Neuroendocrinology Letters Volume 36 No. 3 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

New perspectives in human tear analysis?

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Submitted: 2015-04-03 Accepted: 2015-04-08 Published online: 2015-08-15

Key words: **tear; proteome analysis; neuropsychiatry**

Neuroendocrinol Lett 2015; **36**(3):185–186 **PMID:** 26313380 NEL360315L01 © 2015 Neuroendocrinology Letters • **www.nel.edu**

Abbreviations:
DTT - dithiothreitol
IAA - iodoacetamide
LC-MS/MS - liquid chromatography (nano HPLC) and mass spectrometry
SDS - sodium dodecyl sulphate

The human tear has the primary function to lubricate the cornea and to be the first-line protective surface against pathogens. Due to difficulties with their collection, tears are rarely utilized in clinical medicine. Nevertheless, these exocrine secretions could be very valuable as a source of potential biomarkers. Direct and prompt regulations of the tears production via parasympathetic tract of nucleus lacrimalis (n. VII) in pons Varoli throughout n. maxillaris of n. V enables to reveal changes in tear composition as an indicative response to various neuropsychiatric entities.

Plasma, urine, and cerebrospinal fluid have been studied extensively in clinical medicine, but tears attracted only marginal interest so far. However, due to major advances in proteomics technique we can readily identify and quantify hundreds to thousands of proteins in single experiment. This can contribute to our understanding of complex biological phenomena and diseases.

In our previous studies, we found deviations of human saliva and sweat structures in patients suffering from panic disorder (Kukumberg *et al.* 2009). Currently, we aim to compare proteins of human tears of healthy subjects with tear proteome of patients affected by different neuropsychiatric disorders.

In our pilot study we analysed 54 tear samples from 7 healthy individuals (5 males and 2 females w/ average age 30). Tear proteins were extracted with SDS-Tris-HCl (pH 6.8) containig 2% β-mercaptoethanol, purified via methanol-chloroform precipitation, reduced with DTT, alkylated with IAA, digested with sequencing grade trypsin and analyzed by LC-MS/MS on LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific) (Takáč *et al.* 2014).

We identified 300 proteins, out of which only 59 (19.6%) were detected previously (Zhou *et al.* 2012) while 241 (80.4%) proteins were novel, not reported in the literature yet. To our knowledge, we are first ones to discover the presence of pituitary adenylate cyclase-activating polypeptide (PACAP) in human tear. PACAP and its receptors are expressed in the hypothalamus, the gonadotrope cells of the anterior pituitary gland, and the

gonads, forming an autocrine-paracrine system in these tissues (Zheng *et al.* 2014). It has been observed that levels of PACAP were increased in serum of women with post-traumatic stress disorder, but not in men (Dias & Ressler 2013).

Clusterin is another protein detected in human tear for a first time. Clusterin is multifunctional protein that participates in tissue remodeling, apoptosis, lipid transport and mediated cell lysis. Clusterin was implicated in cancer, neurodegenerative diseases and the earliest stages of the Alzheimer disease neurodegenerative process (Desikan *et al.* 2014).

Additional interesting proteins detected in tear fluid from healthy individuals are cystatin SN and ankyrin 3 gene protein (ANK3). Cystatin SN is a secreted cysteine proteinase inhibitor considered to be a tumor marker for gastrointestinal esophageal squamous cell carcinoma (Chen *et al.* 2013), and colorectal cancer (Yoneda *et al.* 2009). The ANK3 protein plays an integral role in regulating neuronal activity, and have been linked to bipolar disorder and schizophrenia (Logue *et al.* 2013).

Our pilot contribution to functional proteomic analysis of normal human tear supports hypothesis of its potential application in neuropsychiatry. Further research focusing on identification of new neurobiological markers in tears will follow. It is reasonable to expect that utilization of latest proteomics-related hardware and software will bring relevant results that will contribute to better understanding and ultimately lead to more effective treatment of a number of neuropsychiatric disorders.

ACKNOWLEDGEMENTS

This work was supported, in part, by a VEGA 2/0170/10, 2/0109/13, APVT-51-027402, by NATO grants CRG-972173 and LST.CLG-977559, by MVTS-32060600/EC-Instruct-FP7-211252 grant, EEA-Norwegian FM SK-0086 grant to R.F., UK/153/2014 to M.K., and NIH INBRE award USM-GR05067-02 to T.P.

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