Spleen Blood as an Alternative Specimen to Peripheral Blood for Postmortem Toxicological Analysis

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Disclosures

• Kevin Shanks, MS and George Behonick, PhD employed by Axis Forensic Toxicology

Outline

• Background
• Methods
• Results
• Conclusions
Ideal Matrix for Postmortem Toxicologic Analysis

- Widely available reference data
- Easily reproducible collection method
- Less prone to postmortem drug redistribution
- Less complex matrix
  - Minimal sample preparation and pretreatment prior to analysis

- Current specimen of choice for most postmortem toxicological analyses: Peripheral Blood
- However, peripheral blood is a limited resource

Spleen: Reservoir of Peripheral Blood Components

- Blood flows in series through the white pulp and red pulp
- Capillaries enter the open circulation of the reticular meshwork before draining into the venous system
- Abnormal red blood cells are retained by the reticular meshwork
- This causes changes in flow, resulting in the concentration of cellular components within the spleen
- The blood-filled space is a large fraction of the total spleen volume
- Potential source of blood for toxicologic analysis

Spleen Blood

- Spleen tissue has long been used for postmortem toxicological analysis
- However, processing solid tissue for postmortem forensic toxicology requires:
  - Tissue homogenization
  - Hydrolysis or enzymatic digestion
- Blood has the advantage of being a less complex matrix than solid tissue
- The ability to isolate large volumes of non-viscous blood from the spleen prior to toxicologic analysis would:
  - Maximize the volume of fluid available for analysis
  - Limit the loss of analytes due to manipulation and processing
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Specimen Collection and Handling

• Specimens collected prospectively at time of autopsy
• 15 cases based on a high suspicion for drug overdose
• Spleens were removed and manually compressed to collect blood
• Simultaneously, peripheral venous blood and vitreous fluid were collected
• All specimens stored at 2-8°C
Analytical Toxicology Methods

- Volatile testing (alcohol, methanol, isopropanol, acetone) accomplished by headspace gas chromatography-flame ionization detection
- Presumptive testing/screening for opiates, oxycodone, cannabinoids, and barbiturates by Enzyme Linked Immunosorbent Assay (ELISA)
- Comprehensive blood screening for prescription/therapeutic agents and illicit drugs conducted by LC-MS
  - Specific LC/MS/MS methods for drugs or drug classes were employed to confirm presumptively screened positive results

Samples were analyzed for the presence of:

- Amphetamines
- Analgesics
- Anesthetics
- Anticonvulsants
- Antidepressants
- Antihistamines
- Antipsychotics
- Barbiturates
- Benzodiazepines
- Cannabinoids
- Cardiovascular Agents
- Cocaine
- Endocrine Agents
- Ethanol
- Fentanyl
- Gastroenterology Agents
- Methadone
- Narcotics
- Neurology Agents
- Opiates
- Phencyclidine
- Propoxyphene
- Sedatives/Hypnotics
- Tramadol
- Stimulants
- Benzodiazepines
- Cannabinoids
- Cardiovascular Agents
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Drug Classes with Positive Results:

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To Compare Data:

- Postmortem distribution coefficient calculated as:
  - spleen blood concentration / peripheral blood concentration
  - spleen blood concentration / vitreous humor concentration
  - peripheral blood concentration / vitreous humor concentration

- Data expressed as mean ± SEM

Amphetamines

![Bar graph showing postmortem distribution coefficient for amphetamines and methamphetamine with error bars for n=3 and n=4]
Conclusions

- This study is the first, to our knowledge, to directly compare drug concentrations between spleen blood and peripheral blood
- Manual compression method:
  - Easily reproducible
  - Results in sufficient quantities of blood
  - Drugs across a wide spectrum of drug classes can be quantitated
- Limitations of manual compression method:
  - Specimen contamination from surrounding sites
  - Susceptible to postmortem drug redistribution
- Further research is necessary to validate the use of spleen blood as an alternative/complementary matrix for postmortem toxicologic analysis

References
