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Associate Professor
Department of Chemistry and Biochemistry
State Univ. of New York College at Oneonta

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“Chemistry of Death”

“A Review of Postmortem Redistribution in Forensic Toxicology”

Lucas Zarwell, MFS, D-ABFT-FT
Chief Toxicologist
Office of the Chief Medical Examiner
Washington, DC
Postmortem Redistribution

Drug movements within the body after death which cause time-dependent variations in blood and tissue drug concentrations prior to autopsy

Who Cares?

• Medical Examiners may depend on toxicology results to help determine the cause and manner of death

• PMR (Postmortem Redistribution) may be misleading, attributing high drug concentrations with a toxic effect
Our Goal

To understand postmortem redistribution in terms of chemistry, pharmacology, and forensic interpretation

Please Ask Questions
Case Example

55 year old male is found deceased in bed in a secure residence. There is an antidepressant medication on scene next to the bed including the tricyclic antidepressant **imipramine**. In addition there is 1/2 full bottle of **wine** on the floor. At autopsy, the medical examiner can find no immediate anatomical cause of death. The medical examiner submits venous blood, heart blood, vitreous humor and liver to the forensic toxicologist for analysis.
Ethanol

Let's Get Started

Discuss the major contributing elements to PMR

- Drug Chemistry ->
- Drug Pharmacokinetics ->
- Distribution Mechanisms ->
Drug Chemistry

- Acid / Base Properties (pKa)
- Lipophilicity
- Size and Structure

pKa

- What is pKa?
  - It is derived from Ka which is the equilibrium constant for the chemical reaction known as dissociation in the context of acid-base reactions
    - pKa = - log_{10} K_a
  - K_a is a quantitative measure of the strength of an acid in solution
pKa

- pKa is used in practice to avoid the many orders of magnitude spanned by Ka
- The value can be assigned to both acids and bases
- Essentially: the smaller the pKa the stronger the acid, the higher the pKa, the stronger the base
- Thus one can determine the degree of ionization at a given pH

What?

- When we substitute these elements (pH and pKa) into the Henderson-Hasselbalch equation we can mathematically determine how much of a drug is ionized at a biological pH
- The ionization of drug molecules is important with regard to their adsorption into the circulation and their distribution to different tissues.
- It's also handy when you are trying to extract on the bench!
Ok, What about our case?

The pKa of Imipramine: 9.5

The pKa of Ethanol is 15.9

The approximate *antemortem* pH of the small intestine is 6

---

Audience Survey Question

How much imipramine is ionized?

- 30%
- 50%
- 75%
- 90.1%
- 99.9%
The nonionized form of the drug tends to be more lipid soluble.

Normal biological pH is about 7.4

Imipramine is highly lipid soluble
**Size and Structure**

Imipramine:
280 Da
Formula: $C_{19}H_{24}N_2$

Calciseptine (mamba venom):
7000 Da
Formula: $C_{299}H_{476}N_{90}O_{87}S_{10}$
Drug Pharmacokinetics

- Drug Chemistry
- Protein Binding
- Volume of Distribution
- Storage Depots

Drug Chemistry

Passive Transport
Filtration (think Kidney)
Active Transport
Facilitated Diffusion
**Protein Binding**

Drug Chemistry effects drugs ability to bind with plasma proteins and tissues in the blood

- Albumin attracts acidic drugs
- α1-acid glycoprotein attracts basic drugs

---

**Volume of Distribution**

Drug chemistry effects the Volume of Distribution ($V_d$)
Volume of Distribution

$V_d$ is determined experimentally

\[ V_d = \frac{A_p}{C_p} \]

Imipramine has a $V_d$ of 20-40 L/kg

Ethanol has a $V_d$ of <1 L/kg

Storage Depots
Audience Survey Question

Storage Deposits: What is a good specimen to measure lead?

- Liver
- Brain
- Bone
- Hair
- Tongue
These are elements of biochemistry which influence drug blood and tissue concentrations whether an individual is alive or dead....

Dead

- Digestion stops
- Metabolism stops
- Blood flow stops
- Breathing stops
- Decomposition starts
Cell Death

- Aerobic respiration stops
- Oxygen is no longer provided (hypoxia)
- In the mitochondria, oxygen was the final electron receptor of the electron transport system responsible for the synthesis of ATP from NADH
- Thus we no longer have ATP to run cellular operations - and cellular death begins

Audience Survey Question

Cell Death: A decrease in cellular pH is caused by?

- Water moving out of the cell into the surrounding vessels
- Anaerobic glycolysis
- Mitochondrial damage and enzyme activation
- Tiny lemons floating in the intracellular space
Cell Death

- Build up of lactic acid result in decreases in intercellular pH
- Na begins to build up in the cell (ATPase pump has failed)
- Water is osmotically pulled into the cell AND increasing catabolites add the intracellular osmotic load
- Leads to cellular dilation, disruption and lysosomal membrane disruption
- Lysosomal enzymes leak out, become active, and digest cell components and membranes
**Distribution Mechanisms**

- “Micro” Redistribution
- Acidification
- Passive Diffusion
- Blood Coagulation and Hypostasis
- Postmortem “Circulation”
- Putrefaction

**Micro Redistribution**

- Enzymes, proteases, phosphatases, glucosidases all leak into the cytoplasm - further breaking down cellular components
- Macromolecules, proteins, and the drugs bound to them (or detached) drift out into the extracellular space
- This tends to be higher in tissues rich in enzymes (pancreas and gastric mucosa) and slower in the heart, liver, and kidney
Acidification

- Contents of a cell become more acidified after death
- After a cell lyses, progressively ionized drugs will distribute more readily as a result of being transported in the acidic fluid in which they are dissolved

Redistribution

Time Zero

<table>
<thead>
<tr>
<th>Time Zero</th>
<th>Blood</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Redistribution

4 Hours Later

Passive Diffusion

Organs which are close to the heart and major blood vessels

- Liver (Left Lobe)
- Stomach / Esophagus
- Adipose Tissue
- Small Intestine (Duodenum)
- Lungs
- Myocardium
Passive Diffusion

Temperature can effect this
Concentration can effect this

Blood coagulation and hypostasis (livor)

- Blood sediments and clots unevenly after death
- This is due to blood clotting and cell lysis happening simultaneously
- As hours pass, hypostasis occurs when the blood sediments and serum flow, according to gravity, to the lower parts of the body
- Drugs follow according to their respective chemistries
Blood coagulation and hypostasis (livor)

Body Position

- It’s been demonstrated that body position may influence PMR
- Repositioning of a body after death may also influence movement of the blood postmortem
- “New” blood sources may pool near tissues and allow more diffusion to occur
Putrefaction

- Bacteria and microflora can effect drug concentrations and must be considered.
- Bacteria can migrate across the intestinal wall to blood vessels and lymph vessels.
- Enteric Bacteria can metabolize drugs and produce ethanol (as well as yeast).
- Effect can be decreased in cooler temperatures.

“Postmortem Circulation”

- Rigor mortis can cause blood movement by causing systolic pressure through ventricular contractions.
- Putrefactive processes in the abdomen can move blood due to gas swelling.
- These are not strong processes.
Ok, What can we do?

- Store and Obtain Autopsy Specimens Properly
- Understand the Limitations of Interpretation
- Study and Review Reference Literature

Environmental Conditions

- Storing decedents between 2-8 C prior to autopsy
  - Slows redistribution
  - Slows putrefaction
  - Conversely, warmer temperatures have the opposite effect
Obtain proper autopsy specimens

- Take blood and tissue from specific sites during autopsy
  - Central Blood (Heart, Subclavian)
  - Peripheral Blood (Inferior Vena Cava)
  - Vitreous Humor
  - Tissue (Liver, Brain)
Venous Blood Collection

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)

Heart Blood Collection

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)
Liver Collection

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)

Vitreous Humor Collection

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)
Testing Alternative Tissues

- Lung
- CSF
- Bone Marrow
- Skeletal Muscle

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)

Literature Ratios

- Central (Heart) / Peripheral (Venous)
- Peripheral (Venous) / Tissue (Liver)
Literature Ratios

- Cocaine 1.3
- Imipramine 1.8
- Doxepin 5.5
- Zolpidem 2.1

Reference Books


Examples of Drugs which exhibit distribution – reference literature

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
<th>$V_d$ (L/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxin</td>
<td>Treats atrial fibrillation</td>
<td>5.1-7.4</td>
<td>Vorphal, 1978</td>
</tr>
<tr>
<td>Morphine</td>
<td>Analgesic</td>
<td>2-5</td>
<td>Logan, 1993</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Tricyclic Antidepressant</td>
<td>6-10</td>
<td>Hebb, 1982</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Tricyclic Antidepressant</td>
<td>20-40</td>
<td>Jones, 1987</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Drinking Alcohol</td>
<td>0.43-0.59</td>
<td>Prouty, 1987</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>Antihistamine</td>
<td>3-14</td>
<td>Hargrove, 2008</td>
</tr>
</tbody>
</table>

Our Case

- What does our analysis show us?
- Show what the reference literature said (basalt and article)
- Understand the clear differences
### Case – Scenario 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.08</td>
<td>14</td>
</tr>
<tr>
<td>IVC</td>
<td>0.09</td>
<td>4</td>
</tr>
<tr>
<td>Liver</td>
<td>0.06 (g/100g)</td>
<td>61 (mg/kg)</td>
</tr>
<tr>
<td>Vitreous Humor</td>
<td>0.11</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Imipramine c/p = 3.5

### Case – Scenario 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.02</td>
<td>2</td>
</tr>
<tr>
<td>IVC</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>Liver</td>
<td>0.005 (g/100g)</td>
<td>18 (mg/kg)</td>
</tr>
<tr>
<td>Vitreous Humor</td>
<td>0.03</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Imipramine c/p = 2.5
### Therapeutic

<table>
<thead>
<tr>
<th>Reference Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>N/A</td>
<td>0.05-0.10</td>
</tr>
</tbody>
</table>

### Intoxication Fatality

<table>
<thead>
<tr>
<th>Reference Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.42-1.77</td>
<td>6-8.5</td>
</tr>
<tr>
<td>Liver</td>
<td>0.25-1.16</td>
<td>33-381</td>
</tr>
</tbody>
</table>

### Natural Postmortem

<table>
<thead>
<tr>
<th>Reference Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.02-0.50</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Liver</td>
<td>0.01-0.35</td>
<td>13</td>
</tr>
</tbody>
</table>
## Comparison

<table>
<thead>
<tr>
<th>Drug</th>
<th>Imipramine IVC Blood</th>
<th>Imipramine Liver</th>
<th>Ethanol Blood</th>
<th>Ethanol Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapeutic</td>
<td>0.05-0.10</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Intoxication</td>
<td>6-8.5</td>
<td>33-381</td>
<td>0.42-1.77</td>
<td>0.25-1.16</td>
</tr>
<tr>
<td>Natural</td>
<td>&lt;0.5</td>
<td>13</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Scenario 1</td>
<td>4</td>
<td>61</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Scenario 2</td>
<td>0.8</td>
<td>18</td>
<td>0.02</td>
<td>0.005</td>
</tr>
</tbody>
</table>

## Audience Survey Question

**Case Scenarios: What does our analysis show us?**

- Scenario 1 and 2 are likely intoxications
- Scenario 1 is likely an intoxication and scenario 2 is likely a natural
- We can't determine if Scenario 2 was drinking wine
- Scenario 1 is clearly a suicide
References


References


Thank you

“Chemistry of Death”

Lucas Zarwell
Chief Toxicologist,
DC Medical Examiner’s Office

Dr. Darren Griffin
Professor of Genetics,
University of Kent

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