Species Differences in the Formation of Benzo(a)pyrene-DNA Adducts in Rodent and Human Endometrium

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ABSTRACT

The formation of adducts of benzo(a)pyrene metabolites on DNA was investigated in endometrial tissue from humans, hamsters, mice, and rats. Anti-Benzo(a)pyrene-7,8-diol-9,10-epoxide was the predominant adduct identified in all the species studied. The amount of (+)-anti-benzo(a)pyrene-7,8-diol-9,10-epoxide bound to DNA from human endometrium was approximately three times higher than to DNA from hamster tissue. Among the three animal species examined, the level of this adduct was highest in hamsters and lowest in rats. The high pressure liquid chromatography profiles of adducts formed in endometrium from humans and hamsters were similar, but the specific activity (pmol/mg DNA) of each adduct formed was different. Syn-7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene adduct was present in humans, hamsters, and rats but was not detected in mouse endometrium. There was an unidentified adduct present only in rat tissue. Rats had the lowest level of total DNA-bound radioactivity and the largest percentage of this total eluted as an uncharacterized radioactive peak that eluted with water (53%). These results demonstrate that endometrial tissues from humans and three rodent species differ with regard to the quantities and proportions of benzo(a)pyrene-DNA adducts formed from benzo(a)pyrene.

INTRODUCTION

Several studies indicate that qualitative and quantitative analysis of the adducts formed in the reaction between chemical carcinogen and DNA are of major importance for the understanding of initiation of neoplasia. A positive correlation between the carcinogenicity of a series of PAH1 of widely differing carcinogenic potencies and their extent of reaction with DNA has been observed (1–3). The metabolism and DNA binding of PAH benzo(a)pyrene have been studied extensively in many in vitro and in vivo models. Its metabolism has been shown to be stereoselective producing four enantiomers of BPDE that differ in tumorigenic activity; only BPDE I is mutagenic in mammalian cells and highly carcinogenic in mice (4–6).

Recent studies have shown measurable levels of BP binding to DNA in several human tissues, including esophagus (7), bronchus (8, 9), lung (10), endometrium (11), and colon (12). Although this information has been gained from these in vitro studies in human tissues, long-term in vivo carcinogenicity testing is done with experimental animals. In order to extrapolate the results of carcinogenicity testing in laboratory animals to estimates of cancer risks in humans, it is necessary to know how chemical carcinogens are acted upon within a variety of tissues in humans and experimental animals. The present study was designed to compare the quantities and types of carcinogen-
concentrator/dialyzer with a YM 10 membrane. DNA purified in this manner is particularly pure with no more than 0.1% each of RNA and protein. The DNA was enzymatically digested to the deoxyribonucleosides and their metabolite adducts. Hydrolyzed samples were stored frozen (—70°C) prior to HPLC analysis (14).

HPLC Analysis of BP Metabolite(s) DNA-Adducts. The enzymatic degradation products were analyzed by HPLC and liquid scintillation counting. The HPLC system (Waters Associates, Milford, MA) consisted of a precolumn and C18-silica analytical column. The precolumn was then connected to an analytical column. The next four columns were prepared by reacting generally labeled [3H]BPDEI and [3H]BPDE with 3-methylcholanthrene-induced liver microsomes. Other standards were prepared by reacting generally labeled [3H]BPDE I and [3H]BPDE II with DNA (15).

RESULTS

We examined the binding of BP metabolites to DNA in the endometrium of human and rodent species (rat, hamster, and mouse). We also characterized the BP metabolite-DNA adducts and determined their binding levels. To quantitate the specific adducts formed after [3H]BP treatment, we incubated uterine tissue slices and endometrial scrapings (in human and hamsters only) with labeled BP for 18 h in culture and compared the efficacy of the two different modes of maintenance in vitro.

The BP metabolite-deoxyribonucleoside adducts were separated by HPLC (Fig. 1). The first fraction to elute from the column was the WF which contains uncharacterized polar compounds. Base-line activity was obtained before the start of the water-methanol gradients. After the elution of the WF, the 40–70% methanol-water gradient was started; this caused the elution of an uncharacterized radioactive peak appearing early in this gradient. Four peaks, labeled I, II, III, and IV, were obtained in rat endometrial tissue (Fig. 1, upper left). The characterization of these peaks has been reported by Anderson et al. (18) and Stowers and Anderson (16). Peak I has not been fully characterized but it is thought to be either a BP-phenol oxide-DNA adduct or a BPDE I-deoxyxycytidine adduct. Peak II and Peak III have been identified as adducts of BPDE I to the N-2 position of guanosine and are (—)BPDE I-dGuo and (+)BPDE I-dGuo, respectively. Peak IV has been identified as a BPDE II-dGuo adduct (15).

The specific activities (pmol/mg DNA) of adducts of Peaks I–IV and uncharacterized WF radioactivity are given in Table 1. These specific activities are based on pooled samples in the case of rodents and individual samples in the case of humans. The specific activity was calculated only when peaks contained at least 100 dpm above background. The quantity of BP bound was 7, 4, and 2 times higher in humans than in rats, mice, and hamsters, respectively. The amount of uncharacterized radioactive eluted in the WF was similar in human, hamster, and mouse; however, in rats it represented the highest percentage (53%) of the total radioactivity (Table 1). This was a consistent finding in four different experiments.

The results of this study show that there are notable differences in the amount and type of adducts formed in different species. Peak I was detected only in rat tissue but was absent in all the other three species examined. Peak II, identified as (—)BPDE I-dGuo, was detected only in hamsters and human tissues. (+)BPDE I-dGuo (Peak III) is the predominant adduct formed in human, hamster and mouse. The amount of this adduct that is formed in hamster and mouse is very similar, but in humans it is 3–4 times higher than in these two rodent species. In rat endometrium, the level of this adduct is substantially lower. BPDE II-dGuo (Peak IV) adduct was also present in hamsters, humans, and rats but was completely absent in...
BP-DNA ADDUCTS: HUMAN AND RODENT ENDOMETRIUM

Table 1 BP metabolite-DNA adduct formation in endometrium

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>WF†</th>
<th>Peak I</th>
<th>Peak II</th>
<th>Peak III</th>
<th>Peak IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>0.342</td>
<td>0.02 (5.8)</td>
<td>0.014 (4.0)</td>
<td>0.284 (83.0)</td>
<td>0.038 (11.1)</td>
<td>0.013 (6.5)</td>
</tr>
<tr>
<td>Hamster</td>
<td>0.197</td>
<td>0.024 (12.1)</td>
<td>0.013 (6.5)</td>
<td>0.096 (48.7)</td>
<td>0.064 (32.4)</td>
<td>0.0732 (87.9)</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.0832</td>
<td>0.01 (12.0)</td>
<td>0.009 (16.0)</td>
<td>0.013 (23.2)</td>
<td>0.004 (7.1)</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>0.056</td>
<td>0.03 (53.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>WF†</th>
<th>DNA binding (pmol/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>0.02 (5.8)</td>
<td>0.014 (4.0)</td>
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<tr>
<td>Hamster</td>
<td>0.024 (12.1)</td>
<td>0.013 (6.5)</td>
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<tr>
<td>Rat</td>
<td>0.03 (53.5)</td>
<td></td>
</tr>
</tbody>
</table>

* Average of 4 different experiments.
† WF, uncharacterized radioactivity eluted with water wash; Peak I, probably BP phenol adduct; Peaks II and III, (−)BPDE I-dGuo and (+)BPDE I-dGuo adducts, respectively; Peak IV, BPDE II-dGuo adduct.
‡ Numbers in parentheses, percentage of total radioactivity eluted from HPLC.

DISCUSSION

The results of this study show differences in the binding of BP to the DNA of endometrium from various rodents and humans. In addition to comparing the total binding of BP in each species, we have quantitated the amounts of the individual DNA adducts formed by BP metabolite(s). Our previous study (11) has demonstrated that BP binds to the DNA of human endometrium in organ culture and there is an interindividual variation in the level of binding. The present study represents the first report of the analysis of BP-DNA adducts formed in organ culture of human and rodents endometrial tissue. There have been several reports of metabolism and DNA binding of BP in various other systems. These studies include those in cell cultures (4, 17, 18); human lung tissue (10); human and bovine bronchial explants (9); cultured human colon (12); cultured bladder and tracheobronchial explants from humans, monkeys, hamsters, and rats (19); perfused mouse lung and liver slices (20); mouse liver and lung (21); and skin of mice (22). Many previous studies have suggested that the formation of BPDE-DNA adducts is an obligatory step in BP-induced tumor formation (1). However, many other factors such as the capacity of cells to repair the damaged DNA, the rate of cell division and the presence of tumor promoters may be important in determining whether the potentially carcinogenic adduct on DNA will ultimately lead to tumor formation in a particular tissue.

In the studies reported with other human tissues in culture, BPDE I-dGuo was the predominant adduct in bronchial explants (9), lung (10), and colon (12). Our results with fresh human endometrial tissue are in agreement with these reports. In mouse tissue, we detected only BPDE I adduct. This is in contrast with other reports on lung and liver (21) and on forestomach, muscle, colon, and kidney (16), where the predominant adduct was BPDE I-dGuo, but BPDE II (Peak IV) as also detected. However, these studies were done in vivo. In hamster endometrial tissue, the proportion of BPDE I and BPDE II adducts formed was around 60 and 40%, respectively. The higher level of BPDE II-dGuo (Peak IV) compared to other species is in agreement with cell culture studies (18, 23).

Since our results demonstrate that the type and level of adducts formed in rat endometrium are different from human, hamster, and mice, it is of interest to compare the data on rat endometrial tissue with other tissues from rats. In rat endometrium, one of the adducts (Peak I) found was chromatographically identical to adducts formed upon further metabolism of BP phenols. In a previous study (24), Peak I cochromatographed with the BP phenol-derived adduct obtained from microsomal incubations of DNA and BP. Ashhurst and Cohen (25) reported the major adduct in isolated hepatocytes from rat to be BPDE I-dGuo. However, they used a prelution step with Sephadex LH-20 to remove the WF. In their study, the relative amount of BP phenol-generated adduct was very small. In another study, rat trachea and bladder tissues contained a significant amount of BP metabolite-DNA adduct that corresponded to “Peak I” and only small amounts of this adduct were found in human bladder. “Peak I” was also detected in hamster bladder and tracheal tissues (19). However, in our studies this peak was completely absent in endometrial tissue from humans, hamsters, and mice. In a study with isolated rat liver, no DNA adducts were found, but RNA adducts were detected (20). In the present study with rat endometrium, besides the high levels of WF, BPDE I-DNA adducts (Peak III), BPDE II-DNA adducts (Peak IV), and an unidentified Peak I were also present. The specific activity of BPDE I adduct, although it was 23% of the total DNA-associated radioactivity, was small in quantity compared to the other species. This may be a reflection of the low level of reactive metabolites in rat tissue.

We found major differences in the types and levels of BP metabolite-DNA adducts formed in endometrium from various rodent species and humans. These differences of BPDE-dGuo binding levels may be a significant factor contributing to the susceptibility of a target organ to PAH-induced neoplasia. These results emphasize that caution must be exercised in the selection of animal models for carcinogenesis bioassays and for the extrapolation of the data obtained to estimates of endometrial cancer risks in humans.

ACKNOWLEDGMENTS

We thank Drs. Gene P. Siegel and Leslie A. Walton for obtaining human endometrial tissue. We gratefully acknowledge the assistance of Catherine White and the help and encouragement of Dr. Marshall W. Anderson, both of the National Institute of Environmental Health Sciences, Research Triangle Park, NC. We also thank Dale S. Bost for assistance in the preparation of this manuscript.

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