

Mutation, Gene Expression and DNA Adduct Formation in Human Lymphoblast Cells Dosed with Benzo[a]pyrene and 4-aminobiphenyl

Elaine M. Ricicki^{*1}, Wendy Luo¹, Helmut Zarbl² and Paul Vouros¹

¹Barnett Institute & Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA

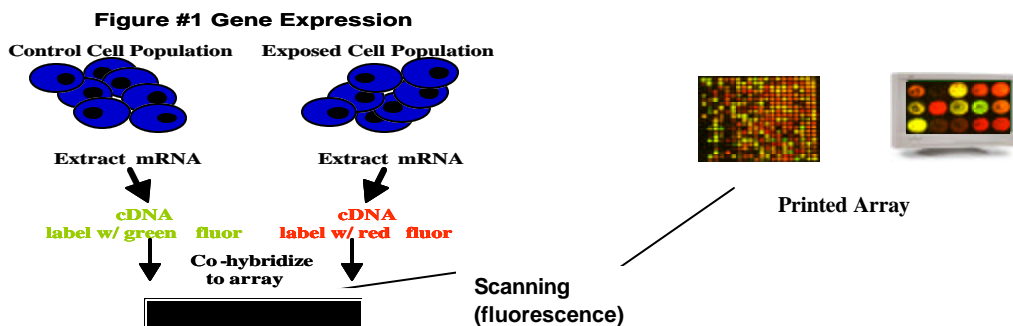
²Fred Hutchinson Cancer Research Center, Seattle, WA

Introduction: In recent years, toxicogenomics has developed into a sophisticated area of research focusing on the integration of the physical effects toxic compounds have on cells through toxicity and DNA adduct analyses, as well as their effect on cell processes through mutation and gene expression analyses. Among various environmental compounds, 4-aminobiphenyl and benzo[a]pyrene have been determined to be carcinogenic and have been isolated as candidates for toxicological studies. Due to the diversity of the information necessary, various analytical techniques can be employed in these studies. Commonly, microarray technology, protein assays, and liquid chromatography/mass spectrometry are useful methods for individual aspects, where afterward, data can be integrated to form a more complete understanding of the chemical and biological effects of these toxic compounds. In this study, human lymphoblast (TK 6) cell lines were dosed with the active forms of 4-aminobiphenyl and benzo[a]pyrene. By utilizing the aforementioned techniques, we have investigated the relationships between toxicity, gene expression, mutagenicity, and DNA adduct formation.

Methods:

Chemical Treatment, Toxicity and Mutation Analysis: Human lymphoblast (TK6) cells were treated with various concentrations of benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) and N-hydroxy-4-acetylamino-biphenyl(OH-AABP). Cells were extracted at 1, 9 and 27 hours after dosing. For toxicity and survival rate, cell counts were taken using a Coulter Counter and survival rate was calculated using a growth curve extrapolation. Mutation analyses were performed at the HPRT and TK loci. The cells were plated in the presence and absence of 6-thioguanine or trifluorothymidine. The results are reported as a mutant fraction, a ratio of the colony-forming efficiency in response to the addition of the compounds compared to the efficiency in their absence.

Gene Expression Profile Analysis: Gene expression analyses were performed on cells where chemical treatments produced a 5, 15 and 40% toxicity, which corresponded to the OH-AABP treated cells (time point: 1 hour) at 0.5, 1.0, and 10 μ M doses and the BPDE treated cells (time point: 1 hour) at 0.015, 0.030, and 0.12 μ M. From treated and untreated cells, mRNA was extracted and used to make cDNA through reverse transcription. The cDNA was then labeled with either Cy3-dUTP (untreated) or Cy5-DUTP (treated) fluorescent dyes. Labeled cDNA strands were then co-hybridized to the probe molecules in the microarray. The expression was determined as the mean ratio of fluorescence intensities relative to a reference sample. Figure #1 shows a schematic of the analysis.



DNA Adduct Analysis: DNA was isolated from OH-AABP treated cells (time point: 27 hours) at levels of 0.5, 1.0, and 10 μ M doses. Following enzymatic digestion to nucleosides, N-(deoxyguanosine-8-yl)-4-aminobiphenyl (dG-C8-ABP) adducts were enriched by C₁₈ solid phase extraction. Chromatographic

separations were performed using an Agilent 1100 Capillary HPLC and New Objective, 75µm ID PicoFrit column packed to 10.5cm with Aquasil C₁₈, 5 µm particles. Mass Spectral analyses were conducted on a TSQ 700 triple quadrupole mass spectrometer equipped with an in-house built interface. Figure #2 shows a schematic of the LC/MS system.

Figure #2: Schematic of Liquid Chromatography and Mass Spectrometry

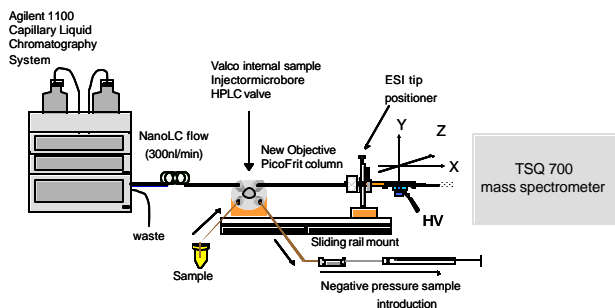
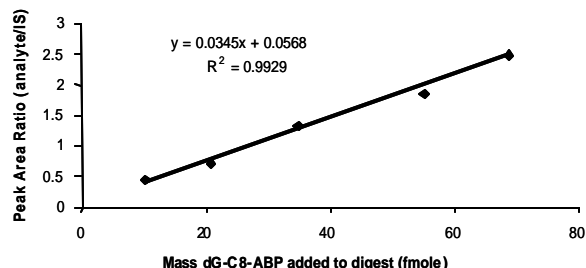


Figure #5: Standard Curve of dG-C8-ABP adduct
internal standard: dG-C8-ABP-d₉
(SRM 435→ 319 and 444→ 328)



Results:

Mutation and Toxicity: In the toxicity studies, BPDE was found to be 1000 fold more toxic than OH-AABP in TK6 cells and at the highest dose, OH-AABP cells exhibited no toxicity. Also, at similar levels of toxicity, mutation levels induced by the compounds at the HPRT and TK6 loci were consistent. Figure #3 shows the toxicity produced by the two compounds, while Figure #4 illustrates the mutation fraction at the HPRT loci.

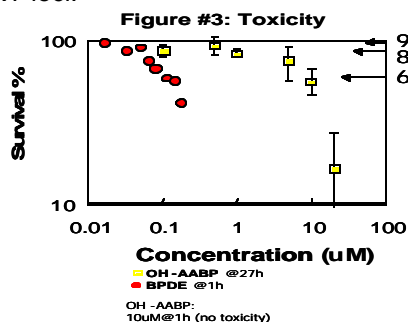
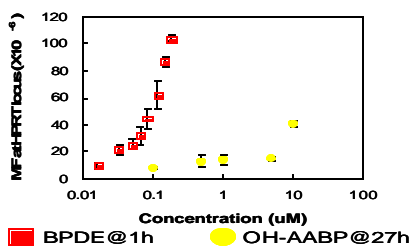


Figure #4: Mutation Fraction at HPRT locus



Gene Expression Analysis: Significant differences in gene expression were observed as a function of type of carcinogen and dose. Using the array readout and hierarchal clustering, differences in gene expression were isolated to specific genes. Some of the genes that had varied regulation in the treated cells are involved in biosynthesis, xenometabolic enzymes, membrane permeability, transcription and DNA repair and replication.

DNA Adduct Analysis: Using selected reaction monitoring of 435→319, the dG-C8-ABP adduct was analyzed. The standard curve was created for quantification of the adduct within the cells. The standard curve shows the analyte to internal standard ratio and the standard mass added to the digestion. The curve proved to be linear ($y=0.0345x+ 0.0568$) with an $r^2= 0.992$ (Figure #6). Analysis was then performed on the DNA isolated from the cells. The mass of adducts in 2.0×10^8 cells was determined to be 32 fmole (3.62 in 10^8 nucleosides), 612.6 fmole (68.2 in 10^8 nucleosides), and 1190.3 fmole (132 in 10^8 nucleosides) fmole for the 0.5, 1.0 and 10.0µM, respectively.

Conclusion: The analyses presented in this paper show three separate experiments that were integrated to show a complete roadmap of the toxic compound effects. A direct correlation is shown between carcinogen exposure, cellular toxicity, mutation fraction at the HPRT and TK loci genes, and DNA adduct formation. The exposure of cells to the two carcinogens leads to identifiable and specific differences in gene expression. Finally, specific changes in gene expression associated with DNA repair suggest a possible relationship between DNA adduct formation, mutation, and cellular toxicity. Continuing studies will further explore these relationships.