UDC 57.017.23+112.7:352.465:151.643

LOCALIZATION AND LEVEL OF THE CELLULAR PRION IN THE KIDNEYS OF DIFFERENT AGE WISTAR LINE RATS

Kushkevych M. V., Vlizlo V. V.
Institute of Animal Biology of NAAS, Lviv, Ukraine, e-mail: m_kushkevych@ukr.net

Transmissible sponhioform encephalopathies (TSEs) are resulting from infection and genetic or/and sporadic cases. The infectious agent of the disease is pathological prion (PrP^{Sc}, Sc – scrapie from). The precursor of PrP^{Sc} is the cellular prion (PrP^{C}, C – cellular from). It is located on the outer surface of the cell membrane and involved in different metabolic processes.

The PrP^{C} localization and its level in the kidney tissue of rats of different age were studied. PrP^{C} was found in the convoluted and straight nephrons tubules and in vascular glomeruli in kidney tissue by immunohistochemical analysis. However, according to tissue age-related changes the localization of PrP^{C} was not changed.

The total amount of cellular prion in the kidneys of different age rats was determined by dot blotting analysis. PrP^{C} level decreased by 44 % in the kidneys of six months animals compared to one month animals. Its level also decreased by40 % in thirty months animals' tissue compared to one month animals.

Keywords: *cellular prion, immunohistochemistry, dot blotting, kidneys, age changes*

Prion diseases are slow neurodegenerative disorders in humans and animals, which have a fatal effect. The disease is a manifestation of molecular pathology in which cell (physiological) prion changes its structure and is transformed in pathological form [1, 2, 3]. The study of the physiological role of PrP^{C} in cellular processes is an important for understanding the causes of neurodegeneration including direct effect of prions or loss of PrP^{C} functionality.

PrP^{C} is localized on the surface of mammalian cells and consists of sialic glycoproteins formed by about 210 amino acids, which is connected to the plasma membrane by glucosil-phosphotidil-inositol fragment [4, 5].

The studies of PrP^{C} functions *in vitro* and *in vivo* have shown that this protein is involved in copper metabolism and protection mechanisms against of oxidative stress and apoptosis and also in cell adhesion, migration, proliferation and differentiation, and interactions with extracellular components. In addition, the cellular prion is involved in the synaptic structure formation and its functioning. It maintains the Ca^{2+}-homeostasis, influencing on the Ca^{2+}-channels activity [6, 7].

Cellular prion as a precursor of pathological prion was founded in different tissues by western blotting [8]. However, there are no information about its localization in the kidneys of animals. Because of the sporadic diseases occur in older age persons, we investigated the PrP^{C} level in the tissue of rats of different ages.

The aim of the study was to determine the ontogenetic changes of cellular prion localization and level in the rats' kidneys.

Materials and Methods. Manipulation with the animals were carried out under the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), the Decision of the First National Congress on Bioethics (Kyiv, 2001) and the Law of Ukraine "On Protection Animals from Brutal Treatment" (Kyiv, 2006; the protocol № 55 of the expert commission meeting from 17.05.2016).

Research was carried out on the males of laboratory rats *Rattus norvegicus var. alba, Wistar line*, which were held under standard vivarium conditions. The animals aged one, six and thirty months were decapitated, the kidneys were selected for this research.

For immunohistochemical studies, the tissue was fixed, washed and dewatered. The paraffin blocks formation was performed using standard techniques [9]. The isolated fragments of the tissues were incubated with monoclonal primary antibodies (Antibody mAB6H4; Prionics, Switzerland) at +4°C for 12 h. Dako firm (Denmark) reagent kit for immunohistochemical studies was used. After washing, the sections of the tissues were stained by Mayer hematoxylin and placed in aqueous permanent mounting medium (Dako, Denmark). Histological studies were performed on the microscope Axioskop 40 (Carl Zeiss, Germany). Tissue sections, which were painted only with hematoxylin, were used as a control. The amount of cellular prion in the tissues sections was determined using a program VideoTest 5.0.

A dot blotting analysis of the kidneys was performed. 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 samples with the same concentration of the protein were deposited on polyvinildiftorid (PVDF) membrane (Millipor, USA), which was incubated with monoclonal primary antibodies (Antibody mAB6H4; Prionics, Switzerland) at +4°C for 12 h, and secondary polyclonal goat anti-mouse antibodies which is conjugated with alkaline phosphatase (Sigma, Germany) at +22 °C during 60 min. 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) [9].

Student coefficient was calculated to assess the probable difference between the statistical characteristics of alternative data set. The accurate approximation was when $P \leq 0.05$ [10]. Statistical analysis of results was carried out using the programs Excel and Origin.

Results. Since the cellular prion is a substrate for the formation of abnormal prion [1], then study of its localization in tissues and organs is important for explaining of the prion diseases pathogenesis mechanism. Therefore, our task was to investigate the localization of PrP^C in different age rats' kidneys tissue.

The localization of PrP^C in the convoluted and straight nephrons tubules and in vascular glomeruli was found. However, according to age-related changes the localization of PrP^C was not changed (fig. 1). There is evidence that PrP^{Sc} was found in the collecting tubes of the kidneys and urine in abnormal prion-infected hamsters, as well as in sick people [11]. That is, the presence of PrP^C in the animals' kidneys indicates the possibility of its conversion into PrP^{Sc} in the case of TSE.

Similar results are obtained by O. A. Peralta et al. [12] exploring different somatic tissues of cattle. The highest PrP^C level was set in neurons, thymocytes and lymphocytes. Furthermore, PrP^C was found in pancreatic islets of Langerhans, myocardium, alveoli, renal glomeruli and skin epithelial cells.

Age dynamics of PrP^C level as an immunohistochemical analysis result is shown in fig. 2 *B*. Quantitative data was set after the digitization of tissue photographs for the same magnification. The PrP^C level did not significantly changed in the different age rats' kidneys.

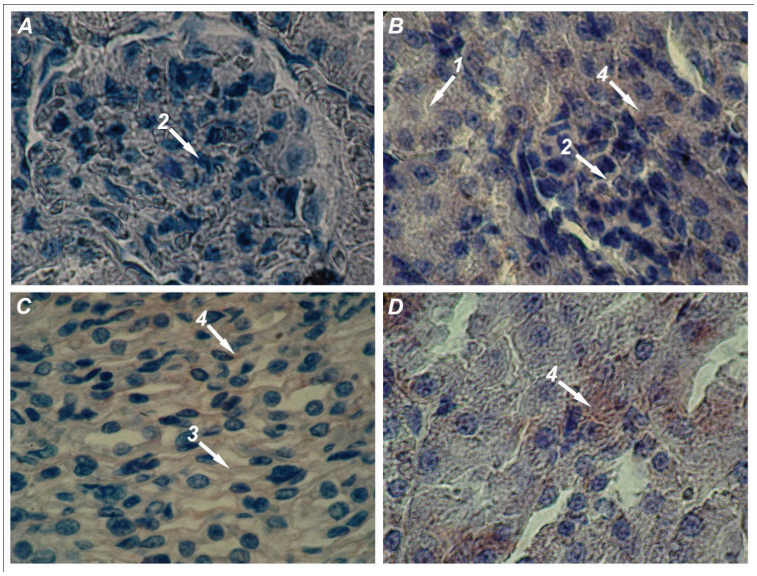

Figure 1. PrP^C localization in kidneys of one month (*B*), six months (*C*) and thirty months (*D*) rats; *a* is control; *1* is the convoluted nephron tubule; *2* is the vascular glomeruli; *3* is the straight nephron tubule; *4* is the localization of the cellular prion (light microscopy, hematoxylin staining, X1000)

The total amount of cellular prion in the kidneys of different age rats was also determined by dot blotting analysis. PrP^C level decreased by 44 % in the kidneys of mature (six months) animals compared to young (one month) animals. Its level also decreased by 40 % in old (thirty months) animals' tissue compared to young (one month) animals (fig. 2 *A, C*).

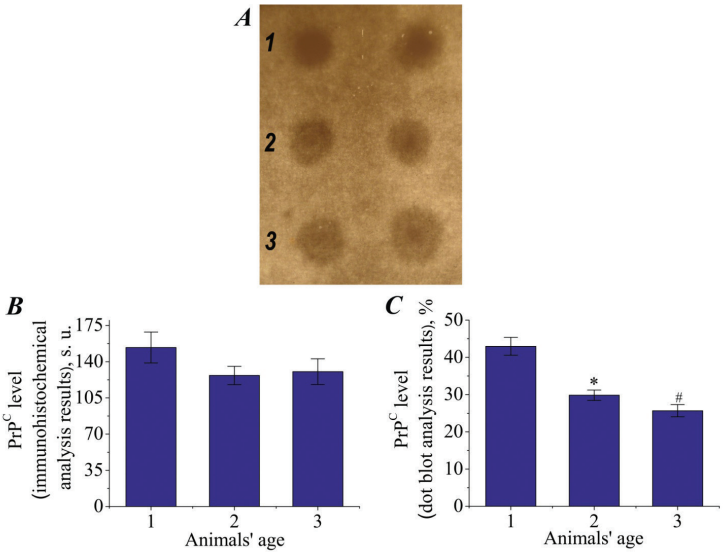

Figure 2. Total PrP^C level in the kidneys of different age rats (*A* is the dot blotting analysis; *B, C* are the histograms): *1* is one month; *2* is six months; *3* is thirty months; (M ± m; * (#) $P < 0.05$; the second (third) age group of rats is compared to the first group)

Similar results are obtained by Mar Cuadrado-Tejedor with collaborators. They analyzed the cortex and hippocampus areas of the rats' brain by the Western blotting analysis [13]. The PrP^C level is increased in both areas in mature animals (38 weeks) compared to young (six weeks), and it is decreased in old animals (56 weeks).

Conclusions. The PrP^C was founded in the convoluted and straight nephrons tubules and in vascular glomeruli in the kidney tissue. The localization of PrP^C was not changed according to tissue age-related changes of laboratory rats.

The amount of cellular prion decreased in the kidneys of six and thirty months animals, compared to one month animals.

Prospects for future research is to found the tissue localization and level of the cellular prion in other organs of rats' prion replication system.

References

1. Verbitsky P.I. Spongioform encephalopathy in cattle and other prion infections / P.I. Verbitsky. Kyiv: Vetinform, 2005. – 240 p. (In Ukrainian).
2. Transmissible and genetic prion diseases share a common pathway of neurodegeneration / R.S. Hegde, P. Tremblay et. al. // Nature. – 1999. – Vol. 402. – P. 822 – 826.
3. Prusiner S.B. Genetic and infectious prion diseases // Arch. Neurol. – 1993. – Vol. 50. – P. 1129–1153.
4. Westergard L., Christensen H., Harris D. The cellular prion protein (PrP^C): its physiological function and role in disease // Biochim. Biophys. Acta. – 2007. – Vol. 1772. – P. 629–644.
5. Kovacs G. Prion Diseases: From Protein to Cell Pathology / G. Kovacs, H. Budka // The American Journal of Pathology. – 2008. – Vol. 172, № 3. – P. 555 – 565.
6. Physiology of the prion protein / R. Linden, V.R. Martins, M.A. Prado et al. // Physiol. Rev. – 2008. – Vol. 88: – P. 673–728.
7. Physiological prion and its role in the functioning of the cell / V.V. Vlizlo, V.V. Stadnyk, Ch.Ya. Major, P.I. Verbitsky // The Animals Biology. – 2008. – vol. 10, N 1–2. – P. 9–23. (In Ukrainian).
8. Major Ch.Ya. The content of physiological prion in peripheral part of prion-replication system under the action of glucoseaminoglycans series drugs. // PhD thesis, Institute of Animal Biology NAAS of Ukraine. – Lviv, 2010. – 6 p. (In Ukrainian).
9. Laboratory methods of research in biology, stockbreeding and veterinary medicine: a guide / V.V. Vlizlo, R.S. Fedoruk et al. Lviv: Spolom. – 2012. – 764 p. (In Ukrainian).
10. Lakin G.F. Biometry / G.F. Lakin. Moscow: HS, 1990. – 352 p. (In Russian).
11. A cell line infectible by prion strains from different species / M. Courageot, N. Daude, R. Nonno, S. Paquet, M. Di Bari, A. Le Dur, et al. // J. Gen. Virol. – 2008. – P. 341 – 348.
12. Peralta O.A. Quantitative and qualitative analysis of cellular prion protein (PrP^C) expression in bovine somatic tissues / O.A. Peralta, W.H. Eyestone // Prion. –2009. – Vol. 3, №3. – P. 161–170.
13. Cellular Prion Protein and Sexual Dimorphic. Areas in Rodents. Correlates with Alzheimer Disease / M. Cuadrado-Tejedor, A. Irujo et al. // Neuroscience & Medicine. – 2011. – Vol. 2. – P. 384–391.

ЛОКАЛІЗАЦІЯ І ВМІСТ КЛІТИННОГО ПРІОНА У НИРКАХ ЩУРІВ ЛІНІЇ WISTAR РІЗНОГО ВІКУ

Кушкевич М. В., Влізло В. В.
Інститут біології тварин НААН, Львів, Україна

Метою роботи було визначення онтогенетичних змін локалізації та вмісту клітинного пріона (PrP^C) у нирках лабораторних щурів.

Методи досліджень. Дослідження виконували на самцях лабораторних щурів Rattus norvegicus var. alba, лінії Wistar. Тварин, віком 1, 6 та 30 місяців, декапітували і відбирали нирки для досліджень. Проводили імуногістохімічний аналіз, використовуючи набір для імуногістохімії фірми Dako й антитіла 6H4 фірми Prionics (Швейцарія). Також виконували дот блот аналіз тканини нирок. Під час проведення цих досліджень застосовували ті ж моноклональні первинні антитіла, а також поліклональні вторинні козячі антимишачі антитіла, кон'юговані з лужною фосфатазою (Sigma, Німеччина)

Результати досліджень. У тканині нирок локуси клітинного пріона виявлено у звивистих та прямих канальцях нефронів, а також у судинних клубочках. Проте, відповідно до вікових змін, локалізація PrP^C не змінилася. За результатами цього аналізу встановлено, що рівень PrP^C вірогідно не змінювався з віком тварин.

Загальний вміст клітинного пріона у нирках щурів різного віку визначали також методом дот блот аналізу. Кількість PrP^C зменшилася на 44 % у нирках зрілих (6 місяців) тварин, порівняно з молодими (1 місяць). Вміст досліджуваного протеїну також знижувався на 40 % у тканині старих (30 місяців) тварин, порівняно з молодими (1 місяць).

Висновки. PrP^C виявлено у нирках у звивистих та прямих канальцях нефронів, а також у судинних клубочках. Локалізація PrP^C не змінювалася відповідно до вікових змін тканини лабораторних щурів.

Кількість клітинного пріона зменшувалася у нирках 6- і 30-місячних тварин, порівняно з 1-місячними.

Ключові слова: *клітинний пріон, імуногістохімія, дот блот, нирки, вікові зміни*