ELSEVIER

Antiviral Research 28 (1995) 291–302

Antiviral Research

Hydrocephalus induction in mice infected with herpes simplex virus type 2 after antiviral treatment

Raymond F. Schinazi [a,b,*], Xuejun Yao [a,b]

[a] *Georgia VA Research Center for AIDS and HIV Infections, Veterans Affairs Medical Center, Decatur, GA 30033, USA*

[b] *Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA*

Received 21 December 1994; accepted 3 July 1995

Abstract

By using antiviral chemotherapy to moderate the lethal effect of wild-type herpes simplex virus type 2 (HSV-2), a new mouse model for herpes simplex virus (HSV)-induced hydrocephalus was developed. Groups of BALB/c mice were infected either intracerebrally (i.c.) or intraperitoneally (i.p.) with a lethal dose of HSV-2. The antiviral agent 2′-fluoro-5-methylarabinosyluracil (FMAU) was administered i.p. 2 days after virus inoculation. By day 21, 80 and 71.4% of the mice infected i.c. or i.p., respectively, survived. The surviving animals were randomly subdivided into different groups and some were challenged i.c. or i.p. with a lethal or superlethal dose of homologous virus. The mice were sacrificed at 2 or 3 months after the initial virus infection. Neuropathological changes of the brains were assessed. Dilation of lateral and third ventricles was noted in the animals initially inoculated i.c., especially in all the animals inoculated i.c. and challenged i.c. with a superlethal virus inoculum, but not in those inoculated i.p. Microscopic examination of hydrocephalic brains revealed evidence of viral meningoencephalitis. Two different mechanisms of ventricular enlargement in this animal model are proposed. This model is relevant since HSV-induced cases of hydrocephalus have been reported to occur in humans and in particular neonates. Issues of virus persistence and expression, long-term evaluation for disease progression, and intervention strategies could be examined with this model.

Keywords: Hydrocephalus; Herpes simplex virus; Antiviral agent; Animal model

* Corresponding author at: Veterans Affairs Medical Center, Medical Research 151, 1670 Clairmont Road, Decatur, GA 30033, USA. Fax: +1 (404) 7287726.

SSDI 0166-3542(95)00052-6

1. Introduction

Our group previously reported that herpes simplex viruses (HSV)-induced retinitis and cataracts could occur in mice surviving intracerebral infection and treated with different antivirals (Schinazi et al., 1984, 1985a). To further assess the secondary effects of intracerebral infections after antiviral treatment, the incidence of hydrocephalus in drug-treated mice surviving infection with HSV type 2 (HSV-2) was examined.

Herpes simplex viruses are now recognized as a leading cause of sporadic encephalitis. The fatality rate in patients is high and many survivors after treatment are left with permanent neuropsychiatric deficits including hydrocephalus (Whitley et al., 1984; Hattori et al. 1993; Soo et al., 1993). More recently, Mansour et al. (1994) reported clinical evidence of retinitis and hydrocephalus in a case of a newborn infected with HSV. In that case, a prenatal diagnosis of hydrocephalus was reported. Hayashi et al. (1986) reported on the development of hydrocephalus in mice using an isolate of attenuated HSV type 1 (HSV-1). Considering the use of attenuated virus may not be clinically relevant and the majority of HSV infections resulting in encephalitis and hydrocephalus in neonates are caused by HSV-2 rather than HSV-1 (Malm et al., 1991), the ability of the wild strain of HSV-2 to cause hydrocephalus was examined. Because HSV-2 strains are usually more neurovirulent than HSV-1 in mice, the virus-inoculated mice were treated with the antiviral drug 2′-fluoro-5-methylarabinosyluracil (FMAU). In previous studies conducted in parallel with the clinically useful antiviral agents acyclovir or vidarabine, FMAU proved to be one of the most potent antiviral agents for the treatment of HSV-2 encephalitis in mice, and non-toxic in mice infected intraperitoneally (i.p.) or intracerebrally (i.c.) (Schinazi et al., 1983, 1986). The incidence and severity of hydrocephalus in surviving animals before and after virus challenge were subsequently assessed. This animal model could have merits for assessing the secondary effects of HSV-2 infections after antiviral treatment, for evaluating novel antiviral agents and their ability to prevent hydrocephalus, and to study the detailed pathogenesis of HSV-induced hydrocephalus in mice.

2. Materials and methods

2.1. Virus

The HSV-2 prototype strain G was supplied by B. Roizman (University of Chicago, IL) (Ejercito et al., 1968). The virus was plaque-purified in mycoplasma-free Vero cells and high-titer pools were prepared in Hep-2 cells as previously described (Schinazi et al., 1982).

2.2. Antiviral drug

The antiviral compound FMAU was provided by J.J. Fox (Memorial Sloan Kettering Cancer Center, New York, NY) and its antiviral effect against HSV-2 strain G was

Table 1
Effect of FMAU on mortality rate in mice inoculated with HSV-2 (strain G)

Group	Virus inoculation		Treatment	No. dead/no. treated [a] (% mortality)	Mean survival day Mean ± S.D. [a]
	PFU/ml	Route			
A	30/0.02	i.c.	FMAU [c]	14/70 (20) [d]	9.9 ± 3.5 [d]
B	1500/0.5	i.p.	FMAU [c]	8/28 (28.6) [d]	19.0 ± 1.9 [d]
C	30/0.02	i.c.	None	18/20 (90)	6.7 ± 2.5
D	1500/0.5	i.p.	None	10/10 (100)	8.2 ± 0.8
E	PBS/0.02 [b]	i.c.	None	0/5 (0)	
F	None	–	FMAU [b,c]	0/5 (0)	

[a] Calculated on day 21.
[b] Mock-infected control.
[c] Administered 10 mg/kg i.p. once daily for 4 days, 48 h after virus inoculation or after the experiment was initiated (for group F).
[d] $P < 0.05$, compared to corresponding untreated control.

confirmed by using plaque and yield reduction assays in Vero cells and in a mouse model described previously (Schinazi et al., 1982).

2.3. Animals and experimental protocol

After acclimatization for 2 weeks, 6-week-old BALB/c female mice (Harlan-Sprague Co., Indianapolis, IN) were divided into 6 groups (Table 1). Two groups (A and B) of 70 and 28 mice were infected with lethal dose of HSV-2, under anesthesia of Metofane (Pitman-Moore Co., Washington Crossing, NJ), either i.c. into the frontal lobe of right cerebral hemisphere (30 PFU/0.02 ml) (Schinazi et al., 1983) or i.p. (1.5×10^3 PFU/0.5 ml). Beginning 2 days later, FMAU, at a non-toxic dose, was administered i.p. (10 mg/kg, in 0.5 ml PBS per dose, once daily for 4 days) to moderate the lethal effects of the virus. Two groups (C and D) of 20 and 10 mice were virus-inoculated either i.c. or i.p., but were not treated with the antiviral drug. In addition, two groups (E and F) of 5 mice each were mock infected i.c. with PBS or treated i.p. with FMAU and served as controls for the potential trauma caused by the i.c. inoculation or the induction of hydrocephalus by the antiviral drug. After virus inoculation, all mice were monitored twice daily for 21 days for untoward effects (ruffled fur, weight loss, or neurological dysfunction) or death. By day 21, all animals died in the i.p. inoculated untreated group (D) and only 2 out of 20 animals survived in the i.c. inoculated untreated group (C). In contrast, 80 and 71.4% of the mice treated with FMAU survived the i.c. or i.p. virus inoculation, respectively (groups A and B).

In order to evaluate the effect of different doses and routes of virus challenge on the induction of hydrocephalus, the surviving animals in groups A and B were randomly subdivided into 7 groups 1 month after initial virus inoculation (Table 2). They were then challenged either i.c. (groups A1, A2, and B1) or i.p. (groups A3 and B2) with a lethal (30 PFU/0.02 ml for i.c. and 1.5×10^3 PFU/0.5 ml for i.p.) or superlethal dose (3×10^4 PFU/0.02 ml for i.c.) of homologous virus. Repeated virus i.c. inoculation had previously been shown to increase the incidence of hydrocephalus (Hayashi et al., 1986).

Table 2
Incidence of hydrocephalus after HSV-2 inoculation or challenge in BALB/c mice

Group	Treatment [a]		No. of mice challenged	No. with hydrocephalus/ no. survivor (%)
	Original	Challenge		
A1	VIC + FMAU	SVIC	10	10/10 (100) [b]
A2	VIC + FMAU	VIC	17	9/16 (56)
A3	VIC + FMAU	VIP	9	2/9 (22)
A4	VIC + FMAU	PBS	15	6/15 (40)
B1	VIP + FMAU	VIC	5	0/3 (0)
B2	VIP + FMAU	VIP	4	0/4 (0)
B3	VIP + FMAU	PBS	6	0/5 (0)
C	VIC			1/2 (50)
D	VIP			0/0 (0)
E	PBS			0/5 (0)
F	FMAU			0/5 (0)

[a] VIC, intracerebral inoculation with a lethal dose of HSV-2 (30 PFU/0.02 ml); SVIC, i.c. inoculation with superlethal dose of virus (3×10^4 PFU/0.02 ml); VIP, i.p. inoculation of virus (1.5×10^3 PFU/0.5 ml).
[b] $P < 0.05$, comparing the incidence of hydrocephalus in group A1 to group A2; $P < 0.01$, comparing A1 to A3 or A4.

The remaining animals were mock-challenged with PBS (groups A4 and B3). The animals were continuously monitored for an additional 1–2 months until sacrifice, when their brains were removed for macroscopic and histopathological examination.

2.4. Gross and microscopic analysis

The 74 mice surviving virus inoculation and challenge, or mock controls, were bled under anesthesia retro-orbitally and then sacrificed as specified by the Animal Welfare Act. Brains (some of them with calvaria) were fixed in PBS-buffered 10% formalin and embedded in paraffin. Before embedding, each sample was incised coronally passing through the lateral and third ventricles to observe macroscopic evidence of hydrocephalus. Coronal sections of whole brain of 5 μm were cut in series at levels passing the lateral and third ventricles, cerebral aqueduct, and fourth ventricles. They were then stained with hematoxylin–eosin (H and E), or with luxol fast blue to demonstrate myelin (Kluver and Barrera, 1953). The hydrocephalus in the gross brains was graded in a masked fashion as follows: 0, no apparent ventricular dilation; 1 +, dilation of unilateral ventricle; 2 +, dilation of bilateral lateral ventricles; and 3 +, marked dilation of bilateral ventricle accompanying with obvious rarefaction of adjacent tissue and/or the third ventricular dilation.

2.5. HSV antibody determination

Before sacrifice, all the mice were bled under Metofane anesthesia retro-orbitally with microhematocrit capillary tubes (Oxford Labware, St. Louis, MO). Several animals were bled additionally before virus challenge. Sera were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of anti-HSV antibody to confirm virus

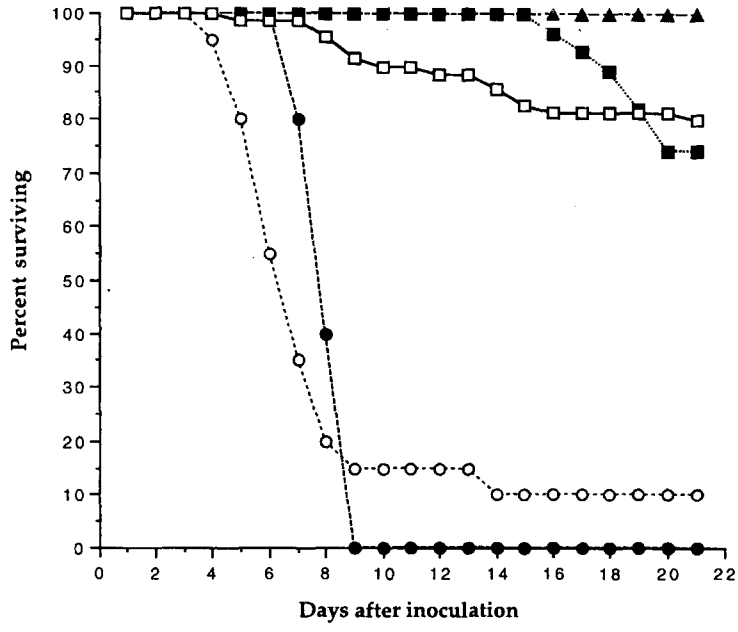


Fig. 1. Survival rate of mice 21 days after virus inoculation. Group A, 70 mice inoculated i.c. with 30 PFU of HSV-2. Two days later, FMAU i.p. treatment was initiated at 10 mg/kg once daily for 4 days (□); group B, 28 mice inoculated i.p. with 1.5×10^3 PFU of HSV-2 and FMAU treated as in group A (■); group C, 20 mice inoculated i.c. with 30 PFU of HSV-2 without FMAU treatment (○); group D, 10 mice inoculated i.p. with 1.5×10^3 PFU of HSV-2 and not treated (●); group E (△) and F (▲), 5 mice each were injected with PBS i.c. or FMAU i.p., respectively, and served as controls.

infection. Absorbencies were measured at 492 nm with a Titertek Multiscan (Flow Laboratories, Malen, VA). Antibody titers were estimated as described previously (Schinazi et al., 1985b).

2.6. Statistical analyses

χ^2- and t-tests (Colton, 1974) were used to compare the differences of mortality rate in mice inoculated with HSV-2, mean survival day, incidence of hydrocephalus, and differences in antibody titers between the various groups.

3. Results

3.1. Clinical observations

Clinical signs began to appear 2–4 days after i.c. or i.p. virus inoculation. The main manifestations were lethargy, anorexia, loss of weight, ruffled fur, and hunched bodies. Paralysis and convulsions were also present in the mice inoculated i.c. As shown in

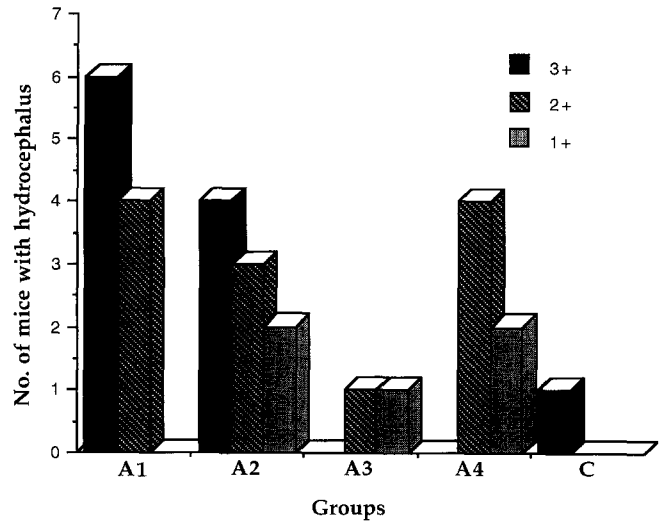


Fig. 2. Incidence and severity of hydrocephalus in various groups. For group number, see Table 2.

Table 1 and Fig. 1, most of the mice with these signs died gradually within 10 days without FMAU treatment (groups C and D) or were humanely sacrificed when moribund. However, in the FMAU-treated animals (groups A and B), most of the mice survived the lethal virus inoculation, even the 1000-fold lethal virus challenge. Only a small proportion of mice showed signs of disease and some died, but their survival time was longer than that of the untreated group (Table 2, group A1). No clinical signs were apparent in the mock-infected mice treated with PBS or FMAU during the period of observation.

3.2. Gross examination and incidence of hydrocephalus

The animals with hydrocephalus (Fig. 2) did not manifest obvious clinical abnormalities. After making a coronal incision of the fixed brains, however, it was easier to observe the degree of ventricular dilation compared with normal brains. In this experiment, the ventricular dilation mainly occurred in the lateral ventricle. Whereas third ventricular dilation was noted in a few of animals, no fourth ventricular dilation was found (Fig. 3a–c). Comparing the left and right sides of the same hydrocephalic brain indicated a more severe dilation on the virus-inoculated side (Fig. 3b, c). As shown in Table 2 and Fig. 2, hydrocephalus of different degrees occurred in animals inoculated initially i.c. (groups A1–A4 and C), regardless of whether or not they were subsequently challenged i.p. or i.c. The incidence and severity of hydrocephalus appeared to be greater in animals inoculated i.c. and challenged i.c. with a 1000-fold lethal dose of virus (group A1). In that group, the incidence of hydrocephalus was 100% ($n = 10$), and 6 out of 10 brains were graded as 3 + . The difference in the incidence of hydrocephalus between groups A2 and A3 was not statistically significant. No hydrocephalus was

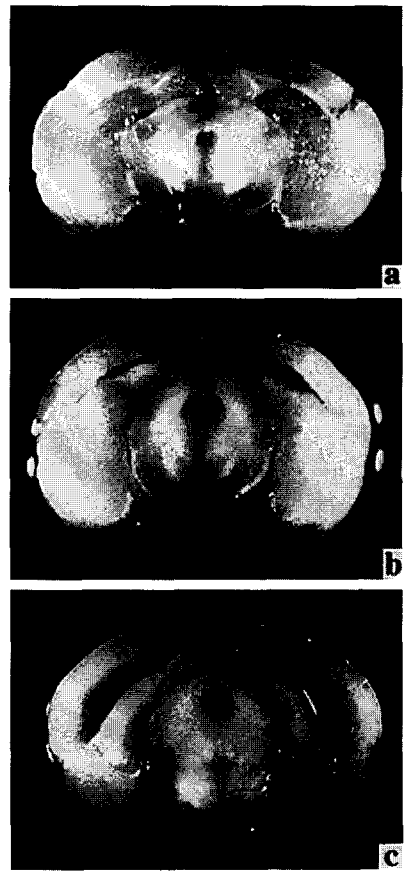
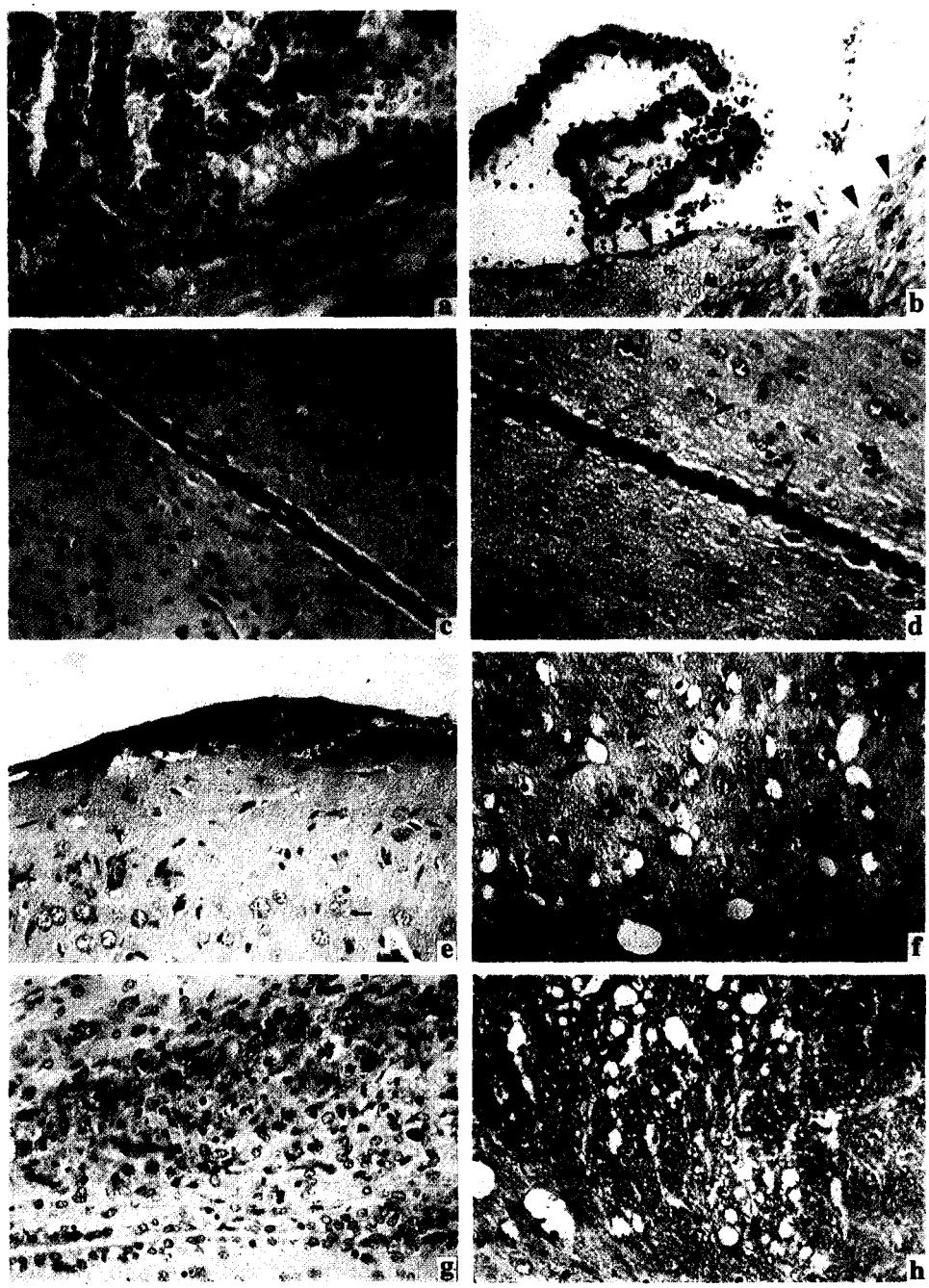


Fig. 3. Coronal sections of brains showing the normal control (a), and the lateral ventricular dilation graded as 2+(b), and 3+(c) of hydrocephalic brains, ×4. A more severe ventricular dilation in the virus-inoculated side (right side in b and c) is apparent.

found in the animals initially inoculated i.p. and challenged i.p. or i.c., and in the mock-infected animals treated with PBS or FMAU.

3.3. Histopathological examination

As mentioned above, the degree of ventricular dilation in hydrocephalic brains was different. Although some differences in the histopathology of the hydrocephalic brains were noted, their general features are described below. The ependymal lining cells on the enlarged lateral ventricular surface were usually flattened and partly or completely denuded (Fig. 4a, b), but in the third ventricles the epithelial cells were uniformly lined. The loops of choroid plexus epithelium within the lateral and third ventricles appeared to be separated because of the ventricular dilation in comparison with controls (Fig. 4a, b). Clusters of red blood cells were often found in the lumen of dilated ventricles (Fig.


a
b
c
d
e
f
g
h

4b). The majority of cerebral aqueducts in hydrocephalic brains did not present histopathologically remarkable changes compared with the normal ones (see Fig. 4d compared to c), but red blood cells could be frequently found inside the narrow aqueduct (Fig. 4d). Only in one animal, in which hydrocephalus was graded 3 +, was the histological structure of the aqueduct prominently destroyed (not illustrated). No obvious morphological changes were found in the fourth ventricles. Similarly, obliteration or fibrosis was not found in the subarachnoid space.

In the vicinity of markedly dilated lateral ventricles, cerebral cortical mantle and corpus callosum were usually rarefied (Fig. 4b). In addition, evidence of viral meningoencephalitis in the hydrocephalic brains was apparent, as suggested by perivascular cuffing with mononuclear cells in the leptomeninges (Fig. 4e), occasional local neuronal vacuolation in the gray matter (Fig. 4f), demyelination in the white matter (Fig. 4h), gliosis (Fig. 4f), and microhemorrhages (Fig. 4g). These changes were relatively widespread or diffusely present through much of the cerebrum and the severity of these lesions seemed to parallel the degree of ventricular dilation. In contrast, no obvious tissue damage, inflammatory response, or ventricular dilation was observed in the control animals.

3.4. Anti-HSV antibodies

The mock-infected animals treated with PBS or FMAU were anti-HSV antibody seronegative as determined by ELISA. All sacrificed mice inoculated i.c. or i.p. with HSV-2, regardless of whether or not they were treated with FMAU, had seroconverted at the end of the experiment. Anti-HSV antibody titers were not significantly different between the animals in various groups (groups A–C) (data not shown). However, the results revealed that the seroconversion had occurred before virus challenge.

4. Discussion

Utilizing delayed antiviral treatment to moderate the lethal effects of the virus on mice inoculated i.c. resulted in 80% survival (Table 1 and Fig. 1); of these animals, 40% developed hydrocephalus (Table 2). However, a 100% incidence of HSV-2-induced

Fig. 4. Coronal paraffin sections of brains stained with H and E (a–g) or luxol fast blue (h), ×200. a: normal pattern of lateral ventricle, as visualized by simple columnar ependymal cells (arrowheads) lining all the ventricular surface, and the choroid plexus (star), consisting of uniform cuboid epithelia threads nearly filling all the ventricular cavity (group B2). b: dilated lateral ventricle with adjacent rarefied corpus callosum (star) showing the flattened ependymal cells (arrow) and partially denuded ventricular surface (arrowheads), as well as separated choroid plexus, and clusters of red blood cells within the ventricle (group A2). c: longitudinal section of the narrow cerebral aqueduct in the normal brain (group B2). d: red blood cells (arrows) in the narrow cerebral aqueduct, although the ependymal cells of the aqueduct are normal (group A2). e: a perivascular mononuclear cell cuffing in the leptomeninges (group A2). f: neuronal vacuolation (arrows), and several rod-shaped microglial nuclei (arrowheads) in the gray matter of the midbrain (group C). g: microhemorrhages marked by hemosiderin-laden macrophages in the cerebral parenchyma of the frontal lobe (group A3). h: demyelination in the white matter of the midbrain (group C).

hydrocephalus was noted after a high dose of virus challenge by the same route (Table 2). This work may help to elucidate the development of hydrocephalus and recurrent HSV encephalitis in humans after effective antiviral treatment. Although there have been several papers reporting the correlation between the intracerebral infection of virus and the development of hydrocephalus (Johnson and Johnson, 1969; Masters et al., 1977; Lagace-Simard et al., 1982; Hayashi et al., 1986; Larsen et al., 1993), our work represents the first report of wild-type HSV-induced hydrocephalus in mice. The most important features of this HSV-2 model are its reproducibility in a high proportion of survivors and that the experimental approach may more faithfully mimic certain CNS diseases seen in human neonatal survivors of disseminated HSV infection than currently available models.

Experiments were performed to determine if i.p. HSV infection could induce hydrocephalus, since neonatal disseminated HSV infection can involve the brain and viscera simultaneously (Nahmias et al., 1989). However, induction of hydrocephalus was not observed, even after i.c. challenge with the same lethal dose of virus 1 month after the initial virus i.p. inoculation. In contrast, the findings revealed that the initial i.c. viral infection played an important role in the development of encephalitis and hydrocephalus. Two reasons could explain the inability of the animals inoculated initially i.p. with a lethal dose of HSV-2 to develop hydrocephalus. First, it is likely that antiviral treatment initiated 48 h after the initial i.p. challenge prevented or moderated virus infection in the brain. It has previously been established that after i.p. infection, virus may take 2–3 days to establish infection in the brain (Schinazi et al., 1986). Second, when challenged, the surviving animals had HSV antibodies which protected them from the pathogenic effects resulting from i.c. virus challenge.

The pathogenesis of ventricular enlargement remains a controversial issue which is not yet fully understood (Miller and Adams, 1992), probably due to the use of different model systems to investigate this issue. For the virus-induced hydrocephalus model described herein, three probable mechanisms of the ventricular enlargement which are *not mutually exclusive* need to be considered: (1) aqueductal stenosis may have occurred resulting in enlargement of the lateral and third ventricles; the aqueduct may be abnormal, whereas the fourth ventricle may be normal in size; (2) obstruction of the subarachnoid space or arachnoid villi damage; in this case, all the ventricles and the aqueduct should be dilated; and (3) a so-called 'ex vacuo' hydrocephalus may have occurred, in which there may be no obstruction of CSF flow and the ventricular dilation is compensatory to loss of brain tissue.

In the HSV-2-induced hydrocephalic samples, ventricle dilation was only found at the lateral and third ventricles, but not at the fourth ventricle, which suggests that the first possibility is a plausible explanation for the mechanism of hydrocephalus induction. However, except for one case of hydrocephalus showing prominent aqueductal destruction, the great majority of aqueducts did not present marked abnormality compared with normal ones. Although red cells were noted in the aqueduct, it is not clear if this resulted in the observed ventricular enlargement. The fourth ventricles were normal in size and no obliteration was found in the subarachnoid space, which would argue against the second possibility. Neurovirological studies in experimental animals have indicated that ependymal cells are susceptible targets for a number of virus infections and suggest that

the destroyed ependymal cells in the early stage of virus inoculation may lead to the subsequent development of hydrocephalus before aqueduct stenosis occurs (Bruni et al., 1985; Marc, 1993; Takano et al., 1993; Takei et al., 1987). However, proof of the correlation between depletion of ependymal cells shown in this experiment and occlusion of CSF pathway in the early stage of HSV infection cannot be ascertained until kinetic histopathological and immunocytochemical studies are performed. In view of the finding that the degree of parenchymal destruction in the hydrocephalic brains seemed to parallel the degree of ventricular dilation and that the lateral ventricular dilation was more severe on the side of virus inoculation, the induction of hydrocephalus is clearly related to the viral encephalitis and ependymitis. Therefore, the third possibility should also be considered to be one of the reasons for the induction of hydrocephalus.

It is not clear why the incidence of hydrocephalus increased after i.c. challenge with a superlethal dose of homologous virus, although it could be related to the immunopathological effect of the disease process (Hayashi et al., 1986) as well as to the effects of acute viral inflammation induced by the virus i.c. challenge.

In summary, we demonstrate for the first time the development of hydrocephalus in HSV-2-infected mice after treatment with a potent antiviral compound. If it is presumed that the third proposed mechanism of ventricular enlargement is associated with CNS disease in human neonates surviving HSV infection, it would be useful to have this mouse model of HSV-2-induced diffuse CNS disease to develop intervention strategies to prevent hydrocephalus, and to study the detailed pathogenesis of HSV-induced hydrocephalus. It is of interest that despite the high potency of drugs such as FMAU, a significant number of animals developed hydrocephalus. These results emphasize the importance for early intervention with antiviral treatment in humans with herpetic encephalitis.

Acknowledgements

We thank R. Taylor Scott for excellent technical assistance. This work was supported in part by the Department of Veterans Affairs and by the Georgia VA Research Center for AIDS and HIV Infections.

References

Bruni, J.E., Dolbigio, M.R. and Clattonburg, R.E. (1985) Ependyma: normal and pathological. A review of the literature. Brain Res. Rev. 9, 1–19.

Colton, T. (1974) Statistics in Medicine. Little, Brown and Company, Boston, pp. 136–142 and 174–176.

Ejercito, P.M., Kieff, E.D. and Roizman, B. (1968) Characterization of herpes simplex virus strains differing in their effect on social behavior of infected cells. J. Gen. Virol. 2, 357–364.

Hattori, S., Isogai, Y. and Morimoto, Y. (1993) Double meningoencephalitis with herpes simplex virus and rubella virus. Rinsho Shinkeigaku 33, 642–645.

Hayashi, K., Iwasaki, Y. and Yanagi, K. (1986) Herpes simplex virus type 1-induced hydrocephalus in mice. J. Virol. 57, 942–951.

Johnson, R.T. and Johnson, K.P. (1969) Hydrocephalus as sequelae of experimental myxovirus infection. Exp. Mol. Pathol. 10, 68–80.

Kluver, H. and Barrera, E. (1953) A method for the combined staining of cells and fibers in the nervous system. J. Neuropathol. Exp. Neurol. 12, 400–404.

Lagace-Simard, J., Descoteaux, J.-P. and Lussier, G. (1982) Experimental pneumovirus infections: 2. Hydrocephalus of hamsters and mice due to infection with human respiratory syncytial virus (RS). Am. J. Pathol. 107, 36–40.

Larsen, P.D., Chartrand, S.A., Tomashek, K.M., Hauser, L.G. and Ksiazek, T.G. (1993) Hydrocephalus complicating lymphocytic choriomeningitis virus infection. Pediatr. Infect. Dis. J. 12, 528–531.

Malm, G., Forsgren, M., el-Azazi, M. and Persson, A. (1991) A follow-up study of children with neonatal herpes simplex virus infections with particular regard to late nervous disturbances. Acta Paediatr. Scand. 80, 226–234.

Mansour, A.M. and Nichols, M.M. (1994) Congenital diffuse necrotizing herpetic retinitis. Graefes Arch. Clin. Exp. Ophthalmol. 231, 95–98.

Marc, R.D.B. (1993) Neuropathological changes caused by hydrocephalus. Acta Neuropathol. 85, 573–585.

Masters, C., Alpers, M. and Kakulas, B. (1977) Pathogenesis of reovirus type 1 hydrocephalus in mice. Arch. Neurol. 34, 18–28.

Miller, J.D. and Adams, J.H. (1992) The pathophysiology of raised intracranial pressure. In: J.H. Adams (Ed.), Greenfield's Neuropathology, 5th edn., Oxford Univ. Press, New York, pp. 69–105.

Nahmias, A.J., Keyserling, H. and Lee, F. (1989) Herpes simplex viruses 1 and 2. In: A.S. Evans (Ed.), Virus Infection of Humans: Epidemiology and Control, 3rd edn., Plenum Medical Book Co., New York, pp. 393–413.

Schinazi, R.F., Peters, J., Williams, C.C., Chance, D. and Nahmias, A.J. (1982) Effect of combinations of acyclovir with vidarabine or its 5′-monophosphate on herpes simplex viruses in cell culture and in mice. Antimicrob. Agents Chemother. 22, 499–507.

Schinazi, R.F., Peters, J., Sokol, M.K. and Nahmias, A.J. (1983) Therapeutic activity of 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine and -thymine alone and in combination with acyclovir and vidarabine in mice infected intracerebrally with herpes simplex virus. Antimicrob. Agents Chemother. 24, 95–103.

Schinazi, R.F., Gammon, J.A., Stulting, R.D., Kuck, J.R.F., Wright, J.D. and Nahmias, A.J. (1984) Evaluation of antivirals in preventing herpes simplex virus (HSV)-induced cataracts in a murine model. Invest. Ophthalmol. Vis. Sci. Suppl. 25, 23.

Schinazi, R.F., Scott, R.T., Gammon, J.A., Stulting, R.D., Kuck, J.R.F., Wright, J.D. and Nahmias, A.J. (1985a) Evaluation of antivirals in preventing herpes simplex virus (HSV) induced cataracts in a murine model. In: R. Kono and A. Nakajima (Eds.), Herpes Viruses and Virus Chemotherapy. Elsevier, Amsterdam, pp. 245–257.

Schinazi, R.F., Scott, R.T., Peters, J., Rice, V. and Nahmias, A.J. (1985b) Antiviral activity of 5-ethyl-2′-deoxyuridine against herpes simplex viruses in cell culture, mice, and guinea pigs. Antimicrob. Agents Chemother. 28, 552–560.

Schinazi, R.F., Fox, J.J., Watanabe, K.A. and Nahmias, A.J. (1986) Activities of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine and its metabolites against herpes simplex virus type 1 and 2 in cell culture and in mice infected intracerebrally with herpes simplex virus type 2. Antimicrob. Agents Chemother. 29, 77–84.

Soo, M.S., Tien, R.D., Gray, L., Andrews, P.I. and Friedman, H. (1993) Mesenrhombencephalitis: MR findings in nine patients. Am. J. Roentgenol. 160, 1089–1093.

Takano, T., Mekata, Y., Yamano, T. and Shimada, M. (1993) Early ependymal changes in experimental hydrocephalus after mumps virus inoculation in hamsters. Acta Neuropathol. 85, 521–525.

Takei, F., Hirano, K.S. and Kohn, I.J. (1987) New ultrastructural changes of ependyma in experimental hydrocephalus. Acta Neuropathol. 73, 400–402.

Whitley, R.J. (1984) Herpes simplex virus infections of the central nervous system. In: F. Rapp (Ed.), Herpesvirus, Proceedings of Burroughs Wellcome-UCLA Symposium. Alan R. Liss Inc., New York, pp. 71–90.