AGE-RELATED CHANGES IN P-GLYCOPROTEIN FUNCTION AT THE BLOOD-BRAIN BARRIER. - A COMPARATIVE PRECLINICAL STUDY.

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Introduction: Blood-brain barrier (BBB) has many age-related dysfunction that might be a cause of many neurodegenerative disorders.[1,2,3] The increased leakage of the BBB can be the result of the alteration of endothelial cells, thickness of basal lamina, and morphology of pericytes and astrocytes, but also due to the changes in expression of different transporter proteins at the endothelial cell layer of the brain capillary.[4] **Our study aimed** to investigate the structural and morphological age-related changes of the BBB and the alteration of function and expression of P-glycoprotein in Wistar rat models.

Methods

Male Wistar rats 2-3 months (young) and 14-16 months (middle aged) were studied. Dual and triple-probe microdialysis techniques were used to compare BBB permeability for quinidine (QND) in young and aged rats in presence and absence of a specific P-gp inhibitor (PSC-833). Concentrations of QND were analyzed by LCMS-MS. Furthermore, comparative MR imaging of the brains was performed to study anatomical changes, and also single photon emission computed tomography (SPECT) imaging was applied for comparison P-gp functionality. For ultrastructural analysis, electronmicroscopy was performed.

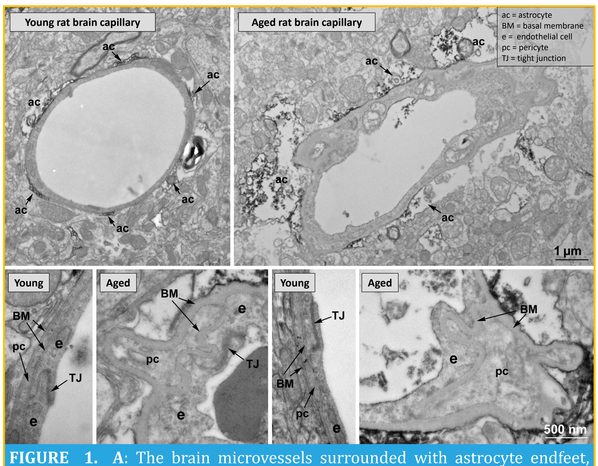

FIGURE 1. A: The brain microvessels surrounded with astrocyte endfeet, basal membrane and pericytes in young and aged Wistar rats.

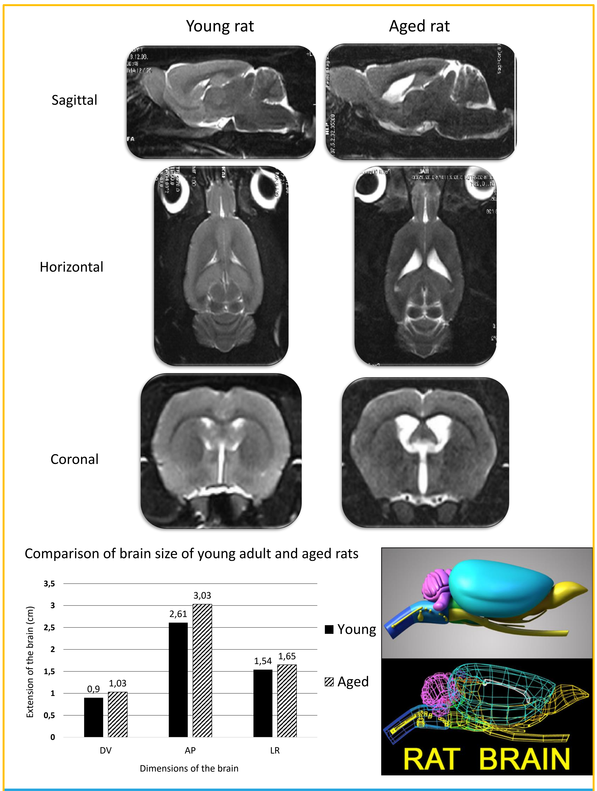

FIGURE 2. A: MR Images of young and aged rat brains to determine the stereotaxic coordinates. The bar graph shows the brain growth in cm; dorsoventral (DV), anterior-posterior (AP), and lateral (LR) extensions of the brain in aged rat (>1 year and 2 months old) are compared to young rat (3-4 months old).

Results

The control level of QND in absence of PSC-833 was higher in aged rats. However, in presence of PSC-833, the brain levels increased less in aged than in young rats suggesting lower expression level or impaired functionality of P-gp in old subjects. In MR imaging the extension of cerebral ventricles increased significantly and there were also characteristic ultrastructural changes at the BBB with aging by electronmicroscopy.

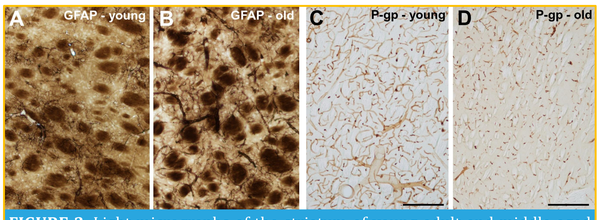

FIGURE 3. Light micrographs of the striatum of young adult and middle aged animals, showing astroglial- (**A, B**) and P-gp-immunostaining (**C, D**). Scale: **A, B**: 200 µm; **C, D**: 250 µm.

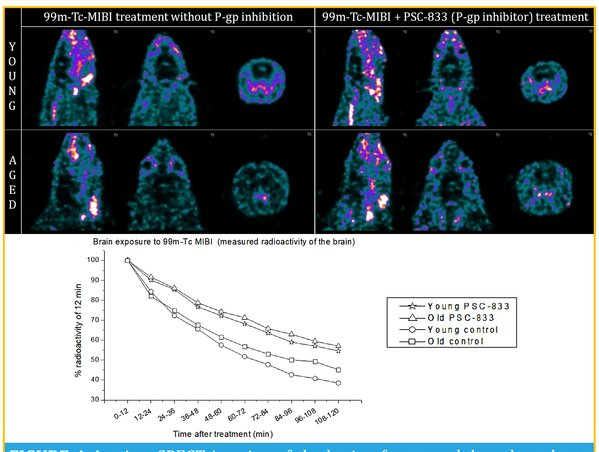

FIGURE 4. In vitro SPECT imaging of the brain of young adult and aged rats after intravenous administration of 99m-Tc-MIBI or PSC-833 + 99m-Tc-MIBI. 99m-Tc-MIBI is a substrate of P-gp and PSC-833 is a specific P-gp inhibitor. The substrate exposure is higher in old than in young rats. After P-gp inhibition the brain levels increase both in young and in aged subjects.

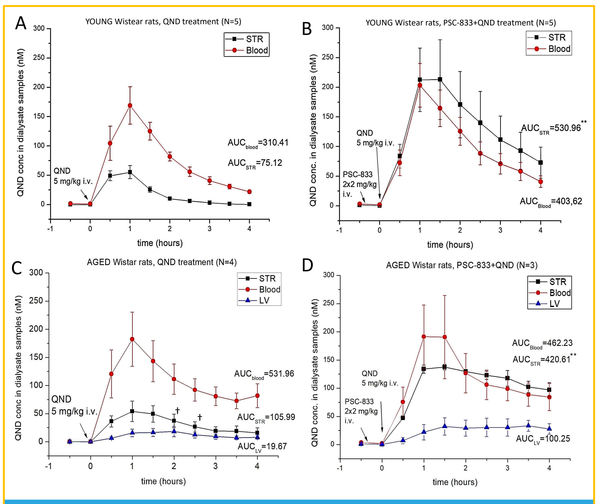

FIGURE 5. Concentration-time profiles of QND in young (A,B) and aged (C,D) Wistar rats, measured by dual- or triple-probe microdialysis. Both the striatal and the ventricular levels of QND increased in a statistically significant manner if the QND treatment was combined with PSC-833 treatment (B,D). The eliminationof of QND seem to be slower in aged animals.

Conclusions

Our results indicate that there are many differences between young adult and aged rats in the structure and function of the BBB. Our findings suggest a lower expression and/or reduced P-gp function with aging leading to enhanced brain entrance of the drugs. And, on the other hand, there are some other morphological changes in the cerebral capillaries acting against the brain uptake of dangerous substances as it was shown by electronmicroscopy.

References

1. **Erdő F, Denes L, de Lange E.**, *Age-associated physiological and pathological changes at the blood-brain barrier: A review.*, J Cereb Blood Flow Metab. 2017 Jan; 37(1) : 4-24.
2. **Zlokovic BV.**, *Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders.*, Nat Rev Neurosci. 2011 Nov 3;12(12):723-38.
3. **Gray MT, Woulfe JM.**, *Striatal blood-brain barrier permeability in Parkinson's disease.*, J Cereb Blood Flow Metab., 2015 May; 35(5) : 747-50.
4. **Peters A, Josephson K, Vincent SL.**, *Effects of aging on the neuroglial cells and pericytes within area 17 of the rhesus monkey cerebral cortex.*, Anat. Rec., 1991; 229(3) : 384-398.

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