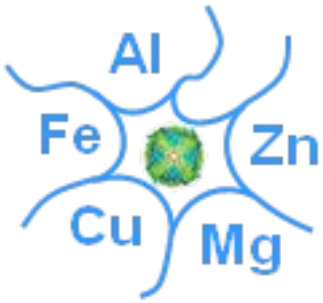
EMPIR EURAMET
The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

PTB
Physikalisch-Technische Bundesanstalt
Braunschweig und Berlin

ReMiND 2019

Biomolecules in Neurodegenerative Diseases



Meet internationally recognised scientists in the field of research, diagnosis and treatment of neurodegenerative diseases such as Alzheimer‘s disease!

26th and 27th June 2019

at Physikalisch-Technische Bundesanstalt (PTB)

ReMiND 2019

Kindly supported by

TauRx Therapeutics Ltd
Innovation in neurodegeneration

Agilent
Trusted Answers

Under the auspices of

Joint Committee for Traceability in Laboratory Medicine

JCTLM
Accurate results for patient care

Agenda

Wednesday 26th June 2019

08:00 - 09:00 Registration

09:00 - 09:20 Welcome Address

Dr. Bernd Güttler (PTB)

1. Session Biomolecules in neurodegenerative diseases

09:20 - 10:00 Amyloid production, clearance and aggregation in Alzheimer's disease (Keynote Lecture)
Gerd Multhaup (McGill University)

10:00 - 10:25 Influenza A virus infection in a mouse model of Alzheimer's disease

Shirin Hosseini (TU Braunschweig)

10:25 - 10:50 The use of Tau-Aggregation-inhibitors to treat Alzheimer's Disease: Being a Tauist in the Land of Amyloid
Franz Theuring (Charité/TauRx)

10:50 - 11:20 Coffee Break / Poster Foyer

11:20 - 11:45 Role of metals and metal containing biomolecules in neurodegenerative diseases

Claudia Swart (PTB)

11:45 - 12:10 Tau-protein quantification using gold and magnetic nanoparticles

Claudia Frank (PTB)

12:10 - 13:30 Lunch Break / Poster Buffet in Foyer

2. Session Biomolecules in neurodegenerative diseases

13:30 - 14:10 The identification and characterisation of small molecule human ABAD inhibitors as therapeutics in Alzheimer's Disease (Keynote Lecture)
Laura Aitken (University of St. Andrews)

14:10 - 14:35 ICP-MS – A useful tool for quantitative phosphoproteomics?
Andrea Raab (University of Aberdeen)

14:35 - 15:00 Development of a method for protein quantification via isotope dilution ICP-MS for application on an Alzheimer's biomarker
Nora Lemke (Charité)

15:00 - 15:30 Coffee Break / Poster Foyer

3. Session Metals in neurodegenerative diseases

15:30 - 15:55 Metal content of Alzheimer's Disease mouse model brains:
Total element determination by ICPMS
Elizabeth Griffin (University of Aberdeen)

15:55 - 16:20 High-throughput and low volume analysis of biologically essential trace elements in cerebrospinal fluid using ICP-TOF-MS
Gunda Köllensperger (University of Vienna)

16:20 - 16:45 Multi element analysis of micro volume biological fluids using ICPMS – method development, validation and interlaboratory comparison
Süleyman Z. Can (TÜBITAK Ulusal Metroloji Enstitüsü)

16:45 - 17:10 Copper, iron, and zinc isotopic distributions in Alzheimer's Disease
Nikolay Solovyev (University Ghent)

19:00 - 22:00 Reception of the City of Braunschweig and Conference Dinner

Thursday 27th June 2019

1. Session Metal containing biomolecules in neurodegenerative diseases

09:00 - 09:40	Quantification of biomolecules in biological matrices (Keynote Lecture) *Albert Sickmann (ISAS)*	
09:40 - 10:05	Preparation and characterization of an isotopically enriched (^{57}Fe) ferritin standard for species specific isotope dilution mass spectrometry *Anastassiya Tchaikovsky (University of Vienna)*	
10:05 - 10:30	Quantitative determination of Cu-containing proteins as potential biomarkers for Alzheimer's Disease *Julia Gleitzmann (PTB)*	
10:30 - 10:55	Determination of exchangeable copper by speciation studies to underpin Wilson's Disease diagnosis *Estela del Castillo Busto (LGC)*	
10:55 - 11:15	Coffee Break / Poster	Foyer
11:15 - 12:45	Visit of laboratories at PTB	
12:45 - 13:00	Groupphoto of all participants	Stairs in front of the seminar centre
13:00 - 14:00	Lunch Break / Poster	Buffet in Foyer

2. Session Clinical diagnosis of neurodegenerative diseases

14:00 - 14:40	Comparability in clinical diagnosis (Keynote Lecture) *Ian Young (Joint Committee for Traceability in Laboratory Medicine)*	
14:40 - 15:05	Innovative measurements for improved diagnosis and management of neurodegenerative diseases *Milena Quaglia (LGC)*	

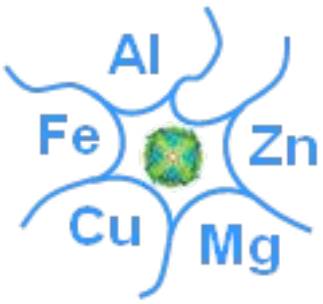
15:05 - 15:35 Future challenges and possible contributions of NMIs

Discussion

15:35 - 15:45 Closure of the conference

Wednesday

Presentations



Amyloid production, clearance and aggregation in Alzheimer's disease

Keynote speaker: G. Multhaup[a]

[a]Department of Pharmacology & Therapeutics and Integrated Program in Neuroscience, McGill University, H3G 1Y6 Montreal, Quebec, Canada
Corresponding author: email: gerhard.multhaup@mcgill.ca

Keywords: Alzheimer disease, amyloid Aβ, copper

The pathogenesis of Alzheimer's disease (AD) involves cleavage of the amyloid precursor protein (APP) into amyloid-beta (Aβ) peptides. These peptides can then form larger assemblies (i.e. oligomers and fibrils) in the brain which ultimately is associated with progressive neurodegeneration and cognitive decline. Different metals, such as zinc, iron, and copper, have been investigated for their impact on AD pathogenesis and it yet remains a controversial area of research.

The beta-site APP cleaving enzyme 1 (BACE1) can transfer cytosolic copper to intracellular compartments through binding to its transmembrane region sulfur-rich core. However, BACE1 is known primarily for its initial cleavage of the amyloid precursor protein (APP), which ultimately leads to the generation of Aβ peptides. Recently, we found that altered BACE1 levels and activity impact the degradation of Aβ40 and Aβ42 into a common Aβ34 intermediate. Using human cerebrospinal fluid (CSF) samples from the Amsterdam Dementia Cohort, we showed that Aβ34 is elevated in individuals with mild cognitive impairment who later progressed to dementia. Furthermore, Aβ34 levels correlate with the overall Aβ clearance rates in amyloid positive individuals. Using CSF samples from cognitively normal individuals at risk for AD, we further demonstrated that the Aβ34/Aβ42 ratio, representing Aβ degradation and cortical deposition, may serve as a marker of amyloid clearance.
Since the discovery that copper can bind directly to synthetic Aβ peptides as well as APP *in vitro*, cell-based studies have shown that copper can inhibit Aβ production and can have beneficial effects, like stimulating the non-amyloidogenic pathway of APP secretion. In mouse models of AD, daily copper supplementation decreased soluble amyloid production and increased their life span. In clinical trials with AD patients, beneficial effects on disease progression have also been observed through long-term oral intake of copper. As misbalanced copper homeostasis continues to play an important role in AD pathogenesis, effects of metal ions on the non-amyloidogenic and the amyloidogenic pathway may add to the complexity of approaches using copper chelators or supplementation as a possible effective therapeutic strategy.

List of references:

1. Liebsch F, Aurousseau MRP, Bethge T, McGuire H, Scolari S, Herrmann A, Blunck R, Bowie D, Multhaup G. Full-length cellular β-secretase has a trimeric subunit stoichiometry, and its sulfur-rich transmembrane interaction site modulates cytosolic copper compartmentalization. J Biol Chem. 2017 Aug 11;292(32):13258-13270. doi: 10.1074/jbc.M117.779165. Epub 2017 Jun 21.
2. Liebsch F, Kulic L, Teunissen C, Shobo A, Ulku I, Engelschalt V, Hancock MA, van der Flier WM, Kunach P, Rosa-Neto P, Scheltens P, Poirier J, Saftig P, Bateman RJ, Breitner J, Hock C, Multhaup G. Aβ34 is a BACE1-derived degradation intermediate associated with amyloid clearance and Alzheimer's disease progression. Nat. Commun. (NCOMMS-18-13252C), accepted March 2019.

Influenza A virus infection in a mouse model of Alzheimer's disease

S. Hosseini[a,b], K. Michaelsen-Preusse[a], A. Holz[a,b], K. Schughart[c], M. Korte[a,b]

[a]Department of Cellular Neurobiology, Zoological Institute, TU-Braunschweig, Germany
[b]Helmholtz Centre for Infection Research, Neuroinflammation and Neurodegeneration Group, Braunschweig, Germany
[c]Helmholtz Centre for Infection Research, Department of Infection Genetics, Braunschweig, Germany

Corresponding author: m.korte@tu-braunschweig.de

Keywords: Influenza infection, Neuroinflammation, Alzheimer's disease, APP/PS1 mice

Influenza viruses until today are a leading cause of worldwide severe pandemics and represent a major threat to human and animal health. Although the primary target of influenza viruses in mammals is the lung, infection can cause neurological disorders, including delirium and encephalopathy. We could show previously that a peripheral influenza A virus infection caused by a non-neurotropic virus variant leads to long-term neuroinflammation and synapse loss together with impaired memory formation in young adult mice (*Hosseini et al. 2018, JNS*). Processes of neuroinflammation have indeed been associated with neurodegenerative diseases such as Alzheimer's disease (AD) and prolonged or excessive innate immune responses are considered a risk factor for AD. Therefore, in order to investigate the role of influenza infection for the development and progression of AD two months old APP/PS1 mice were infected intranasally with non-neurotropic H3N2 (maHK68) influenza A virus. Whereas the infection had no effect on neuronal cell number in the CA1 region analysis of spine density revealed a reduction 120 days post infection in comparison to WT and also to non-infected APP/PS1 mice. A detailed analysis of microglia density and morphology revealed neuroinflammation in the hippocampus already of uninfected APP/PS1 mice but microglia activation was even more pronounced in APP/PS1 mice upon H3N2 infection. Taken together these results demonstrate that influenza infection as a peripheral immune stimulation may exacerbate AD possibly by triggering microglia hyperactivation.

List of references:

1. Hosseini S, Wilk E, Michaelsen-Preusse K, Gerhauser I, Baumgärtner W, Geffers R, Schughart K, Korte M. Long-Term Neuroinflammation Induced by Influenza A Virus Infection and the Impact on Hippocampal Neuron Morphology and Function. *J Neurosci.* 2018 21;38(12):3060-3080.

The use of Tau-aggregation-inhibitors to treat Alzheimer's Disease: Being a Tauist in the Land of Amyloid

F. Theuring[a]

[a]Charité – University Medicine Berlin, Institute of Pharmacology, Berlin; TauRx, Singapore

Keywords: neurofibrillary tangles, tau protein, Alzheimer's disease

Two pathological hallmarks can be found postmortem in the brains of Alzheimer patients: amyloid plaques made out of β-amyloid protein aggregates and neurofibrillary tangles composed of Tau protein aggregates. For the last 30 years the main interest had been on focusing on the development of the amyloid plaques and it was hoped that therapeutic intervention interfering with the underlying processes in amyloid aggregation might result in disease modifying activity. However, all the clinical trials which had used different approaches such as small molecules and/or monoclonal antibodies targeting various aspects in the amyloid hypothesis had failed, despite the fact that thousands of patients had been enrolled in the clinical studies and ca. 16 bn US $ had been spend for R&D by big Pharma.
The involvement and importance of the Tau protein and its aggregation process in leading to the development of Alzheimer Disease had basically been ignored, although sufficient clinical data had already demonstrated the fundamental contribution of this protein to the disease processes.
I'm going to report on preclinical and clinical Phase 2 and 3 data in developing Tau-aggregation-inhibitors and some of the surprising findings in establishing a disease-modifying treatment to fight Alzheimer's Disease.

Role of metals and metal containing biomolecules in neurodegenerative diseases

C. Swart[a], C. Frank[a], G. Köllensperger[b], M. E. del Castillo Busto[c], A. Raab[d]

[a]Physikalisch-Technische Bundesanstalt, Bundesallee 100, 38116 Braunschweig, Germany
[b]University of Vienna, Waehringer Str. 38, 1090 Vienna, Austria
[c]LGC, Queens Road, Teddington, Middlesex, TW11 0LY, United Kingdom
[d]University of Aberdeen, Department of Chemistry, Meston Walk, Aberdeen, UK
Corresponding author: claudia.swart@ptb.de

Keywords: neurogenerative diseases, metals, metalloproteins

Neurodegenerative diseases are one of the major challenges for health care systems in the ageing societies of the Western World, with currently over 6 million people affected in the European Union. According to epidemiological data, only half of the patients suffering from e.g. Alzheimer's disease (AD) are currently identified, and then often only in the advanced stages of the disease. It is suspected that one of the main reasons for this is the lack of accuracy in the results of the biomarker assays used for identification and quantification. The most established biomarkers for AD are β amyloid peptide 1-42 (Aβ 1-42), total tau-protein, hyperphosphorylated tau-protein, and ratios thereof, in cerebrospinal fluid (CSF). Recent epidemiological evidence strongly suggests that several metal ions such as iron, zinc, copper, aluminium, mercury and lead are directly or indirectly involved in the development of AD as they can be found in the plaques formed in the brains of patients. Some metalloproteins might act as shuttles for metals to the brain such as the iron containing proteins transferrin and ferritin as well as albumin, which is known to unspecifically bind a great number of metals.

Potential reference measurement procedures (RMPs) for some of these metals and metalloproteins in CSF and brain homogenate have been developed within the project 15HLT02 "ReMiND", a project in the framework of the European Metrology Programme for Innovation and Research (EMPIR). Most results of the project are presented in various presentations during this conference. RMPs are an important precondition to enable the establishment of a reference system, which provides reliable and comparable results for the biomarkers to clinical laboratories (and, thus, to attending physicians) as well as to research groups seeking to find a cure for neurodegenerative diseases.

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating-States and from the European Union's Horizon 2020 research and innovation programme.

List of references:

1. https://www.ptb.de/empir/remind.html
2. https://www.euramet.org/research-innovation/research-empir/empir-calls-and-projects/call-2015-health-si-broader-scope-normative-research-potential/

Tau-protein quantification using gold and magnetic nanoparticles

C. Frank[a], V. Maurer[b], R. Stosch[a]

[a]Physikalisch-Technische Bundesanstalt, Bundesallee 100, 38116 Braunschweig, Germany
[b]Institute for Particle Technology, TU Braunschweig, Volkmaroder Str. 5, 38104 Braunschweig, Germany
Corresponding author: claudia.frank@ptb.de

Keywords: tau-protein quantification, gold and magnetic nanoparticles, isotope dilution surface-enhanced Raman scattering (ID-SERS)

Metallic and magnetic nanoparticles provide versatile sensing platforms for biological and biomedical applications such as the detection and quantification of large biomolecules serving as diagnostic markers (biomarkers) in human medicine. Here, a new sandwich immuno-assay for surface-enhanced Raman scattering (SERS) based determination in combination with the isotope dilution (ID) approach of tau-protein, one of the established biomarkers for Alzheimer's disease, will be shown.
The developed assay will be used to separate the target analyte from the matrix with the help of magnetic nanoparticles as well as to quantify of the tau-protein by utilisation of a sensitive SERS active marker coupled to gold nanoparticles. The linkage of both nanoparticles to the protein will be ensured by immunoreaction with specific antibodies (Fig. 1 left). Due to the high Raman cross section of the marker DTNB (5,5'-disulfanediylbis(2-nitrobenzoic acid)) this approach makes this method suitable for very small sample volumes and low analyte concentrations typically found in cerebrospinal fluid (CSF) samples for tau-protein on the order of >500 pg/mL while highest accuracy is achieved through the ID approach[1]. Therefore, an isotopic enriched form of the marker was used as perfect internal standard (spike). Due to the higher molecular weight of the spike a specific Raman shift can be observed and used for SI-traceable quantification (Fig. 1 right).

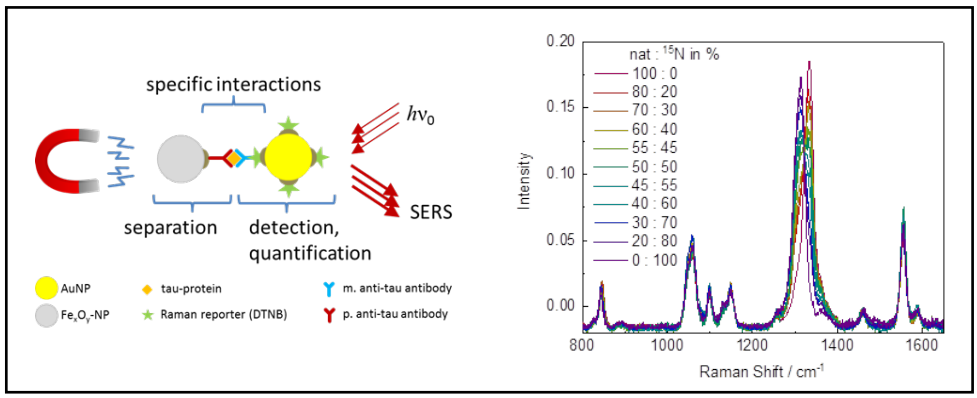


Figure 1: (left) Schematic depiction of the sandwich assay in its natural isotopic composition enriched form, (right) Raman spectra of the hybrid complex of different ratios of the natural (nat) and the isotopic form (^{15}N)

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

List of references

1. Zakel, S., Wundrack, S., O'Connor, G., Güttler, B., Stosch, R. (2013) J. Raman Spectrosc., 44(9) 1246-1252.

The identification and characterisation of small molecule human ABAD inhibitors as therapeutics in Alzheimer's Disease

Keynote speaker: L. Aitken[a], G. Baillie[b], O. Benek[c], K. Musilek[c], T. Smith[a], A. Morrison[b], P. Jones[b], S. McElroy[b], F. Gunn-Moore[a]

[a]School of Biology, University of St. Andrews, Scotland, UK
[b]University of Dundee, European Screening Centre, BioCity Scotland, UK
[c]Department of Chemistry, University of Hradec Kralove, Czech Republic
Corresponding author: la49@st-andrews.ac.uk

Keywords: Drug Discovery, Alzheimer's Disease, ligand-based drug design

Background: The mitochondrial enzyme, amyloid binding alcohol dehydrogenase (ABAD), has been shown to mediate the cytotoxic effects of Amyloid-β within the Alzheimer's diseased brain. Mutational studies have shown that ABAD must be catalytically active for cytotoxicity to be observed and therefore the direct inhibition of ABAD may offer a novel therapeutic strategy to treat the disease [(1,2,3,4)]. We have several collaborations in place to synthesize molecules that will inhibit ABAD from benzothiazole ureas, to repurposed drugs. Significantly, our industrial standard high throughput screening (HTS) strategy [(5)] was accepted into a lucrative pharmaceutical industry backed program that produced a validated hit list of molecules capable of directly inhibiting the ABAD enzyme superior to previous molecules.

Methods: To assess the compounds therapeutic potential we are using many different techniques and have established a screening pipeline to triage the compounds. Briefly, our primary screening strategy utilises recombinant ABAD enzyme extract to measure ABAD activity, followed by cell based and biophysical de-selection assays to verify the inhibitory nature, the cell permeability and cytotoxicity of these compounds. Medicinal chemistry is utilised to optimise the molecules pharmacokinetic properties and potency, whilst x-ray crystallography has been used to visualise the protein-ligand interactions.

Results: We have characterised several series of various types of drug like molecules with our most advanced showing hit molecules at both the recombinant protein level (pM to nM potency) and cellular level (low nM potency). Our molecules exhibit favourable chemical characteristics often seen in drug like molecules and several of the most promising molecules have undergone medicinal chemistry to expand on the structural activity relationships (SAR) further.

Conclusions: With further modifications and evaluation some of these hit molecules have the potential to be applied as therapeutics in AD.

List of references

1. Valasani et al. Curr Alzheimer Res. 2014 Feb; 11(2): 128-136.
2. Hroch, et al. Bioorg. Med. Chem. Lett., 2016 Aug, 26 (15), 3675-3678
3. Hroch, et al. Bioorg Med Chem 2017 Feb, 25 (3), 1143-1152
4. Benek, et al. Medicinal Chemistry, 2017 Jan, 13, 4, 345-358
5. Aitken, et al. SLAS Discovery, 2017, Jul, 22 (6), 676-685

ICP-MS – A useful tool for quantitative phosphoproteomics?

A. Raab[a], L. Griffin[a], B. Platt[b], J. Feldmann[a]

[a]University of Aberdeen, Department of Chemistry, Meston Walk, Aberdeen, UK
[b]University of Aberdeen, The Institute of Medical Sciences, Ashgrove Road, Aberdeen, UK
Corresponding author: A.raab@abdn.ac.uk

Keywords: ICPMS, proteomics

Accurate quantification of proteins and their post-translational protein modifications (PTMs) is an active field in a range of research areas. Especially in the biochemical / medial field significant work in this area is happening.
The major route to quantification so far is “bottom-up” protein identification via databases and quantification via spiking of the sample with the peptide(s) of interest labelled with stable isotope(s). For relative quantification of proteins in different samples the proteins/peptides in each sample can be labelled using different isotope tags. The first approach requires the availability of the labelled peptide(s) of interest which might make it impractical to apply for none-targeted protein quantification.
Elemental determination of proteins /peptides in contrast relies on the presence of generally ICP-MS accessible elements. In the field of proteomics these are predominantly sulphur, from methionine and cysteine, and phosphorus from PTMs. For absolute quantification of these elements simple element solutions can be used, or in the case of sulphur isotope dilution (ID)-MS. Quantification by ICP-MS would also allow a complete mass balance to estimate protein losses during sample preparation. Protein losses during sample preparation are difficult to avoid due to the multiple steps required especially when enrichment methods are used. Results of different phosphopeptide enrichment methods will be presented.

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union’s Horizon 2020 research and innovation programme.

Development of a method for protein quantification via isotope dilution ICP-MS for application on an Alzheimer's biomarker

N. Lemke[a,b], A. H. El-Khatib[b], F. Theuring[a], N. Jakubowski[b], J. Vogl[b]

[a]Charité – Universitätsmedizin Berlin, corporate member of Free University Berlin, Humboldt-University Berlin, and Berlin Institute of Health, Center for Cardiovascular Research, Institute of Pharmacology, Group Molecular Pharmacology, Hessische Str. 3-4, 10115 Berlin, Germany
[b]Federal Institute for Materials Research and Testing (BAM), 1.1 Inorganic Trace Analysis, Richard-Willstätter-Str. 11, 12489 Berlin, Germany
Corresponding author: nora.lemke@bam.de

Keywords: ICP-MS, Isotope dilution, proteins

Inductively coupled plasma mass spectrometry (ICP-MS) is a powerful method for the matrix-independent quantitative analysis of target elements. Developed for the use in inorganic trace analysis, ICP-MS is nowadays emerging as a valuable tool for bioanalytical questions. Especially the use of ICP-MS for quantitative proteomics by measuring heteroatoms has gained popularity in the last decade, considering that established quantification methods like organic mass spectrometry depend on the existence of matched protein and peptide standards or labelling of the target protein. The need for reliable quantification of proteins is constantly growing, but only a limited number of well characterized and quantified protein standards are available so far. Not only in basic research, but also in a clinical context, accurately quantified, traceable protein standards are needed to ensure comparability of measurements between laboratories. One disease with a major impact on our ageing society is Alzheimer's disease (AD), which is still challenging to diagnose. As this is also due to a lack in comparability and accuracy of existing biomarker assays, the community would greatly benefit from well quantified protein biomarker standards.
In this work, we applied isotope dilution analysis (IDA) using ICP-MS to quantify proteins of known stoichiometry via their sulfur content. Sulfur is present in two amino acids, cysteine and methionine, and hence exists in nearly all proteins. Simple strategies were employed for the detection of low molecular sulfur species to correct for sulfur contaminants and allow for reliable quantification of various proteins. We report the protein mass fractions with expanded uncertainties of a standard reference material and commercially available proteins determined by sulfur IDA. The herein developed method can be applied for the reliable and traceable quantification of pure proteins and will be used for the quantification of an AD biomarker. Our target is the tau protein, as brain load and distribution of tau is highly correlated with the clinical progression of AD.

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

Metal content of Alzheimer's Disease mouse model brains: Total element determination by ICPMS.

E. Griffin[a,b], A. Raab[a], B. Platt[b], J. Feldmann[a]

[a]Trace Element Speciation Laboratory (TESLA), Department of Chemistry, College of Physical Sciences, University of Aberdeen, Scotland.
[b]Institute of Medical Sciences, University of Aberdeen, Scotland.
Corresponding author: j.feldmann@abdn.ac.uk

Keywords: Alzheimer's Disease, Mouse Models, ICPMS.

Alzheimer's disease (AD) a common cause of dementia, is the most prevalent neurodegenerative disease worldwide. It is characterised pathologically by amyloid plaques and neurofibrillary tau tangles in the brain. In addition to these pathological hallmarks, evidence suggests that both essential, and non-essential trace metals play a role in disease mechanisms. This includes an association of metals and amyloids, and also the promotion of tau phosphorylation and aggregation. There is therefore motivation to compare essential and non-essential element concentrations in brain tissue of different AD-mouse models.

This work used ICPMS to determine the total element concentrations of a range of elements, with a particular focus on Fe, Cu and Zn, within the brains of different mouse models. The disease models expressed human mutated Tau (L66) and Amyloid (PLB1-Dbl, PLB2-APP, APP/PS1) with varying expression levels and pathology. Freeze dried sheep brain was used as a laboratory reference material to track reproducibility.

Results showed significantly higher concentrations (2 - tailed t-test $p < 0.01$) of Fe, Cu and Zn in disease models compared to WT controls. Concentrations of Fe in PLB1-Dbl, PLB2-APP and APP/PS1 models were all significantly higher than WT control. Concentrations of Cu in PLB1-Dbl and PLB2–APP were significantly higher than WT controls and concentrations of Zn in PLB1-Dbl were significantly higher than WT controls. Concentrations of Fe and Cu were significantly higher in the human tau overexpressing L66 model than WT control.

Elevated levels of Fe, Cu and Zn in AD models compared to WT are likely associated with aggregated proteins as found in plaques and tangles.

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

High-throughput and low volume analysis of biologically essential trace elements in cerebrospinal fluid using ICP-TOF-MS

G. Koellensperger[a], S. Theiner[a], A. Schoeberl[a], L. Fischer[b], A. Tchaikovsky[a] and S. Hann[b]

[a]University of Vienna, Waehringer Str. 38, 1090 Vienna, Austria
[b]University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria
Corresponding author: gunda.koellensperger@univie.ac.at

Keywords: Multi element analysis, cerebrospinal fluid, inductively coupled plasma mass spectrometry

Metal ions are thought to be directly or indirectly involved in the development of the Alzheimer's disease (AD). Therefore, multielement profiles in clinically relevant fluids could give new insights on AD formation. For serum and blood, reference levels are fairly well established for most trace elements and serve as important diagnostic parameters. However, there is limited data available for elemental concentrations in cerebrospinal fluid (CSF) despite its important role in the homeostasis and metabolism of the central nervous system. In this study, we present different state-of-the-art ICP-MS methods for high-throughput multielement analysis in CSF. Due to the lack of certified reference material (CRM), open vessel digestion in combination with sectorfield-ICP-MS analysis was used as gold standard to establish a reference data set. Owing to the low sample volume and trace element concentrations of CSF, flow injection methods with 5 µL sample intake were used in combination with a pseudo-simultaneous mass analyzer time-of-flight ICP-MS. The performance for the ICP-TOF-MS for accurate high-throughput and multielement analysis in CSF was compared to quadrupole-based ICP-MS/MS analysis.

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

Multi element analysis of micro volume biological fluids using ICPMS – method development, validation and interlaboratory comparison

M. Tunc[a], Y. S. Z. Can[a]

[a]TÜBITAK Ulusal Metroloji Enstitüsü, PK 54, 41470 Gebze, Kocaeli / Turkey
Corresponding author: tunc.murat@tubitak.gov.tr

Keywords: Microvolume, Serum, CSF

Method development, validation and interlaboratory comparison studies were carried out in serum, cerebrospinal fluid and pig brain for cadmium (Cd), lead (Pb), magnesium (Mg), calcium (Ca), aluminum (Al), iron (Fe), copper (Cu) and zinc (Zn) using ICPMS within EMPIR 15HLT02 ReMiND project.

A 40 µL serum aliquot was transferred to a polypropylene tube, and 100 µL internal standard solution (containing Y, In, Tl) added. The mixture is diluted to 1000 µL with using 0.1 % (v/v) HNO_3. The measurements were performed using sector-field ICP-MS by standard addition calibration. Recovery values in the range of 90 - 111 %, repeatability values 1.1 – 11 % RSD were obtained in method validation studies for all elements in serum. Intermediate precision has been tested to demonstrate the day-to-day variation parameter of the validation study, and values in the range of 2.8 – 20 % RSD were obtained. For the Cd and Pb at trace levels in biological fluids, the method detection limit (MDL) value of 0.1 and 0.4 µg/kg was calculated, respectively. Correlation coefficient (r^2) range of the calibration curves 0.997 – 0.999 were obtained for all elements.

Multi-element analysis was performed in serum, cerebrospinal fluid and pig brain digests samples in the inter-laboratory comparison studies. According to comparison results, the mesurements in serum and pig brain digests were consistent with the participants

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

Copper, iron, and zinc isotopic distributions in Alzheimer's Disease

N. Solovyev[a], M. Costas-Rodríguez[a], A. Raab[b], F. Theuring[c], F. Vanhaecke[a]

[a]Ghent University, Department of Chemistry, Atomic & Mass Spectrometry – A&MS research unit, Campus Sterre, Krijgslaan 281-S12, 9000 Ghent, Belgium
[b]University of Aberdeen, Department of Chemistry, Meston Walk, Aberdeen, AB24 3UE, UK.
[c]Charité – Universitätsmedizin Berlin, CCR/Institut für Pharmakologie, Hessische Str. 3-4, 10115 Berlin, Germany
Corresponding author: nikolay.solovyev@ugent.be

Keywords: Transgenic mice Alzheimer's models, isotopic analysis, metals

The etiology of Alzheimer's disease (AD) is still quite unclear, which hinders adequate treatment of this widespread neurodegenerative disease. A number of environmental factors, such as exposure to metals or pesticides, are sometimes considered as potential contributors to dementia risk [1]. Additionally, there is still a lack of reliable and effective diagnostic and prognostic biomarkers for AD, which hinders early diagnosis. As a result, AD is usually diagnosed at a rather advanced stage only, when cognitive decline has already occurred and there are obvious signs of dementia.
Essential trace elements, such as copper, iron and zinc, seem to be implicated in AD, potentially affecting pathological processes such as redox dyshomeostasis and amyloidogenesis. The study of natural isotopic signatures of essential metallic elements in neurological research is an emerging trend in inorganic biochemistry [2] as it may reveal even the slightest changes in metal homeostasis in the neuronal microenvironment.
In the current study, the isotopic distributions of Cu, Fe, and Zn were investigated in two independent AD models vs. matched wild-type controls. Two established AD models [3] were explored: β-amyloid overexpressing mice (B6.Cg-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax) and τ-transgenic mice [4]. All experiments were conducted in males. There were 9 animals per group (4groups: 2 transgenic and 2 matching wild-type controls). The animals were sacrificed at the age of 4-5 months, blood plasma/serum and the brain were collected for Cu, Fe, and Zn isotopic analysis via multi-collector inductively coupled plasma-mass spectrometry (MC-ICP-MS) after chromatographic target element isolation. The rodents' chow was also analyzed to document the baseline isotopic composition of the targeted essential elements. The disruption of metal homeostasis in the β-amyloid overexpressing and τ-transgenic mice was evaluated from an isotopic perspective.

Acknowledgments

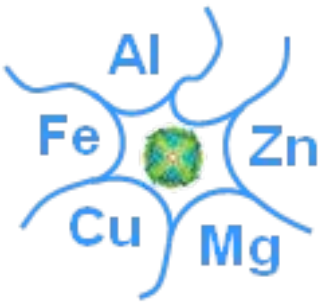
This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

List of references:

1. Killin L.O. et al., BMC Geriatr, 2016, 16, 175-end page?.
2. Costas-Rodríguez M., Delanghe J., Vanhaecke F., TrAC Trend Anal Chem, 2016, 76, 182-193.
3. Kitazawa M., Medeiros R., LaFerla F.M., Curr Pharm Des, 2012, 18, 1131-1147.
4. Melis V. et al., Cell Mol Life Sci, 2015, 72, 2199-222.

Thursday

Presentations



Quantification of biomolecules in biological matrices

Keynote speaker: A. Sickmann[a]

[a]Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V.
Corresponding author: sickmann@isas.de

Keywords: Biomarker, Mass spectrometry, Quantification

To understand and elucidate the molecular basis of a disease is only the first step towards improved diagnosis and therapy concepts. Another important challenge in this context is to find dependable markers that can be used for the reliable identification and discrimination of different diseases. Given the enormous number of potential analytes (= biomarker) in biological systems, it is necessary to employ methods for high precision measurements in biological matrices.

A biomarker is a characteristic feature of a biological system that allows an objective measurement and its measurement result on a normal or pathological process in a cell type, a specific organ or throughout the body or organism. Biomarkers therefore, serve as indicators of the current state of a biological system and can be used, for example, to detect the impact of environmental factors and monitor the effect of drugs, as well as the progress of disease.

The biomarker itself can be of a different nature: it can be different phenotypes cells, common or rare gene variants, altered gene products (for example kinases) and metabolites such as amino acids, lipids and endogenous metabolites as well as degradation products of drugs that make a specific statement about metabolic changes and modulation of protein functions.

The portfolio of biomarkers is thus very heterogeneous and may differ from the determination of individual parameters via a panel of selected markers (for example components of a signalling cascade of different molecular classes) up to the complex function of organs (for example, pumping function of the heart) and characteristic changes of biological structures and their capture by imaging techniques.

During the last decades, mass spectrometry (MS) developed towards an irreplaceable tool for the quantitative detection of biomolecules in diverse biological matrices. However, MS-based bioanalysis has not fully exploited to its many opportunities and advantages and does not yet play a major role in clinical research or routine clinical analysis. MS based approaches allow fast, precise and sensitive detection of biomolecules from any biological materials with low development times for individual molecules. Not to mention, its unsurpassed capability for the identification and quantification of molecular variants (e.g., isoforms and post-translational modifications) as well as the detection of unknown analytes and the precise quantification of several hundreds of biomolecules from one sample at the same time.

Within the presentation an up to date overview about strategies for quantification of biomolecules, selected assay development and current examples for detection of proteins in plasma, blood cells and tissues is given.

Preparation and characterization of an isotopically enriched (^{57}Fe) ferritin standard for species specific isotope dilution mass spectrometry

A. Tchaikovsky[a], A. Schoeberl[a], S. Emin[a], S. Neumayer[a] and G. Koellensperger[a]

[a]University of Vienna, Waehringer Str. 38, 1090 Vienna, Austria
Corresponding author: gunda.koellensperger@univie.ac.at

Keywords: Ferritin, isotope dilution mass spectrometry, size exclusion chromatography

The recently introduced EC-directive covering all in vitro diagnostic medical devices (Directive 98/79/EC, In vitro diagnostic medical devices, IVD) demands to assure “the traceability of the values assigned to calibrators and control materials [...] through reference measurement procedures and/or available reference materials of higher order”. At the same time, we face the situation that especially in the case of clinically relevant metallo-proteins, established diagnostic assays lack traceable standards. We present the production pipeline and the characterization of an isotopically enriched (^{57}Fe) ferritin standard. Moreover, we will discuss analytical figures of merit of the methodological tool set based on liquid chromatography inductively coupled plasma mass spectrometry enabled by the standard. More specifically, we will discuss enrichment strategies together with size exclusion chromatography in the context of species specific isotope dilution analysis of ferritin in biologically relevant samples in studies on neurodegenerative diseases.

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union’s Horizon 2020 research and innovation programme.

Quantitative determination of Cu-containing proteins as potential biomarkers for Alzheimer's Disease

J. Gleitzmann[a], C. Swart[a]

[a]Physikalisch-Technische Bundesanstalt, Bundesallee 100, 38116 Braunschweig, Germany
Corresponding author: julia.gleitzmann@ptb.de

Keywords: Metalloproteins, Alzheimer's disease, ID-HPLC-ICP-MS

According to estimates over 6 million people in the European Union are affected by neurodegenerative diseases, which places a considerable burden on the health care systems. The most common form of dementia is Alzheimer's disease (AD), which is responsible for about 70 % of dementia sufferers. In an ageing population, the diagnosis and treatment of these diseases are gaining more and more importance. Determination of the biomarkers for AD (the most common are β-amyloid peptide and τ-protein) is generally performed using immunoassays or optical methods which often lead to incomparable results. Besides the established biomarkers, metalloproteins are under discussion as potential clinical markers. For example, active ceruloplasmin (CER) or superoxide dismutase (SOD) levels in brain, cerebrospinal fluid (CSF) or serum alter in patients affected by AD compared to healthy individuals. A promising approach for the quantitative determination of these proteins is species-specific isotope dilution inductively coupled plasma mass spectrometry (SS-ID-ICP-MS) as it is a primary measurement method and gives results that are traceable to the International System of Units (SI). To perform SS-IDMS, a protein reference and an adequate spike material are needed. As there are very few protein reference materials commercially available, pure native proteins are characterized in-house for the use as a reference. Isotopically enriched protein spike materials are produced in-house by demetallation and re-metallation procedures.

Acknowledgement

This project has received funding from the EMPIR programme co-financed by the Participating-States and from the European Union's Horizon 2020 research and innovation programme.

List of references:

1. Reiber et al., J. Alzheimers Dis. Parkinsonism, 2014, 4, 147-151
2. G. Torsdottir et al., Dement. Geriatr. Cogn. Dis. Extra, 2011, 1, 366-371
3. G.P. Rodrigues et al., J. Trace Elem. Med. Biol., 2017, 44, 83-87

Determination of exchangeable copper by speciation studies to underpin Wilson's disease diagnosis

M. E. del Castillo Busto[a], I. Abad Alvaro[a], T. Morley[b] and H. Goenaga Infante[b]

[a]LGC, Queens Road, Teddington, Middlesex, TW11 0LY, United Kingdom
[b]Gmp-Orphan, Pépinière Paris Santé Cochin, 75014 Paris, France
Corresponding author: estela.delcastillo@lgcgroup.com

Keywords: Exchangeable copper, Albumin, ICPMS

Wilson's disease (WD) is a genetic disorder of copper metabolism and is characterised by the accumulation of this metal in various body tissues, mostly liver, brain and the cornea of the eye. This overload is caused by mutations in the Cu-transporter gene (ATP7B gene) that participates in Cu metabolism and its removal from the body. An early diagnosis of WD is crucial to prevent the progression of the disease that could lead to irreversible hepatic, neurological and psychiatric damages.

A combination of tests are currently used in the diagnosis of WD: ATP7B mutation testing, clinical diagnosis (e.g. Kayser-Fleischer Ring) and biochemical tests such as serum ceruloplasmin, 24h urinary copper, serum free copper and calculated non-ceruloplasmin copper (NCC) or exchangeable Cu (CuEXC). CuEXC corresponds to the labile fraction of Cu in serum that is not bound to ceruloplasmin (CER). It should include Cu bound to proteins, mostly Albumin (ALB) and, to a lower extent, alpha-2-macroglobulin, low molecular weight Cu species and free Cu. EDTA is used as Cu chelating agent able to complex loosely-bound Cu from ALB and alpha-2-macroglobulin, while preserving Cu bound to CER. Calculated NCC or CuEXC, which is currently determined by subtracting Cu-CER from total serum copper, is considered so far the best guide to WD's treatment efficacy and follow-up[1]. However, negative values have been obtained using this indirect approach and no reference methodology for the accurate quantification of Cu-CER has been reported so far. Therefore, there is an increasing need for reliable methods, enabling quantification of CuEXC to underpin measurements in the clinic.

The aim of this work was to evaluate the performance of speciation methodology to determine CuEXC as a direct method for WD diagnosis. An anion-exchange HPLC-ICP-MS method was developed for the first time for the fractionation of Cu associated with proteins in serum under the frame of the EU ReMiND project. It was evaluated for the detection of Cu bound to Cu-containing species after EDTA treatment in control human and WD patient serum samples. Since Cu released from ALB was found to be the main contributor to CuEXC, further method development focused on the accurate quantification of Cu bound to ALB by using double matching species-specific isotope dilution analysis. The reference methodology developed here will be invaluable to provide CuEXC reference values and Cu speciation data to support clinical measurements. It will also help monitoring efficiency of Cu chelating drugs, aiming at finding more effective therapies.

Acknowledgments

This work derives from the project 15HLT02 ReMiND funded by the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme. Gmp-Orphan is acknowledged for the provision of WD´s patient serum samples.

List of references:

1. Eve A. Roberts et al. Hepatology 2008 (47) 2089-2111.

Comparability in clinical diagnosis

Keynote speaker: I. S.Young[a]

[a]Centre for Public Health, Queen's University Belfast, UK.
Corresponding author I.Young@qub.ac.uk

Keywords: standardization, decision limits, clinical utility

Biomarker measurement plays a critical role in the diagnosis and management of many clinical disorders. In general, patients and clinicians assume that biomarker measurement is consistent over time and between laboratories, and treatment guidelines frequently feature decision limits or diagnostic thresholds which support clinical decisions or trigger a specific diagnosis. In reality, for many established and emerging biomarkers there are considerable differences in values between different assays and laboratories, and as a consequence the pathway followed by a patient may depend on the laboratory where a diagnostic test is performed.

In order to achieve comparability in clinical diagnosis, it is essential that measurement procedures measure the same quantity. In addition, calibration of all measurement procedures should be traceable to a common reference system and reference materials should be commutable across all methods where they are to be used. In practice these requirements present considerable challenges, particularly for new and emerging assays and in the context of reference materials which may have been available for a number of years.

Approximately 4000 measurands are currently used for clinical diagnosis, and of these less than 10% have been fully standardized. In order to prioritise measurands for standardization based on clinical need, the International Consortium for the Harmonization or Clinical Laboratory Results (ICHCLR) systematically considers the extent of between assay differences (based on external quality assessment (EQA) data), the frequency with which a test is performed, and the use of or need for fixed decision limits in clinical guidelines to assign a priority category.

A number of organizations are involved in the standardization activities, particularly National Metrology Institutes and the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC). Reference measurement procedures, materials or services which meet ISO criteria are listed on a publically available database by the Joint Committee on Traceability in Laboratory Medicine (JCTLM).
Surveillance of EQA data from providers who use commutable samples is important to ensure ongoing maintenance of assay comparability.

Clinical laboratories have a vital role in maintaining assay comparability, by ensuring that assays in routine use demonstrate traceability where possible and by bringing pressure to bear on manufacturers to address traceability issues when it is clear that significant between assay differences continue to exist.

Innovative measurements for improved diagnosis and management of neurodegenerative diseases

M. Quaglia[a], Cano[b], K. Dean[c], C. Divieto[d], A. Fillmer[e], L. Göschel[f], S. Lehmann[g], C. Grande[h], J. Melin[i], S. Pang[a], L. Pendrill[i], G. Verona[k]

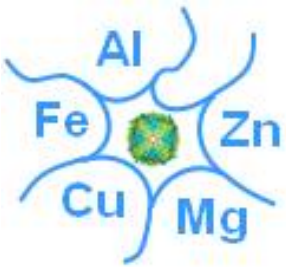
[a]LGC Ltd, United Kingdom; [b]Modus Outcome, France, [c]UEA, United Kingdom, [d]INRIM, Italy, [e]PTB, Germany, [f]Charitè, Germany, [g]Univ Montpellier, INSERM, [h]LNE, France, [i]RISE, Sweden, [k]UCL United Kingdom
Corresponding author: milena.quaglia@lgcgroup.com

Development of novel therapies for Alzheimer's Disease (AD) is constrained by the lack of available methods for preclinical diagnosis, despite extensive research on biomarker identification. The EMPIR NeuroMET project unites National Measurement Laboratories, clinicians and academics, to overcome limitations in measurement methods and provide a better understanding of how to improve, combine and analyse measurements in AD diagnosis and treatment. Comparability through SI (System of International Units) traceability and uncertainty analysis is an, as yet, unmet requirement for regulatory approval of biomarkers, patient centred outcome measures, clinical thresholds and new therapeutic drugs. We will report on:

- Multimodal statistical analysis on blood, CSF and saliva biomarkers data from the NeuroMET cohort generated by using mass spectroscopy and immunoassay platforms, including a novel immunoassay approach to overcome matrix effects when relative quantification is not sensitive enough. A new digital PCR approach was developed to assess microRNAs quantities in blood to compare with established biomarkers.
- Progress towards the development of mass spectrometry reference measurement procedures traceable to the SI for t-tau and α-synuclein in cerebrospinal fluids.
- Development of ultrahigh field Magnetic Resonance Imaging and Spectroscopy protocols for increased spatial and spectral resolution and decreased uncertainty, and their application to the NeuroMET cohort.
- Improved cognitive assessment protocols, with improved metrological evaluation of cognitive performance scores and the development of construct specification equations for various cognitive protocols and biomarkers.
- Potential relationships between volumes of AD-related brain structures and neurometabolite concentrations with measured cognitive function.

Improving measurement uncertainty to enable utilisation of recognised and emerging NDD biomarkers in peripheral biological fluids such as blood or saliva is a prerequisite for the development of routine clinical tests for NDD. We here demonstrate how multidisciplinary metrological approaches can provide useful tools for improved diagnosis and drug development

Poster



Development of traceable quantification method for transferrin at low concentrations expected in CSF and small sample volumes

F. G. Coşkun[a], S. Z. Can[a]

[a]TÜBITAK Ulusal Metroloji Enstitüsü, PK 54, 41470 Gebze, Kocaeli / Turkey
Corresponding author: gonca.coskun@tubitak.gov.tr

Keywords (up to 3): Cerebrospinal fluid, Transferrin, SS-HPLC-IDMS

Iron is one of the metal ions that are though to be involved in development of Alzheimer's Disease (AD). Recent developments revealed that metalloproteins transport the metals to the brain across the blood-brain barrier. Hence, reliable measurement method for determination of TRF in cerebrospinal fluid (CSF) is needed for investigation the influence of TRF in AD development.

Triple species-specific HPLC isotope dilution mass spectrometry (SS-HPLC-IDMS) approach was used for determination of TRF at the levels of ~0.01 mg/L using small sample volumes (100-200 µL). ERM-DA470k/IFCC (IRMM) and pooled CSF sample (obtained from a private clinical testing laboratory in Istanbul) were used for method development and the method was validated using ERM-DA470k/IFCC (IRMM/JRC). In triple SS-HPLC-IDMS approach two calibration blends were prepared with ^{56}Fe-TRF solution and the synthesised ^{57}Fe-TRF spike solution. The sample blend was prepared with ^{56}Fe saturated ERM-DA470k/IFCC (IRMM) (and pooled CSF sample) and ^{57}Fe-TRF spike solution. After isotopic equilibrium was reached all blends were concentrated using Amicon® Ultra-0.5 Centrifugal Filter Devices (30K). The measurement of ^{56}Fe/^{57}Fe ratios in all blends were performed on HPLC-ICP-MS system using bracketing sequence. The instruments used for the measurements were Agilent 1100 Bioinert HPLC and Agilent 8000 ICP-MS Triple Quad (Agilent Technologies). MonoQ 5/50 GL column (5 x 50 mm i.d., GE Healthcare) is used for separation of TRF sialoforms.

In the validation study, 5.3% repeatability has been obtained in measurement of 6 replicate measurements. The trueness of the method at give levels were tested, and recoveries varying in the range of 93% - 105% were obtained.

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

Immunoprecipitation with nanodiamonds for the enrichment and isolation of ceruloplasmin from human plasma

J. L. Völzke[a], A. H. El-Khatib[b], M. Weller[a]

[a]BAM Bundesanstalt für Materialforschung und -prüfung, FB 1.5 Protein Analysis, Richard-Willstätter-Straße 11, 12489 Berlin, Germany
[b]BAM Bundesanstalt für Materialforschung und -prüfung, FB 1.1 Inorganic Trace Analysis, Richard-Willstätter-Straße 11, 12489 Berlin, Germany
Corresponding author: ahmed.elkhatib@bam.de

Keywords: Immunoprecipitation, nanodiamonds, ceruloplasmin

Introduction: To isolate a specific target out of a complex matrix like human plasma is an ongoing challenge in biomedical analysis. Nanodiamonds are an interesting material with many benefits like very high adsorption capacity of proteins on their surface and their excellent chemical stability.

Method: In this study, we present a novel approach of immunoprecipitation of the copper-containing ferroxidase ceruloplasmin (CER) based on nanodiamonds acting as a novel carrier material. We used Protein G as the first coating layer to achieve the oriented immobilization of the CER antibody. To confirm the selective enrichment of ceruloplasmin out of human plasma, SDS-PAGE was performed prior to the copper quantification via ICP-MS following the microwave-assisted digestion of the gel bands.

Results: SDS-PAGE demonstrated the successful CER isolation from human plasma using the proposed method. The isolated CER was quantified with ICP-MS via its copper content following the digestion of the gel bands

Acknowledgments

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

SI traceble reference measurement procedure for CSF amyloid beta $(Aβ)_{1-40}$ and $(Aβ)_{1-42}$

M. Oztug[a], G. Altin Yilmazer[a], B. Vatansever[a], S. Z. Can[a]

[a]National Metrology Institute/TUBITAK UME, Gebze, Kocaeli, TURKEY
Corresponding author: merve.oztug@tubitak.gov.tr

Keywords: CSF, Amyloid Beta, Quantification

Clinical diagnosis of Alzheimer's disease (AD) is now based on the results of general clinical evaluation and cognitive tests. However, the use of amyloid-beta (Aβ) peptide in cerebrospinal fluid (CSF) as a biomarker for amyloid pathology has become increasingly common in clinical trials. Recently, mass spectrometry (MS) reference measurement procedures for absolute quantification of Aβ1-40 and Aβ1-42 measurements in CSF have been published [12]. It has been suggested that the CSF Aβ1-42 / Aβ1-40 ratio improves the detection of cerebral amyloid deposition by compensating for inter-individual variations in total Aβ production. Our aim in this study was to establish a SI traceable reference measurement method for both Aβ1-42 and Aβ1-40 peptides in CSF.

The impurity analysis of the standard material used is of great importance for the accuracy of the weight of the materials. In TUBITAK UME Laboratories, the peptide impurity analysis is performed by PICAA (Peptide Impurity Corrected Amino Acid Analysis) method. PICAA method was applied and validated for both amyloid beta 1-40 and 1-42 peptides to establish the accuracy of the weight of the materials. The mass fractions of the Aβ1-42 and Aβ1-40 peptides were found to be 440.4 ± 27.1 and 517.1 ± 57.1 (mg/g) respectively. Using the peptides analyzed in terms of their mass fraction as standard, an isotope dilution mass spectrometry (ID/MSMS) based reference measurement method was developed for absolute quantification of the Aβ1-42 and Aβ1-40 peptides in CSF. The experiments were performed with Q-Exactive High Resolution (HR) MS coupled with Dionex Ultimate 3000 Ultra Performance Liquid Chromatography (UPLC). Artificial CSF containing 4 mg/ml BSA was used as a surrogate matrix for the study. Linear and reproducible calibration was obtained. The analytical run was assessed determining, linearity, within-run accuracy and carryover. The linearity was obtained for 500-4000 pg/ml and 500-20000pg/ml range for the Aβ1-42 and Aβ1-40 peptides respectively. Matching the acceptance criteria the Correlation coefficient (r) of the calibration curve was found more than 0.995. The accuracy of 90% of the analyzed Quality Control was between $\geq$ 85.0% and $\leq$ 115.0%.

In conclusion, an SI traceable measurement method was developed for the quantification of Aβ1-42 and Aβ1-40 peptides in CSF. This method, due to its effective performance might be used for diagnostic assessments in AD research.

List of references:

1. 1. Shaw, L. M. Qualification of a surrogate matrix-based absolute quantification method for amyloid-β_{42} in human cerebrospinal fluid using 2D UPLC-tandem mass spectrometry. J. Alzheimers. Dis. 41, 441–451 (2014).
2. 2. Pannee, J. et al. Reference measurement procedure for CSF amyloid beta (Aβ)1–42 and the CSF Aβ1–42/Aβ1–40 ratio – a cross-validation study against amyloid PET. J. Neurochem. 139, 651–658 (2016).

Role of the p75 neurotrophin receptor in dendritic spine alterations induced by amyloid-β aggregates

A. Holz[a], A. Patnaik[b], H. Demuth[b], A. Remus[b], J. Baumann[b], M. Zagrebelsky[b] and M. Korte[a,b]

[a]Helmholtz Center for Infection Research, AG NIND, Inhoffenstr. 7, 38124 Braunschweig, Germany.
[b]Zoological Institute, Division of Cellular Neurobiology, TU Braunschweig, Spielmannstr. 7, 38106 Braunschweig, Germany.
Corresponding author: m.korte@tu-bs.de

Keywords: Amyloid receptor, p75^{NTR}, synaptic plasticity

The p75 neurotrophin receptor (p75^{NTR}) is involved in the regulation of several important cellular functions in the central nervous system, including proliferation, differentiation, cell survival and activity-dependent synaptic plasticity. p75^{NTR} has been demonstrated to function as a receptor for amyloid-β_{1-42} aggregates (Aβ). Hence, p75^{NTR} might contribute to the Aβ-induced synaptic dysfunction and loss observed in Alzheimer's disease. Here we examined the influence of Aβ/p75^{NTR} signaling on neuronal structure and function.

At first, we investigated the acute effects of *in vitro* Aβ-application to primary hippocampal neurons which resulted in a dose-dependent decrease in dendritic spine density and the manifestation of a premature spine morphology phenotype. Interestingly, such amyloid-mediated synaptic changes were completely absent when neurons from p75^{NTR} knockout (ko) mice were analyzed. Similarly, applying Aβ to wild type neurons resulted in an increase in the activation level of the RhoA-GTPase which was prevented in p75^{NTR} ko neurons. This is of interest since RhoA signaling regulates spine morphology.

We analyzed individual hippocampal neurons *in vivo* of 3-4 months old animals and observed a significantly lower dendritic spine density in APP/PS1 transgenic mice compared to controls. Importantly, the density of spines in APP/PS1-p75^{NTR} ko mice was higher than in age-matched APP/PS1 animals. These structural alterations were accompanied by a significant reduction in both induction and maintenance of long-term potentiation (LTP) in APP/PS1 transgenic compared to wild type mice. In sharp contrast, hippocampal slices from APP/PS1 animals lacking p75^{NTR} expression generated a stable LTP that was indistinguishable from controls. p75^{NTR} gene ablation in parts also rescued the detrimental consequences of the APP/PS1-transgene on learning and memory in behavioral tests. Finally, we found that microglia of APP/PS1 mice show an activated phenotype, which can be rescued by the removal of p75^{NTR}.

Taken together our results indicate a key role of p75^{NTR} in mediating Aβ-toxicity both *in vitro* and *in vivo*.

Accurate quantification of β-amyloid standards through sulfur on-line isotope dilution using LC-ICP-MS/MS

M. Schaier[a], E. Rampler[a], L. Galvez[a], G. Hermann[a, b], G. Koellensperger[a]

[a]Institute of Analytical Chemistry, University of Vienna, Waehringer Strasse 38, 1090 Vienna, Austria [b]ISOtopic solutions e.U., Waehringer Strasse 38, 1090 Vienna, Austria
Corresponding author:m.schaier@web.de

Keywords: β-Amyloid, LC-ICP-MS/MS, on-line isotope dilution

One of the greatest challenges in medicine today is the fight against neurodegenerative diseases such as Alzheimer's disease. A defining pathological feature is the formation of plaques in the extracellular regions of the brain with the β-Amyloid ($A\beta_{40}$, $A\beta_{42}$) peptide being the main component of this deposit. Therefore, the study of this peptide gives the opportunity to get a better insight into the processes of this complex disease.

For clinical analysis one needs suitable standards, but it is not an easy task to find them, since only a few are commercially available, and these vary in their purity. Furthermore, the determination of absolute concentration in most protein standards is performed in a non-traceable manner, by e.g. Bradford assay in which albumin is used as a calibrant. Hence, accurate methods are needed to characterize these standards.

The aim of this study was to quantify synthetic β-Amyloid standards (purchased from r-Peptide) by analyzing the sulfur-content of its amino acids. For this purpose, an acid-hydrolysis of the peptide was performed. The so obtained individual amino acids were separated by means of a strong anion exchange column and directly analyzed with inductively coupled plasma mass spectrometry (ICPMS/MS) in oxygen mode. By continuously spiking with a ^{34}S standard, species specific isotope dilution was made possible. In order to validate the established method in an appropriate manner for the oxidized amino acids, methionine sulfone and cysteic acid, a ^{34}S-enriched yeast standard was used. In addition, the commercially available proteins lysozyme and myoglobin were successfully quantified by this procedure. To prevent sulfur contamination, all consumables were pre-cleaned with nitric acid. Ultimately the combination of LC-ICP-MS/MS with species-specific IDA allowed us to accurately quantify β-Amyloid standards with high throughput and low volume consumption.

Keynote Speakers

Prof. Gerhard Multhaup is Professor and Chair of the Department of Pharmacology and Therapeutics at McGill University. He holds a Canada Research Chair (Tier 1) in Molecular Pharmacology. His research interests include understanding the APP biology and investigating the molecular events of amyloid aggregation, gain of toxicity, and the causes of neuronal dysfunction. The primary aim is to identify novel targets to develop pharmacological strategies for prevention and therapy.

Dr. Laura Aitken is a post-doctoral research fellow at the University of St. Andrews. Dr Aitken earned her BSc. (Hons) in Pharmaceutical Chemistry from the University of Dundee, 2008 and received her Ph.D in Neurobiology from the University of St. Andrews in 2013. She investigates protein-protein interactions involved in Alzheimer's disease and designs potential therapeutics against them. She has developed several series of different compounds against a key drug target in Alzheimer's disease leading a large HTS European grant collaboration, yielding two distinct analogue series of molecules which will shortly be entering in vivo pre-clinical trials.

Prof. Albert Sickmann is the chairman of the Leibniz - Institut für Analytische Wissenschaften - ISAS - e.V. in Dortmund and Berlin. His research interest is focused on platelet activation and inhibition, which is tightly regulated by the integration of fast and highly complex signaling pathways. He develops OMICS technologies to dissect individual signaling hubs to provide and insight in underlying molecular mechanisms and facilitate future options for better diagnostics and individual treatment.

Prof. Ian Young is Professor of Medicine at Queen's University Belfast, and Deputy Medical Director and Consultant Chemical Pathologist at Belfast Health and Social Care Trust. In addition, he is Chief Scientific Advisor to the Department of Health, Northern Ireland, and Director of Research for Health and Social Care. His main clinical and research interests are in nutrition and lipid metabolism, particularly in relation to cardiovascular disease prevention. He is currently President of the Association for Clinical Biochemistry and Laboratory Medicine, UK, and Chair of JCTLM. He is a member of the UK Government's Scientific Advisory Committee on Nutrition, and the Scientific Advisory Board of the UK National Institute of Biological Standards and Controls.

Reception of the City of Braunschweig and Conference Dinner

The Reception of the City of Braunschweig and conference dinner will take place on Wednesday 26th June 2019 at 19:00h at the historic town-hall in Braunschweig. You will find the official invitation with your conference documents. A map of the city centre is included in the invitation and the location is highlighted.

The historic town hall can be reached from PTB by public transport or taxi. Using public transport take the bus 461, direction “Hauptbahnhof” and leave at “Hildesheimer Straße”. Change to bus 450 direction “Rathaus”. Alternative you can transport take the bus 433, direction “Völkenriede” and leave at “Grasplatz”. Change to bus 416 direction “Kralenriede”. In both cases get off at the stop called “Altstadtmarkt”.

To a limit number of participants, we can offer to take you with us into town by private car. If you need a taxi or want to go into town with us, feel free to contact us at the conference desk.

A link to local public transport, including a link to the bus and tram app, can be found here (https://www.verkehr-bs.de/startseite.html).

Support

We are very happy to be able to welcome you at Physikalisch-Technische Bundesanstalt in Braunschweig and hope you will enjoy your stay here.
If you need anything or have any questions / suggestions, please, contact us at the registration desk or

Dr. Claudia Swart
PTB
Bundesallee 100
38116 Braunschweig
Germany

Email: remind2019@ptb.de or claudia.swart@ptb.de
Phone: 0049-531-592-3220