Mutations Induced in the Hypoxanthine Phosphoribosyl Transferase Gene by Three Urban Air Pollutants: Acetaldehyde, Benzo[a]pyrene Dioleopxide, and Ethylene Oxide

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Provisional mutational spectra at the hypoxanthine phosphoribosyl transferase (HPRT) locus in vitro have been worked out for acetaldehyde (AA) and benzo[a]pyrene dioleopxide (BPDE) in human (T)-lymphocytes and for ethylene oxide (EO) in human diploid fibroblasts using Southern blotting and polymerase chain reaction (PCR)-based DNA sequencing techniques. The results indicate that large genomic deletions are the predominant hprt mutations caused by AA and EO, whereas BPDE induces point mutations that are mainly GC\(\rightarrow\)TA transversions. The mutational spectra induced by the three agents are clearly different from the background spectrum in human T-cells. Thus, the hprt locus is a useful target for the study of chemical-specific mutational events that may help identify causes of background mutation in human cells in vivo. — Environ Health Perspect 102(Suppl 4):135–138 (1994).

Key words: acetaldehyde, benzo[a]pyrene dioleopxide, ethylene oxide, HPRT, human T-cells, molecular analysis, mutation, PCR

Introduction

Humans are exposed to many compounds in ambient air that are known to cause genetic damage in various test systems. The possible consequences of this exposure include somatic mutation in critical target genes that may lead to cancer and germ line mutation that may lead to genetic disease in the offspring. To elucidate the course of events resulting in these severe consequences, it is important to study the frequency and spectrum of background mutation in human cells in vivo. It is also important to determine the relative contribution of mutation induced by environmental agents as opposed to spontaneous mutation caused by endogenous factors. This research should be carried out for each specific gene locus and cell type of interest because genes may differ with regard to mutation rates and the types and phenotypic consequences of mutation. Moreover, the same genes in different cell types may respond differently to mutagens depending on factors such as transcriptional activity and DNA repair activity (1).

The development of methods for the analysis of mutation at the human locus for the purine salvage enzyme hypoxanthine phosphoribosyl transferase (HPRT) has offered several unique possibilities for the evaluation of human in vivo mutagenesis (2–4). Human T-cell clonal can be performed with high cloning efficiency, and selection for HPRT mutants in medium containing 6-thioguanine yields Reliable and reproducible estimates of the mutation frequency in the T-cell population from the peripheral blood of individual subjects (5–9). Information with regard to the clonality and origin of HPRT mutation is obtained by molecular analysis of the clone-specific T-cell receptor rearrangement (10).

A further advantage of the human T-cell mutational system is that the nucleotide sequence of the entire human HPRT gene of more than 44 kb has been worked out (11). This provides a variety of tools and approaches for the molecular analysis of HPRT mutation, including methods for screening of mutations such as ribonuclease mismatch cleavage (12), denaturing gradi-
Table 1. Types of mutation at the human hpert locus and methods for their detection (4).

| Type of mutation                  | Method                                      | Comment                                                                 |
|----------------------------------|---------------------------------------------|--------------------------------------------------------------------------|
| Chromosomal mutation             | Cytogenetic karyotyping                    | No mutation of this type has been detected so far                       |
| Gross structural alterations     | Southern blot analysis about                | Only deletions larger than about 100 bp are detected                     |
| Point mutations                  |                                             |                                                                          |
| Missense mutation                | Sequencing of PCR-amplified cDNA or genomic DNA | Coding error causing an amino acid substitution                         |
| Nonsense mutation                |                                             | Coding error causing a stop codon                                        |
| Frameshift mutation              |                                             | Small deletion / insertion affecting the reading frame                   |
| Splice mutation                  |                                             | Change affecting sequence involved in splicing functions                 |

Table 2. Point mutations at the hypoxanthine phosphoribosyl transferase locus in germ line and somatic cells (20,28,31,37).

| Type of point mutation          | Lesch-Nyhan and gout patients | TG-resistant T-lymphocytes |
|--------------------------------|-------------------------------|---------------------------|
| Amino acid substitution        |                               |                           |
| Missense                       | 46 (53%)                      | 36 (40%)                  |
| Nonsense                       | 9 (9%)                        | 8 (8%)                    |
| Small deletions / insertion (frameshift) | 20 (23%)            | 17 (19%)                  |
| Splice mutations               | 12 (14%)                      | 29 (32%)                  |
| All point mutations            | 86 (100%)                     | 90 (100%)                 |

Results

The Background Spectrum of HPRT Mutation

Gross structural alterations detected by Southern blot analysis account for about 10 to 15% of the background spectrum of HPRT mutations in T-lymphocytes from adult donors (29) and in patients with LNS (30). The background spectrum of point mutation at the HPRT locus in human T-cells was compiled and discussed by Hou et al. recently (26). These data are shown in Table 2 together with data on germ line mutation derived from studies of LNS and gout patients (4,20). It is obvious that the types and relative frequencies of the various types of mutation in germ line and somatic cells are similar, with the possible exception that splicing mutations seem to be less frequent in the germ line than in the somatic cells, as pointed out by Rossi et al. (31).

Although almost 100 somatic and germ line-point mutations have been mapped in the human HPRT gene, very few have been found to recur at the same site within the HPRT coding sequence of 654 base pairs. Thus, the mutations are widely dispersed along the coding sequence, and there are no predominating mutational hot spots (4,20,26).

Acetaldehyde-induced HPRT Mutation

Human exposure to AA occurs regularly because of its production by endogenous metabolism and its presence in the environment (e.g., automobile exhausts, cigarette smoke, ambient air). The genetic toxicity of AA has been demonstrated in a variety of test systems, and there is evidence for its carcinogenicity in experimental animals (32). The ability of AA to induce HPRT mutation in human T-lymphocytes in vitro was demonstrated by He and Lambert (22). Cells treated with 1.2 to 2.4 mM of AA for 24-hr, or 0.2 to 0.6 mM for 48 hr showed a dose dependent, 3- to 16-fold increase of the mutant frequency. At the highest concentration of AA, 40% of the T-cell mutants were found to have large genomic deletions detectable by Southern blot analysis. In contrast, the frequency of large deletions in untreated cultures was only about 10%, which is similar to the frequency of gross structural alterations in the background spectrum of HPRT mutation (discussed above). All of the AA-induced deletions were found to extend into the 3' -flanking region of the HPRT gene, whereas the deletions in the control cultures mainly affected the 5' part of the gene (22). Thus, large 3'-flanking deletions may be an important type of HPRT mutation induced by high dose AA in vitro. Nevertheless, 60% of the mutations in the treated cultures showed normal Southern blot patterns, indicating that the majority of AA-induced mutations in human T-cells are likely to be point mutations. The nature of these mutations are not known yet.

BPDE-induced HPRT Mutation

BPDE is a carcinogenic in vivo metabolite of benzo[a]pyrene, one of the most well-known combustion products in vehicle exhausts, cigarette smoke, and ambient air. There are four different stereochemical forms of BPDE, and the (+)-anti form is the most potent mutagen in mammalian cells (33). The frequency of HPRT mutation in human T-cells treated with 0.3 to 0.6 μM of (+)-antiBPDE for 24 hr was found to increase in a dose-dependent way up to 50 times the frequency in untreated control cultures (23). Thus, BPDE is a potent mutagen in this system. No large alterations of the HPRT gene could be detected by Southern blot analysis in the BPDE-induced T-cell mutants, which shows that BPDE induces mainly point mutations. Direct sequencing of HPRT cDNA and genomic HPRT DNA from the mutant clones showed that the predominating type of base pair substitution induced by BPDE in the coding and splicing sequences was GC>TA transversion. In the coding region of the HPRT gene, these mutations were preferentially located in the sequence context AGG- or GAA- (18). In the concurrent control spectrum as
Table 3. Characteristic features of the hypoxanthine phosphoribosyl transferase mutational spectra induced by acetaldehyde, benzo[a]pyrene diolepoxide, and ethylene oxide as compared to the background spectrum (18,22,26,40).  

|                      | Background | Acetaldehyde | BPDE  | Ethylene oxide |
|----------------------|------------|--------------|-------|----------------|
| Large deletions and rearrangements | 10%        | 40%          | 0%    | 48%            |
| Base substitutions   |            |              |       |                |
| GC>AT                | 17%        | NS           | 0%    | 7%             |
| GC>TA                | 10%        | NS           | 41%   | 4%             |
| Other                | 17%        | NS           | 14%   | 11%            |
| Small deletions and insertions | 17%        | NS           | 5%    | 4%             |
| Splice mutations     | 29%        | NS           | 41%   | 28%            |

NS, not studied. a The background, AA and BPDE spectra were derived from human T-lymphocytes, and the ethylene oxide spectrum is based on fibroblast data (40).

well as in the general background spectrum, GC>TA transversion accounts for a minor proportion of the base pair substitutions. Moreover, there was a strong preference for mutation induction in the nontranscribed DNA strand, suggesting that BPDE adducts are more efficiently removed from the transcribed than from the nontranscribed DNA strand (18). This is likely to be a consequence of preferential DNA repair, as demonstrated previously in the HPRT gene (34,35) and other genes (1). Thus, these data show that there is a clear difference between the BPDE-induced mutational spectrum as compared to the background spectrum (Table 3).

Ethylene Oxide-induced HPRT Mutation

EtO is formed in humans and animals by metabolism of ethene, a widely distributed air pollutant present in car exhausts and cigarette smoke. Ethene also is produced endogenously. The genetic toxicity and animal carcinogenicity of EtO is well documented, and human occupational exposure to EtO has been associated with an increased risk of leukemia (36). The mutagenic effect of EtO in the HPRT gene in human diploid fibroblasts was demonstrated recently by Kolman et al. (25). The mutant frequency was found to increase linearly within the dose range of 2.5 to 10 mM of EO, with a mutagenic potency of \(9.8 \times 10^{-6}\) mutants per mM. Independent mutant cell clones were studied with PCR-based techniques and direct DNA sequencing or Southern blot analysis to unveil the nature of the EO-induced mutations. Among 28 mutants studied, 48% demonstrated large genomic deletions of the whole or part of the HPRT gene. Most of the partial deletions have breaking points in the 5' part of the gene. The EtO-induced point mutations include one bp deletion causing a frameshift mutation, six base pair substitutions causing missense or nonsense mutations or affecting the translational start codon, and seven splicing mutations. Thus, these results (40) indicate that EtO induces both point mutations and gross structural alterations in human fibroblasts, with a strong preference for large intragenic or total HPRT deletions.

Discussion

Our data, summarized above, show that each of the three urban air pollutants studied induces a very specific mutational spectra at the HPRT locus in human cells in vitro (Table 3). The mutants were selected from experiments in which the mutant frequency was increased by a factor of 10 or more. Thus, at least 9 out of 10 mutants should be induced by the chemical compound.

The most informative mutational spectrum was obtained in the BPDE-treated cultures. All of the BPDE-induced HPRT mutants were found to have point mutations, 41% being GC>TA transversions causing coding errors and 41% splice mutations (Table 3). In the normal background spectrum, GC>TA transversions in the coding region account for only 10% of all mutations (Table 3). Benzo[a]pyrene is a ubiquitous urban air pollutant, and it is likely that its metabolite BPDE induces HPRT mutation in T-cells in vivo. For the sake of discussion one may assume that all the background GC>TA transversions in vivo are due to BPDE mutagenesis. Since BPDE-induced splice mutations also should contribute to the spectrum of background mutations, at most 24% (0.4/0.41) of all the background mutations in vivo could be caused by BPDE. However, the true figure is probably much lower because spontaneous mutation due to replication error contributes to the background frequency of GC>TA transversions, and BPDE is not likely to be the only environmental mutagen that gives rise to this type of base substitution. It will be interesting to study whether subjects who sustain high exposure to benzo[a]pyrene and other (PAHs) show any significant increase of base substitution (e.g., GC>TA transversion mutation) or other type of mutation as compared to unexposed controls. Such studies could focus on heavy smokers who are known to have increased frequencies of in vivo HPRT mutation (8,9) and coke oven workers preferentially having concomitant exposure evaluation by measurements of individual PAH–DNA adduct levels (38).

It is possible that a certain fraction of the large deletion mutations in the background spectrum of HPRT mutation is due to in vivo mutagenicity of AA and EtO, considering the predominance of such mutations in the corresponding in vitro spectra (Table 3). To assess this possibility, further studies of individual mutational background spectra and chemical-specific mutational spectra in vitro are needed. Tate et al. (39) recently demonstrated an increased frequency of HPRT mutation in peripheral lymphocytes of factory workers occupationally exposed to EtO. The comparison of mutational spectra in such workers with the background spectrum in unexposed individuals and the spectrum of HPRT mutation induced by EtO in vitro will improve the basis for quantitative estimation of the risk associated with EtO exposure in vivo.

Knowledge about mutational spectra induced by specific chemicals or radiation at the HPRT locus will also be extremely useful in elucidating the mechanisms by which mutation occurs in human cells in vivo and to evaluate the influence of metabolic activation and DNA repair. Because mutation is likely to be the initiating step in many carcinogenic processes, the elucidation of the mutagenic mechanisms also may contribute to the understanding of carcinogenesis. The HPRT gene is a large locus in which many different types of mutation are detectable, from large deletions and rearrangements to single base substitutions (4,26,31). Therefore, the HPRT locus may be a useful target for the identification of in vivo mutations that are specific or typical for a particular carcinogenic agent or exposure. Moreover, the identification of carcinogen-specific mutational spectra may be extremely valuable in human biomonitoring and quantitative risk evaluation.

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