DOT BLOTTING ANALYSIS AS A WAY OF CELLULAR PRION TOTAL LEVEL DETERMINING

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Transmissible spongiform encephalopathies are fatal diseases caused by pathogens of protein nature — prions. Scrapie is diagnosed in sheep and goats and bovine spongiform encephalopathy is diagnosed in cattle. These diseases have a long period of incubation which is required for the replication and accumulation of the pathogen protein. The cellular prion (PrP^C) is a membrane protein of normal cells and it is involved in important processes. But under conditions of neurodegeneration PrP^C becomes a substrate for the formation of abnormal pathological prion. The study of cellular prion level in different tissues is an important for understanding the mechanism of neurodegeneration. The aim of study was the determination of the PrP^C total level in spleen, jejunum, liver, kidneys, muscle and brain of laboratory rats.

Research was carried out using the six months males of laboratory rats *Rattus norvegicus var. alba*, *Wistar* line. The animals were decapitated and the tissues were selected. The dot blotting analysis of tissues was carried out. For that, the tissue was homogenized and lysed in a special buffer with the addition of 0.001 % mixture of proteinase inhibitors (*Sigma*, Germany) as well as centrifuged. The protein level was measured by Lowry method.

The samples with the same concentration of the protein were deposited on polyvinyl diftorid (PVDF) membrane (*Millipor*, USA). The monoclonal primary antibodies (Antibody mAB6H4; *Prionics*, Switzerland) and secondary polyclonal goat anti-mouse antibodies, which are conjugated with alkaline phosphatase (*Sigma*, Germany), were used too. Detection of the immune complexes was carried out using a substrate for alkaline phosphatase CDP-Star (*Tropix*, UK). Visualization was performed using X-ray film *Retina XBM* (*Lizoform Medical*, Ukraine) and film development kit for films (*Kodak*, Japan) (Vlizlo V. V., 2012). The cellular prion total level was 100 % in jejunum, 97.41 % in medulla oblongata, 91.72 % in spleen, 52.63 % in cerebellum, 40.71 % in liver, 29.84 % in kidneys and 17.14 % in femoral muscle.

Prion pathologies arise mainly as a result of oral infection, while eating affected meat products or feed, as evidenced in experiments on monkeys (Verbitsky P. I., 2005). So the highest PrP^C level in jejunum cells contributes to prionopathy. In experimental mice pathological prion was found in the spleen and lymph nodes on 5–13th week after injection, in the spinal cord on 13–17th week, and in the cerebrum on 17–19th week. Pathological changes in the brain appeared on the 25th week, and the clinical symptoms of encephalopathy appeared from 34th week. Pathological changes were observed only in the brain (Yuan J. et al., 2010).

Obviously, due to the cellular prion expressing spleen is the organ of prion replication. Neurons and glial cells express high level of PrP^C too so they are very sensitive to abnormal prion lesions. This may explain a considerable degradation of neurons during prionopathies. Total PrP^C level in other investigated tissues is lower but the infection spared depends on the PrP^C production in this tissues.

The cellular prion is revealed in spleen, jejunum, liver, kidneys, muscle and brain of laboratory rats by dot blotting analysis. This confirms that PrP^C is playing an important physiological function and investigated tissues are potentially dangerous because express cellular prion which is a precursor of pathological prion. The infection spared depends on the production of PrP^C in the tissues.

Keywords: RATS, DOT BLOTTING ANALYSIS, CELLULAR PRION, TRANSMISSIBLE SPONGIFORM ENCEPHALOPATIES