SVK 2015 STUDENTSKÁ VĚDECKÁ KONFERENCE 2015
19.5.2015 | 3. lékařská fakulta Univerzity Karlovy v Praze

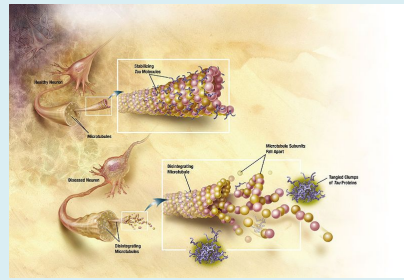
NATURALLY OCCURRING ANTIBODIES AGAINST PROTEIN ASSOCIATED WITH ALZHEIMER DISEASE

Michala Kolářová[a,b*], Lenka Hromádková[a,c,d], Barbora Jankovičová[d], Aleš Bartoš[a,e], Jan Říčný[a], Zuzana Bílková[d] and Daniela Řípová[a]

[a]Department of Neurobiology and AD center, National Institut of Mental Health, Klecany, Czech Republic, [b]Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic, [c]Faculty of Science, Charles University in Prague, Prague, Czech Republic, [d]Department of Biological and Biochemical Sciences, University of Pardubice, Pardubice, Czech Republic, [e]Department of Neurology, Third Faculty of Medicine, Faculty Hospital Kralovske Vinohrady, Charles University in Prague, Prague, Czech Republic

INTRODUCTION:

In past years, large attention was devoted to diagnostic markers as well as therapeutic approaches to neurodegenerative diseases, especially to Alzheimer disease (AD). The newest clinical studies are focused on tau protein. They are using monoclonal antibodies (Abs) against a specific epitope of tau protein or polyclonal Abs like intravenous immunoglobulins (IVIg), which represents a reservoir of naturally occuring antibodies. Some studies found that IVIg products contain Abs against tau protein. According to these findings, we were interested in the isolation of these Abs and their characterization. As another step we used these isolated antibodies as standard in our in-house ELISA for establishing concentrations of specific antibodies in CSF and serum of 4 different groups of patients.



Applied Bioreagents and Methods

Intravenous Immunoglobulin (IVIG) preparation: Flebogamma® 5% dual inactivation and filtration (DIF), 5 g of human IgG / 100 ml, IgG > 99%, IgA < 50 µg/ml

Forms of tau protein: 1) Commercially available recombinant human tau (isoform 2N4R, 1-441, rPeptideBogart, GA, USA); **2)** Prepared recombinant tau forms: A) full-lenght human tau 1-441 His-tag, B) truncated human tau 155-421 HisTag, C) truncated human tau 13-391 HisTag

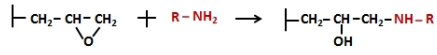
ELISA

Elisa Reader Multiskan EX , Thermo Scientific
0.125 µg of tau/ well
50 µl of 0.05 - 36 µg/ml anti-tau specific antibodies from IVIG; 50 µl of undiluted CSF or 50 of diluted blood serum as follows 1:200, 1:600, 1: 1800
Conjugate: goat anti-human IgG/HRP (Novex, Life Technologies) dilution 1 : 10 000
Colorimetric detection: TMB substrate (Sigma-Aldrich)

Western blot

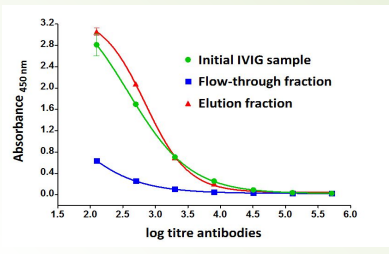
Nitrocellulose membrane (0.2 µm, Bio-Rad)
5 µg of tau protein per well
Non-reducing SDS-PAGE elfo (10% T, 3% C)
Elution fraction – dilution 1 : 125
Conjugate dilution 1 : 15 000
Chemiluminescence detection: Clarity western ECL (Bio-Rad)

Isolation procedure

Solid phase	(Hydroxyethyl)methacrylate copolymer with reactive epoxy groups (50 µm) – 5 ml
Ligand	Tau 1-441 HisTag (25 mg)
Conditions: Coupling of ligand	0.1 M NaH_2PO_4, 1M NaCl, pH 9.2 Overnight, 4°C, gentle stirring Inactivation by 0.2M ethanolamine
Princip of coupling reaction	
Biospecific binding	8 ml of IVIG in PBS buffer / 1 cycle
Elution	0.1M glycin-HCl pH 2.6 by flow 1 ml/ min

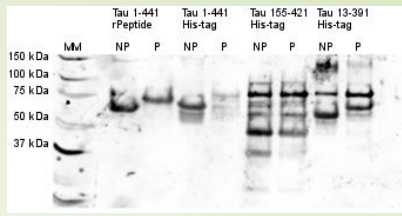
Isolation Efficiency

ELISA of all IgG fractions with binding ligand Tau 1-441 His-tag



Purity	0.852
Concentration (µg/ml)	0.397
Total amount of isolated IgG	3.2 mg
Amount of isolated IgG / 1 ml IVIG	400 µg

Characterization of Isolated IgG

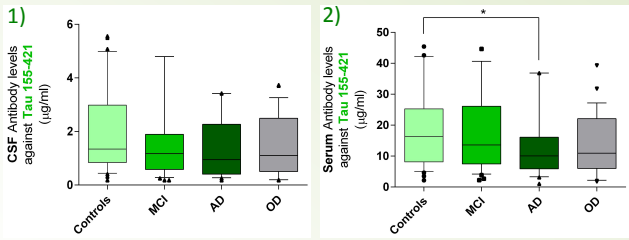


Western blot

P: phosphorylated,
NP: non-phosphorylated

Antibody levels in CSF and serum fluids

Diagnosis	N	Female sex (%)	Age at sampling (years)
Normal controls	44	19 (43%)	66 ± 8
Mild cognitive impairment	32	14 (44%)	69 ± 8
Alzheimer disease	28	23 (82%)	75 ± 7
Other neurodegenerations	26	11(42%)	66 ± 10



Legend: **M**ild **C**ognitive **I**mpairment; **A**lzheimer **D**isease; **O**ther **D**ementias

The box plots are pictured as median (horizontal line) with box of 25th-75th percentiles and whiskers (10th-90th percentiles). Outliers are indicated as circles. For statistical comparison was used Kruskal-Wallis test (* p≤ 0.05).

CONCLUSION:

We found that in the commercial product Flebogamma were specific Abs against tau protein. We were able to isolate antibodies reactive with various forms of tau protein and performed their basic characterization by immunosorbent techniques. It is interesting that from the plasma of healthy donors it is possible to isolate specific Abs against one protein that is associated with AD. This results are in agreement with our measurement of Abs levels in serum samples from healthy controls. We found significant difference between control and AD group in serum antibody levels against truncated form of tau protein. The control group has higher levels of specific antibodies against fragment of tau 155-421 which decrease with progression of disease. (Fig. 2). We can see similar profile of antibodies in CSF and serum fluids (Fig. 1 and 2).

Our hypothesis is that the used fragment can represent pathological form of tau protein and therefore healthy population can have higher levels of naturally occurring Abs against it. During progression of disease, those Abs bind to increasing amounts of pathological tau molecules, including truncated forms, and that leads to lower antibody levels.

Aknowledgements:
This work was supported by the project „National Institute of Mental Health (NIMH - CZ)", grant number ED2.1.00/03.0078 and the European Regional Development Fund, by Czech Science Foundation (project GACR P304/12/G069) and by EU project NADINE (No. 246513), 260168/SVV/2015 and GAČR 13-2660 1S.