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Review

Chromatographic techniques coupled with mass spectrometry for the determination of organic acids in the study of autism☆

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ABSTRACT

Chromatographic methods find application in the diagnostics and prognosis of diseases. They are used in finding new biomarkers, which may result in early medical intervention. Early diagnosis and intervention are especially important in the case of diseases of unknown etiology. One of these is autism. Autism is a neurodevelopmental disorder characterized by severe impairment in reciprocal social interaction and communication and a pattern of repetitive or stereotyped behavior. Organic acids are intermediate metabolites of all major groups of organic cellular components and can play a role in the pathogenesis of autism. This review presents information about abnormal levels of some organic acids observed in the urine of children with autism and determination of acids with the use of chromatographic techniques. 342 literature sources on frequency (2005–2012) of the use of chromatographic methods in the determination of organic compounds in various body fluids were searched.

Contents

1. Scope

This review focuses on the use of chromatographic techniques coupled with mass spectrometry for the determination of organic acids in the diagnosis and intervention in autism.

2. Introduction

Coupled techniques such as gas chromatography–mass spectrometry (GC–MS), gas chromatography–tandem mass spectrometry (GC–MS/MS), liquid chromatography-mass spectrometry (LC–MS), liquid chromatography–tandem mass spectrometry (LC–MS/MS), liquid chromatography–nuclear magnetic resonance-mass spectrometry (LC–NMR-MS) and capillary electrophoresis–mass spectrometry (CE–MS) find wide applications in clinical research. Chromatographic methods are applied in the diagnostics and prognosis of diseases. They are used to find new biomarkers, which may help in early medical intervention. Early diagnosis and quick intervention are especially important in the case of diseases of unknown etiology such as autism. Autism is a neurodevelopmental disorder characterized by severe impairment in reciprocal social interaction and communication and in the pattern of repetitive or stereotyped behavior. Many investigations suggest that biomedical intervention may help in the therapy and recovery of autistic children. Restrictive diets and other nutritional or gastrointestinal therapies such as a gluten-free and casein-free diet, antifungal medications to treat fungal overgrowth in the gut and dietary supplementation with vitamins, minerals, omega-3 fatty acids and others have become very popular in intervention in autism [1]. The analysis of metabolites

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such as carboxylic acids, amino acids, carbohydrates, opioid peptides in body fluids of autistic children plays an important role in the screening, diagnosis, and monitoring of a variety of anomalies in autism. Opioid peptides are a potential marker of autism and thus they are very useful in predicting and monitoring responses to a casein and gluten-free diet. The determination of opioid peptides in the urine of autistic children is possible due to chromatographic techniques such as liquid chromatography coupled mass spectrometry (LC–MS/MS), liquid chromatography and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) [2]. Moreover, a chromatographic method was studied for the quantification of β-carbolines in hair as potential biomarkers in autistic children [3]. Elevated levels of trans-indolyl-3-acrylolglycine (IAcrGly) have been reported in the urine of people with autism. The results suggest that urinary IAcrGly can be a diagnostic indicator of autism. Chromatographic method LC–MS/MS makes it possible to detect and quantify IAcrGly [4]. Biochemical anomalies connected with organic acids have been identified in many people with autism [5]. A profile of the biochemical acid may play an important role in understanding the etiology of autism and different developmental disorders in children. In the case of autistic children, the quantitative organic acid profiling can assess: mitochondrial energy production, fatty acid metabolism, carbohydrate metabolism, B-complex sufficiency, methylation of co-factors, neurotransmitter metabolism, oxidative damage, detoxification status and bacterial and yeast overgrowth.

3. The health problems comorbid with selected organic acids

Since the first use of organic acid profile in the diagnosis of lactic iso-valeric in 1966 [6], the list of disorders resulting from abnormal excretion of organic acids has widely grown [7]. The determination of organic acids is the key for the diagnosis of inborn errors of metabolism (IEM). Organic acidurias are a biochemically heterogeneous group of inborn errors of metabolism. They are characterized biochemically by the accumulation of metabolites which are not present under physiological conditions, produced from the activation of alternative pathways in response to the loss of function of a specific gene product (enzyme), or by the accumulation of pathological amounts of normal metabolites. More than 65 inherited metabolic abnormalities are known to yield a characteristic urinary organic acid pattern, which is essential for diagnosis [8].

In the case of neurological diseases and disorders, homovanillic acid (HVA) and vanillylmandelic acid (VMA) play a crucial role. The analysis of the levels of VMA and HVA in body fluids is used in the diagnosis of various health problems (Table 1), as well as to monitor the progress of therapy.

Studies of metabolic fingerprints present the correlation between the occurrence of bacterial infection and the levels of succinic acid in clinical samples. Although the succinic acid gives information about the bacterial infection of the organism, it cannot serve as an indicator of differentiation of aerobic and anaerobic bacteria [17].

Since the publication of the information (25 years ago) about the deficiency of carnitine and the deficiency of muscle carnitine palmitoyltransferase, the fatty acids oxidation disorders (FAOD) has become one of the most important problems in the group of congenital disorders with a broad spectrum trial (from major malformations or sudden infant death syndrome, to the defects in adulthood nearly asymptomatic) [18]. Among mitochondrial dysfunctions of fatty acids oxidation we can distinguish, for example: very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, mitochondrial trifunctional protein (MTP) deficiency, carnitine palmitoyl transferase (CPT 1) deficiency, carnitine palmitoyltransferase 2 (CPT 2) deficiency, carnitine-acylcarnitine translocase (CACT) deficiency. FAOD disorders affect the functioning of organs with high energy demand and insufficient supply of energy can result in severe clinical symptoms, including neurological dysfunction and risk to life. Despite the relative abundance of new research on FAOD, there is evidence that many of these disorders remain undiagnosed due to the diversity of their symptoms. Thus, there is a need to study the metabolic profiles in blood and urine samples of patients [18]. The diagnosis of FAOD relies mainly on the determination of the levels of organic acids in urine with the application of GC–MS, and on the analysis of the blood acylocarnitine profile using tandem mass spectrometry (MS/MS) [19]. Due to the fact that carnitine is essential for the transportation of long-chain fatty acids into the mitochondrial matrix, which is the place of reaction of the β-oxidation, its lack leads to the degradation of fatty acids through alternative less energy efficient ω-oxidation pathway and, consequently, to increased urinary excretion of adipic acid and suberic acid. These abnormalities are common in children with attention disorders. Among the group affected with dicarboxylic acidosis symptoms of Reye's syndrome are observed, which also is associated with the features that affect mitochondrial toxic metabolic products of viruses [14]. In addition, deficits of vitamin B2 (Riboflavin) contribute to the weakening of the β-oxidation and increased urinary excretion of adipic acid and suberic acid [20]. Studies of levels of dicarboxylic acid in patients suffering from Zellweger syndrome indicate that in the absence of ketoacidosis the complete excretion of adipic, suberic and sebacic acid was significantly higher by approximately 100%, 200%, 350% as compared with the reference group. In the treatment of disorders associated with high levels of adipic acid and suberic acid, the literature reports mainly about application of L-carnitine, acetyl-L-carnitine, vitamin B2 and B5, magnesium, coenzyme Co10. The diet low in fat and protein and high in carbohydrates is also recommended [20,21].

Table 1
Health problems associated with abnormal levels of homovanillic acid (HVA) and vanillylmandelic acid (VMA) in body fluids [9–16].

Health problem connected with high level of metabolites in body fluids	Health problem connected with low level of metabolites in body fluids
HVA, VMA in urine – neuroblastoma	HVA, VMA in urine and cerebrospinal fluid – depression, sleep disturbances, anxiety and fatigue
HVA in plasma – delusional symptoms	HVA in cerebrospinal fluid – bipolar disorder type 1
HVA/VMA in urine – Menkes syndrome	HVA in cerebrospinal fluid – Parkinson's disease
HVA, VMA, HVA/VMA in urine–anorexia nervosa	
VMA in urine – familial neurodegenerative disorder with hypertension and paroxysms of irritability and sweating	
HVA in urine – autism	

4. Chromatographic techniques coupled with mass spectrometry in the determination of selected organic acids

Coupled chromatographic techniques are reliable and suitable to determine organic acids present in biological fluids, especially in urine. Organic acids are mostly analyzed by gas chromatography–mass spectrometry and tandem mass spectrometry (GC–MS and GC–MS/MS, respectively) [22,23]. These methods are characterized by high sensitivity, peak resolution and reproducibility. Compounds analyzed by GC/MS should be volatile and thermally stable. Therefore, polar and non-volatile compounds require chemical derivatization at the polar functional groups to reduce the polarity, increase the thermal stability and volatility of