A140 Utilizing Metabolomics Toward Time-Dependent Metabolite Monitoring in Different Postmortem Specimens During Human Decomposition

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Learning Overview: After attending this presentation, attendees will have a better understanding of how metabolomics of postmortem specimens provide additional knowledge in comparing and contrasting human decomposition processes.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by revealing novel aspects of postmortem metabolomics addressing intra- and inter-human metabolic differences in maggots, decomposition fluid, and soil in proximity to human donors across the early and late stages of decomposition.

The ultimate goal of this ongoing study is to reveal drug impacts on human decomposition and the Postmortem Interval (PMI). This presentation examines how metabolomic analyses provide insights into the highly dynamic postmortem biochemical environment. As small-molecule intermediates of human metabolism, metabolites have a multitude of functions, not only in the living human body, but also after death; however, the complexity of factors affecting human decomposition opens up a large, mostly still-unknown area yielding continuous new questions about postmortem metabolic patterns, including molecular signatures or biomarker discovery.

This research is conducted on human donors, obtained through the Body Donation Program of the Forensic Anthropology Center (FAC), in different microenvironments around the three-acre outdoor laboratory known as the Anthropology Research Facility (ARF) in Knoxville, TN. Samples of soil, maggots, and body fluid were obtained and analyzed for metabolites. Soil (10g) obtained from within the Cadaver Decomposition Island (CDI) from ten enrolled donors and control soil (10g), located at least 1m from the donor, were homogenized, flash frozen, and stored in liquid nitrogen until subsequent metabolomic profiling. Decomposition fluid from the body was collected at various anatomical positions around the body. Hourly records of temperature and relative humidity allowed for the calculation of Accumulated Degree Hours (ADH), a metric that combines temperature and time, to determine specific sampling points for soil and decomposition fluid. Maggots were collected at each instar as well as while migrating from the body. Termination of sampling occurred after the body completed active decay as determined by cessation of secretion of body fluid from the trunk.

Metabolites were extracted from 70mg–80mg soil, 30–50mg maggots, and 70–80mg decomposition fluid using a procedure adapted from Lu et al.[1] Analyses were performed by Ultra High-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC-HRMS) operating in negative Electrospray Ionization (ESI) with a 2.5 micron reverse-phase Hydro-RP 100, 100mm x 2.00mm Phenomenex® Liquid Chromatography (LC) column. The Exactive™ Plus Orbitrap™ Mass Spectrometer was run in full scan mode with a scan window from 80–1,200m/z.[1] A flow rate of 0.2mL/min was maintained throughout the analysis. On average, 90–120 identified and 1,000s of unidentified metabolites were manually selected based on mass accuracy (± 5ppm mass tolerance) and retention times (≤ 2min) using an in-house generated metabolite database.

Donor-, location-, and time-related differences in metabolite signatures were statistically evaluated via Partial Least Squares Discriminant Analyses (PLSDA). Initial results indicate that not only does every cadaver show a unique metabolic signature, but also that each CDI soil is metabolically different from the control soil. Each donor had an individual, time-dependent metabolome based on every sampled ADH throughout the monitored decomposition period. Analyzed maggots and decomposition fluid samples show similar donor- and time-dependent trends, whereas body location seems to impact metabolic patterns.

The current results provide an initial impression of the complexity of the temporal metabolic changes occurring after death in different matrices. Ongoing analyses focus on the identification of possible biochemical biomarkers for decomposition. It is anticipated that with further progression of this study and future toxicological analyses, this study will be able to relate postmortem metabolite signatures to certain peri-mortem drug treatments and answer questions concerning drug-induced decomposition alterations.

Reference(s):

1. Lu, Wenyun, Michelle F. Clasquin, Eugene Melamud, Daniel Amador-Noguez, Amy A. Caudy, and Joshua D. Rabinowitz. Metabolomic analysis via reversed-phase ion-pairing liquid chromatography coupled to a stand-alone orbitrap mass spectrometer. *Analytical chemistry* 82, no. 8 (2010): 3212-3221.

Forensic Chemistry, Postmortem Metabolomics, Biomarker Discovery

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