Characterization of the mutational profile of (+)-7R,8S-dihydroxy-9S,10R-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene at the hypoxanthine (guanine) phosphoribosyltransferase gene in repair-deficient Chinese hamster V-H1 cells

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Earlier studies have shown that the profile of mutations induced by (+)-7R,8S-dihydroxy-9S,10R-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (+)-BPDE] at the hypoxanthine (guanine) phosphoribosyltransferase (hprt) gene of Chinese hamster V79 cells was dependent on the concentration of (+)-BPDE. In the present study, we examined the effect of the concentration of (+)-BPDE on its mutational profile at the hprt gene in repair-deficient V-H1 cells (a derivative of V79 cells) to explore the role of DNA repair in the dose-dependent mutational profile of (+)-BPDE. Independent hprt mutant clones were isolated after exposing V-H1 cells to dimethylsulfoxide (DMSO) or to low (4–6 nM; 95% cell survival) or high (40–48 nM; 31% cell survival) concentrations of (+)-BPDE in DMSO. The mutation frequencies for the DMSO control and for the low and high concentration groups were 0.1, 2.1 and 32.9 mutant colonies/105 survivors, respectively. The profile of mutations at the hprt gene was characterized for 148 (+)-BPDE-induced mutant clones and the results from the present study were compared with those obtained earlier with V79 cells. The data indicated that: (i) V-H1 cells were 9-fold more sensitive to the cytotoxic effects of (+)-BPDE than V79 cells; (ii) the mutation frequency in V-H1 cells was similar to that observed in V79 cells following exposure to similar concentrations of (+)-BPDE; (iii) (+)-BPDE-induced mutations at guanine on the transcribed strand of the hprt gene were common in V-H1 cells but were extraordinarily rare in V79 cells; (iv) (+)-BPDE-induced mutations at adenine on the transcribed strand of the hprt gene were common in both V-H1 and V79 cells; (v) although exposure of V79 cells to different doses of (+)-BPDE resulted in a dose-dependent mutational profile at the hprt gene, this was not observed in V-H1 cells. Our observations indicate a defect in the transcription-coupled repair of (+)-BPDE–DNA adducts in V-H1 cells and that the repair activity deficient in V-H1 cells is essential for the dose-dependent mutational profile observed with (+)-BPDE in V79 cells.

Introduction

Human populations are commonly exposed to low levels of environmental mutagens/carcinogens, but most experimental mutagenesis and carcinogenesis studies in animals or cultured cells are done with high, cytotoxic doses of these chemicals. Since experimental studies with low dose levels of chemicals may be more relevant to human populations, we compared the mutational profiles of high and low doses of several chemicals at the endogenous hypoxanthine (guanine) phosphoribosyltransferase (hprt) gene in Chinese hamster V79 cells (1–4).

(+)-7R,8S-Dihydroxy-9S,10R-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene [(+)BPDE] and (–)-(1R,2S,3S,4R)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene [(+)B[c]PhDE] are ultimate carcinogens that are formed during the mammalian metabolism of the polycyclic aromatic hydrocarbons benzo[a]pyrene and benzo[c]phenanthrene, respectively (5–9). (+)-BPDE covalently binds predominantly to guanine in DNA (10,11 and references therein) whereas (-)B[c]PhDE binds preferentially to adenine in DNA (12,13). Nucleotide excision repair is a major mechanism for the removal of such bulky, helix-distorting lesions from DNA (14), but unrepaired DNA adducts may result in mutations during cellular replication (15,16).

Earlier studies from our laboratory demonstrated that exposure of Chinese hamster V79 cells to a low, non-cytotoxic concentration of (+)-BPDE or (–)-B[c]PhDE resulted in a mutational profile at the hprt gene that differed from that observed after exposure of the cells to a higher, cytotoxic concentration of the carcinogen (1–4). When the concentration of (+)-BPDE or (–)-B[c]PhDE was decreased, the proportion of base substitutions targeted at GC base pairs decreased and the proportion of base substitutions targeted at AT base pairs increased (1–4). A possible explanation for these dose-dependent differences in mutational profiles induced by (+)-BPDE and (–)-B[c]PhDE might involve dose-dependent differences in DNA repair activities for the removal of diol epoxide-induced guanine and adenine adducts from DNA in the hprt gene.

In order to enhance our understanding of the role of DNA repair for dose-dependent mutagenesis by (+)-BPDE in Chinese hamster V79 cells, we examined the effect of the concentration of (+)-BPDE on its mutational profile at the hprt gene in a repair-deficient Chinese hamster V79 cell mutant, V-H1 (17). The V-H1 cell line belongs to the second complementation group of UV-sensitive mutant cell lines of rodent origin (18). This mutant is defective in the XPD/ERCC2 gene (19), which encodes for an ATP-dependent DNA helicase. The ERCC2 protein is a subunit of the BTF2/TFIIH complex that is essential for transcription initiation and nucleotide excision repair (20). The V-H1 cell line is extremely sensitive

Abbreviations: (+)-BPDE, (–)-(1R,2S,3S,4R)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene; DMSO, dimethyl sulfoxide; hprt, hypoxanthine (guanine) phosphoribosyltransferase.
to UV radiation and deficient in cyclobutane pyrimidine dimer repair, but shows intermediate levels of (6–4) photoproduction repair (21). In addition, an earlier mutational study of UV-induced hprt mutant clones from V-H1 cells suggested a deficiency in the preferential repair of UV-induced mutagenic lesions from the transcribed strand (22). The results of our studies in V-H1 cells indicate a defect in the transcription-coupled repair of (+)-BPDE–DNA adducts at the hprt gene and a lack of dose dependence in mutation profile caused by (+)-BPDE at the hprt gene.

Materials and methods

Materials

All reagents, PCR primers, sequencing primers and enzymes were obtained and used as previously described (1–3). (+)-BPDE was synthesized as previously described (23). [Methyl-14C]thymidine (59.2 mCi/mmol, >99% pure) was obtained from NEN Life Science Products (Boston, MA). (+)-[3H]BPDE (975 mCi/mmol, >98% pure) was obtained from Chemsyn Science Laboratories (Lenexa, KS).

Tissue culture and characterization of mutations at the hprt gene in 8-azaguanine-resistant mutant clones

The Chinese hamster V-H1 cell line (17) was derived from V79 cells by a replica plating technique for isolating ethyl nitrosourea-induced mutant clones that demonstrate sensitivity to various mutagens. The procedures described previously for V79 cells [culturing and exposing cells to (+)-BPDE, assessing cytotoxicity, generating 8-azaguanine-resistant mutant clones and characterizing mutations in the coding region and flanking intron sequences of the hprt gene] were used for V-H1 cells (1–4).

Repair of (+)-BPDE-induced DNA damage in V-H1 and V79 cells

The nucleotide excision repair activity of V-H1 and V79 cells was determined from the time-dependent removal of covalently bound (+)-BPDE from genomic DNA. Briefly, exponentially growing V-H1 or V79 cells were 3H-labeled with medium containing 0.25 nCi/ml [methyl-14C]thymidine for 2 days. The cells were then trypsinized, dispersed, filtered through a cell strainer, plated at 500×10^3 cells/100 mm dish in 10 ml of medium containing 10% fetal bovine serum and cultured for 20 h. The cells were then treated under subdued yellow light with (+)-[3H]BPDE in 40 μl of dimethyl sulfoxide (DMSO) for 1 h at 37°C. After rinsing with phosphate-buffered saline, the cells were harvested either immediately or after further incubation in fresh medium for 6 or 24 h to allow for repair of the DNA damage. The procedures for isolating genomic DNA and determining DNA adduct levels were described previously (24). The genomic DNA samples were then subjected to 3H and 14C dual counting. The percent of original adduct level remaining in parental DNA was determined by comparing the 3H:14C count ratio from genomic DNA of cells that were allowed to repair for 6 or 24 h to the 3H:14C count ratio from genomic DNA isolated from the cells immediately after treatment with (+)-[3H]BPDE for 1 h. Measuring the percent of original adduct level remaining in parental DNA eliminated the overestimate in the amount of DNA repair caused by the generation of damage-free DNA following cellular replication during the repair incubation.

Statistical analysis

The software package from SAS Institute Inc. (Cary, NC) was used to perform Fisher’s exact test on the mutational data from this study (25).

Results

Repair of (+)-BPDE–DNA adducts in Chinese hamster V-H1 and V79 cells

The (+)-BPDE–DNA adduct level immediately after exposing V-H1 cells to 30 nM (+)-[3H]BPDE for 1 h was 22 adducts/10^7 nucleotides, while the (+)-BPDE adduct levels immediately after treatment of V79 cells with 30 and 480 nM (+)-[3H]BPDE for 1 h were 27 and 309 adducts/10^7 nucleotides, respectively. The rates for the removal of (+)-BPDE adducts from DNA were determined from the percentage of adduct level remaining on parental DNA after the cells were allowed a period of 6 or 24 h for repair (Figure 1). The extent of repair in V-H1 cells exposed to 30 nM (+)-[3H]BPDE was ~56% of that in V79 cells after 24 h (Figure 1). In two additional experiments, the repair in V-H1 cells was ~46% of that in V79 cells.

Cytotoxicity and mutagenicity of (+)-BPDE in Chinese hamster V-H1 cells

The cytotoxic effect of (+)-BPDE in V-H1 cells was determined by measuring the colony-forming ability of treated cells relative to that of cells treated with the vehicle control, DMSO. Treatment with a high concentration (40–48 nM) or with a low concentration (4–6 nM) of (+)-BPDE for 1 h resulted in 31% and 95% cell survival, respectively. The concentration of (+)-BPDE that resulted in ~30% cell survival in V79 cells was ~9-fold higher and ranged from 300 to 480 nM (Table I). Thus, V-H1 cells were extremely sensitive to the cytotoxic effects of (+)-BPDE treatment when compared with V79 cells (Table I).

In the present study, we only analyzed data from experiments in which the mutation frequency for the (+)-BPDE-treated groups was at least 10 times higher than the spontaneous mutation frequency to reduce the chance of including in our data a spontaneously generated mutation. The mutation frequencies for cells treated with DMSO or with a low or high concentration of (+)-BPDE in DMSO were 0.1, 2.1 and 32.9 8-azaguanine-resistant colonies/10^5 surviving cells, respectively (Table I). Thus, the mutation frequency at the hprt gene, but not cell survival, was similar in both V-H1 and V79 cells following exposure to similar concentrations of (+)-BPDE (40–48 nM for V-H1 cells and 40–100 nM for V79 cells) (Table I).
Table I. Mutagenicity and cytotoxicity of (+)-BPDE in Chinese hamster V-H1 and V79 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mutations/10^5 surviving cells</th>
<th>Percent cell survival</th>
<th>Mutations/10^5 surviving cells/nmol diol epoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V-H1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dose (40–48 nM)</td>
<td>32.9 ± 5.9</td>
<td>31 ± 2</td>
<td>0.74</td>
</tr>
<tr>
<td>Low dose (4–6 nM)</td>
<td>2.1 ± 0.2</td>
<td>95 ± 1</td>
<td>0.42</td>
</tr>
<tr>
<td>DMSO control</td>
<td>0.1 ± 0.1</td>
<td>100 ± 0</td>
<td></td>
</tr>
<tr>
<td>V79 cells</td>
<td>0a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dose (300–480 nM)</td>
<td>514 ± 112</td>
<td>32 ± 3</td>
<td>1.32</td>
</tr>
<tr>
<td>Intermediate dose (40–100 nM)</td>
<td>52 ± 13</td>
<td>100 ± 3</td>
<td>0.74</td>
</tr>
<tr>
<td>Low dose (10–20 nM)</td>
<td>10 ± 1</td>
<td>97 ± 1</td>
<td>0.67</td>
</tr>
<tr>
<td>DMSO control</td>
<td>1 ± 0.2</td>
<td>100 ± 0</td>
<td></td>
</tr>
</tbody>
</table>

Cells were exposed to the indicated dose of (+)-BPDE for 1 h. Independent 8-azaguanine-resistant mutant clones were examined. Each number represents the mean ± SE from at least eight experiments.

aTaken from Wei et al. (2).

Table II. Kinds of mutations observed in the coding region of the hprt cDNA in (+)-BPDE-induced 8-azaguanine-resistant mutant clones obtained from Chinese hamster V-H1 and V79 cells

<table>
<thead>
<tr>
<th>Mutant clones</th>
<th>V-H1</th>
<th>V79</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High dose (40–48 nM)</td>
<td>Low dose (4–6 nM)</td>
</tr>
<tr>
<td>No. analyzed</td>
<td>73</td>
<td>75</td>
</tr>
<tr>
<td>Percent with base substitutions</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>Percent with exon deletions</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>Percent with ± 1 frameshifts</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Percent with tandem mutations</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

Cells were exposed to the indicated dose of (+)-BPDE for 1 h. Independent 8-azaguanine-resistant mutant clones were examined.

aTaken from Wei et al. (2).

bUnpublished observations.

Kinds of mutations in the coding region of the hprt gene in (+)-BPDE-induced 8-azaguanine-resistant clones

Mutations in the coding region (654 bp) of the hprt gene were characterized for 148 (+)-BPDE-induced mutant clones from V-H1 cells by reverse transcription–PCR amplification of the mRNA in cell lysates followed by direct DNA sequencing of the PCR products (Table II). The most common mutations observed in the coding region of the hprt gene in the (+)-BPDE-induced mutant clones were base substitutions (~58% of the mutant clones), followed by exon deletions (~28% of the mutant clones), ±1 frameshift mutations (~14% of the mutant clones) and tandem base mutations (~4% of the mutant clones) (Table II). For V-H1 cells, altering the concentration of (+)-BPDE did not affect the percentage of these four kinds of mutations (Table II). Previous results with V79 cells were strikingly similar (2).

Effect of (+)-BPDE concentration on exon deletions in the coding region of the hprt cDNA of Chinese hamster V-H1 cells

Of the 42 (+)-BPDE-induced V-H1 mutant clones with an exon deletion in their hprt cDNA, 20 exon deletion mutations were observed in the high concentration group and 22 in the low concentration group (Table III). The profile of exon deletions was not influenced by the concentration of (+)-BPDE (P = 0.91, Fisher’s exact test). Forty-one (+)-BPDE-induced V-H1 mutant clones with an exon deletion in their hprt cDNA were further characterized by PCR amplification of their genomic DNA using primer sets flanking the deleted exons followed by direct sequencing of the PCR products. The mutant clones capable of generating a PCR product of the same size as those of parental V-H1 cells were aberrant splicing mutants. For the high concentration group, 14 of 20 (70%) of the mutant clones with exon deletions resulted from aberrant splicing whereas six (30%) resulted from deletions in the genomic DNA. For the low concentration group, 14 of 21 (67%) of the mutant clones with exon deletions resulted from aberrant splicing whereas seven (33%) resulted from deletions in the genomic DNA. A lack of dose dependence was observed in the percentage of V-H1 mutant clones with an exon deletion in the hprt cDNA that resulted from either aberrant splicing or deletions in the genomic DNA.

Effect of (+)-BPDE concentration on base substitutions in the coding region and flanking intron sequences of the hprt gene

The mutation spectra induced by high and low concentrations of (+)-BPDE in the coding region and flanking intron sequences of the hprt gene in V-H1 mutant clones revealed that the predominant base substitutions observed were GC→TA transversions (Table IV and Figure 2). Throughout the course of the study, a total of 12 8-azaguanine-resistant V-H1 mutant clones arose spontaneously. None of the spontaneously generated mutations (type/site) were observed in the mutation...
The strand distribution of premutagenic lesions for base substitutions in the coding region and flanking intron sequences of the *hprt* gene in (+)-BPDE-induced 8-azaguanine-resistant mutant clones

Table IV. Kinds of base substitutions in the coding region and flanking intron sequences of the *hprt* gene in (+)-BPDE-induced 8-azaguanine-resistant mutant clones

<table>
<thead>
<tr>
<th>Base substitution target</th>
<th>V-H1*</th>
<th>V79b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High dose (40–48 nM)</td>
<td>Low dose (4–6 nM)</td>
</tr>
<tr>
<td>GC base pairs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC→TA</td>
<td>31 (51)</td>
<td>25 (50)</td>
</tr>
<tr>
<td>GC→CG</td>
<td>11 (18)</td>
<td>9 (18)</td>
</tr>
<tr>
<td>GC→AT</td>
<td>10 (16)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Total</td>
<td>52 (85)</td>
<td>40 (80)</td>
</tr>
<tr>
<td>AT base pairs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT→GC</td>
<td>3 (5)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>AT→TA</td>
<td>5 (8)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>AT→CG</td>
<td>1 (2)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (15)</td>
<td>10 (20)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses represent the percentage of total base substitutions.

Discussion

While human populations are commonly exposed to low doses of chemical mutagens/carcinogens, we have been concerned that experimental mutagenic and carcinogenic studies with high doses of chemicals may not be relevant to human populations (1). In earlier studies, we compared the profile of mutations induced by high and low doses of the carcinogenic enantiomers (+)-BPDE and (-)-B[c]PhDE at the *hprt* gene of V79 cells (1–4). These studies demonstrated that exposure of V79 cells to a low, non-cytotoxic concentration of (+)-BPDE or (-)-B[c]PhDE resulted in a profile of mutations at the *hprt* gene that differed from that observed after exposure of the cells to a higher, cytotoxic concentration of the carcinogen. When the concentration of (+)-BPDE or (-)-B[c]PhDE was decreased, the proportion of mutations at GC base pairs decreased and the proportion of mutations at AT base pairs increased (1–4). A recent study by Chen and Thilly (26) demonstrated that the mutational spectra induced by benzo[a]-pyrene in a human lymphoblastoid cell line with a cytochrome P-450 metabolite activation system was dependent upon the conditions of exposure. A low concentration of benzo[a]-pyrene and a long exposure time resulted in a different profile of mutations than a high concentration and a short exposure time (26). All of these investigations emphasized that mutation...
Fig. 2. Mutations induced by high and low concentrations of (+)-BPDE in V-H1 cells. Upper case letters indicate the DNA sequence in the coding region of the hprt gene, whereas lower case letters indicate the intron sequence of the exon–intron junction of the hprt gene in V-H1 cells. High dose group mutations and low dose group mutations are shown above and below the wild-type sequence, respectively. The open triangle and open diamond indicate a single base deletion and a single base addition, respectively. The boxed letters indicate a tandem mutation.

studies using low doses of mutagens are more relevant to human exposure conditions. In the present study, we explored the possible influence of DNA repair on dose-dependent changes in mutation profile by asking whether there was a dose-dependent difference in the profile of mutations induced by (+)-BPDE at the hprt gene in V-H1 cells, a nucleotide excision repair-deficient cell line (17) derived from V79 cells.

The (+)-BPDE adduct level in total genomic DNA immediately after a 1 h exposure of V-H1 and V79 cells to 30 nM (+)-[3H]BPDE was 22 and 27 adducts/10^7 nucleotides, respectively. These observations indicate that the amount of (+)-BPDE that is available to react with DNA in the cellular microenvironment is similar for both V-H1 and V79 cells following exposure to the same concentration of (+)-BPDE.

The nucleotide excision repair activities of V-H1 and V79 cells were determined for the removal of (+)-BPDE adducts from the overall genome during a 24 h time interval. About 60% of (+)-BPDE adducts were removed from V79 cells after 24 h (Figure 1). This observation agrees with the earlier data by Brookes and Osborne in which 60% of (+)-BPDE adducts were excised from V79 cells after 24 h repair (27). V-H1 cells exhibited an ~50% decrease in repair activity for the removal of (+)-BPDE adducts when compared with V79 cells (Figure 1). This observation agrees with earlier findings demonstrating that the repair activity for UV-induced (6–4) photoproducts in V-H1 cells was ~50% of that in V79 cells (21). Our data suggest that there may be similarities in the repair of (+)-BPDE adducts and the repair of (6–4) photoproducts in V-H1 cells. In contrast to the ability of V-H1 cells to repair (6–4) photoproducts, V-H1 cells are completely deficient in cyclobutane pyrimidine dimer repair (21).

The concentration of (+)-BPDE that kills 70% of V-H1 cells was 9-fold lower than that required to exert the same effect in V79 cells. This observation is consistent with an earlier finding demonstrating that V-H1 cells were ~10-fold more sensitive to UV-induced killing than V79 cells (28). The high sensitivity of V-H1 cells to the cytotoxic effects of (+)-BPDE treatment observed in our study may be attributed...
to the reduced nucleotide excision repair activity present in V-H1 cells when compared with V79 cells. Our results indicate that a 50% decrease in nucleotide excision repair activity in V-H1 cells relative to V79 cells (Figure 1) is associated with a significant increase in the sensitivity to (+)-BPDE-induced toxicity (Table I). It is likely that V-H1 cells repair those (+)-BPDE–DNA adducts associated with cell killing much less effectively than overall (+)-BPDE–DNA adducts. The data in Table V suggest that V-H1 cells are profoundly impaired in the preferential removal of premutagenic (+)-BPDE-induced guanine adducts from the transcribed strand of the hprt gene. It is possible that the high (+)-BPDE-induced toxicity observed in V-H1 cells may be caused by a decreased ability to preferentially repair (+)-BPDE-induced guanine adducts from the transcribed strand of active genes in the V-H1 genome.

Although V79 cells repaired (+)-BPDE adducts about twice as fast as V-H1 cells, the mutagenic activity of (+)-BPDE is comparable in V-H1 and V79 cells following exposure to a similar dose of (+)-BPDE that is cytotoxic to V-H1 cells (40–48 nM) but not to V79 cells (40–100 nM) (Table I and Figure 1). Following DNA damage, DNA lesions in the original cell population may be removed by DNA repair mechanisms or eliminated by cellular responses that lead to cell death (29). In all cases, the same pattern of adducts was seen after cells were treated with the same dose of (+)-BPDE. DNA repair might be the predominant mechanism for the removal of DNA adducts from V79 cells and cell death might be the major pathway for the loss of DNA adducts in V-H1 cells.

The kinds of mutations observed in (+)-BPDE-treated V-H1 cells were similar to those observed in (+)-BPDE-treated V79 cells, but V-H1 cells had some tandem base mutations that were not observed in V79 cells (Table II). Although our results indicate that exposure of V-H1 and V79 cells to a similar concentration of (+)-BPDE resulted in a similar distribution of base substitutions (the high dose group of V-H1 cells and the intermediate dose group of V79 cells in Table IV), the global mutation spectra (type/site of mutations; Figure 2, and ref. 2) was significantly different between these two groups ($P = 0.039$, Fisher’s exact test).

V-H1 cells exhibited many (+)-BPDE-induced base substitutions at guanine on the transcribed strand of the hprt gene (32% of the mutations at guanine) (Table V). These results are in marked contrast to our earlier observations with V79 cells in which only 0.5% of the base substitutions targeted at guanine occurred on the transcribed strand of the hprt gene (Table V) (2,3). The results indicate a deficiency in the preferential removal of (+)-BPDE–guanine adducts from the transcribed strand of the hprt gene in V-H1 cells, suggesting that these cells are deficient in transcription-coupled repair of (+)-BPDE adducts. Our results are consistent with a previous finding (22) suggesting that UV lesions on the transcribed strand of the hprt gene were not preferentially repaired in V-H1 cells.

The results from the present study indicate that altering the concentration of (+)-BPDE did not cause a significant change in the proportion of base substitutions at GC and AT base pairs in the coding region and flanking intron sequences of the hprt gene in V-H1 cells (Table IV) ($P = 0.61$, Fisher’s exact test). In addition, our data demonstrated a lack of dose dependence in the global mutation spectra (type/site of mutations), the pattern of base substitutions or in the proportion of base substitutions at GC and AT base pairs between these two groups at the same dose (~40 nM) of (+)-BPDE in repair-deficient Chinese hamster V-H1 cells. The lack of dose dependence in mutation profile induced by (+)-BPDE in the coding region and flanking intron sequences of the hprt gene in repair-deficient Chinese hamster V-H1 cells differs from our earlier observations indicating that (+)-BPDE induced a highly significant dose-dependent difference in the mutation profile at the hprt gene in repair-proficient V79 cells (1–3). The present data suggest that the ERCC2 protein, which is defective in V-H1 cells, may play a role in transcription-coupled repair of (+)-BPDE adducts and in the dose-dependent mutational spectra previously observed for (+)-BPDE in repair-proficient V79 cells.

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