Natural Infection with Rabies Virus: A Histopathological and Immunohistochemical Study of Human Brains

Firouzeh Farahtaj a, Leila Alizadeh b, Alireza Gholami a, Alireza Tahamtan c, Sadegh Shirian d, Maryam Fazeli a, Amir Sasan Mozaffari Nejad e, Ali Gorji b,f, Hamid Mahmoudzadeh Niknam g, Amir Ghaemi h,*

a Collaborating Center for Reference and Research on Rabies, Pasteur Institute of Iran, Tehran, Iran
b Shefa Neuroscience Research Center, Khatam Alainbia Hospital, Tehran, Iran
c Department of Virology, Golestan University of Medical Sciences, Gorgan, Iran
d Department of Pathology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
e Molecular Research Center, Student Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
f Department of Neurosurgery and Neurology, Westfälische Wilhelms-Universität Münster, Münster, Germany
g Department of Immunology, Pasteur Institute of Iran, Tehran, Iran
h Department of Virology, Pasteur Institute of Iran, Tehran, Iran

ABSTRACT

Objectives: Despite all the efforts and increased knowledge of rabies, the exact mechanisms of infection and mortality from the rabies virus are not well understood. To understand the mechanisms underlying the pathogenicity of rabies virus infection, it is crucial to study the tissue that the rabies virus naturally infects in humans.

Methods: Cerebellum brain tissue from 9 human post mortem cases from Iran, who had been infected with rabies virus, were examined histopathologically and immunohistochemically to evaluate the innate immune responses against the rabies virus.

Results: Histopathological examination revealed inflammation of the infected cerebellum and immunohistochemical analyses showed an increased immunoreactivity of heat shock protein 70, interleukin-6, interleukin-1, tumor necrosis factor-alpha, caspase-3, caspase-9, toll-like receptor3 and toll-like receptor4 in the infected brain tissue.

Conclusion: These results indicated the involvement of innate immunity in rabies infected human brain tissue, which may aggravate the progression of this deadly disease.

©2019 Korea Centers for Disease Control and Prevention. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Rabies virus (RV) is an enveloped bullet-shaped virus belonging to the Rhabdoviridae family, genus Lyssa virus, and is known to be the causative agent in rabies infection in many mammalian species [1]. The widespread dissemination, public-health concern, veterinary implications and economic burdens set rabies as the most important viral zoonosis worldwide [2]. Rabies accounts for over 59,000 deaths annually as reported by the World Health Organization (WHO) and millions more undergo post-exposure prophylaxis [3]. Most of the cases of rabies-infected humans occur in developing countries, where canine rabies remains the main source for human exposure [4]. Despite many attempts toward medical intervention, rabies continues to present as a public health concern worldwide with the highest case fatality rate and the numbers of cases have been increasing, mainly due to the large global rabies reservoirs in both domestic and wildlife animals [5].
RV is transmitted through the bite from a rabid animal and enters the peripheral nervous system via the neuromuscular junctions or via a sensory nerve through nerve spindles where it infects neurons [6]. The virus moves rapidly from one neuron to another, along the spinal cord, up to the central nervous system (CNS) and the salivary glands [7]. In the CNS, dissemination of the virus occurs rapidly accompanied by serious pathological and pathophysiological changes in the brain, such as neuronal necrosis, neuronophagia, satellitosis and perivascular cuffing [8,9]. Patients eventually die of circulatory insufficiency, cardiac arrest as well as respiratory failure [10]. However, the exact mechanisms underlying RV infection in human and the pathogenesis of rabies are not completely understood [11]. To develop an effective therapeutic approach, it is important to fully understand the mechanism by which rabies causes lethal neurological disease.

Due to the neurotropic nature of RV infection, understanding rabies pathogenesis requires knowledge of histopathological and immunohistochemical characteristics of the disease in the CNS [12]. Studies on RV neurovirulence have been mainly conducted on animal models that were infected with laboratory strains with various degrees of pathogenicity [13]. There is often little or no histopathological evidence of neural destruction in animals dying of rabies and the functional changes in RV-infected neurons in vitro are minimal [14]. Additionally, limited studies have investigated the histological relationship of direct infection of RV with immune responses in human [15]. For a precise understanding of the mechanisms underlying the pathogenicity of RV infection, it is crucial to study tissue from infected humans.

The aim of this research was to study RV-infected human brains histopathologically and immunohistochemically in order to investigate innate immunity against rabies infection.

Materials and Methods

This study was conducted on 9 human cases of rabies with brain necropsy. Post mortem samples (formalin-fixed and paraffin-embedded) were supplied by the WHO collaborating center for Reference and Research on Rabies, Pasteur Institute of Iran and this study was performed in accordance with the national experimental guidelines (Ethic no.: et- 91/0201/4549). The cerebellum tissue blocks were stored at -80°C until analysis was performed. All 9 cases had a history of rabid animal exposure and a clinical history of confusion, hydrophobia, agitation and eventually seizures and coma were present in all cases for a period of 30 to 60 days. All 9 rabies’ cases had been previously confirmed by the fluorescent antibody test (FAT), which is the gold standard test in the diagnosis of rabies and is recommended by both the WHO and the World Organization for Animal Health. In the FAT, the presence of specific aggregates of the RV nucleoprotein antigen (N) in brain smears (Negri bodies) is detected by a direct immunofluorescence technique.

All the tissue samples were stored in 10% neutral buffered formalin for 72 hours thenceforth were treated for histological studies. The paraffin blocks were cut into 4-6 µm sections which were mounted on glass slides. The serial sections were stained with hematoxylin and eosin (H&E) for histopathological analyses and immunohistochemical studies. All the samples were examined under a light microscope for histopathological changes including immunohistochemical studies. All the samples were examined under a light microscope for histopathological changes including immunohistochemical studies. All the samples were examined under a light microscope for histopathological changes including immunohistochemical studies.

For immunohistochemical analyses, 3 paraffin embedded sections were selected from each sample. The selected sections were deparaffinized in xylene and rehydrated using alcohol series. Endogenous peroxidase activity was inhibited by incubating the samples in 3% hydrogen peroxide in absolute methanol. Antigen retrieval was performed by heating the tissue sections in 10 mM citrate buffer (pH6.0) for 10 minutes at 95°C.

The cooled sections were washed with phosphate buffered saline (PBS)and incubated at 4°C overnight with primary antibody against a series of markers which included; mouse polyclonal antibody against interleukin-6 [IL-6 (Santa Cruz Biotechnology)] diluted to 1:350, mouse polyclonal antibody against interleukin-1beta [IL-1β (Abcam, Cambridge, United Kingdom)] diluted to 1:400, mouse monoclonal antibody against tumor necrosis factor-alpha [TNF-α (Abcam, Cambridge, United Kingdom)] diluted to 1:400, mouse monoclonal antibody against caspase-3 [Abcam, Cambridge, United Kingdom)] diluted to 1:400, mouse monoclonal antibody against heat shock protein 70 [Hspa70 (Santa Cruz Biotechnology)] diluted to 1:300, mouse monoclonal antibody against caspase-9 [Abcam, Cambridge, United Kingdom)] diluted to 1:50, mouse monoclonal antibody to caspase-9 [Abcam, Cambridge, United Kingdom)] diluted to 1:50, mouse monoclonal antibody against toll-like receptor 3 [TLR3 (Santa Cruz Biotechnology)] diluted to 1:50, and rabbit polyclonal toll-like receptor 4 [TLR4 (Santa Cruz Biotechnology)] diluted to 1:200 in a solution containing 1-5% normal goat serum in 0.3% Triton X-100 and 0.1M PBS (pH 7.4).

The primary antibodies were then removed by washing 3 times with PBS. Sections were then incubated with goat anti-mouse (or goat anti-rabbit for TLR4) and rabbit horseradish peroxidase conjugated secondary antibody (Santa Cruz Biotechnology) diluted to 1:350 and samples were left to incubate for 1hour at room temperature.

The secondary antibody was then removed by washing the slides 3 times with PBS prior to be incubated in 0.5 µL 3-3′-diaminobenzidine (Roche) and 1.5 µL peroxide buffer for 5-10 minutes at room temperature. The sections were
counterstained with hematoxylin and images were taken using a ×40 objective lens (BX71; Olympus, Japan).

Data were presented as mean ± SEM. Statistical significance between the 2 groups was analyzed by independent samples T test, followed by an appropriate post-hoc test to compare differences among all the data. Differences were considered statistically significant when the \( p < 0.05 \).

**Results**

1. RV FAT

Negri bodies were detected by specific fluorescence of bound conjugate in all samples (Figure 1).

2. Histopathological findings

The tissue samples were examined for changes, such as microglial proliferation, perivascular inflammation, neuronophagia and presence of Negri bodies. Astrocytic proliferation and swelling associated with enlarged nuclei were observed in stained sections (Figures 2A and 2B). Perineuronal satellite oligodendroglia surrounded degenerated neurons with condensed chromatin and little cytoplasm (Figure 2C). Neuronal cell bodies were red, angular and shrunken. Furthermore, their nuclei were contracted and dense. The necrotic neuron cell bodies were surrounded by macrophages. Perivascular cuffing associated with neuronal degeneration was also prominent (Figures 2D, 2E and 2F). Eosinophilic and sharply outlined inclusion bodies (Negri bodies) were observed in the cytoplasm of certain nerve cells infected with RV (Figures 2G and H).

3. Immunohistochemical findings

The immuno-reactivity of inflammatory mediators such as IL-6, IL-1β and TNF-α in RV-infected brain tissue (IL-6 = 21.6 ± 1.16, IL-1β = 18.2 ± 0.9 and TNF-α = 24 ± 1.39) were statistically significantly higher \( (p \leq 0.01) \) than those detected in normal brain tissue (IL-6 = 2.5 ± 0.5, IL-1β = 3.4 ± 0.6 and TNF-α = 4.5 ± 1.29). Immunohistochemical examination revealed that Hsp70 immuno-reactivity in RV-infected brain tissue was significantly increased compared to normal brain tissue \( (p \leq 0.01) \). The mean number of Hsp70 reactive cells, per square millimeter, in RV-infected and in normal brain tissue was 20.6 ± 1.45 and 1 ± 0.28, respectively. Moreover, expression levels of caspase-3 and caspase-9 were significantly increased in RV-infected tissue \( (\text{caspase-3} = 21.1 ± 1.4 \text{ and caspase-9} = 30.3 ± 1.09) \) compared to those detected in normal tissue \( (\text{caspase-3} = 1.5 ± 0.5 \text{ and caspase-9} = 2.1 ± 1.8) \) \( (p \leq 0.01 \text{ and } p \leq 0.001, \text{ respectively}) \). Furthermore, expression levels of TLR3 and TLR4 were significantly increased in RV-infected tissue \( (\text{TLR3} = 26.1 ± 1.07 \text{ and TLR4} = 24.1 ± 6) \) compared to those detected in normal brains \( [\text{TLR3} = 3 ± 1.15 \text{ and TLR4} = 6 ± 1.22; p \leq 0.001 \text{ and } p \leq 0.01 \text{ respectively} \) (Figure 3)].

**Discussion**

Although rabies is inevitably a fatal disease and presents a horrifying clinical picture, little is known about the
The exact mechanism of infection and mortality due to rabies-associated encephalitis [12]. The involvement of the inflammatory response in the pathogenesis of the virus is yet to be well characterized [15,16]. Some studies have demonstrated that virus distribution alone cannot explain the pattern of symptoms or the clinical diversity of rabies [15]. Therefore, other mechanisms such as immune responses other than direct viral infection may be involved in CNS pathogenesis [15]. To date, no detailed histopathological and immunohistochemical study on human brain tissue infected with RV has been published. Previous studies have contributed to understanding of the pathogenesis and diagnosis of rabies, which highlights the importance of conducting these types of study in humans. In this current study, histopathological and immunohistochemical examinations of human brain tissue were performed on individuals infected with RV. All the rabies-infected tissue analyzed in this study presented with Negri bodies as the main marker of RV infection, as well as an inflammatory response as evidence. Although the presence of Negri bodies in neurons is a pathologic hallmark of rabies, these inclusion bodies in the infected neurons may also be absent. The main purpose of this study was to assess the distribution of rabies viral antigen and inflammatory changes within the human brain.

The results in this study showed that astrocyte nuclei are somewhat enlarged and appear more numerous than expected. A long-held belief is that when neurons are injured or some kind of perturbation of the perineuronal microenvironment is present, oligodendroglia around the neurons hypertrophy and proliferate in a process referred to as satellitosis [17]. Microscopic lesions of the CNS including perivascular cuffing with lymphocytes, macrophages and plasma cells, are typically lymphomonocytic (non-suppurative), such as microgliosis which is sometimes prominent and variable but often not severe neuronal degeneration and ganglioneuritis. These findings support the notion that RV could induce inflammation in the CNS and is usually involved in the infection. It is hypothesized that the induction of inflammatory responses, on one hand can lead to the clearance of RV from the CNS when the virus dose is low. However, extensive inflammation in the CNS can result in disease and death when large fixed doses of virus are administered to infect mice [18]. Nevertheless, there are limited data as to whether RV could induce an inflammatory response in the CNS [16]. Consistent with pathological evidence of inflammation in the CNS, immunohistochemical examination showed an increased immuno-reactivity of IL-1β, TNF-α, IL-6, TLR3, and TLR4. Upregulation of these combined factors may play an important role in coordinating the dramatic inflammatory responses associated with rabies-encephalopathy [19]. These factors can modify the hippocampus and other limbic-system functions, including electrical cortical activity, hypothalamic-pituitary-adrenal axis and serotonin metabolism [20]. These factors may also trigger astrocytosis and inflammation of the brain in the CNS [21]. Uproregulation of IL-1β and TNF-α, are two important pro-inflammatory cytokines that have been shown to be associated with meningitis and damage to the blood-brain barrier [22]. These factors also prime macrophages and microglial cells to release reactive nitrogen intermediates, which are associated with CNS inflammation and the severity of rabies disease [23]. Solanki et al who examined 25 brains of patients with either furious or paralytic rabies, showed no correlation between inflammation or viral antigen distribution, or expression of IL-1β and TNF-α (in microglia, macrophages and lymphocytes)
Increased modulation of microglia/macrophages and lymphocyte infiltration in the brain has been suggested to play a role in the treatment of neurological disorders (such as multiple sclerosis, Alzheimer’s disease, and amyotrophic lateral sclerosis) by preventing neuronal damage, improving repair, and eliminating toxic protein [24-26]. In addition, Miao et al [27] described the innate immune response in the brain during rabies infection using wild type (street) and attenuated rabies strains in a mouse model and concluded that either intracerebral or intramuscular infection of mice with street strains, failed to induce both the innate and adaptive immune responses in the CNS, leading to death.

It has previously been demonstrated that human T-cell immunity to RV, where high concentrations of serum IL-6 have been detected, cannot reduce encephalitic rabies, whereas those without this response survive longer and present with paralytic rabies [16].

Hsps are involved in several normal cellular functions (ex. Innate immune responses and apoptosis) and also involved in the replication process of several viruses [28,29]. Over-expression of Hsp70 in this study confirmed the efficiency of RV replication. This protein was shown to be upregulated as early as 4 hours post-infection and has been shown to bind specifically with the RV N protein in the nucleocapsid, resulting in a dramatic increase in P protein synthesis and increased viral production [30].

TLR3, a Type I intracellular transmembrane protein is a negative regulator of axonal growth that has been found in glial cells and neurons in brain disorders, neurodegenerative diseases and viral infections [31,32]. Some studies have shown that TLR3 plays a crucial role in the core of Negri body formation surrounded by a ring of viral N and P proteins [33]. Induction of such factors by RV infection may be involved in the CNS inflammation which consequently could contribute to the subsequent process of pathological changes. Préhaud et al [34] reported that human postmitotic neurons (NT2-N cell line) expressed TLR-3 and could mount an innate immune response that was characterized by the production of IFN, chemokines, and inflammatory cytokines, in response to RV and dsRNA [poly I:C)]. They demonstrated for the first time that human neurons constitutively express TLR-3. It was proposed that viral components other than dsRNA trigger the innate immune response. They also suggested that in the absence of glia, these viral components have the intrinsic machinery to trigger a typical innate immune response, including the 2-step interferon response during RV infection [34].

Apoptosis plays an important biological role in the development and homeostasis of cell populations, as well as in the pathogenesis and expression of many disease processes [35]. Apoptosis has been proposed to be the principal cause of neuronal death [11], limiting the spread of viruses [36-38]. An important regulatory event in the apoptotic process is the activation of caspases, a family of cysteine proteases [39]. Activation of caspase-9 and -3 was detected in this study, suggesting that apoptosis induced by RV was also involved in the activation of a caspase-dependent pathway as observed previously [40].

Conclusion

The results observed in this study through detailed histology of human cerebellum following infection with wild-type street rabies, showed insight into the immunology behind the infection indicating innate immunity may aggravate disease progression. These findings further our understanding of viral pathogenesis, and may aid the development of novel strategies for rabies treatment.

Conflicts of Interest

The authors declare that there were no conflicts of interest associated with this paper.

Acknowledgments

The authors would like to acknowledge the Institute Pasteur of Iran for the financial support. We are indebted to the WHO Collaborating Center for Reference and Research on Rabies, Tehran, Iran for making their human brain tissue available.

References

[1] Jackson AC. Rabies pathogenesis. J Neurovirol 2002;8(4):267-9.
[2] Meltzer ML, Rupprecht CE. A review of the economics of the prevention and control of rabies. Part 2: Rabies in dogs, livestock and wildlife. Pharmacoeconomics 1998;14(5):481-98.
[3] Martinez L. Global infectious disease surveillance. Int J Infect Dis 2000;4(4):222-8.
[4] Fu ZF. Rabies and rabies research: past, present and future. Vaccine 1997;15(Suppl):S20-4.
[5] Hemachudha T, Laframantest J, Rupprecht CE. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. Lancet Neurol 2002;1(2):101-9.
[6] Watson HD, Tignor GH, Smith AL. Entry of rabies virus into the peripheral nerves of mice. J Gen Virol 1981;56(2):371-82.
[7] Tsang H, Lycke E, Ceccaldi P-E, Ermine A, Hiradot X. The antegrade transport of rabies virus in rat sensory dorsal root ganglia neurons. J Gen Virol 1989;70(8):2075-85.
[8] Jamadagni S, Singh C, Sandhu B. Histopathological alterations in brains of rabies infected buffaloes and cattle. Ital J AnimSci 2007;6(suppl 2):872-4.
[9] Bennett JE, Dolin R, Blaser MJ, Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 8th ed. Philadelphia (PA):Saunders; 2014.
[10] Tirawatnpong S, Hemachudha T, Manusuthit S, Shuangshoti S, Phanthumchinda K, Phanuphak P. Regional distribution of rabies viral antigen in central nervous system of human encephalitic and paralytic
rabies. J Neurol Sci 1989;92(1):91-9.
[11] Jamalkandi SA, Mozhgani S-H, Pourbadie HG, et al. Systems Biomedicine of Rabies Delineates the Affected Signaling Pathways. Front Microbiol 2016;7:1688.
[12] Hemachudha T, Ugolini G, Wacharapluesadee S, Sungkarat W, Shuangshoti S, Laethamatas J. Human rabies: neuropathogenesis, diagnosis, and management. Lancet Neurol 2013;12(5):498-513.
[13] Kojima D, Park C-H, Satoh Y, Inoue S, Noguchi A, Oyamad T. Pathology of the spinal cord of C57BL/6J mice infected with rabies virus (CVS-11 strain). J Vet Med Sci 2009;71(3):319-24.
[14] Iwata M, Unno T, Minamoto N, Ohashi H, Komori S. Rabies virus infection prevents the modulation by α 2-adrenoceptors, but not muscarinic receptors, of Ca 2+ channels in NG108-15 cells. Eur J Pharmacol 2000;404(1-2):79-88.
[15] Hooper DC. The role of immune responses in the pathogenesis of rabies. J Neurovirol 2005;11(1):88-92.
[16] Hemachudha T, Phanuphak P, Sriwanthana B, et al. Immunologic study of human encephalitic and paralytic rabies: preliminary report of 16 patients. Am J Med 1988;84(4):673-7.
[17] Wesseling P, Kros JM, Jeuken JW. The pathological diagnosis of diffuse gliomas: towards a smart synthesis of microscopic and molecular information in a multidisciplinary context. Diagnostic Histopathology 2011;17(11):486-94.
[18] Hemachudha T, Wacharapluesadee S, Mitrabhakdi E, et al. Pathophysiology of human paralytic rabies. J Neurovirol 2005;11(1):93-100.
[19] Solanki A, Radatra BD, Vasishtha RK. Correlation of cytokine expression with rabies virus distribution in rabies encephalitis. J Neuroimmunol 2009;217(1-2):85-9.
[20] Hemachudha T, Phuapradit P. Rabies. Curr Opin Neurol 1997;10(3):260-7.
[21] Murphy F, Harrison A, Winn W, Bauer S. Comparative pathogenesis of rabies and rabies-like viruses: infection of the central nervous system and centrifugal spread of virus to peripheral tissues. Lab Invest 1973;29(1):1-16.
[22] Marquette C, Van Dam A-M, Ceccaldi P-E, Weber P, Haour F, Tsiang H. Induction of immunoreactive interleukin-1β and tumor necrosis factor-α in the brains of rabies virus infected rats. J Neuroimmunol 1996;68(1-2):45-51.
[23] Koprowski H, Zheng YM, Heber-Katz E, et al. In vivo expression of inducible nitric oxide synthase in experimentally induced neurologic diseases. Proc Natl Acad Sci 1993;90(7):3024-7.
[24] Kreutzberg GW. Microglia, the first line of defence in brain pathologies. Arzneimittel-Forschung 1995;45(3A):357–60.
[25] Schwartz M, Moalem G, Leibowitz-Amit R, Cohen IR. Innate and adaptive immune responses can be beneficial for CNS repair. Trends Neurosci 1999;22(7):295-9.
[26] Lucin KM, Wyss-Coray T. Immune activation in brain aging and neurodegeneration: too much or too little? Neuron 2009;64(1):110-22.
[27] Miao FM, Zhang SF, Wang SC, Liu Y, Zhang F, Hu RL. Comparison of immune responses to attenuated rabies virus and street virus in mouse brain. Arch Virol 2017;162(1):247-57
[28] Mayer MP. Gymnastics of molecular chaperones. Mol Cell 2010;39(3):321-31.
[29] Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 2005;62(6):670-84.
[30] Lahaye X, Vidy A, Fouquet B, Blondel D. Hsp70 protein positively regulates rabies virus infection. J Virol 2012;86(9):4743-51.
[31] Ghaemi A, Sajadian A, Khodaie B, et al. Immunomodulatory Effect of Toll-Like Receptor-3 Ligand Poly I/C on Cortical Spreading Depression. Mol Neurobiol 2016;53(1):143-54.
[32] Sajadian A, Tabarraei A, Soleimanjahi H, Fotouhi F, Gorji A, Ghaemi A. Comparing the effect of Toll-like receptor agonist adjuvants on the efficiency of a DNA vaccine. Arch Virol 2014;159(8):1951-60.
[33] Menager P, Roux P, Megret F, et al. Toll-like receptor 3 (TLR3) plays a major role in the formation of rabies virus Negri Bodies. PLoS Pathog 2009;5(2):e1000315.
[34] Préhaud C, Mégret F, Lafage M, Lafon M. Virus Infection Switches TLR-3 Positive Human Neurons To Become Strong Producers of Beta-Interferon. J Virol 2005;79(20):12893–904.
[35] Saikumar P, Dong Z, Mikhallov V, Denton M, Weinberg JM, Venkatachalam MA. Apoptosis: definition, mechanisms, and relevance to disease. Am J Med 1999;107(5):489-506.
[36] Gougeon ML. Apoptosis as an HIV strategy to escape immune attack. Nat Rev Immunol 2003;3(5):392-404.
[37] Morimoto K, Hooper DC, Spitsin S, Korpowski H, Dietzschold B. Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression in infected primary neuron cultures. J Virol 1999;73(1):510-8.
[38] Fu ZF, Jackson AC. Neuronal dysfunction and death in rabies virus infection. J Neurovirol 2005;11(1):101-6.
[39] Nicholson DW, Thornberry NA. Caspases: killer proteases. Trends Biochem Sci 1997;22(8):299-306.
[40] Rutherford M, Jackson AC. Neuronal apoptosis in immunodeficient mice infected with the challenge virus standard strain of rabies virus by intracerebral inoculation. J Neurovirol 2004;10(6):409-13.