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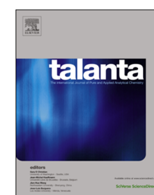

Liquid chromatography–tandem mass spectrometry method for determination of panel of neurotransmitters in cerebrospinal fluid from the rat model for tauopathy

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ABSTRACT

Alzheimer's disease (AD) is still being recognized today as an unmet medical need. Currently, there is no cure and early preclinical diagnostic assay available for AD. Therefore much attention is now being directed at the development of novel methods for quantitative determination of AD biomarkers in the cerebrospinal fluid (CSF). Here, we describe the liquid chromatography–tandem mass spectrometry method for determination of 5-hydroxytryptamine (SER), 5-hydroxyindoleacetic acid (5-HIAA), homovanilic acid (HVA), noradrenaline (NADR), adrenaline (ADR), dopamine (DA), glutamic acid (Glu), γ-aminobutyric acid (GABA), 3,4-dihydroxyphenylacetic acid (DOPAC) and histamine (HIS) in cerebrospinal fluid (CSF) from the rat model for human tauopathy. The benzoyl chloride was used as pre-column derivatization reagents. Neurotransmitters and metabolites were analysed on ultra performance liquid chromatography (UPLC) on C18 column in combination with tandem mass spectrometry. The method is simple, highly sensitive and showed excellent linearity with regression coefficients higher than 0.99. The accuracy was in a range of 93–113% for all analytes. The inter-day precision ($n=5$ days), expressed as % RSD, was in a range 2–10% for all analytes. Using this method we detected significant changes of CSF levels of two important neurotransmitters/metabolites, ADR and 5-HIAA, which correlates with progression of neurodegeneration in our animal model.

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1. Introduction

Alzheimer's disease (AD) is a chronic, irreversible neurodegenerative disease that affects higher structures of the brain [1]. Prominent neuropathologic features of AD are senile plaques, neurofibrillary tangles, neuroinflammation, synaptic and cell loss [2–4]. AD is a leading cause of dementia worldwide. Currently 20–30 million individuals suffer from dementia today, with 4.6 million new cases of dementia every year [5]. In spite of intensive research there is no disease modifying drug for AD treatment available on the market. Currently, most prevalent is symptomatic therapy, which is not able to stop progression of the disease [6].

It has been proposed that therapeutic intervention is most effective when administered early in the disease's progression, before neurodegeneration is too severe and widespread. There is unmet need for novel diagnostic tools that accurately identify Alzheimer's disease in the very early phase of the disease. An ideal diagnostic tool must be sensitive to earliest changes in AD and should differentiate among preclinical AD, normal aging and other brain disorders that cause memory loss [7]. It has been shown that valid clinical biomarkers could be obtained from detail metabolomic studies of body fluids of transgenic animal models of the disease [8]. Major advantage of metabolomics over other 'omics' is that metabolism is conserved during evolution and metabolic networks are essentially very similar in animals and human beings. Therefore the findings from animal models are directly transferable to humans [9].

One group of metabolites that is of particular interest in neuroscience research is a group of neurotransmitters (NT) together with their bioprecursors and metabolites. Several methods have been developed and used for analysis of NT and metabolites in cerebrospinal fluid (CSF) from rat. The most common method is HPLC with electrochemical detection [10–13]. Although very sensitive, this

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; NT, neurotransmitters; UPLC, ultra-performance liquid chromatography; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; IS, internal standard; Glu, glutamic acid; GABA, γ-aminobutyric acid; HIS, histamine; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanilic acid; SER, 5-hydroxytryptamine; DOPAC, 3,4-dihydroxyphenylacetic acid; NADR, noradrenaline; ADR, adrenaline; DA, dopamine

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method is not applicable for analysis of NT with low intrinsic electroactivity (e.g. histamine) [14]. Radioenzymatic methods have been used for analysis of adrenaline or noradrenaline in rat CSF [15,16]. The radienzymatic methods are selective and very sensitive, however their major disadvantage is a use of the radioactive chemicals. Combination of HPLC with UV [17] or fluorimetric [18] detection was also used for NT/metabolites in rat CSF. Pre-column derivatization with N-hydroxysuccinimide esters followed by HPLC with electrochemical detection is another method that was used previously. However this method is very laborious and requires separate HPLC runs for non-electroactive and electroactive compounds [19].

LC/MS methods are becoming popular in neuroscience research due to their sensitivity, specificity and applicability for complex matrices such as tissues and body fluids. Ultra performance liquid chromatography (UPLC) is a modern method that runs chromatographic separations using columns packed with small size particles. The small size particles allow for increased speed with superior resolution and sensitivity. In combination with mass spectrometry, UPLC has been previously used for analysis of few NT/metabolites in various biological matrixes [20–22].

Chemical derivatization is often used to enhance the detection sensitivity in ESI/MS. Many derivatization reagents previously used for UV or fluorescence detection were found to be useful also for MS [23]. Recently, the derivatization with dansyl chloride was used for analysis of panel of NT in human plasma [24]. However, derivatization with dansyl chloride has several disadvantages such as need for increased temperature during the derivatization reaction, longer time for derivatization and photosensitivity of derivates. Furthermore, there is no stable isotope labelled dansyl chloride available commercially and must be synthetically prepared for analysis. Derivatization with benzoyl chloride was hence used as a better alternative for neurochemical monitoring of NT and metabolites in brain microdyalisate [25] or brain tissue and plasma samples [26].

Here we developed rapid and sensitive method for analysis of panel of neurotransmitters and metabolites in rat CSF. Benzoyl chloride was used as pre-column derivatization reagent. All selected analytes were separated and quantified within the single chromatographic run. The use of UPLC/MS offered an advantage of short analysis time and therefore very good sample throughput, applicability for all analytes of interest and selectivity. To demonstrate its suitability in neuroscience research, the method was subsequently used for analysis of CSF samples from the transgenic rat model for tauopathy.

2. Experimental

2.1. Instrumentation

A Waters (Waters, Praha, CZ) Quattro Premier XE mass spectrometer coupled to a Waters ACQUITY UPLC system was used. Mass spectra were acquired using positive electrospray ionization and SRM. The capillary voltage was 3 kV and the source temperature and desolvation temperature were 120 and 450 °C, respectively. The cone gas and desolvation gas flowed at 50 and 750 L/h, respectively. Argon was used as collision gas at a manifold pressure of 2.8×10^{-3} mbar. The collision energies and source cone voltages were manually optimized for each SRM transition. Data were acquired with MassLynx 4.0 and calibrated and quantified by QuanLynx software.

2.2. Chemicals and materials

Glutamic acid, γ-aminobutyric acid, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, homovanilic acid, noradrenaline, adrenaline, dopamine, 3,4-dihydroxyphenylacetic acid, histamine, ^{12}C-benzoyl chloride, $^{13}C_6$-benzoyl chloride, ammonium formate and LC/MS grade acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). D6 4-aminobutyric acid was from C/D/N isotopes (Quebec, Canada). Water was purified using a Millipore system (Bedford, MA, USA). All other reagents used in the study were of analytical grade.

2.3. Preparation of standard solutions

Individual stock solutions of 5-hydroxytryptamine (SER), 5-hydroxyindoleacetic acid (5-HIAA), homovanilic acid (HVA), noradrenaline (NADR), adrenaline (ADR), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), histamine (HIS) were freshly prepared in methanol/water mixed solution (1:1, v/v). Glutamic acid (Glu) and γ-aminobutyric acid (GABA) stock were prepared in water. For NADR, ADR and DA, 250 μM ascorbic acid was added. For every sample set, working solutions were prepared from the freshly prepared stock solution every day. The benzoyl chloride (^{12}C and $^{13}C_6$) solution (2% in acetonitrile, v/v) was prepared fresh before each analysis. Calibration curves of the analytes were prepared by diluting the stock solutions with 50% methanol/water (v/v) to the concentration ranging from 0.25 to 4000 ng/ml and spiking into matrix. Internal standard was prepared by derivatization of standard mixture with $^{13}C_6$ benzoyl chloride using the same procedure as ^{12}C reagent, then diluted in DMSO.

2.4. Ultra-performance liquid chromatography

Acquity UPLC BEH C18 column (2.1 mm × 150 mm, 1.7 μm particle size) with VanGuard pre-column was used for analysis. Column temperature was set for 30 °C. Mobile phase A consisted of 10 mM ammonium formate/0.15% formic acid in water, and mobile phase B was acetonitrile. Mobile phase gradient program was as follows: 10% B for 2 min; increased to 90% B from 2 min to 10 min; 90% B from 10 min to 11 min, and then 10% B from 11 min to 13.3 min. The flow rate was 0.3 mL/min and the injection volume was 5 μL.

2.5. Method validation [27]

The inter-day precision and accuracy of the method was determined by analysing the 3 different concentrations over 5 days. Intra-day accuracy and precision was calculated from 6 repeat injections. The limit of detection (LOD, S/N = 3:1) and the limit of quantification (LOQ, S/N = 10:1) were calculated from standard chromatograms. Stability of derivatized standards was tested by analysing the sample stored at RT, 4 °C and −80 °C for up to 24 h after derivatization.

2.6. Animals

The generation and characterization of a transgenic rat model for tauopathy expressing human truncated tau151–391 was described in details elsewhere [28,29]. For this study, heterozygous transgenic rats (6–7 month old) and non-transgenic SHR age-matched controls were used. All animals were housed under standard laboratory conditions with free access to water and food and were kept under diurnal lighting conditions (12 h light/dark cycles with light starting at 7:00 am). All experiments on animals were carried out according to the institutional animal care guidelines conforming to international standards and were approved by the State Veterinary and Food Committee of Slovak Republic and by Ethics Committee of Institute of Neuroimmunology. Efforts were made to minimise the number of animals utilised and to limit discomfort, pain or any other suffering of the experimental animals used in this study.

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