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Investigation of the retinal and cortical vascular barriers' permeability along the onset and progression of neurodegenerative pathologies in a rat model

Peunov D.A.*, Telegina D.V., Stefanova N.A.
Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia
** d.peunov@g.nsu.ru*

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Motivation and Aim: Age-related macular degeneration (AMD) and Alzheimer's disease (AD) are neurodegenerative disorders, serving the main cause for central blindness and dementia in modern aging population respectively. High comorbidity of the diseases also worsen condition of patients [1]. Both pathologies are known to be associated with a vasculature dysfunction and recently with abnormalities of blood-retinal (BRB) and blood brain (BBB) barriers. It's considered that healthy aging is accompanied by decline of BRB and BBB and they are disrupted in both AMD and AD. However it remains unclear whether barriers' dysfunction cause or follows development of the diseases. The purpose of this study was to trace changes in BRB and BBB permeability along onset and progression of AMD-like and AD-like pathology, possibly revealing some common patterns. *Methods and Algorithms*: The experiments was performed on senescence-accelerated OXYS rats, which simulate key characteristics of both sporadic AD and dry AMD [2]. During the periods, when clinical signs of AD and AMD in OXYS rats are absent (age 20 and 45 days), manifest (~5 months), and progress (at 12 and 18 months), we assessed the permeability of BRB BBB for Evans blue dye (EBD) and the retinal and prefrontal cortex (PFC) level of the tight-junctional proteins occludin (Ocln) and claudin-5 (Cldn5) by Western blot analysis as described early [3]. Age matched Wistar rats served as controls.
Results: Two-way ANOVA shown that in retina concentration of EBD depended on strain as well as age ($F_{1, 43}$=15.5, p<0,001; $F_{3, 43}$=4,8, p<0,01) and was lower in OXYS rats compared to the Wistar. Post-hoc test confirmed that at the age of 20 days, 5 and 18 month it was significantly lower (p<0.05). At the age of 18 month, observed only in OXYS rats decrease of EBD concentration (p<0.05) from the age of 12 month, was the cause (Fig. 1, A).
Since the data on the concentration of the EBD in PFC didn't met the assumption of normality we performed Kurskal-Wallis test followed with pair by pair t-test comparison. At the first stage we shown that the concentration of the dye in PFC was significantly dependent on age (Chi-squred$_3$=19.2, p<0.001) but not on strain. Post-hoc comparison revealed tendency in the OXYS rats at the age 1.5 month to have higher level of the EBD, compared to the Wistar. Both strains shown significant gradual decrease (p<0.05) of the EBD concentration from the age of 45 days until 18 month (p<0.05). In OXYS rats the tendency of the dye content to drop was detectable earlier than in Wistar (Fig. 1, B).

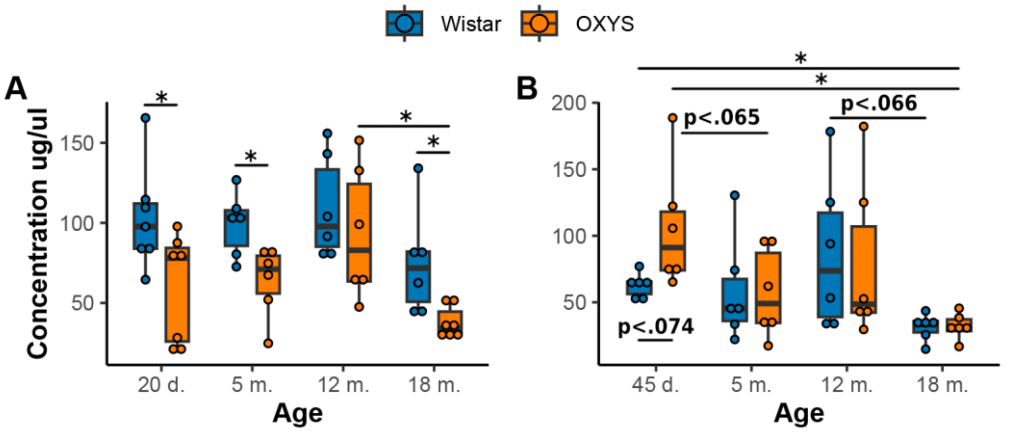

Fig. 1. Concentration of retained EBD in retina (A) and prefrontal cortex (B) of OXYS and Wistar rats along the progression of pathologies. * – significant at p<0.05. Boxes state for Q1–Q3 range, line for median, whiskers end on the min/max values. d – days, m – months

The regulation and restriction of the blood-to-tissue and reverse transports considered to be the key function of BBB and BRB and is carried out by tight junctions (TJ). Among others claudin-5 (Cldn5) and occludin (Ocln) are considered to be those of importance for TJ functioning in BRB and BBB. While Cldn5 is directly forms intercellular bonds, Ocln serves regulatory functions. The level of Cldn5 in retina depended on the strain ($F_{1,24}$=4.4, p<0.05), specifically tended to be higher in the OXYS compared to the Wistar rats. (fig. 2, A). The amount of Ocln in retina was higher in the OXYS rats relatively to the Wistar at the age of 18 month (p<0.05). It was caused by decrease from the age of 20 days, detected only in the Wistar rats (fig. 2, B). In PFC We haven't detected any changes nor in Cldn5 (fig. 3, A) neither Ocln levels (Fig. 3, B).

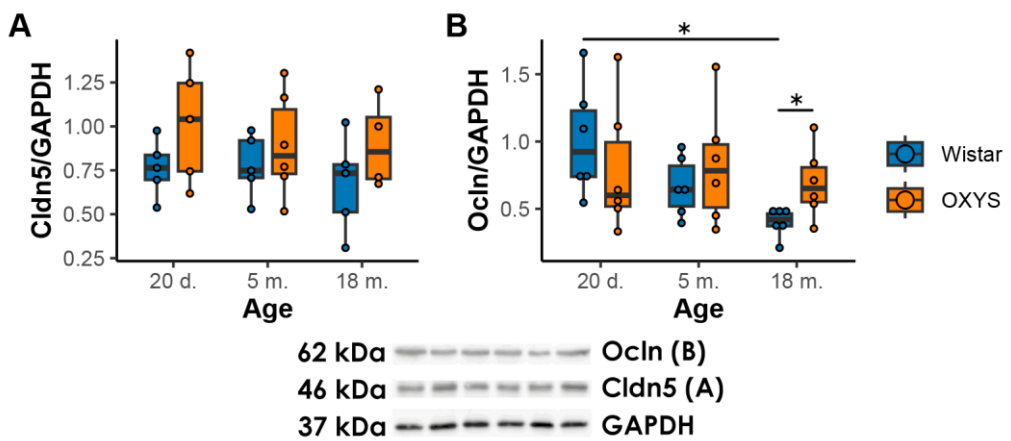

Fig. 2. level of Cldn5 (A) and Ocln (B) in retina of Wistar and OXYS rats along the progression of pathologies. * – significant at p<0.05. Boxes stand for Q1–Q3 range, line for median, whiskers end on the min/max values. d – days, m – months

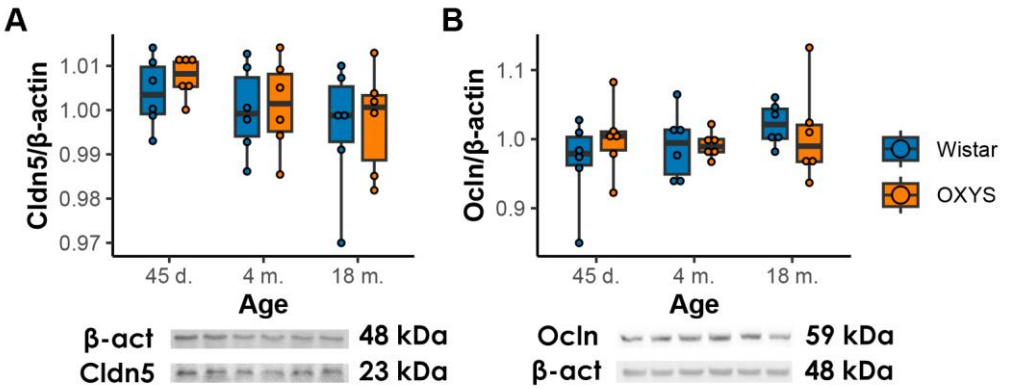

Fig. 3. level of Cldn5 (A) and Ocln (B)in cortex of Wistar and OXYS rats along the progression of pathologies. Boxes stand for Q1–Q3 range, line for median, whiskers end on the min/max values. d – days, m – months

Conclusion: According to our data, BRB and BBB permeability for the EBD weakly altered with age in both retina and PFC in Wistar and OXYS rats. But in PFC of OXYS rats the permeability for the dye tended to decrease earlier than in Wistar due to the higher level of BBB permeability at the age of 45 days in OXYS rats. In OXYS rats AMD-like retinopathy develops against the lowered permeability of the BRB, which may result from the decreased level of vascular growth and increased activity of Müller cells, which was shown earlier [4, 5]. Vascular growth factor deficit could dampen vesicular transport, which is an alternative way, how albumin enters the tissue. Thus it may impact transport of the albumin-binded EBD. Slight elevation in overall Cldn5 content in retina also could be explained with upregulated activity of Müller cells, as they are able to bring up expression of Cldn5 in retinal pigment epithelium, where it is normally absent, as a compensatory reaction to a pathology progression.

Overall the obtained data supports a role of BRB dysfunction in AMD progression and assumes that leakiness of barriers is not immanent to the AMD and AD development at least the portion could be measured with EBD.

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