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METABOLOMICS: CONCEPT, TECHNIQUES AND PHARMACOLOGICAL RELEVANCE

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ABSTRACT

Objectives: To review the concept of metabolomics, the methods and analytical techniques employed in metabolic profiling and applications of metabolomics in pharmacology.

Data Sources: Primary literature and review articles were obtained via PUBMED, Science Direct and BioMed Central search using the key term metabolomics. Additional studies were identified from bibliography of reviewed literature.

Data Synthesis: Metabolomics is the study of metabolism at a global level in a cell, tissue or organism mostly by means of analyzing biological fluids. Thousands of molecules known and unknown can be identified and quantified using various analytical techniques, the widely used being mass spectrometry and nuclear magnetic resonance spectroscopy. The large amount of data generated through these methods is managed through bioinformatic tools that help to simplify the complex data. Lastly, this review discusses the application of metabolomics in various aspects of pharmacology such as drug discovery, disease diagnosis and therapeutic monitoring. The potential of pharmacometabolomics to achieve the goal of individualized drug therapy has also been studied.

Conclusion: Metabolomics will have an impact on pharmaceutical research and development. Along with pharmacogenomics, pharmacometabolomics will aid in better understanding of variations in individual response to drug treatment and also adverse drug effects.

Keywords: *Metabolomics; Mass Spectrometry; Bioinformatics; biomarkers; Pharmacometabolomics.*

INTRODUCTION

Metabolism is the conversion of food consumed into energy that can be utilized by the body. [1] Drug metabolism involves the same enzymatic pathways and transport systems that are used for the metabolism of food. As a result the drug may be converted into a form that can be easily eliminated as a more potent or a poisonous moiety. Metabolic changes are thus the most important markers of alterations in the body in response to a disease or drug treatment. [1] The number of metabolites may range from a few thousands to tens of thousands of known and unknown structures.[2] Metabolomics is defined as a comprehensive collection of metabolites in a cell or organism not including the proteins and nucleic acids.[1] The term metabolomics and the related term metabonomics were coined in the late 1990s to describe the development of approaches which aim to measure all the metabolites which are present in a cell, tissue or organism.[3] Metabolomics is the technology which involves global analysis of all metabolites, their regulation and fluctuation in a sample such as individual cells and metabonomics is the analysis of metabolic responses to drugs or disease by analyzing body fluids or tissues and thus creating a systematic biochemical profile of the metabolites in the organism.[4,5] The basic analytical tools employed in metabolomics are Nuclear Magnetic Resonance (NMR), Mass Spectroscopy(MS), Infra Red (IR) and various hyphenated techniques like Liquid Chromatography (LC) -MS, GC(Gas Chromatography)-MS, Capillary Electrophoresis (CE)-MS and Electron Capture (EC) -MS.[4,6] This generates a vast amount of data which requires the application of biostatistics and new mathematical methods.[4] This data is managed with the various tools of bioinformatics as explained below. The study of genome constitutes genomics which has been amplified to the level of transcriptome – the study of which is called transcriptomics.[2,3&7] There are thousands of metabolites, many of them still undiscovered to the scientific world and many of them under scrutiny such as xenobiotics, nutritional compounds, microbial metabolites in the gut whether to be considered as a part of the human metabolome or not.[1]

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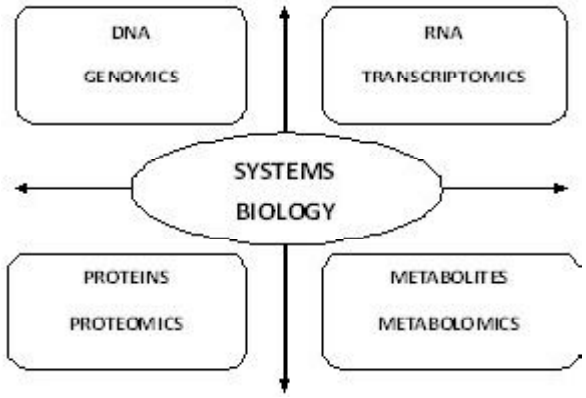

Fig. 1: *The science of 'omics' with systems biology and its various branches*

Techniques used in Metabolomics

Metabolomics is measurement of concentration of most of the metabolites in a particular sample such as serum, plasma, urine or a collection of cells. [2] This requires specialized methods of sample preparation because conventional methods may lead to loss of some metabolites thus affecting the objective of metabolomic research.[8] For instance; studies have shown that when urine samples are subjected to procedures like centrifugation and pH adjustment, the metabolomic results get negatively affected.[8] A mixture of techniques is used as one technique is not enough for analyzing the diverse kinds of metabolites constituting the metabolome.[4] The techniques that are used in metabolomics consist of two stages (a) a separation stage usually gas chromatography, liquid chromatography or electrophoresis (b) an identification stage consisting of mass spectrometry.[9] The techniques used include NMR, Fourier transform infra-red (FTIR) spectroscopy, Ultra-Performance Liquid Chromatography (UPLC) and LC-Electrochemistry array (LCECA) detection. [2,10]

Figure 2 shows how a typical metabolomic study is carried out:

Liquid Chromatography

Liquid chromatography is mostly used coupled to mass spectrometry.[4] Stationary phases such as normal phase, reverse phase or ion exchange phase can be used to separate analytes based on their hydrophilicity, lipophilicity or charge[4] HPLC can be used for separating a large variety of metabolites and derivatization is not required as in gas chromatography.[11] Higher resolutions in liquid chromatography can be obtained by means of ultra performance (UP) LC, which makes use particles of 1-2 microns.[12] UPLC along with MS, has been used in metabolic profiling of urine in rodent studies. [10]

Gas chromatography

Gas chromatography is mainly used for separation of metabolic components in samples prior to their analysis by mass spectrometry. GC is used widely for

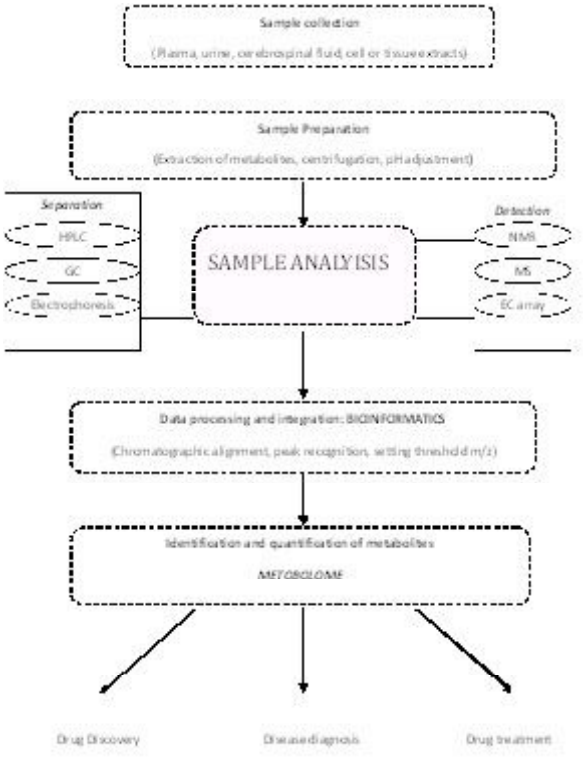

Fig. 2: *Schematic representation of metabolomic study*

toxicological analysis where as LC is used mainly for routine analytical work when quantification of already identified metabolites is to be done.[13] One of the main limitations with this technique is that in most cases derivatization of metabolites is required and some of them may not be thermostable enough to undergo volatalization before analysis.[11] Recently the technique of two dimensional gas chromatography (GCxGC) developed in early 1990, uses two consecutively coupled columns has been found to possess enhanced resolution and is faster than conventional gas chromatography. [14,15]

NMR spectroscopy

NMR spectroscopy is widely utilized in metabolomic research. It uses the differences in magnetic properties of the nuclei in the molecule. NMR can be used for identification of structure of both known and unknown metabolites, as there is no requirement of a dependable standard as is the case in MS and IR spectroscopy. [2] It is non destructive thus samples can be used for further analysis. Another advantage is its widespread coverage of various types of chemical moieties and gives the accurate structure of metabolites. [1] By using isotopic labels like ^{13}C, isotopomers (isomers with isotopic atoms) having ^{13}C atom can be generated which can be used to reveal biochemical pathways within cells. The limitations of NMR include its low sensitivity and very high initial costs. [1]

MS based techniques

MS is much more sensitive as compared to NMR. MS can be used to analyze samples directly or after separation using chromatography or electrophoresis.[11] MS is used as a hyphenated technique in various ways – LC-MS, GC-MS, EC-MS and LC-MS-MS.[12] The development of mass analyzers like time of flight, triple quadruple and ion trap mass analyzers help in accurate identification of compounds. [4]

LC-MS is increasingly being used in metabolomic analysis. HPLC is the most widely used. LC-MS techniques do not require derivatization as in case of GC-MS. LC-MS-MS can be used for quantification of drugs in plasma, serum, blood and saliva. Examples of drugs quantified in this manner include benzodiazepines, beta-blockers and sulphonylurea type antidiabetics.[13,14&15]

GC-MS is suitable for analysis of urine samples as most of the metabolites in urine have polar groups that are easily amenable to trimethysilylation and thus facilitating successive analysis.[8] This technique is used for analysis of amino acids, sugars, aromatic amines and fatty acids. [11]

Role of bioinformatics

Metabolomics generates large amounts of data. The generated data is generally multivariate i.e. the data consists of many variables (different metabolites) for many objects (individuals or cells). These require specialized bioinformatics tools in addition to the conventional statistical methods. The methods of data analysis in metabolomics can be broadly classified into two categories (Figure 3): supervised and unsupervised analysis.[11]

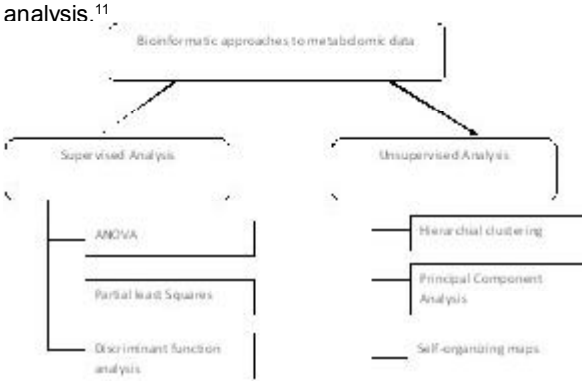

Fig.3 : *Bioinformatics in Metabolomics*

Supervised analysis can be used when it is known that the value of response that is to be predicted (Y-parameters) is related with each of the sample inputs (X-parameters). [16] Artificial neural networks, soft-independent modeling of class analogy (SIMCA) and k-nearest neighbor analysis constitutes alternative supervised approaches. [4]

Unsupervised analysis helps to find out how the data are organized, it helps to identify patterns in the data. [2] This algorithm greatly aids in simplification of the data with minimal bias. Unsupervised methods of data analysis are basically of two forms: dimension reduction and cluster analysis. Principal component analysis comes under dimension reduction and involves converting multidimensional data sets into lower dimensions through generation of linear combination of variables called the principal components.[4] Cluster analysis involves finding clusters of similarly characterized samples. Hierarchical clustering is an example of this method. [16]

APPLICATIONS

Analytical techniques like GC-MS, NMR and LC-MS are capable of identifying hundreds of chemical moieties in biological samples. Metabolomics technology finds widespread application in fields like pre-clinical and clinical assessment of new drug molecules, toxicology, and disease diagnosis and in therapeutics also in the form of pharmaco-metabolomics.

Metabolomics and drug discovery

Metabolomics is used in various phases of drug discovery. This includes pre-lead prioritization, assessment of preclinical efficacy and safety assessment, clinical safety assessment and toxicology profile.[17] Metabolomics is also helpful to find out the mechanism of action of drugs and also the mechanism of its toxicity.

NMR based metabolomic technology is an example of a method that is used in drug discovery to determine both safety and efficacy of drugs. In order to identify and assess the safety of drug candidates biological fluids from animals or patients are analyzed both before and after treatment by one dimensional NMR and Principal Component Analysis. Any difference in the metabolite profile produced as a result of toxicity associated with the drug can be identified. [18]

Differential NMR metabolomics is a technique used to determine the *in vivo* efficacy and specificity of new drugs. This involves the comparison of the metabolome of different cell lines namely wild type and mutant cell lines under various environmental conditions including drug treatments. Mostly four different cell lines are used; these include (a) wild type cells (b) mutant cells (c) wild type cells with drug candidate and (d) mutant cells with drug candidate. The mutant cells have the drug's protein target inactivated. The cell lysates of different cell lines are collected and subjected to proton NMR, followed by Principal Component Analysis where in the clustering pattern gives information regarding the activity and selectivity of the drug. [18]

Another aspect in drug discovery where metabolomics is useful is to study drug mechanisms. The ^{1}H-NMR was used to study the response of mice to a single injection of anti-neoplastic agent cisplatin.

Nephrotoxicity is a side-effect observed in patients treated with cisplatin. However, in the experimental animals noticeable changes in the urinary metabolic profile was observed after drug exposure that appeared before changes in the usual biomarkers of nephrotoxicity such as blood urea nitrogen and serum creatinine. Urinary metabolite profile showed the presence of glucose, amino acids and trichloroacetic acid cycle metabolites. Accumulation of non-esterified fatty acids and triglycerides in serum, urine and kidney tissue was observed. An increased level of plasma insulin was also observed. An interesting finding was that these metabolic alterations were ameliorated by administration of a PPARα ligand. Although it is unclear which of these or rather if any of these metabolic changes might be related to nephrotoxicity, this work may help in defining biomarkers that help to predict whether nephrotoxicity may occur. [2]

Metabolomics in disease diagnosis

An important application of metabolomics is in the discovery of chemical biomarkers that help in disease diagnosis and understanding animals of disease. Examples of disease where this has been done include atherosclerosis, coronary artery disease, ocular diseases, cancer and neurodegenerative diseases.

Cardiovascular diseases

The application of metabolomics to cardiovascular biomarkers is based on the principle that various metabolites and intermediates in different biochemical pathways such as the Tricarboxylic Acid Cycle (TCA) may function as regulatory signals with hormone like function. For instance, α-ketoglutarate and succinate may bind to certain orphan G-protein coupled receptors in the kidney leading to activation of renin-angiotensin system.[1]

Comparison of blood samples from exercised patients with inducible ischemia and those without through LC-MS led to the identification of numerous biomarkers of ischemia. For instance, the levels of lactic acid and its metabolite, metabolites of adenosine monophosphate catabolism in skeletal muscle correlate with cardiac ischemia which was statistically significant.[19] Metabolomics and proteomics together have been used to characterize metabolic profiles of atrial tissue that predispose individuals for development of atrial fibrillation (AF). For this purpose, cardiac tissues from patients with AF were analyzed using both metabolomic and proteomic approaches. NMR studies of the cardiac tissues revealed an increase in beta-hydroxybutyrate, a major substrate in ketone body metabolism. There was also a rise in ketogenic amino acids and glycine. The proteomic studies demonstrated the difference in the levels of expression of *3-oxoacid transferase*, an important enzyme involved in ketolytic energy expression between the controls and AF patients. [20]

Apoptosis in tumor

Metabolomics has been applied in cancer research to understand apoptosis in tumours. The spectroscopy method used for analysis of tumor metabolism is high resolution magic angle spinning (HRMAS) ^{1}H NMR spectroscopy. This approach is capable of producing high-resolution spectra from intact tissue. Metabolomics was applied to investigate the role of hypoxia inducible factor (HIF)-1β in tumor metabolism and growth. The expression of HIF-1β is increased in many cancers, which in turn causes up regulation of proteins in a number of metabolic pathways like the glucose transporters, glycolytic pathways and vascular endothelial growth factors. *In vivo* studies indicated that hepatoma cells deficient in HIF-1β grown a solid tumors in mice have reduced rates of growth. [3]

Central Nervous System Diseases

The application of metabolomics in the study of CNS diseases is done for various purposes. This includes identification of disease mechanism and diagnostic markers for the disease state. Metabolomic studies have been done on neurodegenerative diseases like Parkinson's disease PD and Huntington's disease, and also in psychiatric disorders like depression and schizophrenia. [21]

Metabolomic analysis of the plasma profile of PD patients and control subjects showed observable differences. The metabolites 8-OHdG and glutathione were significantly increased in PD patients and the uric acid levels were reduced. Higher uric acid levels have been found to lower the risk of PD and slow the progression of the disease. Increased oxidized glutathione was found in the plasma of PD patients and changes in ratio of oxidized to reduce glutathione might reflect a response to oxidative damage. It has been found that treatment with anti-parkinsonism drugs causes reversal of the metabolic profile of PD patients where in they become more similar to that of controls.[21] Lipidomics is a branch of metabolomics that specifically focuses on a range of polar and non polar metabolites, thus leading to a comprehensive assessment of lipid biochemistry. It is branch that is made use of in the metabolomic study of schizophrenia. Prior to drug treatment, major changes were observed in the baseline levels of two phospholipids-phosphatidylethanolamine and phosphatidylcholine, the finding suggested that these phospholipids which play a critical role in membrane structure and function seem to be impaired in schizophrenia patients. It was observed that treatment with antipsychotic drugs like olanzapine, risperidone and aripiprazole lead to the elevation of phosphatidylethanolamine. [2]

Metabolomics in drug treatment - Pharmacometabolomics

Individualized drug therapy requires an understanding of the various factors and mechanisms that are responsible for variation in drug response among patients. Pharmacogenomics involves understanding the genetic makeup of individuals so as to predict their response to drugs both in terms of efficacy and adverse reactions. Pharmacotranscriptomics involves the analysis of gene transcripts using cDNA microarrays. Microarray analyses of some tumors have shown that there is a correlation between the gene-expression pattern and patient's response to therapy. However, when it comes to healthy individuals, there arise questions of how reasonable and ethical it is to predict drug response after obtaining biopsies of tissues like liver or brain. Pharmacoproteomics uses the approach of identifying certain protein profiles that predict the efficacy or safety of drugs. Metabolomics can complement the other aspects of systems biology-genomics, transcriptomics and proteomics to achieve personalized drug therapy.[19] However, for this purpose it is the metabonomics that is taken into consideration rather the metabolome.

While pharmacogenomics is based on the principle of gaining an understanding of the human genome (genotype) to tailor the drug therapy for an individual, metabolomics uses metabolic phenotype of the individual to predict the metabolism or toxic effects of the drug. The metabolic phenotype provides an integrated picture of physiological, chemical, genetic and environmental influences that can affect the drug metabolism in an individual. [20] To understand how the metabolomics can be useful in drug therapy, a study involving pharmacometabonomic analysis of paracetamol was conducted in healthy volunteers. In this study the pre dose metabolite and post dose metabolite profile in urine was analyzed using proton-NMR. It was found that individuals excreting higher concentration of *p*-cresol-*O*-sulphate were more likely to excrete relatively less acetaminophen-*O*-sulphate and larger amounts of acetaminophen-*O*-glucorunide than individuals excreting less amount of *p*-cresol-*O*-sulphate. Acetaminophen and p-cresol (produced by bacteria in the gut) are both aromatic phenols that are structurally similar and compete for sulphonation. Thus, it is possible that increased production of p-cresol in the colon will make a person more vulnerable to acetaminophen-induced hepatotoxicity. [22,23] This type of pharmacometabolomics association may be of relevance in case of other drugs as well and further studies in this area will greatly aid in improvement of personalized drug therapy.

CONCLUSION

Metabolomics will definitely have a major impact on pharmaceutical research and development. It can provide information regarding pharmacokinetic and pharmacodynamic properties of a drug and also the mechanism responsible for individual variation in drug response. However, some of the limitations of this approach include use of multiple analytical techniques with different sensitivities and the complexity of the resulting data. However, combining the mathematical models of metabolism and statistical assessment of the data obtained a better understanding of metabolomic profiling is possible.

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