental agents. However, other factors such as accuracy of DNA repair processes, pharmacokinetics and epidemiology must be considered to establish a carcinogenic effect of chemicals on HMEC. These studies support the role of environmental agents in human breast cancer and aid in the validation of proposed animal models by determining whether chemicals are DNA reactive or metabolized to DNA reactive species in this critical target tissue.
**Table 1: DNA Repair Responses in Human Mammary Epithelial Cells**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration</th>
<th>Case B402</th>
<th>Case B499</th>
<th>Case B708</th>
<th>Case 3014</th>
<th>Case 3085</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NG 4</td>
<td>% in repair</td>
<td>NG 2</td>
<td>% in repair</td>
<td>NG 4</td>
</tr>
<tr>
<td>Control</td>
<td>-0.4</td>
<td>4</td>
<td>0.1</td>
<td>2</td>
<td>0.1</td>
<td>4</td>
</tr>
<tr>
<td>BP</td>
<td>0.001 mM</td>
<td>2</td>
<td>-0.3</td>
<td>0</td>
<td>26c</td>
<td>86d</td>
</tr>
<tr>
<td></td>
<td>0.01 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMBA</td>
<td>0.001 mM</td>
<td>-0.2</td>
<td>12d</td>
<td>0.6</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0.001 mM</td>
<td>1.9</td>
<td>25d</td>
<td>0.5</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>AFB₁</td>
<td>0.001 mM</td>
<td>-0.1</td>
<td>8</td>
<td>1.2</td>
<td>10d</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>0.01 mM</td>
<td>23c</td>
<td>83d</td>
<td>22c</td>
<td>87d</td>
<td>22c</td>
</tr>
<tr>
<td>TSC</td>
<td>0.25%</td>
<td>0.9</td>
<td>3</td>
<td>1.0</td>
<td>15d</td>
<td>1.5</td>
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<tr>
<td></td>
<td>2.5%</td>
<td>1.6d</td>
<td>19d</td>
<td>3.9d</td>
<td>33d</td>
<td>2.3</td>
</tr>
<tr>
<td>UV</td>
<td>100 J/m²</td>
<td>92c</td>
<td>100d</td>
<td>96c</td>
<td>100d</td>
<td>102c</td>
</tr>
</tbody>
</table>

*a*NG for at least 30 cells counted for each 2 slides.

*b*An individual cell with ≥6 NG is considered in repair.

*c*Significantly greater than control at P<0.01; Student's *t* test.

*d*Significantly greater than control at P<0.05; *χ²* test.

*e*Significantly greater than control at P<0.05; Student's *t* test.
REFERENCES


