UNIVERSITY OF PNG
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL SEMINAR
OVERVIEW OF ALCOHOL (ETHANOL & METHANOL)

ETHANOL:

Distribution of Ethanol:

- Absorbed ethanol is distributed in body fluids in proportion to their water content
- Ethanol easily crosses the Blood-Brain Barrier, to affect cerebral function
- Ethanol may be detected and quantitatively measured in Blood, Urine, Cerebrospinal Fluid and Expired Air
    - They are usually used in Forensic or Medico-legal practice,
        - Commonly used are Blood Alcohol Concentration (BAC) and Expired Air
- BAC is most useful parameter for accessing alcohol consumed, because alcohol rapid equilibrates across the Blood-Brain Barrier
- BAC reflects the concentration of alcohol currently affecting the brain

How is Ethanol Metabolized in the Body?

- Liver is mainly responsible for the metabolism of Ethanol
- Between 5 – 8% of alcohol is excreted unchanged by the Kidneys, Sweat and Breath

Ethanol is metabolized in the liver by TWO main pathways:

Alcohol Dehydrogenase (ADH):

- Operational when BAC is in the range 1 – 5mmol/L (4.6 – 23 g/dl)

Ethanol metabolism can be separated into two steps:

Alcohol Dehydrogenase
Ethanol + NAD ==========➔ Acetaldehyde + NADH + H^+

Acetaldehyde Dehydrogenase
Acetaldehyde + NAD ==========➔ Acetate + NADH + H^+

Microsomal Ethanol Oxidizing System (MEOS):

- MEOS is also called Cytochrome **P450** system (CYP2E1) that utilizes NADPH and molecular Oxygen
- MEOS is activated when BAC is higher than 5.0mmol/L (23 g/dl)
- MEOS increases in activity in Chronic alcoholism and may account for the increased metabolic clearance of alcohol indicated by increased blood levels of both Acetaldehyde and Acetate

MEOS (Cytochrome P 450)

Ethanol + NADPH + H^+ ==========➔ Acetaldehyde + NADP

Acetaldehyde + NAD ========➔ Acetate + NADH + H^+

How is the Ethanol content of a drink calculated?

- Ethanol content of a given drink can be calculated from its Percent Alcohol by Volume (% ABV), which by law should be clearly indicted on the container of all alcoholic drinks

% ABV x 0.78 = gram Ethanol/100ml
(0.78 is the specific gravity of ethanol)

- Absolute amount of ethanol in drink is then calculated by reference to Volume of drink
- Exact number of Units of ethanol in given Volume of drink can be calculated from % ABV using the following information:
    - 10 ml (or 7.8g) of Absolute ethanol is Equivalent to 1.0 unit of alcohol

Example:
Questions:

- A patient seen at A&E was said to have consumed 250ml of homebrew. If % ABV of the homebrew is 80 how many units of ethanol did the patient consume?
- Calculate the BAC of this patient

Answer to first part of question:

Number of units of ethanol = % ABV x Volume of drink (ml) / 1000

Number of units of ethanol = 80 x 250 = 20000

20000/1000 = **20 units of ethanol**

The patient consumed **20 units of ethanol**

Answer to second part of question:

To calculate BAC (mmol/L) of the patient, you need to know the number of gram of ethanol in the 20units of ethanol consumed by the patient.

- Not that BAC can be expressed either as g/dl or mmol/L

Number of grams of ethanol = % ABV x Specific gravity of ethanol

80 x 0.78 = 62.4g/dl

Since 1.0 unit of ethanol = 7.8g absolute alcohol
Thus 62.4g absolute alcohol = 8.0units of ethanol
This patient consumed 20units, which is equivalent to 156g/dl of absolute alcohol
To convert to mmol/L: (not that Mol Wt of ethanol = 45.87):

156g/dl x 10 = 1560g/L

1560 / 45.87 = 114.7mmol/L

BAC of this patient = 114.7mmo/L

Significance of Ethanol intoxication:

- Amount of ethanol in blood and other tissues can be measured
  - Largest single use of such determination arises from the need to identify individuals intoxicated by ethanol
- Definition of drunkenness (Inebriation) in not precise
- Relationship between ethanol concentration and stage of intoxication for an average adult can be summarized as follows (MW of ethanol = 45.87)

Stages of Intoxication:

Normal:

- No impairment of mental or physical skills;
- Blood or Urine ethanol from 1 to 3mmol/L (4.6 to 14 mg/dl)

Social stage:

- Blood concentration up to 11mmol/L (50 mg/dl),
- Urine from 2 to 13mmol/L (9 to 60 mg/dl);
- Effects are slight, mild euphoria or a feeling of well being with normal behavior tests

Pre-intoxication stage:

- Blood from 11 to 33mmol/L (50 to 150 mg/dl),
- Urine fom13 to 43mmol/L (60 to 197 mg/dl);
  - Release of social inhibition with some signs of instability;
  - Diminished neuromuscular coordination;
  - Judgment and control required for quick response may be impaired

Intoxicated (confused) stage:

- Blood concentration from 33 to 65mmol/L (150 to 298 mg/dl),
- Urine from 43 to 87mmol/L (197 to 400 mg/dl);
    - Symptoms and behavior are such that all individuals in this stage would not be regarded as sober
    - Speech is usually impaired and motor skills are uncoordinated

Stuporous stage:

- Blood concentrations from 65 to 87mmol/L (300 to 400 mg/dl)
- Urine from 82 to 109mmol/L (375 to 500 mg/dl);
- All individuals in this stage respond only to strong stimuli

Comatose stage:

- Blood concentrations from 87 to 130mmol/L (400 to 596 mg/dl) or more;
- Subject cannot be easily aroused, show depressed reflexes, hypothermia, and stertorous breathing; death may result

METHANOL:

- Methanol: Colourless, Volatile liquid, Easily mixes with water,
- Methanol is listed as "Poison-Class B"
- Methanol is not normally consumed by individuals,
- Accidental or deliberate ingestion of methanol is not common
- Major routes of exposure: Inhalation and dermal exposure
    - Methanol is readily absorbed by: Inhalation, Ingestion and Dermal Exposure

- About 60 – 80% of inhaled methanol is absorbed in the lungs
- Distribution of methanol is rapid and occurs throughout the body
- Highest concentration occurs in tissues with high water content, lowest concentration occurs in fatty tissues
- Plasma concentration reaches peak 30 – 90 minutes after absorption of Methanol

How is methanol metabolized?

- Primary site of metabolism is Liver

Alcohol Dehydrogenase

Methanol + NAD ==========➔ Formaldehyde + NADH + H^+

Acetaldehyde Dehydrogenase

Formaldehyde + NAD ==========➔ Formic Acid + NADH + H^+

Folate dependent mechanism (one carbon transfer reaction)

Formic Acid + Folate == ==== ==➔ CO_2 + H_2O

{Note: Formaldehyde is the same as Methanal; Formic Acid is the same as Methanoic Acid}

How is methanol excreted from the body?

- Major route of excretion of Methanol is via the urine mainly as **Formic Acid**
- Maximum excretion of Formic acid is about 48 hours after absorption/ingestion
- Small quantity (about 2%) of Methanol is excreted unchanged in the lungs and urine
- Concentration of methanol in urine may be about 20 – 30% higher than in blood

Toxic effect of Methanol:

- Both inhalation and transdermal exposure can result in toxicity
- Trans-dermal exposure can result in Systemic toxicity
- Methanol causes inebriation but by itself it is almost completely non-toxic
- Clinical findings correlate better with Formic acid levels than with Methanol level
- Formaldehyde and Formic acid metabolites are responsible for toxicity
    - Formic acid is more responsible for toxicity
- Formic acid causes profound metabolic acidosis that is typical of methanol poisoning
- Formic acid readily crosses the blood brain barrier leading to CNS toxicity:
    - Damage to CNS is in form of lesions in Basal Ganglia especially Putamen,
        - Resulting in long term Neurological deficits ranging from
            - Moderate Poly-neuropathy to tremors, Rigidity, Spasticity, Parkinsonian-like Extra-pyramidal syndrome with mild Dementia, etc.

- Formic acid can inhibit Oxidative Phosphorylation by blocking Cytochrome C, causing increase in Anaerobic respiration with increase in Lactic acid production, leading to Lactic acidosis

Methanol Toxicity can be separated into Phases:

- **First Phase**: CNS depression:
    - On set about one hour after exposure
        - May occur as intoxication, but of shorter duration compared to ethanol

- **Second Phase**: Asymptomatic period following CNS depression
    - Patient may appear normal up to 24hrs after exposure
        - Metabolism of Methanol to Formic acid is slow, can be accumulative in tissues

- **Third Phase**: Severe Metabolic Acidosis:
    - Symptoms may include: Vomiting, Severe Headache, Visual Toxicity

- **Fourth Phase**: Ocular Toxicity of varied severity: (caused by systemic not local exposure)
    - In severe cases may occur about 24hrs after ingestion of about 4 – 10 ml of methanol
    - Conditions range from:
        - Mild Photophobia and Misty or Blurred Vision to markedly reduced Visual Acuity and Complete blindness

What is the Biochemical basis for the therapeutic use of ethanol in methanol and ethylene glycol toxicity?

- Therapeutic use of ethanol in neutralizing Methanol and Ethylene Glycol toxicity is because of the high affinity of Ethanol for Alcohol Dehydrogenase
- Methanol is metabolized to Formic acid, causing metabolic acidosis
- Ethylene Glycol is metabolized to Oxalic acid, which is very toxic to Mitochondrial membrane and also causes severe metabolic acidosis
- ADH has a higher affinity for Ethanol than for Methanol and Ethylene Glycol
- If the individual is administered Ethanol then Methanol or Ethylene Glycol will not be metabolize, because of the high affinity of ADH for Ethanol the enzyme will metabolize Ethanol instead, while Methanol and/or Ethylene Glycol will pass out in the urine
- Methanol will not be converted to Methanal (Formaldehyde) and then to Formic acid, which is toxic to the mitochondria membrane and can also cause severe Metabolic acidosis
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Summary of metabolism of Ethylene Glycol and Methanol indicating ADH reactions inhibited by Ethanol

Ethylene Glycol ===#==➔ Aldehyde =====➔ Oxalic Acid

Methanol ====#==➔ Formaldehyde =====➔ Formic Acid

{**NB: Fomepizole (4-Methyl-Pyrazole) is an inhibitor of Alcohol Dehydrogenase that appears to have fewer side effects than ethanol**}

How can methanol intoxication be monitored?

- Ethanol is used to decrease the metabolism of Methanol
- Alcohol Dehydrogenase (ADH) is the rate-limiting step in the metabolism Methanol
- ADH has a greater affinity for Ethanol than Methanol
- Additional therapeutic intervention for methanol poisoning includes:
    - Controlling the degree of metabolic acidosis by administering Sodium Bicarbonate to the patient
        - Should be considered in acidotic patients with a bicarbonate less than 15mEq/L
    - Plasma electrolyte level: Na^+, K^+, Cl, HCO_3^-, and Plasma pH, must be monitored
- Folic acid (Folate) to be given to the patient
    - Enzymes that metabolize Formic acid into CO_2 in humans are Folic acid dependent
- Whether Folic acid is beneficial in a person who does not have Folic acid deficiency is not clear but it is probably prudent to administer this agent

Ethylene Glycol:

- Ethylene Glycol (Antifreeze) is metabolized to Oxalic acid and a number of other products such as Glyoxylic acid, and Glycoaldehyde
- Patients that consume Ethylene Glycol can develop severe metabolic acidosis and severe Hypocalcemia, due to precipitation of Calcium by Oxalate
- Such patient should be given therapeutic dose of Ethanol, since ADH preferentially metabolize ethanol, because it has a higher affinity for ethanol that ethylene glycol
- Unchanged ethylene glycol will be eliminated in the urine