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USING HIPPOCAMPAL SLICE CULTURE FOR MODELING OF PERIVENTRICULAR LEUCOMALACIA *IN VITRO*

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Perinatal damage of central nervous system, namely periventricular leucomalacia, PVL (lesion of white matter in prematurely born children) is among major contemporary medical and social problems in pediatrics and neurology. PVL results in different neurological disorders, including motor and cognitive impairments and with 90 % probability leads to development of cerebral palsy.

The development of *in vitro* models of periventricular leukomolation is important for finding ways of neuroprotection in case of this pathology. Our aim was to simulate PVL using organotypic brain slice cultures. This type of cell culture was chosen because in this case cell and layer types, intercellular connections, synaptic organization and receptor distribution typical of intact nervous tissue are preserved. Apart from that organotypic slice culture provides a direct access to the intercellular liquid enabling strict control of cultivate conditions and immediate influence of studied compounds.

PVL was modeled via oxygen-glucose deprivation (OGD) and addition of endotoxin lipopolisaccharid (LPS) to the cultural medium. The next step was the evaluation of the tissue state via estimation of the lactatedehydrogenase content changes in the cultural medium and immunohistochemical staining. Following antibodies were used: anti-GFAP (marker of astrocytes), anti-Iba-1 (microglial marker), anti-Rip (marker of ologodendrocytes). Primary antibodies were visualized using secondary antibodies conjucated with AlexaFluor fluorohrom. OGD and LPS effects were studied after applying them both separately and simultaneously.

The change of LDH concentration in the culture medium indicates the degree of cell membrane damage. Spectrophotometric assay revealed an increase in LDH content in the cultural medium after OGD and LPS, and their combination led to the most pronounced tissue damage.

After PVL modeling considerable changes in imunohistochemical staining were observed. Rip-immunoreactivity dropped indicating the decrease in number of oligodendrocytes. Integral density of fluorescence for Iba-1 and GFAP increased indicating micro- and astrogliosis and being the sign of inflammation.

Taking into account peculiarities of the changes in organotypic brain slice cultures described above, it can be concluded that an adequate model of periventricular leucomalacia that can be used to test the various means of neuroprotection was developed.