In vitro mutagenicity and genotoxicity study of PAHs and nitro-PAHs using the bacterial revertant (Ames) test and alkaline single cell gel electrophoresis (Comet) assay

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In previous studies, we demonstrated that ambient PM collected from urban site of Korea air could induce DNA damage. Various mutagens and carcinogens present in the urban air differ according to the source of the pollutants. Polycyclic aromatic hydrocarbons (PAHs) and their nitrated compound are produced in the combustion of fossil fuels as diesel emission exhausts. In recent, PAH and nitro-PAH have been identified in urban air particulate matter (PM), and some of them were found to be tumorigenic in experimental animals and humans. Detecting of DNA damage in cells exposed to genotoxic agents is being used to assess the carcinogenic potential of environmental agents. In this study, we examined mutagenicity and genotoxicity of PAH and nito-PAH contained in PM using Ames test and Comet assay with presence or absence of an exogenous metabolic activation system (S9 mixture). Ames test. Salmonella mutagenicity test was conducted using TA98 and TA100 and comet assay, which is the technique for measuring DNA-damage was conducted in human placcial alveolar epithelial (A549) cells. From the results, among 14 PAHs and nitro PAHs tested, most of PAH and nitro PAH tested showed DNA mutation in Ames test and comet assay. It showed that comet assay in vitro was more sensitive than Ames test. Therefore, we suggested that the comet assay in vitro is a useful, sensitive, fast screening system in mammalian cells that can be used as a test to identify genotoxicity of mutagens and environmental complex samples.

Poster Presentations – Field A4. Toxicology

Comparative Studies on the Detection of Drug-Toxic Substances in the Formalin Fixed and Unfixed Tissue Specimens

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Gastric contents and blood samples are generally analyzed for the detection of the Drug-toxic substances(DTS) in the postmortem specimens, but tissue specimens from postmortem for the detection of the DTS are especially, required in the cases that analysis of DTS in blood or gastric contents is impossible because of insufficient or inaccessible specimens in special cases. Generally, the tissues are fixed in the formalin, so that the detection of the toxic substances from them is not popularly performed due to its unvalidated accuracy. Therefore, we performed this comparative study for the detection of the DTS in the formalin fixed and unfixed tissue specimens. The tissue specimens(liver, spleen, heart, lung and kidney) were obtained from the postmortem by autopsy, and fixed with formalin. And we analyzed the amount of the DTS from the formalin fixed tissues, on that day, and after 1, 3, and 7 days, as well as from the formalin unfixed tissues. This study can give us the helpful data for the detection of some DTS in formalin fixed specimen in future.

Development of rapid, sensitive and reproducible paraquat analysis method in the postmortem
Paraquat (methyl viologen) is a bis-quaternary ammonium compound as a wide range herbicide, which was first introduced as an oxidation-reduction indicator dye. When paraquat (fatal dose, 1–2g) was administered to men, the oxido-reduction system of cell was repeatedly acted to perpetuate the cell membrane system. Many death cases had been occurred after ingestion of paraquat around Daion area for the last six months. Therefore, development of more rapid, simpler, and more sensitive paraquat detection method in biological specimens than the conventional methods was indispensable. The most important step of paraquat analysis in the biological specimens is its extraction from the specimens because paraquat is very insoluble in organic solvent due to its strong polar property. As a most common extraction method, solid phase extraction (SPE) has been used for paraquat extraction from biological specimen. However, SPE procedures were somewhat time-consuming and resulted unsatisfactory recovery in our laboratory. We developed simple, sensitive and reproducible Liquid-Extraction method of high recovery for paraquat in the biological specimens. A 0.5 mL of blood was extracted with 0.5 mL of chloroform-ethanol 7:3 mixture solvent. After centrifugation at 13000 rpm for 3 minutes, the ethanol layer (upper layer) was directly injected into HPLC. For qualitative test the ethanol layer was evaporated and the residue was color tested by adding Na₂S₂O₄ and ammonium water. The recoveries of paraquat in 6 blood samples which were already spiked with paraquat standard in this method were average 102% but recovery in SPE was about 80%. Linearity in the range of 1.05–67.3 μg/mL was obtained with correlation coefficient (r² > 0.999). Limit of detection (LOD, with S/N ≥ 3) and limit of quantitation (LOQ, with S/N ≥ 10) were 0.5 μg/mL and 1.0 μg/mL, respectively. Seven postmortem specimens, bloods, were analyzed by this validated LLE method for paraquat determination. The concentration ranges was from 1.5 μg/mL to 335.9 μg/mL. The published paraquat concentrations from bloods of 32 fatalities were in the ranges of 0–60 μg/mL. This LLE extraction method was much time saved and recovery, sensitivity, and reproducibility were significantly improved when compared to those of SPE.

Simultaneous Determination of Underivatized Diazepam and Nordiazepam in Plasma Using Gas Chromatography/Mass Spectrometry.

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Diazepam (DZ) is one of the most frequently prescribed drugs as an antianxiety agent, muscle relaxant, and anticonvulsant and sometimes causes intoxication due to accidental overdose, misuse or abuse. DZ is metabolized to nordiazepam (NDZ, desmethyldiazepam), oxazepam (OX) and temazepam (TM) which are also pharmacologically active, although OX and TM do not accumulate in blood or plasma to an appreciable extent. Screening or confirmation methods for DZ and NDZ in plasma are very important for clinical and toxicological studies and in forensic cases.

To human thawed plasma was added internal standard solution and various amounts of DZ and NDZ. Plasma samples were adjusted to pH 9 and were extracted with ethyl acetate. GC/MS analysis was performed using an Agilent MSD 5973 mass spectrometer and the column was a DB-5MS. The detection limit was 0.5 ng/mL and the assay was sensitive to 1 ng/mL and linear to 500 ng/mL with correlation coefficients of >0.99 for both DZ and NDZ. The recoveries of DZ and NDZ were 89.6 % and 88.4 %. This sensitive and simple method is useful for plasma samples of forensic toxicological interest and in clinical studies when low concentrations of DZ are to be detected. Preliminary studies extended this approach to additional benzodiazepines. The results suggested that sensitive assay methods that do not require derivatization can be developed for midazolam, prazepam and flurazepam. The method appeared to be less well suited for the development of methods for lower concentration of oxazepam, temazepam, lorazepam, flunitrazepam, alprazolam and triazolam.