INTERACTIONS BETWEEN RELEVANT NON-DIOXIN LIKE TOXINS AND DIOXIN-LIKE TOXINS IN H4IIE RAT HEPATOMA CELLS BY SELDI-TOF PEPTIDOMIC SCREENING.

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Introduction

Historical incidents of dioxin contamination of the human food chain usually involve complex mixtures of dioxin-like and non dioxin-like chemicals produced by combustion events or from PCB-oils. [1, 2, 3, 4, 5.] Many of these compounds have distinct toxic modes of action and the global response of an organism to these multiple toxic challenges is of importance to understanding the risk posed by dioxins to human and animal health.[6, 7] Furthermore, elucidation of the biochemical responses to these toxins could enable the identification of serum or urine biomarkers which could be used to detect dioxin exposure without the need for slaughter and analysis of fat for dioxin content.[8,9,10]

Materials and Methods

Three distinct toxic compound classes, typically co-present during dioxin contamination events, were investigated in this study: dioxin, ortho-PCBs and polyaromatic hydrocarbons (PAHs). Prototypical compounds from each class (2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), PCB-52 and benzo-a-pyrene (BaP) respectively) were applied in media to near-confluent flasks of hepatoma H4IIE cells overnight, both individually and in each possible combination at the following concentrations: 0.2 μM 2,3,7,8-TCDD, 1.5 μM PCB-52, and 2 mM BaP.

Post-treatment cell lysates were obtained and applied to CM10 ProteinChip surfaces and analysed using Surface Enhanced Laser Desorption/Ionisation-Time of Flight Mass Spectroscopy (SELDI-TOF) on a Ciphergen Biosystems ProteinChip SELDI-TOF instrument. Each cell treatment regime was analysed in 12 replicate spots using sinapinic acid as the energy absorbing molecule and peak spectra produced indicating the relative abundance of peptides and proteins in respective lysates (MW <10KD).

The resulting spectra were baseline corrected and normalised using the dedicated ProteinChip software, peak information was then exported to the SIMCA-P+ Statistical software package (Umetrics) and subjected to principle component analysis and partial least squares-discriminant analysis.

Results and Discussion

The treatment of the SELDI spectral data by multivariate statistical analysis as described above demonstrated some relationships between the treatment groups. Clear separation was noted between 2 treatment groups - Cells exposed to a combination of 2,3,7,8-TCDD and PCB-52 and cells exposed jointly to 2,3,7,8-TCDD and BaP were separated clearly from each other and also distinguishable from the control group and those treated with the individual

chemicals in the PCA and PLS-DA scores plots obtained. The loading scores plot identified the peak clusters of importance and evidence of synergistic effects in their response to the compounds was apparent. For example peak cluster 39, with a M\Z ratio of 8465, was found to have an intensity of 7.85 +/- 0.41 (SEM, n=7) in the control group, 10.5 +/- 0.52 (SEM, n=12) in the PCB-52 group and 6.29 +/- 0.39 (SEM, n=12) in the 2,3,7,8-TCDD + PCB-52 group, indicating a complex relationship between the modes of action of these compounds on this peptide produced by H4IIE. A number of statistically significant peak differences are noted between the spectra of each group. The data suggests that the presence of 2,3,7,8-TCDD may have an exacerbating effect upon the action of these other toxic substances which may cast important light on the biochemical response to mixtures of these substances in real exposure scenarios.

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