Herpes simplex virus types 1 and 2 infect the mouse pituitary gland and induce apoptotic cell death

Brief Report

K. Aita and J. Shiga
Department of Pathology, Teikyo University School of Medicine, Tokyo, Japan

Received March 31, 2004; accepted July 2, 2004
Published online August 30, 2004 © Springer-Verlag 2004

Summary. Herpes simplex virus (HSV) infected the anterior lobe of the pituitary gland resulting in cytopathic changes following intravenous (i.v.) inoculation of male mice. Both HSV type 1 (HSV-1) and type 2 (HSV-2) were isolated from pituitary gland following i.v. infection, but not after intraperitoneal inoculation. HSV-infected pituitary cells were microscopically visible beginning at 24 h or 48 h following i.v. inoculation and were localized in the anterior pituitary. In both HSV-1 and -2 infections the pituitary lesions were apoptotic, as determined by light and electron microscopy, TUNEL, and DNA gel electrophoresis. However, the pituitary infection does not appear to be life-threatening since pituitary lesions were also observed following i.v. infection with HSV-1 strain −GC which possesses low virulence. These results suggest that the pituitary gland is one of the target organs of HSV infection.

The natural cycle of herpes simplex virus (HSV) infection involves reactivation of latent infection. However, in acute systemic infection of human newborns, both HSV type 1 (HSV-1) and type 2 (HSV-2) generally spread to the liver and adrenal glands by viremia [11]. Likewise, in experiments of acute HSV infection of mice, the adrenal gland becomes infected with HSV, regardless of the route of inoculation [10, 15, 20]. The authors of these reports speculated that local immune suppression due to the presence of glucocorticoids makes the adrenal cortex highly susceptible to HSV infection. Thus, a relation between hormones and immune function has been suggested. For instance, it is well established that the hypothalamic-pituitary-adrenocortical (HPA) axis acts as a feedback mechanism for the immune system [16], and Noisakran et al. [14] reported that activation of the HPA axis plays an...
important role in stress-induced reactivation of latent HSV-1. In the present study, we found that HSV infected the pituitary gland and that HSV-infected pituitary cells subsequently underwent apoptosis. Only one report has described pituitary infection with HSV in human newborns [9], and the present study is the first report detailing this type of infection in an animal model. Here we describe in detail the histopathological features of pituitary lesions infected with HSV-1 and -2.

Three viral strains were used in this study. HSV-1 strains Miyama +GC and −GC [12] were supplied by Professor K. Kumagai (Tohoku University School of Dentistry, Sendai, Japan) and HSV-2 strain 186 was a gift from Professor Y. Nishiyama [13]. In all assays, both the intravenous (i.v.) and intraperitoneal (i.p.) inoculation doses given were as follows: 5 × 10^5 plaque forming units (pfu) per mouse for HSV-1 strains +GC and −GC, and 1 × 10^6 pfu per mouse for HSV-2 strain 186. Incidentally, it has been shown that both +GC and 186 are highly virulent strains while −GC is a nonlethal strain [13, 19]. Male C3H/HeN mice were around 8 to 10 weeks old when used in the experiments. The handling and treatment of the experimental animals was performed according to the Guideline for Animal Experimentation at Teikyo University School of Medicine.

The plaque assay for virus isolation and titration was performed as previously described [6] with modifications. Briefly, mice were sacrificed at the indicated times (2 or 3 mice at each time point) following virus inoculation (i.v. or i.p.). Pituitary glands were removed, and to each was added 200 μl PBS before being homogenized aseptically with a glass homogenizer. The supernatants were collected and diluted serially with RPMI 1640 medium. GMK cells were placed in 24-well plates and grown as monolayers. A 50 μl aliquot of the diluted tissue suspension was incubated with the GMK cells for 1 h at 37 °C. Cells were then incubated in RPMI 1640 containing 2% FBS and 5% methylcellulose for 48 h at 37 °C. The cells were then stained with 1% crystal violet in methyl alcohol. The appearance of plaques was checked and a mouse was determined as a virus-isolated mouse when a plaque was observed. Furthermore, viral growth (log(10) pfu/ml) in the pituitary was estimated by counting the number of plaques. The sensitivity level of the assay was more than 100 pfu/ml.

Histopathological investigations were performed in an effort to characterize the pituitary lesion infected with HSV. Mice were sacrificed at 14, 24, 48, 72 and 96 h (2 to 3 mice at each time point) following i.v. or i.p. inoculation of HSV-1 or -2. For the investigation of samples by light microscopy, tissues were fixed in 10% buffered formaldehyde and embedded in paraffin. Sections (3.5 μm) were stained with hematoxylin and eosin (H.E.) according to routine methods. Immunohistochemistry was performed on paraffin-embedded tissue sections (3.5 μm) by a previously described procedure [2]. Briefly, HSV antigen was detected with rabbit anti-HSV-1 polyclonal antibody (diluted 1:100; DakoCytomation) or rabbit anti-HSV-2 polyclonal antibody (DakoCytomation), and immune complexes were visualized using a peroxidase reaction.

Three different analyses (TUNEL, electron microscopy and DNA gel electrophoresis) were performed in an effort to verify apoptosis of infected pituitary cells. The in situ detection of apoptosis was performed on paraffin-embedded