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Mass spectrometry as a potential technique for clinical medicine

Spektrometria mas jako potencjalna technika w medycynie klinicznej

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mass spectrometry, metabolomics, proteomics, clinical medicine

Słowa kluczowe

spektrometria mas, metabolomika, proteomika, diagnostyka laboratoryjna

Summary

Metabolomics, proteomics and genomics are popular with scientists, including those in the field of medical science. The discovery of new drugs of their metabolites, their mode of action, metabolism or eventually their absorption in the body have a direct impact in the treatment of diseases. The discovery of new metabolic pathways involved in pathogenic processes may lead to a better understanding of phenomena at the cellular level. Genome sequencing, understanding its structure and its impact on protein coding are new areas that provide information on the course of biochemical and metabolic processes, including many diseases. Metabolomics allows to obtain information about intracellular processes, study of the influence of pharmacological agents on the body, identification of new, unknown metabolic pathways involved in pathogenic processes, monitoring of known metabolic diseases or pharmacokinetic study of new drugs. The task of knowing the entire human metabolome is a huge challenge for medical sciences. It is difficult to know the metabolisms of simple organisms. Key data, mechanisms of the body's activity, biochemical processes, etiology of diseases are still not unraveled, therefore metabolomics as a field will continue to develop. Proteomics is a complicated and demanding field of research. This is related to the dynamic and heterogeneous character of the proteome and its variable. Proteins as the basic building block of living organisms play simultaneously a series of roles in biochemical processes. Understanding processes in a healthy human is a challenge for proteomics, and the more the recognition of biological processes in the state of pathology is particularly important and also crucial for this field of science. In addition to metabolomics and proteomics, classical, but also significant, toxicology, forensic, or pharmacokinetic studies remain. This type of research is the most common, where mass spectrometry is used as the basic analytical tool.

Streszczenie

Metabolomika, proteomika i genomika cieszą się zainteresowaniem naukowców, w tym również z dziedziny nauk medycznych. Odkrycie nowych leków, ich metabolitów, sposobu ich działania, metabolizmu czy ostatecznie ich wchłaniania w organizmie mają bezpośrednie przełożenie w leczeniu chorób. Odkrycie nowych szlaków metabolicznych zaangażowanych w procesy chorobotwórcze może prowadzić do lepszego zrozumienia zjawisk na poziomie komórkowym. Sekwencjonowanie genomu, poznanie jego struktury czy jego wpływu na kodowanie białek to nowe dziedziny, które dostarczają informacji na temat przebiegu procesów biochemicznych i metabolicznych, w tym wielu chorób. Metabolomika pozwala na pozyskanie informacji na temat procesów wewnątrzkomórkowych, badanie wpływu środków farmakologicznych na organizm, na identyfikację nowych, nieznanych szlaków metabolicznych biorących udział w procesach chorobotwórczych, monitorowanie znanych chorób metabolicznych czy badanie farmakokinetyki nowych leków. Poznanie całego metabolomu człowieka to ogromne wyzwanie dla nauk medycznych. Ciężko jest poznać metabolom prostych organizmów. Kluczowe dane, czyli mechanizmy działania organizmu, procesy biochemiczne, etiologia chorób nadal pozostają nierozwikłane, dlatego też metabolomika jako dziedzina będzie się wciąż rozwijała. Proteomika jest skomplikowaną i wymagającą dziedziną badań. Związane jest to z dynamicznym i niejednorodnym charakterem proteomu oraz jego zmiennym. Białka jako podstawowy budulec żywych organizmów pełnią jednocześnie szereg ról w procesach biochemicznych. Poznanie procesów u zdrowego człowieka jest wyzwaniem dla proteomiki, a tym bardziej poznanie procesów biologicznych w stanie patologii jest szczególnie ważne i również kluczowe dla tej dziedziny nauki. Poza metabolomiką i proteomiką pozostają jeszcze klasyczne, ale również istotne badania z zakresu toksykologii, medycyny sądowej czy badania farmakokinetyczne. Ten rodzaj badań jest najbardziej powszechny, gdzie wykorzystuje się spektrometrię mas jako podstawowe narzędzie analityczne.

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

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INTRODUCTION

In the last decade, omics techniques (i.e. genomics, metabolomics and proteomics) have commonly entered the world of science as a field of key importance for humans and the environment. Along with the development of knowledge about the information contained in the genetic material, the emphasis was placed on the search for markers in physiological disorders. The markers can be small molecular compounds (metabolomics) or proteins (proteomics). In particular, proteomics is currently the direction of research by doctors, geneticists, biotechnologists and chemists. Compared to genomics that only studies the sequence of genes, proteomics allows us to track changes in protein activity as post-translational modifications (PTMs). In addition to proteins, small-molecule compounds that perform important functions in the body (e.g. inhibitors of biochemical transformations) remain to be tested. Therefore, the second area that is also important for science is metabolomics. The results of metabolic testing give information about the mechanisms already taking place at the cellular level. Studying metabolomics allows us to obtain information about intracellular processes, study the influence of pharmacological agents on the body, identify new and unknown metabolic pathways involved in pathogenic processes, and monitor known metabolic diseases or pharmacokinetic study of new drugs. Proteomics is a complex and demanding field of research. This is related to the dynamic and heterogeneous character of the proteome and its variable and often low concentration (pmol/ml). The technique that found application in mass genomics, proteomics and metabolomics is mass spectrometry. Mass spectrometry is a widely used measuring technique in many medical, natural and exact sciences. It is used for quantitative and qualitative determination of chemical compounds and elements and their isotopes. Obtained mass spectrum provides a lot of information about the composition of the sample, and in many cases, clearly identifies the components of the mixture, in particular biological ones, where the matrix's influence on the obtained result is observed. Although this method is constantly updated, nevertheless, thanks to its advantages, it is considered an extremely important measurement tool. In practice, it is possible to use different measuring systems, selected depending on the type of sample and the type of information sought. In each case, however, several basic components of the measurement system are distinguished.

Sample preparation, in particular if it is a biological material (i.e. organs, cerebrospinal fluid, cell cultures, saliva, blood or urine) is the key to correct determinations of a selected group of compounds. In areas of science such as medicine, access to biological material that is the subject of the analysis is limited, therefore each quantity is valuable. In addition, it should be remembered that the acquisition of material is associated with the disruption of tissue continuity. Therefore, the consent of the appropriate bioethics committee and the consent of the examined person is required (as in this case). It is imperative to be especially careful when collecting biological material, working with it and disposing of residues. The result of the analysis depends on the type of biological material and its preparation. Therefore, an appropriate method of sample preparation should be chosen for the determination of a selected group of compounds (proteins or small molecule compounds < 2000 Da). The method of collecting biological material and its storage has a key impact on the results of analyses. From the moment of collection, the material should be stored at low temperatures, depending on the chemical nature of the material being tested and the schedule of planned analyses. It is important that the biological material is not thawed several times, because the whole structure and the number of analytes determined may change, which will ultimately affect the test result. This would have an effect on obtaining false positives and/or false negative results.

REVIEW

The attention of scientists for a long time is focused on standard qualitative research and quantitative biological material (e.g. urine, blood, serum, plasma, hair, cerebrospinal fluid) focusing on the search for disease markers and metabolism studies (1, 2). Thanks to its very good sensitivity and selectivity, mass spectrometry is considered a great achievement of modern technology.

The term metabolomics, defining a comprehensive analysis of small molecules involved in the metabolic pathways of living organisms, was coined by Oliver Fiehn in 2002. The first group of compounds on which the analysis of mass spectrometry is worth attention are anabolic-androgenic steroids (SAA). Quantitative determination of selected SAAs is carried out routinely using the enzyme immunoassay method (due to the speed of analysis and its price), however, in order to fully confirm the quantitative steroid profile, the GC-MS or GC-MS/MS technique is used (fig. 1a, b). These techniques are particularly important in the study of congenital adrenal hyperplasia in children, where a steroid profile, consisting of about 40 different steroids, is formed, and the presence of each steroid is associated with the activity of a specific enzyme (e.g. 21-hydroxylase, 3β-hydroxysteroid dehydrogenase – type 2, 17α-hydroxylase) in the steroidogenesis pathway. Methodology for the preparation of urine samples for the determination of SAA, in which hydrolysis, solid-phase extraction (SPE) is used, and ultimately the derivation of salicylate derivatives (TMS) is a well-known diagnostic laboratory. Thanks to studies on the metabolism of anabolic-androgenic steroids in children with congenital diseases, effective treatment of patients with accurate diagnosis can be undertaken (3, 4).

Another application for mass spectrometry is the determination of vitamin D and its metabolites. The popularity of vitamin D has increased significantly. It should be emphasized that the term vitamin D was defined by two compounds belonging to the 9,10-sterosteroid group (ergocalciferol – vitamin D_2 and cholecalciferol – vitamin D_3). Research has confirmed the role of vitamin D in systemic

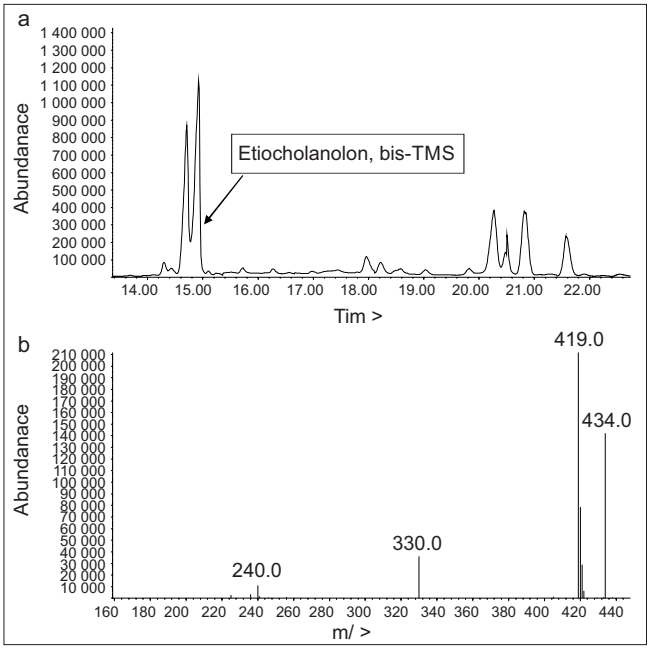

Fig. 1a, b. Example (A) chromatogram (GC-MS) and (B) MS spectrum in SCAN mode for androsterone, bis-TMS and etiocholonolone, bis-TMS

homeostasis in two ways. As a calcemic factor, it is responsible for the absorption of calcium and phosphates in the small intestine, regulates the level of calcium in the blood thus acting on the bones, parathyroid glands and kidneys. The second role is the effect on cell differentiation, antiproliferative effects on bone marrow cells, breast, muscle, intestine and lymphocytes. Vitamin D is essential in the process of normal calcium and bone homeostasis. A deficiency of vitamin D and its metabolites can lead to osteoporosis and hence fractures. The most important diagnostic significance of vitamin D is determined by determining the concentration of 25-hydroxyvitamin D due to the long half-life (2-4 weeks) and its concentration is associated with effective skin synthesis and intestinal absorption. Due to analytical problems (qualitative and quantitative) in the determination of vitamin D and its metabolites (5). Like other steroid hormones, it binds to the binding protein (DPB) and before each determination, the protein-vitamin D complex (and its metabolites) must be released in the sample preparation process. In addition, vitamin D is a hydrophobic compound, which increases the possibility of interference, which can often be undefined. The reference method for the determination of vitamin D and its metabolites was high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS) (fig. 2). It is extremely important, in particular if we perform vitamin D and its metabolites determination in laboratory diagnostics, the selection of appropriate reference materials and participation in interlaboratory comparisons to ensure measurement consistency and ensure high quality results throughout the analytical process. The confirmation of the high sensitivity and speed of MS is the use in newborn screening. Screening of newborns allows for early detection and initiation of treatment of congenital diseases and defects that, if untreated, result in mental or physical impairment. For each newborn, a few drops of blood are collected in a maternity ward for special examination. After drying, the paper is sent to one of the screening centers. In Poland, one of such centers is the Department of Screening Research of the Institute of Mother and Child in Warsaw, which conducts research performed using tandem mass spectrometry, which allows to detect rare disadvantages of metabolism. In Poland, screening tests cover the three most common congenital diseases: phenylketonuria, congenital hypothyroidism and cystic fibrosis. In other countries (e.g. USA), in screening for one analysis using tandem mass spectrometry (MS/MS), over 20 different markers of congenital metabolism defects (fatty acid oxidation disorder, organic acidosis or urea cycle defect) are identified (6-8). An interesting application of mass spectrometry in medicine and laboratory diagnostics is to determine the concentration of immunosuppressive drugs using LC-MS/MS. The use of these drugs is becoming more and more common due to numerous clinical trials and increasing knowledge about this group of medicines. A necessary condition for therapy with immunosuppressive drugs is regular monitoring of the drug concentration in the patient's blood for individual dosing due to the low concentration of the indicated drugs (ng/ml of whole blood), low and variable bioavailability as well as possible numerous interferences. The LC-MS/MS technique became the reference method for studying the kinetics of immunosuppressive drugs. This method ensures high specificity and ensures the linearity of the result, compared to immunochemical techniques, which are not as specific and may cause overestimated results in the concentrations of the drugs to be determined (9, 10).

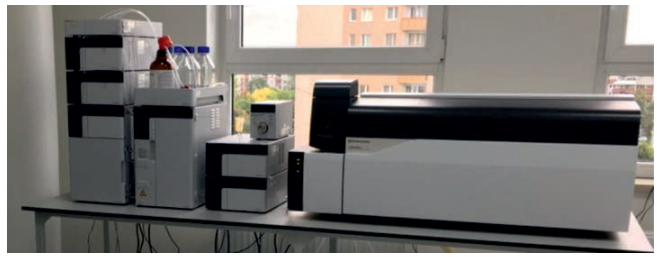

Fig. 2. Example of high performance liquid chromatography coupled with triple quadrupole mass spectrometry

In proteomics research, mass spectrometry finds more and new applications for use. An example is the study of the molecular weight of recombinant monoclonal antibodies (mAbs). Monoclonal antibodies are an important group of biopharmaceuticals with a wide range of applications in laboratory diagnostics and clinical trials. These antibodies are derivatives of glycoproteins obtained from mammalian cells. These cells, after purification and concentration, are stored in a suitable buffer. The great advantage of recombinant monoclonal antibodies is their high stability and wide application. By using nano-LC-Q-TOF (liquid nanopowpling combined with a quadrupole and time-of-flight analysis), the entire molecular weight of the recombinant monoclonal antibody can be determined

for its identification. The mass and peptide sequence of the Fab and Fc fragments of the antibody can also be determined (11, 12).

Mass spectrometry found its place in laboratory diagnostics due to high sensitivity and repeatability. This technique provides strong evidence for physiological disturbances, also helping to track the entire metabolism of endogenous metabolites. It also allows you to determine the concentration of drugs in various types of therapies and toxicology, where the concentration of drugs or stimulants persist at the level of pg-ng/ml. MS may have two applications in diagnostics: as a screening method and targeted analysis of known compounds. One should remember about the important role of specialized software, which is an indispensable element of any analysis related to mass spectrometry. They help to determine the patterns of test compounds, the amino acid sequence in peptide molecules, establish protein conformation, and above all, help to optimize the analytical parameters of the apparatus for accurate qualitative and/or quantitative measurement.

CONCLUSIONS

Mass spectrometry (MS) has found its place in medicine and medical diagnostics thanks to the possibilities of particle analysis as well as high sensitivity and repeatability. This technique provides strong evidence for physiological disturbances, also helping to track the entire metabolism of endogenous metabolites. It allows you to determine the concentration of drugs in various types of therapies or toxicology, where the concentration of drugs or stimulants persist at pg or ng/ml. MS may have two applications in diagnostics: as a screening method and targeted analysis of known compounds. One should remember about the important role of specialized software, which is an indispensable element of any analysis related to mass spectrometry. They help to determine the patterns of test compounds, the amino acid sequence in peptide molecules, establish protein conformation, and above all help to optimize the analytical parameters of the apparatus for accurate qualitative and/or quantitative measurement.

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