An acyclovir-resistant strain of herpes simplex virus type 2 
which is highly virulent for mice

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Summary. Herpes simplex virus type2 (HSV-2), strain YS-4 C-1, isolated by 
plaque cloning from a clinical isolate was found to be resistant to acyclovir 
(ACV; acycloguanosine) in vitro. It was sensitive to phosphonoacetic acid and 
9-β-D-arabinofuranosyladenine. Thymidine kinase (TK) activity of YS-4 C-1 
was less than 1% of that of other strains from the same clinical source. However, 
thymidine plaque autoradiography showed that YS-4 C-1 was not completely 
deficient in TK activity. YS-4 C-1 showed high virulence for mice like other 
HSV-2 strains which were sensitive to ACV. YS-4 C-1 was able to establish 
laten infection in mice. Virus isolated from the brain of a mouse died after 
being inoculated with YS-4 C-1 was also resistant to ACV. ACV was not effective 
in mice inoculated with YS-4 C-1. This study shows that not all ACV-resistant 
strains are avirulent for mice.

Introduction

Acyclovir (ACV; acycloguanosine) is being increasingly used for prophylaxis 
and therapy of herpes simplex virus (HSV) infections. However, recent reports 
have warned that resistant strains may emerge during therapy [1, 2, 6, 11, 20, 
29, 31]. ACV behaves as a nucleoside analog which is selectively phosphorylated 
by HSV-specified thymidine kinase (TK). The monophosphate form of ACV 
is then converted to the triphosphate which is a potent inhibitor of HSV-specified 
DNA polymerase. Three mechanisms of resistance to ACV have been described: 
(i) loss of TK activity, (ii) alteration in TK substrate specificity, and (iii) al-
teration of DNA polymerase [3–5, 8, 11, 14, 17, 20, 24, 29, 30]. Mutants lacking 
TK activity are usually of extremely low virulence [13, 16, 21, 31]. Mutants 
which have altered substrate TK activity show slight attenuation of neurovi-
rulence [7, 8]. The mutants which have altered DNA polymerase also have 
attenuated pathogenicity [12, 23].

We have a series of HSV type2 (HSV-2) strain isolated sequentially from 
recurrent vesicular skin lesions on the face of an infant from 1972 to 1974 [34].
One of these strains, YS-4, was slightly resistant to ACV, and it contained viruses which were highly resistant to ACV. We isolated three clones (YS-4 C-1, YS-4 C-2, YS-4 C-3) through plaque cloning of YS-4. One cloned strain, YS-4 C-1, which was resistant to ACV, was highly virulent for mice, like the ACV-sensitive wild type strains. In this paper, the characteristics of the strain are reported on the basis of TK activity, virulence for mice, sensitivity to ACV in vivo and in vitro and sensitivity to other antitherpes drugs.

Materials and methods

Virus

HSV-2 strains YS-1, YS-2, YS-3, YS-4, and YS-5 are isolates from recurrent vesicular skin lesions on the face of an infant [34]. YS-4 C-1 is a cloned strain previously obtained through plaque cloning of YS-4 in our laboratories [27]. YS-4 C-2 and YS-4 C-3 were also obtained through plaque cloning of YS-4. HSV-2 strain 8620 KN (abbreviated as KN) was isolated from a genital herpetic lesion of an adult female at Kyushu University Hospital, Fukuoka, Japan. KNA-3 and KNA-4 are ACV-resistant mutants obtained by infecting Vero cells with the KN strain in the presence of 5 µg of ACV per ml and cloned through plaque purification for five times.

Cells

Vero cells grown in Eagle’s minimal essential medium supplemented with 5% calf serum were used for virus propagation, titration, and plaque reduction assay.

LTK− cells grown in Dulbecco’s modified Eagle’s minimal essential medium supplemented with 10% fetal bovine serum were used for measuring virus-specified TK activity. The LTK− cells, originally obtained from mouse L cells, are deficient in TK activity and are a gift from Dr. Y. Kino, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan.

Antiviral drugs

ACV was purchased from Nippon Wellcome Co., Ltd., Osaka, Japan. Phosphonoacetic acid (PAA) and 1-β-D-arabinofuranosylthymine (ara-T) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. 9-β-D-arabinofuranosyladenine (ara-A) was purchased from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan.

Plaque-reduction assays

The sensitivity of virus to antitherpes drugs was assessed by plaque-reduction assays using Vero cells. Twelve well plates (Costar 3512, Cambridge, MA, U.S.A.) of Vero cells were inoculated with 150–75 pfu of virus per well. The plates were incubated at 37°C for 60 min to allow virus adsorption, after which 1 ml of medium containing 1% methylcellulose and the required drug concentration was added. Plaques were counted after incubation for two days. The ED50, defined as the dose required for 50% plaque reduction, was obtained from a plot of the reduction in plaque number (relative to the untreated control) against drug concentration.

Restriction endonuclease analysis of viral DNA

Viral DNA was prepared from viral particles obtained after glycerol gradient centrifugation as described by Denniston et al. [10]. The viral DNA was digested with one of five restriction