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Chromatographic and mass spectrometric techniques in studies on oxidative stress in autism

Joanna Kałużna-Czaplińska*, Jagoda Jóźwik-Pruska

Department of Chemistry, Institute of General and Ecological Chemistry, Lodz University of Technology, Lodz, Poland

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ABSTRACT

Healthy body is characterized by the presence of a dynamic and balanced equilibrium between the production of reactive oxygen species (ROS) and the antioxidant capacity. In oxidative stress this balance is switched to reactions of oxidation leading to increased production of ROS, exceeding the capacity of physiological antioxidant systems. Oxidative stress is known to be linked to many disturbances, disorders and diseases. One of these is the autism spectrum disorder (ASD). ASD is a neurodevelopmental disorder manifested by abnormalities in social communication and interaction, as well as by occurrence of repetitive, restricted patterns of behavior or activities. It is believed that adequate knowledge about the oxidative stress biomarkers and the possibility of their reliable measuring could be useful in broadening knowledge on various diseases including ASD. A high number of compounds have been proposed as biomarkers of oxidative stress. Some of these are connected with the severity of ASD. The present review gives a summary of the chromatographic techniques used for the determination of biomarkers for oxidative stress in autism, and of other compounds important in this context. The first part of the review focuses on the correlation between oxidative stress and autism. The second part describes applications of chromatographic and mass spectrometric methods to the analysis of different metabolites connected with oxidative stress in biological fluids of autistic children. Advantages as well as disadvantages of the application of these methods for the analysis of different types of oxidative stress biomarkers are discussed.

1. Introduction

Chemically, an atom is usually composed of a central nucleus with pairs of electrons orbiting around it. Yet, some atoms in molecules have unpaired electrons which tend to form pairs with other electrons, thus making free radicals highly reactive and unstable [1].

In a normal and healthy body there is a dynamic balanced equilibrium between the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), peroxyl, hydroxyl, alkoxy, and nitric oxide (NO) free radicals [2], and the antioxidant capacity of the cell [3]. In states of elevated oxidative stress this balance is disturbed and switched in favor of ROS [4].

ROS are inactivated within the cell by antioxidant defense mechanisms. Primary antioxidant enzymes include catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx). These enzymes are directly involved in the elimination of ROS. Secondary antioxidant enzymes include glutathione reductase and glucose-6-phosphate dehydrogenase which help maintain a steady concentration of NADPH and glutathione (GSH), which are essential for the proper functioning of the whole antioxidant defense system [5,6]. To ensure effective antioxidative mechanism and optimal catalytic activity of these enzymes, the presence of micronutrients, including selenium, copper, iron, zinc and manganese, is necessary. Glutathione, ceruloplasmin, transferrin, vitamin E and vitamin C also participate in the antioxidant defense system [7–9].

ROS have many harmful effects on numerous biomolecules including lipids and proteins, which result in structural and functional disorders at molecular and cellular levels [10,11]. The mitochondrial respiratory chain is a natural endogenous source of ROS in the human body. ROS may also have exogenous origin, e.g., toxins present in the environment or cigarette smoke, drugs, xenobiotics, or radiation [1].

Nevertheless, it should be highlighted that oxidative stress is desirable in some cases. First of all oxidative stress induces apoptosis to prepare the birth canal to delivery. Additionally, oxidative stress affects strengthening the biological defense mechanisms during ischemia [1].

* Corresponding author. Fax: +48 426313103.
E-mail address: jkaluzna@p.lodz.pl (J. Kałużna-Czaplińska).

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Nomenclature

3CT	3-Chlorotyrosine
3NT	3-Nitrotyrosine
5cxP	Pentacarboxyporphyrin
8-OHdG	8-Hydroxy-2'deoxyguanosine
ASD	Autism spectrum disorder
ATEC	Autism treatment evaluation checklist
cP	Coproporphyrin
DLI	Direct liquid introduction
EC	Electrochemical
ECD	Coulometric electrochemical detection
ELISA	Enzyme-linked immunosorbent assay
EP	European Pharmacopoeia
F2-IsoPs	F2-Isoprostanes
GABA	Gamma-aminobutyric acid
GC/NICI-MS/MS	Chromatography/negative ion chemical ionization-tandem mass spectrometry
GC–MS	Gas chromatography-mass spectrometry
GC–MS/MS	Gas chromatography-tandem mass spectrometry
GID	Gastrointestinal dysfunction
GLC–MS	Gas liquid chromatography
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
HCY	Homocysteine
Hg	Mercury
HOCl	Hypochlorous acid
HPLC	High-performance liquid chromatography
HPLC-TOF-MS	High-performance liquid chromatography-time of flight- mass spectrometry
LC–MS	Liquid chromatography-mass spectrometry
LC–MS/MS	Liquid chromatography-tandem mass spectrometry
LC-TOF-MS	Liquid chromatography-time of flight- mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
PDD-BI	Pervasive development disorder behavior inventory
prcP	Precoproporphyrin
ROS	Reactive oxygen species
SAH	*S*-Adenosylhomocysteine
SAM	*S*-Adenosylmethionine
SAS	Severity of autism scale
SIM	Selected-ion monitoring
SOD	Superoxide dismutase
SST	System-suitability Test
tGSH	Total glutathione
TOF	Time of flight
UPLC	Ultra-performance liquid chromatography
UPLC–MS	Ultra-performance liquid chromatography-mass spectrometry
WHO	World Health Organization

Considering all the above, sound knowledge about oxidative stress biomarkers and the availability of reliable analytical methods for the measurement in biological samples are indispensable for scientists from various disciplines to gain deep insights into the pathological features of diverse diseases as well as to assess the efficiency of medications.

Human brain consumes about 20% of metabolic oxygen and the main portion of energy is used by neurons [12]. These cells are characterized by a limited antioxidant capacity and require high amounts of energy, lipids and iron [13]. Therefore, it is prone to oxidative stress. The main role of antioxidants is to counter attacks of neurons by ROS, which is particularly important in the early critical period [14]. It can be assumed that children who have lower GSH levels are exposed more often to oxidative stress [9]. Additionally, other external factors such as environmental and metabolic factors may intensify this process.

The role of oxidative stress is considered important in the context of many diseases, including neuropsychiatric diseases [10,15,16], anxiety disorders [17], and major depressive disorder [18]. According to the Diagnostic and Statistical Manual of Mental Disorders (5th edition, DSM-5), the autism spectrum disorder (ASD) is manifested by abnormalities in social communication and interaction as well as by the occurrence of repetitive and restricted patterns of behavior or activities. ASD represents a single continuum of impairments with varying degrees of severity [19]. It is suggested that this disorder may result from an interaction between immunological, environmental and genetic factors, with oxidative stress being the linking mechanism [20].

Oxidative stress can be detected by the examination of antioxidant status, antioxidant enzymes, protein/DNA oxidation, and lipid peroxidation. All of them are considered significant in children with ASD, including the glutathione system (reduced glutathione-oxidized glutathione), methionine, cysteine, transferrin, urine 8-OHdG, ceruloplasmin, 3-chlorotyrosine (3CT), 3-nitrotyrosine (3NT) [21], and F2t-isoprostanes (F2-IsoPs) [22].

This review focuses on the use of chromatographic techniques coupled with mass spectrometry for the determination of biomarkers of oxidative stress in autism.

2. Biomarkers of oxidative stress

2.1. Biomarkers

As long as the main cause of ASD is not clear, there is a need for searching for biomarkers being specific to this disorder. A limited understanding of its etiology makes it difficult to diagnose in its earliest stage. However, studies on the determination and identification of ASD biomarkers are believed to be a powerful tool in predicting the development of this disorder. This assumption raises the question of the definition of a biomarker. This term refers to a wide range of medical sings. According to the definition presented by The National Institute of Health Biomarkers Definition Working Group [23], a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention". Another definition assumes that a biomarker or a biological marker is a measureable substance, a structure or process which occurs in the body, or its products having an influence or making it possible to predict a disease [24]. WHO describes the biomarker as "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction" [25].

Searching for new biomarkers is currently a very popular direction in research. Hence, the question about abusing this term should be asked. Literature presents some requirements that should be met in order to name a potential factor "biomarker" [26].

A wide range of compounds occurring in the human body are characterized by reference ranges. Disturbances in their concentrations may lead to abnormal functioning of the body, various