Acyclovir Susceptibility of Herpes Simplex Virus Isolates at King Chulalongkorn Memorial Hospital, Bangkok

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Objective: *To determine the ACV susceptibility in Thai HSV clinical isolates.*
Material and Method: *One hundred thirty HSV isolates from the Virology Laboratory Unit, King Chulalongkorn Memorial Hospital, Bangkok, Thailand had typing done by immunofluoresent assay using monoclonal antibody specific to either HSV-1 or HSV-2. Their sensitivity to ACV (IC_{50}) was determined by plaque reduction assay.*
Results: *The IC_{50} of 77 HSV-1 isolates ranged from 0.07-0.97 μg/ml and that of 53 HSV-2 isolates was 0.13-1.66 μg/ml. The standard HSV-1 (KOS) and HSV-2 (Baylor 186) were included in each run. The mean $\pm$ standard deviation (SD) of ACV IC_{50} among HSV-1 and HSV-2 isolates were 0.38 $\pm$ 0.23 and 0.50 $\pm$ 0.32 μg/ml while that of standard HSV-1 and HSV-2 were 0.45 $\pm$ 0.13 and 0.57 $\pm$ 0.04 μg/ml. Statistically significant difference between IC_{50} of HSV-1 and HSV-2 isolates was indicated ($p = 0.02$).*
Conclusion: *No ACV^r HSV has been detected and ACV susceptibility of HSV-2 has more resistance than that of HSV-1.*

Keywords: *HSV, Acyclovir susceptibility, King Chulalongkorn Memorial Hospital*

J Med Assoc Thai 2008; 91 (6): 908-12
Full text. e-Journal: *http://www.medassocthai.org/journal*

Acyclovir (ACV) has been widely used over the past two-decades as an effective and safe drug for the treatment of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) infection[1]. However, a number of reports have raised a problem of ACV resistant HSV (ACV^r HSV)[2-6], which became an increasing therapeutic problem for clinicians. Therefore, the aim of resistance monitoring is to provide the necessary information to enable the physician to prescribe the most optimal drug combination for individual patient.

In this present study, the susceptibility to ACV of HSV isolates from Thai patients was determined by Plaque Reduction Assay (PRA) in order to search for the presence of ACV^r HSV.

Material and Method

Cell and Viruses: Vero cells were grown in M199 growth medium supplemented with 10% fetal bovine serum and antibiotics. Standard viruses of HSV-1 strain KOS and HSV-2 strain Baylor 186 were used throughout the present study.

Clinical isolates: One hundred fifty five HSV clinical samples, obtained from the Virology Laboratory Unit, King Chulalongkorn Memorial Hospital, Bangkok, Thailand during the years 1998 to 2003 were isolated in Vero cells culture system and typed by an indirect immunofluorescence assay (IFA) using monoclonal antibody (MAb) specific to HSV-type (NOVO cratra Laboratory, UK). The viruses were propagated to yield high titers in Vero cell. The number of passages used in the present study varied from the second to the sixth.

ACV susceptibility test: All isolates were determined IC_{50} for their sensitivity to ACV (Acycloguanosine, > 99% HPLC, Sigma, USA) by PRA in Vero cell using 96 well-plate (Nunclon, Denmark). Two-fold dilution of ACV from concentration of 5 to 0.08 μg were added and run in quadruplicate wells. After three to four days, the number of plaques was counted and IC_{50} was calculated. HSV isolates were considered resistant to ACV when the IC_{50} was $\geq$ 3 μg/ml. The standard

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HSV-1 strain KOS and HSV-2 strain Baylor 186 were tested in each assay. Descriptive statistics were used to summarize the all clinical HSV isolates.

Results

One hundred fifty five clinical HSV isolates were from 148 immunocompetent patients; 19 (12.84%) males and 129 females (87.16%) suspected first episode and recurrent herpetic lesions. These clinical specimens were mainly from genital lesions (83.87%; 130/155). Only 25 (16.13%; 25/155) isolates were from non-genital areas, i.e., labia, eye, and skin lesion. All clinical isolates were typed by indirect IFA using MAb HSV type specific. HSV-1 was found predominately 52.26% (81/155), followed by HSV-2, 37.42% (58/155) and mixed infection of HSV-1 and HSV-2, 10.32% (16/155). HSV-1 and HSV-2 were detected in 88% (22/25), and 12% (3/25) of non-genital lesion. While in genital specimens, 45.38% (59/130) were HSV-1, 42.31% (55/130) were HSV-2, and 12.31% (16/130) were mixed infection. Only 139 isolates of HSV monotype were assayed for ACV susceptibility since 16 isolates with mixed infection were excluded and nine isolates were lost during viral propagation. Finally, 77 HSV-1 and 53 HSV-2 isolates were recruited.

The IC_{50} range of 77 HSV-1 isolates was 0.07-0.97 μg/ml and that of 53 HSV-2 isolates was 0.13-1.66 μg/ml. In each assay, the standard HSV-1 (KOS) and HSV-2 (Baylor 186) were included in each run. Therefore, the IC_{50} range of 14 times of HSV-1 (KOS) was 0.20-0.63 μg/ml and that of eight times of HSV-2 (Baylor 186) was 0.53-0.62 μg/ml. The mean and standard deviation (SD) of ACV IC_{50} among HSV-1 and HSV-2 isolates were 0.38 $\pm$ 0.23 μg/ml and 0.50 $\pm$ 0.32 μg/ml while that of HSV-1 (KOS) and HSV-2 (Baylor 186) were 0.45 $\pm$ 0.13 μg/ml and 0.57 $\pm$ 0.04 μg/ml, respectively. Statistically significant difference between IC_{50} of HSV-1 and HSV-2 isolates was indicated ($p = 0.02$). The results of IC_{50} values are shown as scattering plot (Fig. 1, 2).

Discussion

Although HSV infections in normal hosts are usually self-limited, patients with impaired immune systems may suffer chronic, debilitating and even fatal infection. In the early 1980's ACV first became available for the treatment of HSV. Besides the efficacy of ACV in suppressing HSV replication, ACV is recognized as safe and effective treatments for the management of HSV infections in immunocompetent and immunocompromised population[7]. As the clinical utility of ACV became apparent, ACV was then widely used.

For many years, HSV diseases have been successfully treated with ACV. Unfortunately, after long-term treatment of patients with ACV, the emergence of drug-resistant virus variants has been observed[8], and there have been an increasing number of ACV treatment failures that were associated with ACV-resistant viruses in these patients, especially in immunocompromised hosts[9]. Thus, the screening of ACV^r HSV has become increasingly important for choosing the appropriate therapy.

In Thailand, ACV is also used for the treatment of HSV infected patients. Therefore, the authors were interested to determine the sensitivity of HSV isolates

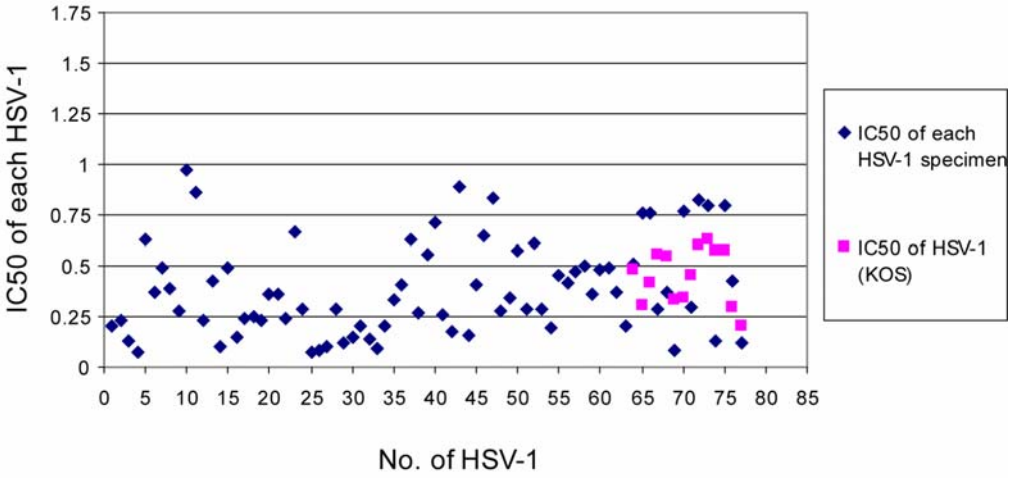

Fig. 1 Scatter plot of ACV IC_{50} of HSV-1 isolates (n = 77) and HSV-1 (KOS)

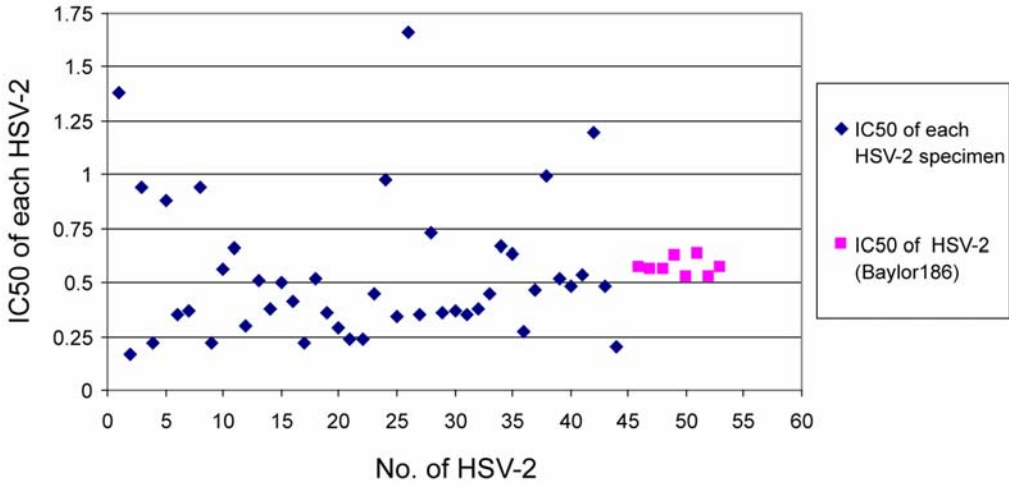

Fig. 2 Scatter plot of ACV IC_{50} of HSV-2 isolates (n = 53) and HSV-2 (Baylor 186)

to ACV by PRA expecting to get some ACV^r HSV. All 191 clinical samples were recruited in the present study. They were re-cultured for HSV isolation and typing. Only 155 samples were successfully re-propagated. Among 155 isolates, 139 isolates showed single or unique HSV type. i.e., 81 HSV-1 and 58 HSV-2 but only 130 samples could be further analyzed for ACV susceptibility test. Interestingly, in genital lesions, approximately half of the genital herpes was caused by HSV-1 (45.38%; 59/130) while HSV-2 were found 42.31% (55/130). These results suggested a population of individuals with a high incidence of genital HSV-1. The predominance of HSV-1 in genital infection has been variably reported worldwide as between 4-60%[10-12]. The susceptibility of those HSV isolates to ACV was exhibited in a wide spectrum from the range of IC_{50} both types, were 0.07-1.66 μg/ml. The range of IC_{50} of HSV-1 isolates was more narrow than that of HSV-2 isolates (0.07-0.97 μg/ml vs. 0.13-1.66 μg/ml). The mean IC_{50} of HSV-1 isolates, 0.38 (SD = 0.23), and that of HSV-2 isolates, 0.50 (SD = 0.32), were statistically significantly different (p = 0.02). The presented data indicates that HSV-1 isolates were more susceptible to ACV than HSV-2 isolates. This finding is correspondent to a previous study of Yoosook et al, in 1989 who reported the mean IC_{50} value range from 0.044-0.162 μg/ml for HSV-1 isolates and 0.008-0.504 μg/ml for all HSV-2 isolates[13]. Comparing the range susceptibility between Yoosook's study and the authors work, our IC_{50} range of both types were wider and higher. Lipipun et al in 2000 demonstrated antiviral activity of ACV against HSV-2 isolates with the effective dose 50% value (ED_{50}) in the range of 0.38-0.87 μg/ml and the mean ± SD was 0.585 ± 0.1 μg/ml[14]. Together with the presented results, this may suggest the increasing of ACV resistance in both types of HSV isolates. However, the measurement of the sensitivity of HSV strains to ACV was standardized by chosen threshold values for ACV-resistance. The cut off value of 3 μg/ml has been used to discriminate between ACV-sensitive and ACV-resistance isolates[15]. According to this criteria, no ACV^r HSV was detected in the present study.

To validate the presented system, standard HSV-1 (KOS) and HSV-2 (Baylor 186) were run in each time of assay. The mean IC_{50} of HSV-1 (KOS) and HSV-2 (Baylor 186) were 0.45 (SD = 0.13) and 0.57 (SD = 0.04) μg/ml. These IC_{50} values were almost the same as the previous report of Parris and Harrington, for standard HSV-1 strain KOS, is 0.4 μg/ml[16] and Kost et al, for standard HSV-2 strain Baylor 186, is 0.6 μg/ml[17].

The majority of ACV^r HSV has been reported to occur essentially in immunocompromised hosts, such as those infected with HIV or receiving transplants undergoing a prolonged course of acyclovir chemotherapy[9,18-20]. In contrast, the recovery of ACV^r HSV from immunocompetent hosts has been uncommon, and median sensitivities of HSV strains isolated before and after therapy have no significant difference[21]. In the present studies, all clinical HSV specimens were obtained from immunocompetent hosts thus, ACV^r HSV may rarely be detected since the prevalence of ACV^r HSV in immunocompetent hosts was previously reported only 0-7%[22-24]. Unfortunately, no clinical samples from immunocompromised hosts were obtained

in this study. However, the presented results could convince that in Thailand, ACV remains to be a drug of choice, which is a very safe and effective treatment to immunocompetent patients.

Acknowledgements

The authors wish to thank the Rachadapisek Sompoj China Medical Board, Faculty of Medicine, Chulalongkorn University for their support of this project. This work has been approved by the Ethics Committee of Faculty of Medicine, Chulalongkorn University No. 128/2001.

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ความไวรับต่อยาอะไซโคลเวียของเชื้อไวรัสเฮอร์ปีส์ซิมเพล็กซ์ที่แยกได้จากโรงพยาบาลจุฬาลงกรณ์ กรุงเทพมหานคร

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วัตถุประสงค์*: ทำการศึกษาความไวรับต่อยาอะไซโคลเวียของไวรัสเฮอร์ปีส์ซิมเพล็กซ์ที่แยกได้จากผู้ป่วยคนไทย*

วัสดุและวิธีการ*: เชื้อไวรัสจำนวน 130 ตัวอย่างที่แยกได้จากตัวอย่างสิ่งส่งตรวจของห้องปฏิบัติการไวรัสวิทยา โรงพยาบาลจุฬาลงกรณ์ กรุงเทพมหานคร ทำการจำแนกไทป์ด้วยวิธี Immunofluorescent assay ใช้โมโนโคลนัลแอนติบอดีจำเพาะต่อไทป์ 1 หรือ 2 นำเชื้อไวรัสมาทดสอบตรวจหาความไวรับต่อยาอะไซโคลเวียด้วยวิธี plaque reduction assay*

ผลการศึกษา*: ไวรัสเฮอร์ปีส์ซิมเพล็กซ์ไทป์ 1 จำนวน 77 ตัวอย่างมีความไวรับร้อยละ 50 อยู่ระหว่าง 0.07 ถึง 0.97 ไมโครกรัมต่อมิลลิลิตร และ ไวรัสเฮอร์ปีส์ซิมเพล็กซ์ไทป์ 2 จำนวน 53 ตัวอย่าง มีความไวรับร้อยละ 50 อยู่ระหว่างช่วง 0.13 ถึง 1.66 ไมโครกรัมต่อมิลลิลิตร ทุกครั้งที่จะทำการทดสอบความไวรับของไวรัสมาตรฐานสองชนิด คือ ไวรัสเฮอร์ปีส์ซิมเพล็กซ์ไทป์ 1 สายพันธุ์ KOS และ ไวรัสเฮอร์ปีส์ซิมเพล็กซ์ไทป์ 2 สายพันธุ์ Baylor 186 ร่วมด้วย ค่าเฉลี่ยและส่วนเบี่ยงเบนมาตรฐานของความไวรับร้อยละ 50 ของไวรัสเฮอร์ปีส์ซิมเพล็กซ์ที่แยกได้ไทป์ 1 และ 2 คือ 0.38 $\pm$ 0.23 และ 0.50 $\pm$ 0.32 ไมโครกรัมต่อมิลลิลิตร ขณะที่ไวรัสมาตรฐานไทป์ 1 และ 2 คือ 0.45 $\pm$ 0.13 และ 0.57 $\pm$ 0.04 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ พบความไวรับร้อยละ 50 ของไวรัสเฮอร์ปีส์ซิมเพล็กซ์ที่แยกได้ทั้งไทป์ 1 และ 2 มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p = 0.02$)*

สรุป*: ไม่พบไวรัสเฮอร์ปีส์ซิมเพล็กซ์สายพันธุ์ที่ดื้อต่อยา แต่เป็นที่สังเกตว่าความไวรับของไทป์ 2 สูงกว่าไทป์ 1*