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ACYCLOVIR RESISTANT GENITAL HERPES VIRUS INFECTION IN A PATIENT WITH AIDS

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Summary. - Three acyclovir resistant strains of HSV-2 were isolated from mucoulcerative lesions in a patient suffering from AIDS in whom the oral and intravenous acyclovir treatment was unsuccessful. All the isolates were classified by monoclonal antibodies and showed no differences in DNA restriction patterns.

Key words: herpes simplex virus; acyclovir resistance; acquired immunodeficiency syndrome

Recent reports warned that resistance may emerge during acyclovir (ACV) therapy in herpes simplex virus infection (McLaren and Corey, 1983).

Acyclovir resistant strains of HSV can be selected *"in vitro"* by increasing the drug concentration (Field and Darby, 1980) and the resistance appears to be associated with a mutation in genes coding for viral thymidine kinase or DNA polymerase (Schniper and Crumpacker, 1980). This paper describes the clinical course of an Acquired Immunodeficiency Syndrome (AIDS) patient who developed extensive ulceration on the penis from which HSV was repeatedly isolated in spite of ACV therapy. The restriction enzyme pattern and the resistance to ACV *in vitro* are desribed.

Samples were obtained from the lesion with a cotton-tipped applicator and inoculated into tubes containing Vero cell monolayers; these were examinated every two days for HSV related cytopathic effect (CPE). Positive cultures were frozen at -70 ^{0}C, propagated in Vero cells, and typed with fluorescein-labelled monoclonal antibodies to HSV-1 and HSV-2 (Biomeriux, France).

Sensitivity of the viruses to ACV was assessed by plaque reduction assays in Vero cells. Confluent monolayers of Vero cells were inoculated with 50 - 100 PFU of HSV, incubated in the presence of different drug concentrations, 2% foetal calf serum and 1% methylcellulose. After three days incubation the cells were fixed and stained with 1% crystal violet, and the plaques were counted. The median inhibitory dose (ID_{50}) of the drug which reduced 50% of plaque number as related to untreated control was determined. Viruses at passage level 2 or 3 were used for determination of drug sensitivity. The reference strains of HSV-1 (622) and HSV-2 (933) were kindly provided by P. Mas (Nationale Institute of Hygiene and Epidemiology, Havana City).

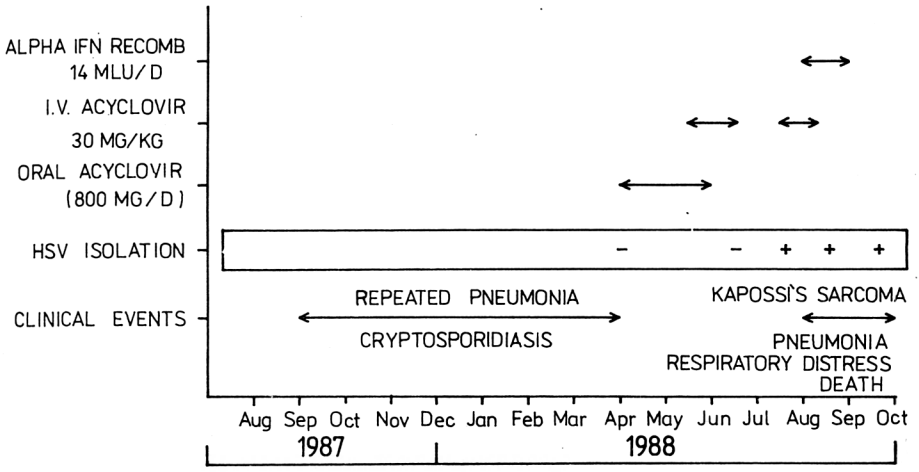

Fig. 1
Clinical and virological events

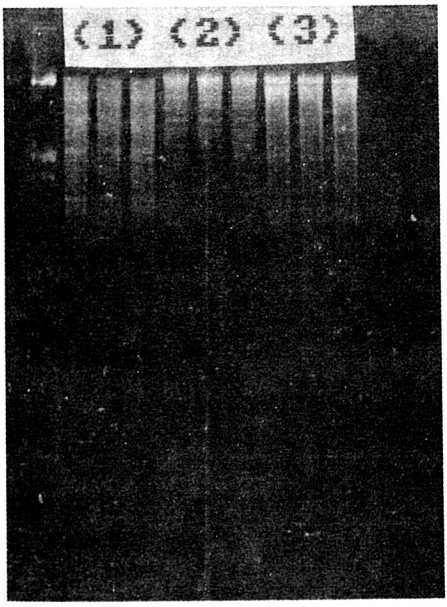

Fig. 2
Restriction endonuclease analysis of the HSV-DNA cleaved with *Bam*HI (1), *Xho*I (2), *Eco*RI (3) Lambda DNA digested by *Hind*III/*Eco*RI was used as control

Viral DNA was extracted from infected Vero cells showing 90% of CPE, according to the Hirt procedure (Hirt, 1967). Purified DNA was digested with an excess of restriction enzymes *Bam*HI, *Eco*RI, and *Xho*I (Ingebiot, Havana, Cuba) and further subjected to 0.8% agrose gel electrophoresis. The analysis was repeated at least twice for each clinical isolate with different DNA preparations.

A 34-year-old homosexual male with diagnosis of AIDS (since August 1987 according to the surveillance criteria of the Centers for Disease Control MMWR, 1987) developed an ulcerated lesion of the penis in April 1988. HSV was recovered from the lesion; oral ACV resulted in a slight decrease in the size of the ulcer but the lesion did not heal completely. When some weeks later, still during oral ACV therapy, the ulcer increased in size and new lesions appeared in the same area, intravenous ACV was applied. Three weeks of latter therapy exerted no evident clinical effect so that ACV was withdrawn. Finally, the patient developed pneumonia, respiratory distress and died in August 1988. Different interferons applied in other specific protocols had no effect on the evolution of the herpetic lesion.

Three HSV isolates were obtained at different intervals (Fig. 1); all were classified as HSV-2 by monoclonal antibodies. The ID_{50} for the HSV-2 reference strain was about 1μg/ml of ACV and for the tested isolates the ID_{50} it was more than 10μg/ml. The first isolate (A1) (April 1988) had the ID_{50} in 10μg/ml but the two others (A2, A3) even 100 μg/ml. Restriction endonuclease analysis of the viral DNA by *Bam*HI, *Eco*RI, and *Xho*I from the three isolates revealed identical patterns (Fig. 2).

Last two HSV-2 isolates (A2, A3) showed higher *in vitro* resistance to ACV than the first isolate (A1); in spite of this difference restriction endonuclease analysis of the serial isolates showed an identical pattern. This phenomenon has been found by others who suggest that all the genital isolates resulted from an initial infection with a single strain, and the selection of resistant virus occurred under the selective pressure of acyclovir therapy (Chatis, 1989). The genomic alteration causing resistance to ACV is not expressed in the cleavage pattern as observed by others (Schinazi, 1986).

Resistence to ACV *in vitro* can develop within a single passage of the virus in the presence of the drug, and reduction in sensitivity to ACV can be demonstated in patients whose sequencial isolates were analysed (Svennerholm, 1985). No correlation has been observed between the high *"in vitro"* ID 50 and the subsequent clinical response to ACV therapy in immunocompetent patients (McLaren, 1983); nevertheless, progressive disease due acyclovir-resistant HSV in immunocompromised patient has been observed frequently (Erlich, 1989). AIDS patient with dermatological HSV infection that did not respond to ACV should be evaluated promptly for the presence of a resistant virus and alternative experimental therapy with others drugs like Forscarnet or Vidarabin can be recommended.

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References

Chatis, P. A., Miller, C. H., Schrager, L. E., and Crumpacker, C. S. (1989): Successful treatment with Foscarnet of an acyclovir resistant mucocutaneous infection with herpes simplex virus in a patient with acquired immunodeficiency syndrome. *N. Engl. J. Med* **320**, 297 - 300.

Erlich, K. S., Mills, J., Chatis, P., Mertz, G., Bush, D. F., Follansbe, S. E., Grant, R. M., and Cumpacker, C. S. (1989): Acyclovir resistant herpes simplex virus infections in patient with the acquired immunodeficiency virus. *N. Engl. J. Med.* **352**, 293 - 296.

Field, H. J., and Darby, G. (1980): Pathogenicity in mice of strains of herpes simplex virus which are resistant to acyclovir *in vitro* and *in vivo*. *Antimicrob. Agents Chemother* **17**, 209 - 216.

Hirt, B. (1967): Selective extraction of polyoma DNA from infected mouse cell cultures. *J. mol. Biol.* **26**, 365 - 369.

McLaren, C., Corey, L., Dekket, C., and Barry, D. W. (1983): *In vitro* sensitivity to acyclovir in genital herpes simplex viruses from acyclovir-treated patients. *J. infech. Dis.* **148**, 868 - 880.

Revision of the CDC surveillance cese definition for acquired immunodeficiency syndrome. M. M. W. R., 1987, Suppl. **36**, 35 - 95.

Svennerholm, B., Vahlne, A., Lowhagen, G. B., Widell, A., and Lycke, E. (1985): Sensitivity of HSV strains isolated before and after treatment with acyclovir. *Scand. J. infect. Dis.* (Suppl.) **47**, 149 - 154.

Shinazi, R. F., Del Vene, V., Taylor Scott, R., and Dubley-Thorpe, J. B. (1986): Characterization of acyclovir resistant and sensitive herpes simplex viruses isolated from a patient with an acquired immune deficiency. *J. Antim. Chemot.* (suppl B) **18**, 127 - 134.

Schnipper, L. E., and Crumpacker, C. S. (1980): Resistance of herpes simplex virus to acycloguanosine: role of viral thymidine kinase and DNA polymerase loci. *Proc. natn. Acad. Sci. U. S. A.* 77, 2270 - 2273.