PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 147, 201–204 (1974)

Different Sensitivities of Type 1 and Type 2 Herpes Simplex Virus to Sodium *p*-Chloromercuribenzoate and Chlorhexidine Gluconate[1] (38311)

KENKICHI SHINKAI
(Introduced by K. Yoshino)

Central Research Laboratories, Sankyo Co., Ltd., Tokyo 140, Japan

Herpes simplex virus strains can be classified into type 1 and type 2 on the basis of antigenical difference (1). The two types also differ in DNA density (2), constituents of protein moiety (3) and various biological properties (4–17). Among these, the different sensitivities to 7,12-dimethylbenz[α]anthracene, a carcinogenic agent, (5) seemed to be a good marker for type differentiation. The author intended to examine whether all known virus-inactivating agents could differentiate the types or only special compounds could do this. Thus, several compounds were tested for their effects of inactivating type 1 (HSV-1) and type 2 herpes virus (HSV-2). As reported herein, two of four compounds tested, sodium *p*-chloromercuribenzoate and chlorhexidine gluconate, differentiated the two types by their different effects on HSV-1 and HSV-2, while the others did not.

Materials and Methods. Viruses. As standard HSV-1 and HSV-2 viruses, HF and UW-268 strains were selected. HF strain had received more than 500 chorioallantoic passages. UW-268 strain was isolated and passaged in human embryo lung cells by Chiang *et al.* (18); after reception, it has received five chorioallantoic passages in this laboratory. Other strains used were isolates from genital and nongenital sites of patients with herpetic infections. Type determination of these isolates was based on neutralization by specific hyperimmune rabbit Ig*M* in the presence of complement (19). Passage histories of all the strains are detailed elsewhere (20). Virus stocks were prepared by culturing in Vero cells and harvesting culture fluids, which were shell-frozen in glass ampoules and stored at −70°.

Chemicals. Sodium *p*-chloromercuribenzoate (PCMB), iodoacetamide (IA) and acrinol (AC) were purchased from Tokyo Chemical Industry Co., Ltd., Tokyo. PCMB was dissolved in a small volume of 0.1 *M* sodium hydroxide and diluted with 0.01 *M* phosphate buffered physiological saline of pH 7.2 (BS). The final pH was 7.4. IA and AC were directly dissolved in BS and diluted with the same diluent. Chlorhexidine gluconate (CHG) was a product of Imperial Chemical Industries, England. This compound was dissolved and diluted with 0.0025 *M* Tris–HCl buffered physiological saline of pH 7.5, because it became turbid when mixed with phosphate.

Direct inactivating test. An undiluted stock virus suspension was mixed with an equal volume of a test compound solution or, as a control, the same quantity of diluent alone, and incubated at 37° or 0° (in an ice bath), as indicated, for 60 min. The mixtures were then serially diluted with BS containing 0.1% egg-yolk for plaque assay of residual infectivity using primary chick embryo cell monolayers (21). The sensitivity was expressed by titer reduction in log of the test mixture minus that of the control. The initial titer of the mixtures was usually 10^7 plaque-forming units (PFU)/ml, and titer reduction in the control mixture averaged 0.2 log.

Photodynamic inactivation test. A stock virus suspension and an equal volume of an AC solution were mixed, while a control mixture was made replacing AC with BS. These mixtures in Pyrex tubes were exposed to light emitted from a 15-W "white" fluorescent lamp at a distance of 5 cm for 60 min at 20°. Calculation of sensitivity

[1] This work was performed in Department of Virology, Institute of Medical Science, the University of Tokyo, under the guidance of Professor K. Yoshino.

was the same as above.

Results. Inactivating effects of various compounds upon standard HSV-1 (HF) and HSV-2 (UW-268) strains. Varying concentrations of PCMB, CHG, IA and AC were tested for inactivation of HF and UW-268 strains at 0°, 20° or 37°, and the results obtained are summarized in Table I. Sensitivity to PCMB and CHG markedly differed between the two viruses, type 1 being less sensitive to the drugs than type 2. The difference was most pronounced with 50–100 μg/ml of PCMB and 200 μg/ml of CHG when the test was carried out at 37°. At 0°, however, the viruses were not inactivated at all by either compound even at the highest concentration used, 200 μg/ml. This absence of effect after reaction at 0° also testified that the above effects of PCMB and CHG were not due to blocking or destruction of receptors in titration cells.

On the other hand, IA, a sulfhydryl reagent, did not inactivate either type even when 400 μg/ml of it was reacted with the viruses at 37° for 60 min. AC, a photodynamic inactivator, did inactivate both strains at 20° depending upon the concentration used, but the extents to which the two viruses were inactivated by this drug paralleled perfectly, showing no difference between the two types. This effect of AC required light, since there occurred no inactivation when the test was performed in a dark room.

Susceptibilities of HSV-1 and HSV-2 strains to PCMB, CHG and AC. Whether the above-observed result could be generalized to all HSV-1 and HSV-2 strains was next to be determined. Fourteen HSV-1 and 17 HSV-2 strains were selected for this purpose, and they were tested against two concentrations of PCMB, 50 and 100 μg/ml, and 200 μg/ml of CHG. In addition, five strains of each type were tested against 10 μg/ml of AC. The results are presented in Table II. It was found that 50 μg/ml of PCMB reduced infectivities of type 1 and type 2 viruses by 0.7–1.5 and 1.4–3.4 logs, respectively. Thus, although type 2 strains showed averagely higher sensitivities to the drug than type 1 strains, there was some overlapping between the two types. This overlapping was eliminated at 100 μg/ml of this drug, the titer reduction of type 1 strains being approximately 1–2 logs and that of type 2 strains more than 2 logs. The difference in sensitivity between the two types was clearer with CHG; at 200 μg/ml, titer reduction of type 1 strains was less than 2 logs whereas that of type 2 strains invariably exceeded 3 logs. It was again confirmed that the photodynamic inactivation by AC occurred to equal degrees with all the HSV-1 and HSV-2 strains tested.

TABLE I. Effects of Four Compounds on the Infectivities of HF(HSV-1) and UW-268(HSV-2) Strains.

Compound	Temperature (°)	Final concentration of drug in mixture (μg/ml)[1]	Sensitivity to drug[a]	
			HF(HSV-1)	UW-268(HSV-2)
PCMB	37	200	2.3	3.5
	37	100	1.8	3.1
	37	50	0.7	2.4
	37	25	0.1	1.2
	0	200	0	0
CHG	37	200	1.1	3.1
	37	100	0.3	1.0
	37	50	0.1	0.6
	0	200	0	0
IA	37	400	0	0
AC	20	200	1.9	1.5
	20	100	2.4	2.4
	20	50	2.3	2.3
	20	10	2.3	2.5
	20	5	1.7	2.1
	20	2.5	1.2	1.3
	20	1.3	0.8	0.4

[a] Expressed by titer reduction in log of test mixture minus that of control mixture as estimated after 60 min incubation.

TABLE II. Effects of PCMB, CHG and AC on Various HSV-1 and HSV-2 Strains.

Type	Strain name	Sensitivity to drug[a] PCMB 50 μg/ml	PCMB 100 μg/ml	CHG 200 μg/ml	AC 10 μg/ml
1	TVS-1	0.9	1.9	1.7	2.4
1	TVD-10	1.2	1.8	1.6	2.5
1	TVN-14	0.8	1.2	2.0	2.3
1	TVD-16	0.7	1.6	2.1	2.6
1	TVS-21	0.8	1.6	1.9	2.3
1	TVH-22	0.7	0.9	2.0	—[b]
1	TVF-25	1.1	1.7	1.9	—
1	KII	1.0	1.3	2.2	—
1	TS	0.5	1.1	2.1	—
1	HM	1.5	1.9	1.9	—
1	MT	1.5	1.9	1.9	—
1	UZ	1.1	1.8	2.0	—
1	ST	0.7	1.5	1.6	—
1	WD	1.1	1.7	1.7	—
2	TVI-4	2.9	3.1	3.9	2.2
2	GCO-9	3.1	3.3	> 4.5	2.3
2	GCN-13	1.8	3.0	3.2	2.3
2	TBS-17	2.4	3.3	3.3	2.5
2	BPA-18	2.5	3.3	3.3	2.4
2	TVH-20	2.2	3.2	3.3	—
2	TVI-24	2.1	3.3	3.9	—
2	TVK-26	2.0	3.3	4.3	—
2	TVT-28	2.2	2.8	3.4	—
2	UR	1.8	2.4	3.2	—
2	UC	3.0	3.3	3.8	—
2	Mori	1.9	2.5	5.1	—
2	NO	3.4	3.5	4.3	—
2	MS	2.1	2.9	3.0	—
2	TH	2.2	2.4	3.7	—
2	Ogiwara	1.5	2.6	> 3.8	—
2	OH	1.4	2.7	4.0	—

[a] See Table I.
[b] Not tested.

Discussion. Earlier investigations demonstrated that HSV-2 was more sensitive to 7,12-dimethylbenz[α]anthracene (5) and heparin (4) than HSV-1. The present experiments have added two more compounds, PCMB and CHG, to the spectrum of drugs which can differentiate the two types. This fact does not necessarily mean an overall vulnerability to inactivating agents of type 2 strains as compared with type 1, since IA has no effect on either type and photodynamic inactivation by AC was similar for both types.

An inspection of the present data may tell a subtle difference between the effects of PCMB and CHG; namely, some HSV-2 strains, as exemplified by the Ogiwara strain, exhibited a relatively higher resistance to PCMB, and at the same time a relatively higher sensitivity to CHG, than the other HSV-2 strains. On the other hand, another HSV-2 strain, Mori, showed an exceptionally high sensitivity to CHG while having an average sensitivity to PCMB. These facts may suggest that different sites of HSV are attacked by PCMB and CHG.

The present results differ from those of Bailey and Longson (22) who described that 200 μg/ml of CHG did not differentiate the two HSV types. The reason for this discrepancy may be that the

drug they used contained a surface active agent. In my limited experience, the type differentiation by CHG was consistent only when the Tris–HCl buffered saline was used as the diluent. They also stated that CHG had no effect on nonenveloped viruses such as polio or adenovirus (22). It is conceivable, therefore, that the site on which the drug acts is on the viral envelope. Consequently, it is suggested that different make-ups of the viral envelope may be responsible for the discrimination between HSV-1 and HSV-2. This is in line with the finding that HSV-1 and HSV-2 glycoproteins were differentiated by serological techniques (23, 24).

Another point worthy of noting is that our standard HSV-1 and HSV-2 strains failed to be inactivated by IA, which was found by Allison *et al.* (25) to exert virucidal effects on various viruses at 10^{-3} *M*.

Differing from PCMB and CHG, photodynamic inactivation by AC did not differentiate the HSV types. It has been known that the mechanism of this inactivation is binding of acridine derivatives to nucleic acid (26, 27). This may be another evidence to substantiate that the action of PCMB and CHG is directed to the protein rather than the nucleic acid moiety of the virus.

Since CHG possesses the ability to clearly differentiate the HSV types, it can be utilized for type determination of HSV isolates as well as for genetic studies of this virus. An advantage of this compound is that it is water-soluble, in contrast to 7,12-dimethylbenz[α]anthracene which should be dissolved in dimethylsulfoxide, and therefore easier for manipulation.

Summary. Degrees of inactivation of HSV-1 and HSV-2 by four compounds were compared. The sensitivity of HSV-2 to *p*-chloromercuribenzoate and to chlorhexidine gluconate was found to be higher than that of HSV-1. Chlorhexidine gluconate could differentiate the HSV types more clearly than PCMB. This was ascertained with 15 HSV-1 and 18 HSV-2 strains. The inactivation occurred at 37° but not at 0°. On the other hand, iodoacetamide did not inactivate either type even at a concentration of 400 μg/ml. Photodynamic inactivation by acrinol was similar for both types.

1. Nahmias, A. J., and Dowdle, W. R., Prog. Med. Virol. **10**, 110 (1968).

2. Kieff, E., Hoyer, B.,Bachenheimer, S., and Roizman, B., J. Virol. **9**, 738 (1972).

3. Gibson, W., and Roizman, B., J. Virol. **10**, 1044 (1972).

4. Schneweis, K. E., Sommerhäuser, H., and Huber, D., Arch. Ges. Virusforsch. **38**, 338 (1972).

5. Docherty, J. J., Goldberg, R. J., and Rapp, F., Proc. Soc. Exp. Biol. Med. **136**, 328 (1971).

6. Kleger, B., and Prier, J. E., J. Inf. Dis. **120**, 376 1969).

7. Figueroa, M. E., and Rawls, W. E., J. Gen. Virol. **4**, 259 (1969).

8. Ratchiffe, H., J. Gen. Virol. **13**, 181 (1971).

9. Couch, E. F., and Nahmias, A. J., J. Virol. **3**, 228 (1969).

10. Dundaroff, S., and Falke, D., Arch. Ges. Virusforsch. **36**, 56 (1972).

11. Thoules, M. E., J. Gen. Virol. **17**, 307 (1972).

12. Ogino, T., and Rapp, F., Virology **46**, 953 (1971).

13. Fischer, T. N., and Fischer, E., Jr., Proc. Soc. Exp. Biol. Med. **143**, 208 (1973).

14. Lowry, S. P., Bresnick, E., and Rawls, W. E., Virology **46**, 958 (1971).

15. Lowry, S. P., Melnick, J. L., and Rawls, W. E., J. Gen. Virol. **10**, 1 (1971).

16. Hutton, R. D., Ewert, D. L., and French, G. R., Proc. Soc. Exp. Biol. Med. **142**, 27 (1973).

17. Rodgers, F. G., J. Gen. Virol. **21**, 187 (1973).

18. Chiang, W., Wentworth, B. B., and Alexander, E. R., J. Immunol. **104**, 992 (1970).

19. Hampar, B., Martos, L. M., Chakrabarty, M., and Burroughs, M. A. K., J. Immunol. **104**, 593 (1970).

20. Kawana, T., Shinkai, K., and Yoshino, K., Japan. J. Microbiol., in press.

21. Taniguchi, S., and Yoshino, K., Arch. Ges. Virusforsch. **14**, 537 (1964).

22. Bailey, A., and Longson, M., J. Clin. Pathol. **25**, 76 (1972).

23. Wildy, P., and Watson, D. H., Cold Spring Harbor Symp. Quant. Biol. **27**, 25 (1962).

24. Savage, T., Roizman, B., and Heine, J. W., J. Gen. Virol. **17**, 31 (1972).

25. Allison, A. C., Buckland, F. E., and Andrewes, C. H., Virology **17**, 171 (1962).

26. Sastry, K. S., and Gordon, M. P., Biochim. Biophys. Acta **129**, 32 (1966).

27. Lerman, L. S., J. Mol. Biol. **3**, 18 (1961).

Received April 2, 1974. P.S.E.B.M., 1974, Vol. 147.