Auris Nasus Larynx xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Facial paralysis induced by ear inoculation of herpes simplex virus in rat

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ARTICLE INFO

Article history:
Received 22 January 2016
Accepted 1 April 2016
Available online xxx

Keywords:
Bell's palsy
Facial palsy
Disease models
Herpes simplex virus

ABSTRACT

Objective: Bell's palsy is caused by the reactivation of herpes simplex virus type 1 (HSV-1). Using Balb/c mice inoculated with the KOS strain of HSV-1, we previously developed an animal disease model that simulated mild Bell's palsy. The current study developed an animal disease model of more severe facial palsy than that seen in the mouse model.

Methods: Three-week-old female Wister rats weighing 60–80 g were inoculated on the auricle with HSV-1 and acyclovir was administered intraperitoneally to deactivate the infected HSV-1. Instead of HSV-1, phosphate-buffered saline was used for inoculation as a negative control. Quantitative polymerase chain reaction (PCR), behavior testing (blink reflex), electroneuronography, histopathology of the peripheral nerve, and immunohistochemistry of the facial nerve nucleus were evaluated.

Results: Facial palsy occurred 3–5 days after virus inoculation, and the severity of the facial palsy progressed for up to 7 days. Quantitative PCR showed an increase in HSV-1 DNA copies in the facial nerve from 24 to 72 h, suggesting that HSV-1 infection occurred in the nerve. Electroneuronography values were $33.0 \pm 15.3\%$ and $110.0 \pm 18.0\%$ in HSV-1-inoculated and control rats, respectively. The histopathology of the peripheral nerve showed demyelination and loss of the facial nerve, and the facial nerve nucleus showed degeneration.

Conclusion: Facial palsy developed in Wister rats following inoculation of the KOS strain of HSV-1 onto the auricles. The behavioral, histopathological, and electroneuronography data suggested that the severity of facial palsy was greater in our rats than in animals in the previous mouse disease model.

1. Introduction

Bell's palsy, characterized by acute facial nerve paralysis, is the most common cause of peripheral facial palsy, with an annual incidence of 20–30 per 100,000 persons [1]. In 1972, McCormick hypothesized that the herpes simplex virus type 1 (HSV-1) is present in the latent state in the geniculate ganglion, and reactivation of HSV-1 from the geniculate ganglion causes Bell's palsy [2]. Recently, HSV-1 deoxyribonucleic acid (DNA) was detected in clinical specimens, i.e., in endoneural fluid from Bell's palsy patients [3]. The reactivation of latent HSV-1 infection in the geniculate ganglion is considered a major cause of Bell's palsy. Bell's palsy is a non-fatal disease, and 70% of patients regain normal facial function without treatment. About 90% of Bell's palsy patients are treated successfully with

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http://dx.doi.org/10.1016/j.anl.2016.04.002
0385-8146/

steroid and antiviral drugs [4], while approximately 10% of patients develop complete facial paralysis and sequelae, such as synkinesis, contractures, and spasm [5].

Due to the difficulty of obtaining clinical specimens from Bell's palsy patients, basic research using animal models is necessary to develop and investigate novel treatment strategies. Sugita et al. successfully introduced acute and transient facial palsy by inoculating HSV-1 onto the auricles of Balb/c mice [6]. In their animal disease model, HSV-1 infected and spread transaxonally to the descending root, geniculate ganglion and facial nucleus, induced demyelination in the descending root, and caused transient facial palsy [7]. This model is useful for elucidating the mechanism underlying the disease [7,8].

However, these animal disease models simulated mild Bell's palsy. Kumagami et al. induced facial palsy by injecting HSV-1 into the facial nerve at the stylomastoid foramen of rabbits [9]. Their model produced complete facial palsy; however, direct virus injection into the nerve is an unusual infection route and probably caused traumatic damage. No animal disease models of severe/complete Bell's palsy produced by inoculating HSV-1 onto the skin or mucosa, which is the initial infection site in humans, have been reported. In addition, the model mice are too small for surgical experiments, such as facial nerve decompression, which represents the optional treatment for complete Bell's palsy. Therefore, models using larger animals are needed.

Our ultimate goals are to develop an animal disease model of severe/complete Bell's palsy and new treatments using the animal disease model. In this study, we induced acute facial palsy in a rat disease model using HSV-1, and assessed functional and histopathological outcomes.

2. Materials and methods

2.1. Virus inoculation and age of rats

The KOS strain of HSV-1 was used at a titer of 1.0×10^6 plaque-forming units (PFU) per mL. Wister rats were inoculated with HSV-1. The procedure was performed under general anesthesia with 3% halothane. After scratching the surface of the auricle with a 27-gauge needle, 0.1 mL of HSV-1 solution was placed on the auricle. To assess the influence of age, we inoculated HSV-1 into 2- to 6-week-old female rats. Instead of HSV-1, phosphate-buffered saline (PBS) was used for inoculation as a negative control.

2.2. Effect of acyclovir on HSV-1 infection

To evaluate the influence of acyclovir, acyclovir (100 mg/kg/body) was administered intraperitoneally to 3-week-old rats inoculated with HSV-1. The acyclovir administration was started 24, 48, or 72 h after HSV-1 inoculation and administrated for 5 days. The incidence of facial palsy was evaluated.

2.3. HSV-1 induced facial palsy in rats

Three-week-old female Wister rats weighing 60–80 g inoculated with HSV-1 were evaluated using quantitative PCR, blink reflex, histopathology, electromyography, and immunohistochemistry. To deactivate HSV-1, acyclovir (100 mg/kg/body) was administered intraperitoneally on days 3–7 after virus inoculation.

2.4. Blink reflex

To evaluate facial nerve function, the blink reflex was assessed 1, 3, 5, and 7 days after virus inoculation. The blink reflex was evoked twice by blowing air onto the eye through a Frazier aspirator at 1 L/min. The 'ratio of optic fissure' was calculated as follows: [1 − (vertical-axis (distance between the upper and lower eyelids) at eye closure/vertical-axis at eye opening)] × 100 (%).

2.5. Quantitative polymerase chain reaction

Quantitative PCR methods were used to determine the DNA copies of HSV-1. Intratemporal portions of the facial nerve were dissected 24, 48, and 72 h after virus inoculation. The facial nerve was cut with a pair of scissors and placed in a sample tube containing 1.0 mL of PBS. After three freeze–thaw cycles, DNA was extracted from 0.1 mL of the sample supernatant using a DNA Purification Kit (TV8-100FRT; Sacace Biotechnologies, Como, Italy). Then, 10 μL of extracted DNA was used for quantitative real-time PCR of HSV-1.

2.6. Histopathology of the peripheral nerve

On day 5 after inoculation, extratemporal portions of the facial nerve were collected carefully and preserved in 3% glutaraldehyde. These specimens were postfixed with 2% osmium and 2% uranyl acetate, dehydrated, and embedded in epoxy resin. The facial nerves were cut into 70-μm slices using a micro-slicer and were observed under a light microscope (Nikon Optiphot; Nikon, Tokyo, Japan).

2.7. Immunohistochemistry of facial nerve nuclei

On day 5 after inoculation, the brain was collected to assess the facial nerve nuclei. Immunofluorescence staining of brain sections was performed as described elsewhere [10]. Briefly, rat brain was fixed with a 10-min transcardial perfusion of fixative containing 4% paraformaldehyde and 0.2% glutaraldehyde in PBS.

The dissected brains were immersed in 15% sucrose in PBS at 4 °C overnight, rapidly frozen in dry ice powder, and sliced into 10-μm-thick coronal sections at the level of the facial nerve nucleus. The brain sections were incubated with the primary antibodies followed by incubation with DyLight 488- and DyLight 649-labeled secondary antibodies. NeuN, which is a marker for neurons, was labeled with DyLight 488 to label the facial nucleus. CD11b, which is a marker of microglia and is also expressed by leukocytes, including monocytes, neutrophils, natural killer cells, and granulocytes, was labeled with DyLight 649. Hoechst 33258 (Sigma–Aldrich, St. Louis, MO, USA) was used for nuclear staining. The immunostained specimens were observed using a Nikon A1 confocal laser scanning microscope (CLSM; Nikon).

Please cite this article in press as: Fujiwara T, et al. Facial paralysis induced by ear inoculation of herpes simplex virus in rat. Auris Nasus Larynx (2016), http://dx.doi.org/10.1016/j.anl.2016.04.002