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Metabolomics in Methanol Toxicity

Rida Tabassum[1], Supraja O D[1], Harshitha J Y [1], Nithyashree S[1], Prashanth Kumar H P[2]

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Abstract:

The domain of "omics" is about the metabolite (small molecule) analysis within the living cells, tissues or organisms is called as Metabolomics. These metabolites are still able to interact with biological system and such interaction is understood as Metabolome. The product of metabolism are affected by genes and environmental factors. Metabolomics best represents the molecular phenotype. This field studies small molecules at the range of 50-1500 Da. It is evaluated that, in plants there are approximately 200,000 metabolites while in humans it is evaluated that there are 3,000 common metabolites. These evaluations are approximates since it is laborious to work out the low-abundance molecules. Overall, it provides, valuable information about what causes changes in our health. Metabolomics plays an important role in forensic toxicology, since it provides an in-depth analysis of altered metabolic pathways that are targeted by harmful chemicals. One such chemical is discussed here is methanol toxicity. Methanol is an extremely weak base and it exists in all life forms starting from bacteria to humans. The metabolites of methanol such as, formic acid and formaldehyde are responsible for toxicity, rather than methanol itself. It is potentially toxic when the concentration is more than 340 mg/L. The blood methanol concentration in sober condition is around $3mgL^{-1}$, which is 400-1000x < lethal concentration. Metabolomics plays a vital role in identifying these metabolites through a number of the methods of detection which includes, NMR, GC-MS, HPLC, etc. The methanol toxicity shows visible signs during a postmortem. Thus, metabolomics is involved in toxicology testing, drug compliance, genetic disorder tests, drug phenotyping and eventually, it also facilitates the understanding of direct cellular phenotypes that are induced by the toxic chemicals such as methanol, arsenic, cyanide, etc.

Keywords: *Omics, Metabolomics, Metabolites, Methanol Toxicity, Postmortem Appearances.*

Authors:

1. *Seventh semester BE Biotechnology, Sapthagiri College of Engineering (Affiliated to VTU, Belagavi), Bengaluru, Karnataka, INDIA*
2. *Assistant Professor, Sapthagiri College of Engineering (Affiliated to VTU, Belagavi), Bengaluru, Karnataka, INDIA*

Introduction

Methanol intoxication is caused by accidental or purposeful ingestion. According to a nationwide study conducted in 2018 regarding subsequent mortality due to methanol toxicity around 1999 - 2013, it was estimated that the probability of survival rate decreased as the time increased. The survival rate was high during the first few months and when the condition prolonged, the survival rate started to fall below 50%. Methanol toxicity in the postmortem process is detected using "metabolomics" **(Chung *et al.*, 2018).**

Overview on Metabolomics

Metabolomics is a field which concentrates on metabolites that are produced from the metabolic activity in a living organism. It is useful to study about the metabolites when it is combined with high-throughput analytical chemistry. Generally, viruses are preferred to study the steps of metabolism since they can utilize and recourse to the host's metabolic pathway. All small molecules that are produced after metabolism in a cell is defined as "metabolome". Metabolomics represents the molecular phenotype **(Manchester and Anand, 2017).**

Process of Metabolomic Study

The analysis of data can be approached by two methods:

- Targeted Approach: In this approach, a specific metabolite is analyzed. This approach is helpful in pharmacokinetics and the activity of modified enzymes.
- Untargeted Approach: In this approach, many metabolites are analysed from different biological samples. It is also called as global approach **(Salek *et al.*, 2020).**

Both the above mentioned approach follow the same procedures. The process that is discussed further is mass spectroscopy based, however, an untargeted approach can also be performed using NMR. This approach is useful to reveal new association between disease and metabolite and also allows to understand more about metabolic pathways and functions **(Roberts *et al.*, 2012).**

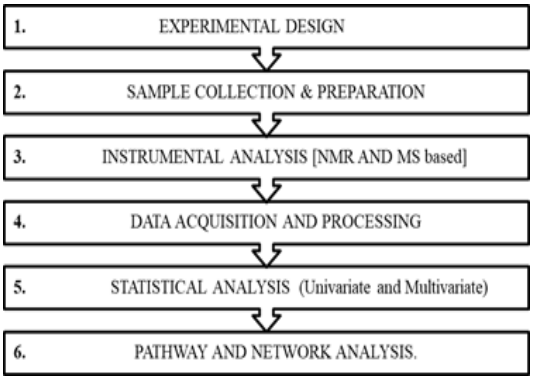

Figure No. 1: Process of Metabolomic Study

The metabolites were extracted from samples such as tissues, plasma, cells, saliva, urine etc. and put through the Instrumental analysis. During analysis, quantification of metabolites using liquid chromatography/Gas chromatography integrated with NMR Spectroscopy or Mass Spectroscopy is performed. Data acquisition and analysis are conducted to identify the particular metabolites that could potentially serve as biomarkers for diseases. The data thus obtained is used for metabolite characterization & processed before Statistical Analysis.

The Statistical analysis is done to reduce the probability of occurrence of variables and examine the difference between groups **(Bartel *et al.*, 2013).** These analyses can be classified into:

(i) Univariate analysis of t-test and Analysis of Variance (ANOVA)
(ii) Multivariate analysis such as the widely used Principal Components Analysis (PCA), Partial Least Squares (PLS) regression, and PLS-Discriminant Analysis (PLS-DA).

In addition, Metabolomics data have also been analyzed using Multivariate Analysis of Variance (MANOVA), ANOVA-Simultaneous Component Analysis (ASCA), Orthogonal-PLS (OPLS), OPLS-Discriminant Analysis (OPLSA-DA), Soft Independent Modeling of Class Analogies (SIMCA), Hierarchical Cluster Analysis (HCA), Self-Organizing Maps (SOMs), Support Vector Machines (SVM) and Random Forest **(Sugimoto *et al.*, 2012; Bartel *et al.*, 2013)**.

The last step is tracing the metabolite pathway. Once all the analysis is done, the metabolites pathway is studied such that the metabolites responsible for phenotypic changes are counteracted with respective antidotes or treatments **(Dabhi, 2015).**

Significance of Metabolomics

In-vitro metabolomic approach helps in achieving large-scale screening of drugs or chemical compounds and also in the rapid understanding of drug toxicity level by obtaining information of metabolites in a single run. As mentioned earlier, it is involved in drug compliance, toxicology testing, and blood analysis and drug phenotyping. The advancements in analytical technologies are making metabolomics a potential tool in pathological mechanism elucidation, novel drug targets discoveries, drug response prediction, identification of diagnostic biomarkers for diseases, drug action interpretation mechanism and also enables precision treatment of patients. The factors influencing the phenotype of the individual include: genetic factors, age, environmental stress and conditions, drugs consumed and its effect in the body which results in the phenotypic changes, disease that individual has conquered in their lifetime, diet and lifestyle of that individual. All these pathways can be identified using metabolomic study. Metabolomic also has direct relation with the abnormalities caused **(gov.uk).**

General Application of Metabolomics (Salek *et al*., 2020)

1. **Agriculture:**

- Development of pesticides
- Improving genetically modified plants
- Due to the range of 1° and 2° metabolites in plants, plant metabolomics is an interesting and developing field

2. **Biomarker Discovery:**

- To investigate the biology underlying a drug or a disease
- To identify novel therapeutic drugs

3. **Systems Biology:**

- Dynamics in biological systems can be studied
- Explore metabolic networks

4. **Toxicology:**

- Toxicity assessment.
- Clinical Trial Testing
- Effects of drug toxicity
- Phenotypic changes due to metabolites

5. **Enzyme Discovery:**

- Discovery of biochemical pathways
- Improve the efficiency of enzymes
- Link changes in metabolite levels to catalytic activity

6. **Human Diseases:**

- Diagnosis purposes
- Determination of disease state
- Genetic disease tests
- Biomarker discovery
- Risk determination

7. **Microbial Biotechnology:**

- Microbial improvement
- Fermentation

Overview on Methanol Toxicity

Methanol(CH3OH), commonly known as methyl alcohol/wood alcohol, is a polar solvent due to (OH) group dominance, one among the class of organic compounds (primary alcohols) and has a molecular weight of 32.04g/mol. It consists of 90% of by volume of ethanol, 9.5% of wood naphtha and 0.5% of crude pyridine **(Reddy and Murty, 2017).**

Properties: Colourless liquid, odour is similar to ethyl alcohol, pH - 7.2, flammable and toxic.

Uses: Paints and paint removers, dyes, resins, gas line de-icers, windshield wiper solutions, varnishes, adhesives and ethanol denaturants. Methanol can commonly be utilized as a suicidal agent but using it as a means of murder is extremely rare. If methanol is accidentally or purposefully ingested, it gets rapidly absorbed through the stomach and intestines (10 minutes). Methanol gets accumulated in the circulatory system with repetitive small doses at a rate of oxidation approximately one-fifth to the oxidation rate of ethanol. Methanol toxicity is linked with severe complications including visual disturbances, neurological deficit and metabolic acidosis. Ingestion of little amount as 4-10 ml of methanol, in adults, cause permanent ocular damage (photophobia, clouded or dim vision and complete blindness). Permanent CNS damage is caused due to the severe intoxication, leading to parkinsonian-like condition and permanent blindness. Diagnosis depends on the clinical picture of the patient, neuroimaging, high osmolar gap and increased acid concentration in body fluid. Neuroimaging focuses on the white matter in the

subcortical region of the brain and basal ganglia. However, brain lesions may occur at other sites. This is an important fact as diligent diagnosis and well-timed treatment provision which utilizes ethanol or fomepizole apart from other ancillary therapy can be life-saving **(gov.uk).**

Mechanism of Methanol Metabolism

Once methanol is ingested, it is immediately absorbed through the gastrointestinal tract within 10 minutes. Methanol is broken down by the liver by the enzyme alcohol dehydrogenase which releases the toxic metabolites, formaldehyde (33 times more lethal than methanol) which in turn is oxidized to form formic acid (6 times more lethal than methanol) by aldehyde dehydrogenase. Formic acid (formate) is accountable for increase in acid in body fluids and retinal toxicity and is also responsible for deaths pertaining to methanol toxicity. Formate may inhibit the Complex IV involved in electron transport chain, increasing lactate production and metabolic acidosis. Oxidation of formaldehyde to formic acid occurs when NAD is reduced to NADH (crucial coenzyme in making ATP). Formate is distributed in the tissues according to their water content, and an increased concentration is reported in the vitreous body and optic nerve. Formate is not completely eliminated and may accumulate.

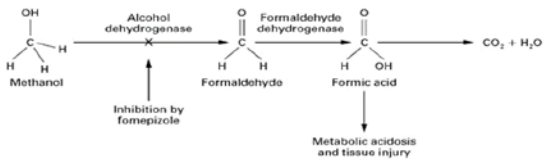

Figure No. 2: General Metabolism of Methanol by Alcohol and Aldehyde Dehydrogenase

The potential lethal dose is around 30-240ml or 1g/kg. Methanol does not completely disappear from the blood for 3 or 4 days, and hence it becomes compatible for the postmortem findings of metabolites of methanol until 3-4 days in the human body. The liver metabolizes methanol very slowly. 3-5% is excreted through the lungs and upto 12% through kidneys. Few health issues are listed in the following table regarding the abnormal methanol concentrations **(Reddy and Murty, 2017).**

Table1: Concentration of methanol in normal and abnormal conditions (Kim *et al.*, 2015)

Biospecimen	Age	Sex	Condition	Methanol concentration	Health issues (if abnormal)
Blood	Adult (>18 years)	Both	Normal	61.1 - 93.7µM	-
Blood	Adult (>18 years)	Both	Abnormal	1548 (624 - 5557)µM	Alcohol intoxication
Faeces	Adult (>18 years)	Both	Normal	Not quantified	-
Faeces	Adult (>18years)	Both	Abnormal	Not quantified	Non-alcoholic fatty liver disease, Crohn's disease, Ulcerative colitis.
Breast milk	Adult (>18years)	Female	Normal	23.6 ± 16.1 µM	-
Urine	Adult (>18 years)	Male	Abnormal	15427 ± 10168 µM/mM	Drunk

Signs and Symptoms

Symptoms may be visible within an hour of methanol contact or sometimes may appear after 24 hours.

1. Nausea, Headache, Drowsiness, Vomiting, Abdominal pain, Gastric distress, Kussmaul Respiration, neck stiffness, depressed cardiac action, hypothermia, cyanosis, Dilation of pupil [Mydriasis], Coma, Death.
2. Presence of odour in the breath. The effect on the CNS is more intense and persistent than ethanol.
3. An increased Osmolal gap accompanied by visual symptoms suggests methanol poisoning.
4. Fatal Dose: 60 to 200 mL
5. Fatal Period: 24 to 36 hours; may be delayed for 2 to 4 days **(Dabhi, 2015).**

Case Study

A 32-year old individual was seen retching. A witness reported that the victim drank a car washer solution by mistake confusing it for alcohol. Soon after the victim complained of headache and stomach pain. He was admitted to a hospital and received treatment in ICU, but was reported dead after 20 days.

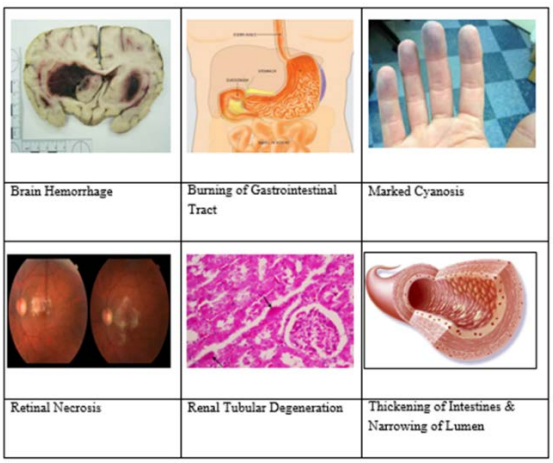

Figure No. 3: Postmortem Appearance

Analysis: Arterial blood gas analysis result was metabolic acidosis state (pH 6.99, pCO_2 25, pO_2 148, HCO_3 6.2 mEq) and blood tests reported high anion gap; an autopsy was performed and the brain showed softening and about 34 g hematoma in the bilateral putamen and 3rd ventricle.

Results: In this case, the methanol concentration testing in blood was not done in the initial stages. Absence of methanol or surfactant (components of washer solution) was reported due to first-aid and long hospital stay**.**

Conclusion: Though analysis of the concentration of methanol, in this case, was not necessary since it was accidental ingestion of washer liquid. It might prove useful in the crime scene. But, to comprehend the exact degree of toxicity, determining the methanol concentration alone is not enough. Thus, determination of the concentration of formic acid (metabolite of methanol metabolism) plays an important role to understand the toxicity level and confirm the cause of death **(Kim *et al.*, 2015).**

Detection of Metabolites of Methanol:

Due to the high sensitivity and accuracy of GC-MS, it is used to verify the method of esterification for the methyl formate production. Furthermore, it was necessary to investigate the correlation between the quantity of methanol and formate in vitreous and blood sample. Formic acid is undetectable in gas chromatography. Thus, conversion of methanol by esterification (derivatization) is a necessary step before quantifying it in blood and vitreous samples. Formic acid esterification follows transferring of formic acid sample (1mL) to headspace vials (Restek) which has a microfilm coating. Addition of conc. sulphuric acid (97%, 500 µl) to act as a catalyst and the mixture is shaken, which is then followed by sample incubation at room temperature for 20mins. Pure methanol (99.9%, 30 µl) is added at a constant interval so that formic acid is converted to methyl formate and acetonitrile (0.197M, 30 µl). This mixture was shaken. The sample was ready for injection finally after 20mins incubation at room temperature.

Preparation: The retention time and amount of carbon in the analyte were detected using GC/MS and GC/FID respectively, from the sample of pure methyl formate. Thereafter, according to the procedure, standard methyl formate sample was prepared. After this, liquid-liquid extraction was carried out using di-chloromethane and water (1:1, *v*/v), and infusion of organic layers to the device.

- About 1mL sample is injected as analyte for the gas chromatography set-up and separated on various types of chromatographic columns. Helium is used as Carrier gas and is eliminated after chromatography by a jet separator or a diffusion membrane.
- As in all mass spectrometers, neutral molecules from the gas chromatograph enters ion source and are ionized by a stream of electrons (e.g. El-

electron Impact Mass Spectrometry) (**Das *et al.*, 2020).**

Analysis

- Usually, a chromatogram is plotted, in which the **x-axis** corresponds to retention time of the analyte to reach the detector, passing through the column.
- Typically, the **y-axis** in the gas chromatogram corresponds to the amount of analyte that is present.
- The concentration of the compound is found by calculating the area under the obtained peak [peak area count]. The integration and calculation of area value is automatically done by the computer data station. The mass spectroscopy detector then allows recognition of a compound by mass spectrum obtained at the time of testing **(Kim *et al.*, 2015).**

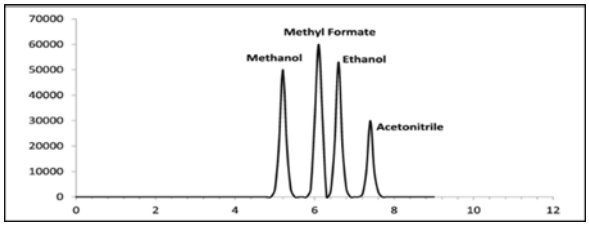

Figure No. 4: Chromatogram Result of Methanol Poisoning

Conclusion

Metabolomics is a thriving field which proves to be quite useful and informative. Since it concentrates on metabolites in general, its application is vast and diverse. One such application deals with forensic science. Using metabolomics for methanol toxicity fetches the information on metabolism before the death of victim with ease and certainty since the effective half-life of methanol is 45-90 hours in the human system.

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