UDC 57.017.23+112.7:352.465:151.643

KUSHKEVYCH M.V., PhD Biol. Sc., e-mail: m_kushkevych@ukr.net,
KOZAK M.R., PhD Biol. Sc., e-mail: mariya_kozak@yahoo.com,
PETRUH I.M., PhD Vet. Sc., Senior Researcher, e-mail: irapetruh@ukr.net,
VLIZLO V.V., Prof., Academician of the NAAS, e-mail: vasyl.vlizlo@inenbiol.com.ua
Institute of Animal Biology of NAAS

CELLULAR PRION LEVEL IN THE ANIMALS' TISSUES

Prion infections cause brain damage in humans and animals with lethal outcome. Cellular prion (PrP^C) is a precursor of pathological prion. Infection development depends on the level of its production in the different tissues.

Comparative data on the relative level of molecular isoforms of the cellular prion in the brain, spleen and small intestine of various animals, in particular cows, laboratory rats and mice, are demonstrated.

Obtained data are important for understanding of the pathogen spreading mechanisms in the case of infection. It can be used as one of the indices for prion infection strains determination and in TSE diagnostics.

Keywords: *cellular prion, brain, spleen, intestine, animals.*

Introduction. Prion infections or transmissible spongiform encephalopathies (TSE) are group of neurodegenerative diseases affecting humans and animals [1]. The causative agent of prion infections is the pathological prion (PrP^{Sc}). Human TSEs includes Creutzfeldt-Jacob disease (CJD), fatal familial insomnia and kuru. Prion infections include scrapi of sheep and goats, transmissible encephalopathy of minks, chronic depressing illness of deer and elks, spongiform encephalopathy of cattle, spongiform encephalopathy of cats [2].

TSEs in humans are rare but they are interest to researchers and practitioners. They are transmissible diseases, the pathogens of which has a unique nature. This is a protein without nucleic acids. When it enters the body's immune response is absent, the transformation of pathogen occurs. The disease ends lethally [1].

Human TSEs are rare but provide opportunities to study human physiology and biomedical science unique perspective.

All prion diseases of humans and animals have common histopathological features. The classic diagnostic triad is spongiform brain vacuolization, loss of neurons and astrocytes proliferation, the formation of amyloid plaques [2].

The precursor of pathological or infectious form is the cellular (physiological) prion protein (PrP^C), which is encoded by the Prnp gene [3]. A key event in the pathogenesis of TSE is the conformational transformation of the PrP^C into a PrP^{Sc} protease-resistant form. Experimental data confirm that PrP^C plays a major role in the replication of prions and prion-induced neurodegeneration [1, 3].

Detection of cellular prion and identification of its isoforms in animal tissues is important for the scientific understanding of pathogen distribution mechanisms. It is also necessary for creation of methods for prion infections diagnosing, in particular bovine spongiform encephalopathy.

The goal of the work was to determine the level of PrP^C in brain, spleen, small intestine of laboratory rats, laboratory mice and cows.

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).

Research was carried out on the males of white non-linear mice *Mus Musculus* and laboratory rats *Rattus norvegicus var. alba, Wistar line,* which were held under standard vivarium conditions. The laboratory animals were decapitated under ether anesthesia, the brain, spleen, small intestine were selected for this research. Cattle of black and white dairy breed were used for the researches too. The same tissues were taken from the cattle after the slaughtering.

A western blotting analysis of the tissues was carried out. For that, the tissue was homogenized and lysed in a special buffer as well as centrifuged at 12.000 × g for 2 min at 4 ºC. The proteins were fractionated by electrophoresis in 12% gradient polyacrylamide gels (PAGE). The electro blotting of proteins on PVDF-membrane was carried out (*Millipore*, USA). The samples with the same concentration of the protein were deposited in each PAGE well. The membranes were incubated with monoclonal primary antibodies (Antibody mAB6H4; *Prionics*, Switzerland) at +4 °C for 12 h, and secondary polyclonal goat anti-mouse antibodies, which are 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) [4].

Student's coefficient was calculated to assess the probable difference between the statistical characteristics of alternative data set. The accurate approximation was when $P \leq 0.05$ [5]. Statistical analysis of the results was carried out using Excel and Origin software.

Results of research and discussion. PrP^C was found in different tissues and organs of the cattle, rats and mice. Using a western-blot analysis three forms of cellular prion were found in the tissues of cattle and rats. They included the diglycosylated form (35–38 kDa), partially (mono) glycosylated form (23–27 kDa) and nonglycosylated form (19–21 kDa). Two forms of cellular prion (glycosylated (29 kDa) and nonglycosylated (19kDa)) were observed in mice tissues.

In relation to PrP^C glycoforms in cattle brain and spleen the diglycosylated forms were predominated and were respectively 62% and 66%. Nonglycosylated form was represented in the smallest amount. It was 18% in cattle brain and 11% in cattle spleen. However, in the small intestine of cows the ratio of glycoforms of cellular prion was different. In this tissue nonglycosylated isoform was 67%, monoglycosylated form was 20% and diglycosylated form was presented in the lowest level – 13%.

In rats' tissues, the ratio of PrP^C glycoforms was the same as in cattle brain and spleen. The level of nonglycosylated isoform was the lowest in brain – 10% and the

highest level was in small intestine – 19%. In brain tissue the level of di- and monoglycosylated forms was almost identical 47 and 49% respectively (fig. 1).

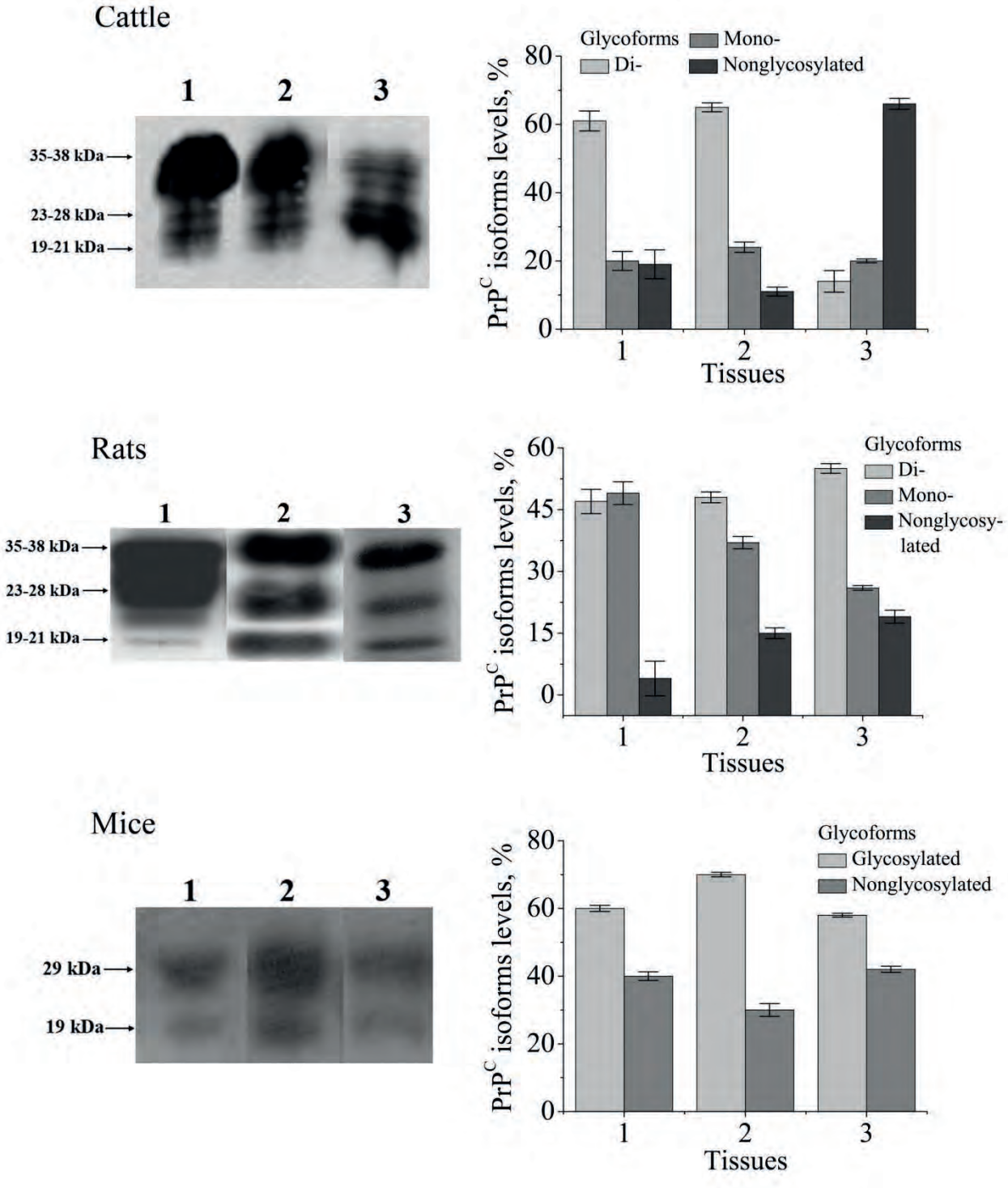

Fig. 1. The level of PrP^C isoforms in tissues of animals:
1 – brain, 2 – spleen, 3 – small intestine.

In mice' tissues glycosylated forms were predominated. Its content was 60%, 70% and 58% respectively in brain, spleen and small intestine. The lowest level of nonglycosylated isoform was found in spleen – 30% and the highest level was in

small intestine – 42% (fig. 1).

Prion pathologies arise mainly as a result of oral infection, while eating affected meat products or feed, as evidenced in experiments on monkeys [2]. In the case of infection, the pathological prion penetrates through the mucosa of the small intestine. It binds with the apical laminin of epithelial cells [1]. It may also interact with the lymphocytes PrP^{C} of solitary and grouped follicles (Peyer's patches). The lymphoid cells spread PrP^{Sc} with the blood to the lymph nodes and spleen, where it enters to the neurons of the sympathetic nervous system, which innervate these organs. In transgenic mice with inhibited production of B-lymphocytes the reduction of disease after intraperitoneal introduction of the PrP^{Sc} was demonstrated. At the same time the intracerebral introduction of animal does not affect on the rate of infection spread in the experimental and control groups [6]. Peripheral infection of the mice leads to the accumulation of the pathogen in the spleen even before it appears in the brain. Under these conditions, the spleen does not perform the protective function, but is the prion replication organ [2]. There is no immune response, since both forms of prions pathogenic and cellular are similar and encoded by the same gene.

This interrelation is characteristic for the brain and shows structural and functional condition of the system of post-translation cellular prion modification. The degree of glycosylation affects the ability of the cellular prion to be transformed into a pathogenic form. At prion infections especially at spongiform encephalopathy in cattle the number of nonglycosylated isoforms increases in the brain, and the level of diglycosylated form decreases [7]. A similar picture is observed during a western blot analysis of brain of patients who died from sporadic CJD [8, 9].

In the intestines of cows, the highest level of the deglycosylated form of cellular prion is established. This can play a key role in the development of prion diseases, since rodents in nature do not suffer from these pathologies. Determination of correlation between prion isoforms and detection of changes is prospect. It can be used as one of the indices for prion infection strains determination and in TSE diagnostics.

Conclusion and prospects for further research. Cellular prion is synthesized in brain, spleen and small intestine of cattle and laboratory animals. This confirms the involvement of these organs in the development of prionopathy and explains the mechanism of the pathogen spreading in case of infection.

Prospects for further research are the determination of the PrP^{C} level in other organs of the prion-replicating systems of animals.

REFERENCES

1. Prusiner, S.B. (1993). Genetic and infectious prion diseases. *Arch. Neurol, 50,* 1129–1153.

2. Verbitsky, P.I. (2005). *Spongioformna encefalopatiya velykoyi rohatoyi hudoby ta inshi prionni infekcii [Spongioform encephalopathy in cattle and other prion infections]*. Kyiv: Vetinform [in Ukrainian].

3. Westergard, L., Christensen, H., & Harris, D. (2007). The cellular prion protein (PrP^{C}): its physiological function and role in disease. *Biochim. Biophys. Acta*, *1772*, 629–644.

4. Vlizlo, V.V. (2012). *Laboratorni metody doslidzhennya u biologiyi, tvarynnytstvi ta veterynarniy medycyni [Laboratory methods of research in biology, stockbreeding and veterinary medicine: a guide]*. Lviv: Spolom [in Ukrainian].

5. Lakin, G.F. (1990). *Byometryya [Biometrics]*. Moscow: HS [in Russian].

6. Kingsbury, D.T., Smeltzer, D.A., Gibbs, C.J., & Gajdusek, D.C. (1981). Evidence for normal cell-mediated immunity in scrapie-infected mice. *Infect. Immun, 32,* 1176–1180.

7. Aguzzi, A., & Heikenwalder, M. (2006). Pathogenesis of prion diseases: current status and future outlook. *Nature Reviews Microbiology*, *4,*765–775.

8. Aguzzi, A., & Christian, H. (2003). Games played by rogue proteins in prion disorders and Alzheimer's disease. *Science, 302*, 814–818.

9. Hill, A. F., Joiner. S., Wadsworth, J. D. F. et al. (2003). Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain, 126*, 1333–1346.

СОДЕРЖАНИЕ КЛЕТОЧНОГО ПРИОНА В ТКАНЯХ ЖИВОТНЫХ / Кушкевич М.В., Козак М.Р., Петрух І.М., Влизло В.В.

Прионные инфекции вызывают повреждение мозга у людей и животных со смертельным исходом. Клеточный прион (PrP^C) является предшественником патологического приона. Развитие инфекции зависит от уровня его экспрессии в разных тканях.

Показаны сравнительные данные об относительном уровне молекулярных изоформ клеточного приона в головном мозге, селезенке и тонком кишечнике различных животных, в частности коров, лабораторных крыс и мышей.

Полученные данные объясняют механизм распространения патогена в случае инфекции. Его можно использовать в качестве одного из показателей для определения штаммов прионной инфекции и диагностики ТСЕ.

***Ключевые слова**: клеточный прион, мозг, селезенка, кишечник, животные.*

ВМІСТ КЛІТИННОГО ПРІОНА В ТКАНИНАХ ТВАРИН / Кушкевич М.В., Козак М.Р., Петрух І.М., Влізло В.В.

***Вступ.** Прионні інфекції або трансмісивні спонгіоформні енцефалопатії (ТСЕ) є групою нейродегенеративних захворювань людей та тварин [1]. Збудником пріонних інфекцій є патологічний пріон (PrP^{Sc}). Попередником патологічної форми є клітинний (фізіологічний) пріонний протеїн (PrP^C), який кодується геном Prnp. Ключовою подією в патогенезі ТСЕ є конформаційне перетворення PrP^C у протеїнорезистентну форму PrP^{Sc}.*

Виявлення клітинного пріона та ідентифікації його ізоформ у тканинах тварин є важливим для наукового розуміння механізмів розповсюдження збудника. Це також необхідно для створення методів діагностики пріонних інфекцій, зокрема губчастоподібної енцефалопатії ВРХ.

***Мета роботи** полягала у визначенні рівня клітинного пріону у мозку, селезінці та тонкому кишечнику лабораторних щурів, мишей та корів.*

***Матеріали і методи досліджень.** Дослідження проводили на самцях білих нелінійних мишей Mus Musculus та лабораторних щурів Rattus norvegicus var. alba, лінії Вістар, яких утримували в стандартних умовах віварію. Лабораторних тварин декапітували під ефірним наркозом, були відібрані мозок, селезінка, тонкий кишечник. Також використовували корів чорно-білої молочної породи, після забою яких було відібрано такі ж тканини.*

Проведено вестерн блот аналіз тканин тварин.

***Результати досліджень та їх обговорення.** PrP^C виявлено в різних тканинах та органах великої рогатої худоби, щурів та мишей. Використовуючи вестерн блот аналіз, в тканинах великої рогатої худоби та щурів було встановлено три форми клітинного пріона.*

Серед них диглікозильована форма (35-38 кДа), частково (моно) глікозильована (23-27 кДа) та неглікозильована (19-21 кДа). У тканинах мишей виявили дві форми клітинного пріона (глікозильовану (29 кДа) та неглікозильовану (19кДа)). Співвідношення глікоформ відрізнялося у різних тканинах, проте у тканинах мишей, щурів, а також у селезінці і мозку корів переважала диглікозильована форма, тоді як вміст деглікозильованої був найнижчим. У тонкому кишечнику корів переважав уміст деглікозильованої форми, що може відігравати ключову роль під час пояснення механізмів виникнення і поширення збудника в організмі.

Висновки та перспективи подальших досліджень. *Клітинний пріон синтезується в мозку, селезінці та тонкому кишечнику ВРХ та лабораторних тварин. Це підтверджує залучення цих органів до розвитку пріонопатії та пояснює механізм розповсюдження збудника у разі інфікування.*

Ключові слова: *клітинний пріон, мозок, селезінка, кишечник, тварини.*

СПИСОК ЛІТЕРАТУРИ

1. Prusiner S.B. Genetic and infectious prion diseases / S.B. Prusiner // Arch. Neurol. – 1993. – Vol. 50. – P. 1129–1153.

2. Вербицький П.І. Губчастоподібна енцефалопатія великої рогатої худоби та інші пріонні інфекції / П.І. Вербицький. – К.: Ветінформ, 2005. – 240 с.

3. Westergard L. The cellular prion protein (PrP^C): its physiological function and role in disease / L. Westergard, H. Christensen, D. Harris // Biochim. Biophys. Acta. – 2007. – Vol. 1772. – P. 629–644.

4. Лабораторні методи досліджень у біології, тваринництві та ветеринарній медицині: довідник / В.В. Влізло, Р.С. Федорук. І.Б Ратич та ін.; за ред. В.В. Влізла – Львів: Сполом, 2012. – 764 с.

5. Лакин Г. Ф. Биометрия / Г. Ф. Лакин. – М.: Высш. шк., 1990. – 352 с.

6. Evidence for normal cell-mediated immunity in scrapie-infected mice / D.T. Kingsbury., D.A. Smeltzer, C.J. Gibbs, D.C. Gajdusek // Infect. Immun. – 1981. – Vol. 32. – P. 1176–1180.

7. Aguzzi A. Pathogenesis of prion diseases: current status and future outlook / A. Aguzzi, M. Heikenwalder // Nature Reviews Microbiology. – 2006. – Vol. 4. – P. 765–775.

8. Aguzzi A. Games Played by Rogue Proteins in Prion Disorders and Alzheimer's Disease / A. Aguzzi, H. Christian // Science. – 2003. – V.302. – P. 814–818.

9. Hill A.F. Molecular classication of sporadic Creutzfeldt-Jakob disease / A.F. Hill, S. Joiner, J.D.F. Wadsworth et al // Brain. – 2003. – Vol. 126. – P. 1333–1346.