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

Ashley Lukefahr, MD[1];
Wendy MacKerricher, MD[1];
Kevin Shanks, MS[2];
George Behonick, PhD[2];
David Winston, MD, PhD[1,3]

[1]The University of Arizona, Department of Pathology
[2]Axis Forensic Toxicology
[3]Pima County Office of the Medical Examiner-Forensic Science Center

Disclosures

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

Outline

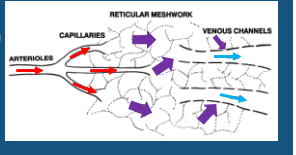
- **Background**
- Methods
- Results
- Conclusions

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

Outline

- Background
- **Methods**
- Results
- Conclusions

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

Manual Compression Method of Spleen Blood Extraction

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

Outline

- Background
- Methods
- **Results**
- Conclusions

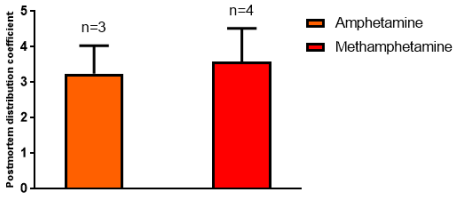
Drug Classes with Positive Results:

- 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

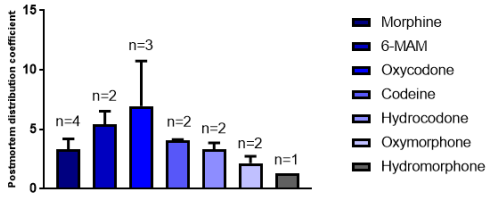
To Compare Data:

- 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

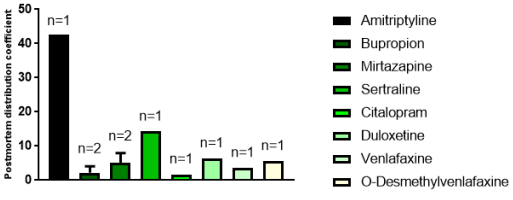
Amphetamines



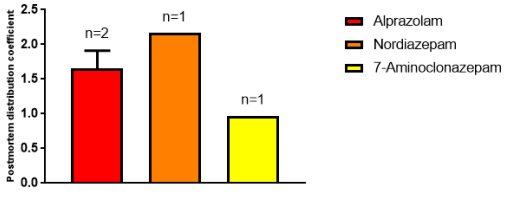
Opiates



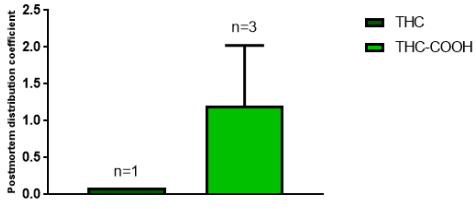
Antidepressants



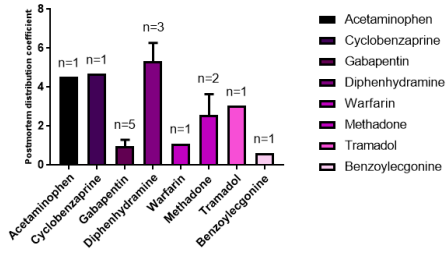
Benzodiazepines



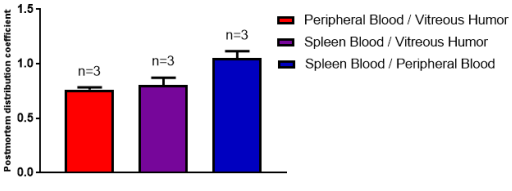
Cannabinoids



Miscellaneous



Ethanol



Outline

- Background
- Methods
- Results
- **Conclusions**

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

- Bergman RA, Heidger PM, Scott-Conner CEH. The Anatomy of the Spleen. In: Bowdler AJ, editor. The Complete Spleen: Structure, Function, and Clinical Disorders. 2 ed. Totowa, NJ: Humana Press; 2002.
- Kerrigan S (2008) Sampling, storage and stability. In S Jickells and A Negrusz (Eds.). Clarke's Analytical Forensic Toxicology (2nd ed., pp 335-356). London:Pharmaceutical.
- Pélissier-Alicot AL, Gaulier JM, Champsaur P, Marguet P. (2003). Mechanisms underlying postmortem redistribution of drugs: a review. J Anal Toxicol 27(8): 533-44.

Thank You