Journal of Pharmaceutical and Biomedical Analysis xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Metabolomics studies in brain tissue: A review

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ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form 5 July 2016
Accepted 7 July 2016
Available online xxx

Keywords:
Metabolomics
Brain
Alzheimer's disease
LC–MS
NMR
Fingerprinting

ABSTRACT

Brain is still an organ with a composition to be discovered but beyond that, mental disorders and especially all diseases that curse with dementia are devastating for the patient, the family and the society. Metabolomics can offer an alternative tool for unveiling new insights in the discovery of new treatments and biomarkers of mental disorders. Until now, most of metabolomic studies have been based on biofluids: serum/plasma or urine, because brain tissue accessibility is limited to animal models or *post mortem* studies, but even so it is crucial for understanding the pathological processes. Metabolomics studies of brain tissue imply several challenges due to sample extraction, along with brain heterogeneity, sample storage, and sample treatment for a wide coverage of metabolites with a wide range of concentrations of many lipophilic and some polar compounds. In this review, the current analytical practices for target and non-targeted metabolomics are described and discussed with emphasis on critical aspects: sample treatment (quenching, homogenization, filtration, centrifugation and extraction), analytical methods, as well as findings considering the used strategies. Besides that, the altered analytes in the different brain regions have been associated with their corresponding pathways to obtain a global overview of their dysregulation, trying to establish the link between altered biological pathways and pathophysiological conditions.

1. Introduction

In the human body, brain has been the last organ to be studied. Nowadays most of its disorders still remain unclear without deep knowledge about either their pathogenesis or the treatments to attenuate their progress. One of the big challenges in advancing in therapeutic treatments of pathologies related to brain has been the combination of different analytical techniques and data analysis strategies to discover the biochemical bases under the development of mental disorders and neurodegenerative diseases. Metabolomics studies metabolites, small molecules, end products of the cascade that starting in genes includes all the interactions in a biological system including exogenous and environmental factors. Therefore, metabolomics can be considered a useful tool to discover the effects of a drug on the central nervous system (CNS), recognize different stages of a disease, or understand some of the brain functions, such as receptor-ligand interactions and new points of view about the mechanism of CNS disorders. This review outlines the principal aspects related to metabolomics in brain tissue with a dual approach in terms of describing methodological strategies and findings, going over the main articles published in recent years, and getting focused on the different possibilities considering analytical platforms and sample treatments and furthermore altered pathways based on the described findings. All the information enclosed will result very useful for researchers to extend the metabolites coverage for diagnosis, monitoring, development of potential drug targets and novel therapeutic interventions, which will eventually benefit patients with mental disorders.

1.1. Metabolomics and brain tissue

In the literature, several articles have been recently published applying different analytical platforms to determine the concentration and distribution of metabolites in different brain regions for understanding the biochemical changes produced in brain under different physiological conditions or therapeutic treatments. The number of scientific articles published each year in the XXIst century in the field of metabolomics related to brain tissue is presented in Fig. 1 and, as can be seen, is increasing exponentially. The main topics covered are neurodegenerative diseases and mental disorders.

The term dementia includes a set of symptoms, such as decline in memory, language, thinking behaviour and other cognitive skills that affect a person's ability to perform everyday activities, depend-

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http://dx.doi.org/10.1016/j.jpba.2016.07.008
0731-7085/

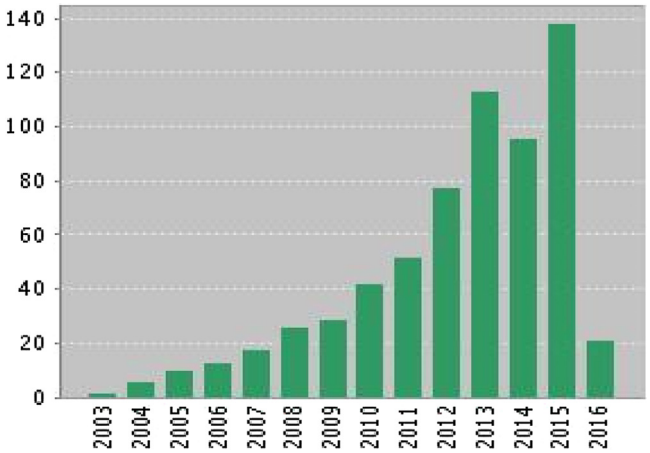

Fig. 1. Chart representing the amount of scientific articles published in the field of metabolomics research related to neurodegenerative diseases during the last years. The search terms were "metabolomics" or "metabonomics", "neurodegenerative diseases", and "brain". The date of publication was not limited, and only articles reporting metabolomics data were selected for this review. Information available at Web of Science.

ing on the disease and stage of illness and it could be acute or progressive. Today, over 46 million people live with dementia worldwide, and this number is estimated to increase to 131.5 million by 2050 according to the World Alzheimer Report 2015 [1]. However, dementia is an umbrella term that comprises different etiologies, including neurodegenerative, metabolic, vascular and infectious diseases [2]. Neurodegenerative disease is a commonly used term that encloses a diverse spectrum of chronic neurological disorder, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) as the most prevalent disorders, affecting people in middle to late age. Although their origins are different, neurodegenerative diseases are characterized by a slowly progressive nervous system dysfunction directly associated with neuronal degeneration [3].

AD is the most common cause of dementia and it accounts for an estimated 60% to 80% of dementia cases. The most frequent brain manifestations believed to contribute to the development of AD include deposits of extracellular amyloid β-peptide, and intracellular deposits of abnormally hyperphosphorylated τ protein resulting in the form of neurofibrillary tangles. Both changes lead to a decline of the number of synapses due to neuronal death, causing the main symptoms of AD, such as progressive memory loss, challenges in planning or solving problems, confusion with time and place, difficulties to write and talk and changes in mood and personality, including apathy and depression, among others [4]. PD is recorded as the second most common neurodegenerative disorder in adults over the age of 65, caused by a progressive alteration of the nervous system that produce a unilateral resting tremor in an arm or leg, although bradykinesia, inability to move, rigid limbs, gait imbalance, or postural instability can also be presented as early symptoms [5]. These symptoms are the result of the progressive degeneration of a majority of the dopamine neurons of the substantia nigra, resulting in loss of dopamine signalling to the striatum. Lewy body inclusions in degenerating neurons are a characteristic neuropathological feature of PD when present in the brainstem and neocortex. Additionally, PD is characterized by T-cell infiltration and accumulations of microglia cells and astrocytes correlated with alterations in glial cell morphology and function [6].

HD is an autosomal dominant neurodegenerative disorder involving uncontrolled movements, psychiatric abnormalities, and cognitive deterioration, induced by an expanded CAG-repeat expansion in the huntingtin (*HTT*) gene, which encodes a polymorphic polyglutamine (PolyQ) repeat in the huntingtin (HTT) protein [7]. It is considered as a rare disorder with a mean age of onset of 40 years, causing the death of patients 20 years after onset. HD prevalence is located between 5–10 cases per 100,000, being slightly lower in East Asian countries and in the black population. The annual incidence varies between 1–4 cases per million inhabitants. The neuropathology is mainly characterized by the neuronal dysfunction and death within brain, predominantly in the cortex and striatum.

Although CNS alterations are the most outstanding clinical aspects, metabolomics changes are also remarkable, together with immune alterations, cardiac failure, skeletal-muscle deteriorating, or weight loss, among others [8]. Finally, ALS is described as a progressive neurodegenerative disease that affects nerve cells in the brain and the spinal cord, resulting in a degeneration of motor neurons. This condition affects people between 40 and 60 years old, and men population is affected more often than women. The initial symptoms can include muscle twitching, cramping, muscle weakness affecting arm or leg, slurred and nasal speech, or difficulty chewing or swallowing [5].

1.2. Targeted and non-targeted metabolomics

In 1998, the word "metabolome" was introduced for the first time, referring to the complete set of low-molecular-weight molecules (<1500 mDa) or metabolites synthesized by a cell, whose shifts give the unique chemical fingerprints that specific cellular processes leave behind [9]. In consequence, metabolomics emerged as a new field in analytical biochemistry research, concerned with the identification, quantification and characterization of the small metabolites in the metabolome [10]. Metabolomics has joined genomics, proteomics and transcriptomics as one of the new "omics", all of them employed to understand the global systems biology. Systems biology applies holistic approaches to inspect the dynamic collection of proteins, genes, transcripts and metabolites present in the human body. Metabolites are chemically transformed during metabolism in response to a particular stimulus or a disease state, giving the *in vivo* information about the actual status of the body [11] (Fig. 2).

Covering the entire spectrum of the metabolome is not possible with one single instrument in currently available technologies. As a consequence, a multiplatform analytical approach is needed in order to extend the coverage of metabolites detection in a biological system. A classification of different approaches to metabolomic/metabonomic analysis was proposed by Fiehn and Nicholson [12,13]; however in practice it can be considered mainly targeted and non-targeted metabolomics. While the first one is described as "the quantitative or semi-quantitative measurement of a defined group of metabolites known to be involved in a specific biochemical pathway or metabolic reaction", the second one is described as "a global unbiased analysis of all the metabolites present in a biological system, including chemical unknowns, under a set of circumstances" [14]. Nowadays, neurodegenerative diseases have no clear biomarkers for their early diagnosis or treatment. Metabolites could be biomarkers of a broad range of central nervous system disorders serving as molecular drivers and by-products of disease pathobiology. Their analysis in precisely dissected regions of brain is an appropriate strategy to follow and some studies with brain tissue have been based on targeted analysis of a restricted number of metabolites [15–18]. However, this strategy reduces the possibility to find possible biomarkers for different pathologies related to brain.

A non-targeted metabolomics analysis seems to be an interesting strategy to follow for these pathologies providing broader metabolite coverage [19] in a discovery phase. Due to the great complexity of the data obtained, non-targeted metabolomics must

Please cite this article in press as: C. Gonzalez-Riano, et al., Metabolomics studies in brain tissue: A review, J. Pharm. Biomed. Anal. (2016), http://dx.doi.org/10.1016/j.jpba.2016.07.008