2723 Cytotoxicity of Peracetic Acid Vapor Exposures on Human Bronchial Epithelial Cells

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Peracetic acid (PAA) is a highly reactive peroxygen compound that is widely used as a disinfectant in healthcare settings and poultry processing plants. PAA is irritating and corrosive to the eyes, skin, and mucous membranes of the respiratory tract. Workers exposed to PAA vapors from the spraying and fogging of disinfectant solutions have reported symptoms such as headaches, lacrimation, coughing, wheezing, and blurred vision. Using a commercially available solution of PAA (32 weight % in dilute acetic acid), we aimed to identify the possible cytotoxicity associated with vapor exposures on normal human bronchial epithelial cells (NHBEs) *in vitro*, differentiated on porous, transwell membranes at the air-liquid interface. Using an in-house exposure chamber, NHBEs were exposed to filtered air (FA) for 1 hour (h), 2 h, and 4 h. This was to account for differences in temperature, relative humidity (RH) and carbon dioxide (CO_2) as compared to the cell culture incubator. Temperature in the chamber was 74.4 °F, RH was 90.4%, and CO_2 in ambient air was 0.04%. Using the water soluble tetrazolium assay, no significant differences in viability were detected between FA-exposed cells and incubator controls. Next, NHBEs were exposed to either FA (controls), 12 or 22 ppm of PAA for 4 h and then returned to incubators for an additional 4 or 24 h recovery period. Cellular viability, lactate dehydrogenase (LDH) production, cytokine production (IL-1β, IL-6, IL-8, and TNFα) and microscopic changes in cells were assessed at 4 and 24 h post exposures. Compared to FA controls, PAA (22 ppm) reduced cellular viability and increased LDH release, denoting acute cytotoxicity upon exposure at both time points. Cytokine production in FA-treated cells was not significantly different than PAA-treated cells, except for IL-6 at both time points. Microscopic changes were assessed using H&E staining of cells. Compared to controls at 24 h, PAA caused cell necrosis and loss of cilia. Our studies show that exposure of NHBE cells to PAA causes significant changes in viability, LDH production, IL-6 production and microscopic abnormalities when compared to FA. Further studies are needed to delineate the exact mechanism(s) by which this occurs.

2724 Development of a Highly Accurate Genome Sequencing Method and Its Application in the Genome-Wide Analysis of Chemical Mutation Signatures

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Direct detection of rare and mutagen-induced *mutations* using a genome sequencer will facilitate a more accurate understanding of the mutational profile of mutagens and their relationships with cancer. However, this is difficult due to a higher frequency of errors associated with the existing genome sequencers. Thus, we developed a highly accurate genome-sequencing method utilizing double-strand DNA sequence and analyzed genome-wide mutations caused by exposure to chemicals. We optimized our sequencing method and achieved high accuracy with an error frequency of less than one per 10^7 -10^8 bp. We analyzed DNA samples of mutagen (i.e., *N*-methyl-*N*-nitrosourea (MNU), *N*-ethyl-*N*-nitrosourea (ENU), diethylnitrosamine (DEN), benzo[a] pyrene (B[a]P), and aristolochic acid (AA))-exposed *Salmonella* typhimurium TA100 cells and *gpt* delta mice, and obtained large-scale genome-wide somatic mutation data. In *gpt* delta mice, approximately 2500-63000 mutations per material were detected depending on the materials. Meanwhile, in *S. typhimurium*, approximately 1300-95000 mutations per material were detected. The six-type mutational spectra showed clear mutation patterns that represented the action mechanisms of each mutagen (e.g., G:C > A:T for MNU). In ENU or AA-exposed samples, significantly different mutational patterns were obtained from *S. typhimurium* and *gpt* delta mice, which suggested the differences in mutagenesis between these species. In MNU-exposed, but not ENU- or DEN-exposed *gpt* delta mice, the 96-trinucleotide mutation signature analysis revealed patterns similar to a known signature of an alkylating agent in human cancer (i.e. signature 11 in the COSMIC database). Furthermore, the signatures for ENU and DEN exhibited a high similarity with each other, suggesting that these mutagens have a common mutagenic mechanism. These results indicated that our sequencing method is useful for precisely characterizing chemical mutagenicity and establishing the link between mutagens and cancer.

2725 Development of a Novel Genome-Wide Mutagenicity Assay Based on Highly Accurate Genome Sequencing: A Case Study Using *Salmonella typhimurium* TA100

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We developed a highly accurate genome sequencing method that comprehensively detects rare mutagen-induced mutations. To evaluate its applicability and utility, we analyzed DNA samples of *Salmonella* typhimurium TA100 exposed to mutagens. We evaluated 12 mutagens of various structural groups, including those requiring metabolic activation (*i.e.* alkylating agents, aldehydes, aromatic amines, and polycyclic aromatic hydrocarbons (PAHs)), and assessed the detection limit of mutation frequency and the ability to analyze various mutation patterns. Our method detected mutations caused by all 12 mutagens, including those with low mutation frequency such as aldehydes (c.a. 7-fold increase compared to the control), which induced nearly 2-fold increase in the number of His^+ revertants compared to control in Ames test. Although PAH-induced mutations were detected, the sensitivity was not sufficient to assess mutagenic mechanisms. TA100 may not have been sufficiently exposed to these mutagens in the liquid culture due to their extremely low water solubility. Therefore, we modified the exposure conditions for these chemicals. For this, TA100 was allowed to form His^+ revertants on agar plates post mutagenic exposure. Then, genomic DNA was extracted from these cells for mutational analyses. Thereby, 7,12- dimethylbenz[a]anthracene- and 3-methylcholanthrene-induced mutations on the major spectrum (G:C > T:A) increased by ~5.4 and 4.1 times relative to liquid culture samples. Therefore, our method can sensitively detect most TA100-positive mutagens. Furthermore, mutagens from the same structural group exhibited similar mutation spectra (*e.g.* G:C > A:T for alkylating agents), which reflected the mutagenic mechanism of each group. Next, we evaluated the 96-trinucleotide mutational signatures using the obtained mutational data. Signatures of the alkylating agents (*e.g. N*-methyl-*N*-nitrosourea) were similar. Similarly, those of aldehydes, aromatic amines, and PAHs were similar to each other (broad spectra of G:C > T:A). These signatures were also similar to known mutational signatures of alkylating agents and tobacco in human cancer, respectively (*i.e.*, signature 11 and 4 in COSMIC database). Our method provides detailed data on mutagen-induced mutations and promotes understanding of their mechanism of action and relationship with human cancer.

2726 Development of an Intracellular Microtubule Stability Assay Using GFP-α-Tubulin

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Aneuploidy, the presence of an abnormal number of chromosomes, is often observed in cancer and several classes of chemicals are known to cause aneuploidy. To detect aneugenicity as result of chemical exposure, typically the micronucleus assay is used. However, both broken DNA fragments caused by clastogenic agents and mis-segregated chromosomes caused by aneugenic agents can lead to the formation of micronuclei. To confirm an aneugenic mode of action, a laborious centromere or FISH staining is needed to assess whether broken chromosomes or complete chromosomes are present in the micronucleus. Aneugenicity can be caused by any process that interferes with chromosome segregation during mitosis, including microtubule disruption or inhibition of cell cycle kinases like Aurora A/B. Here we established an assay to study the effect of substances on tubulin stability, to provide more insight into the cause of aneuploidy upon substance exposure. Several *in vitro* and cellular assays are available to measure tubulin stability. However, most of these assays are dependent on a tubulin labelling step on fixed cells. Here we developed an assay using GFP-tubulin, allowing the direct visualisation of microtubuli, using either live cell microscopy to follow the dynamics during the cell cycle or flow cytometry. First a GFP-Tubulin reporter was stably integrated into mouse embryonic stem cells. We confirmed that the GFP-tubulin was properly integrated in microtubuli. To measure the effect of microtubule disrupting substances, cells were treated with various tubulin poisons and the amount of GFP-tubulin signal in microtubules was quantified using flow cytometry. A DNA staining was included to assess the effect of the agent on the cell cycle distribution. In the assay, both tubulin stabilising and tubulin destabilising substances could be detected. Treatment with both types of substances resulted in an accumulation of cells in G2/M phase. Treatment with agents that affect cell cycle progression but not microtubule stability, such as DNA damaging agents or Aurora kinase inhibitors, did not affect tubulin stability. In conclusion, we developed a microtubule stability assay in mouse embryonic stem cells that can efficiently detect both stabilising and destabilising microtubule disruptors and can be used to detect an aneugenic mode-of-action of genotoxic compounds.

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the 59th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 15–19, 2020.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 542.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 580.

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