JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1983, p. 374–376
0095-1137/83/020374-03$02.00/0

Vol. 17, No. 2

Photodynamic Inactivation of Pseudorabies Virus with Methylene Blue Dye, Light, and Electricity

JANINE A. BADYLAK, GAIL SCHERBA, AND DONALD P. GUSTAFSON*

Department of Veterinary Microbiology, Pathology, and Public Health, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana 47907

Received 2 September 1982/Accepted 9 November 1982

Pseudorabies virus was photoinactivated with a combination of methylene blue dye, light, and electricity. Viral suspensions were mixed with variable amounts of methylene blue dye and then were placed in a current source apparatus. Total inactivation of pseudorabies virus (1.7×10^6 50% tissue culture infective doses per ml) was achieved with constant mixing, a methylene blue dye concentration of 10^{-4} M, and an electrical current of 12 μA for 12 min.

The phenomenon of photodynamic inactivation was discovered in 1900, when Raab inactivated paramecia with low concentrations of acridine dye and visible light (4). The first evidence that viruses were photosensitive was presented by Herzberg, who reported in 1931 that illumination in the presence of methylene blue (MB) dye suppresses or greatly diminishes vaccinia virus eruptions on the skin of rabbits (1). Melnick and Wallis have intensively studied the use of photosensitive compounds to inactivate animal viruses, with major emphasis on the inactivation of herpes simplex virus (2, 3, 8, 9).

Pseudorabies virus (PrV) has been shown to be photosensitive. Using acridine dye to photoinactivate PrV, Sun et al. (6) observed a marked reduction in infective titers, whereas no reduction in infectivity was observed in the control. The antigenicity of the photoinactivated virus was preserved, as evidenced by the use of specific antibody in the immunodiffusion tests and through immunoelectrophoresis (6). In the present study, photodynamic inactivation of PrV was investigated for use in the production of skin test antigens for pseudorabies. An inactivation method was sought which would totally and consistently inactivate the virus and be relatively easy, quick, and inexpensive. In a study by Swartz et al., herpes simplex virus was inactivated with MB dye, light, and electricity (7). A low concentration of MB, when electrically reduced in the presence of herpes simplex virus and irradiated with visible light, resulted in an efficient loss of plaque-forming ability. A methyl methacrylate cell fitted with platinum electrodes was used for the photoinactivation process. Only small quantities (approximately 10 ml) of virus could be inactivated at a time. For the current study, a similar apparatus was designed on a larger scale by L. F. Silva and Edmund C. Strauss (Department of Electrical Engineering, Purdue University, West Lafayette, Ind.) so that mass quantities of viral antigen could be produced (Fig. 1). The electrochemical cell of this apparatus provided a 10-fold increase in volume capacity over the cell used by Swartz et al. (7). The current source was designed so that it was capable of maintaining current densities equal to that produced in the smaller cell, i.e., 1 $\mu A/cm^2$. Cell dimensions (length by height by width) were 11.95 by 2.89 by 2.89 cm, resulting in a length/cross-sectional area ratio of 0.7, the same as that specified by Swartz et al. Transparent acrylic was used to make the cell, thus allowing light, also important in the inactivation process, to enter the cell from all directions. The volume capacity of the cell was 100 ml. A higher voltage difference between electrodes was needed to push current through the increased resistance of the lengthened cell. All power was derived from a conventional 110-V AC source. The cell was fitted with platinum electrodes which were detachable from the cell and detachable from the cables leading to the current source. The current source was a closed loop system providing well-regulated adjustable current directly to the cell. The amount of current flowing between the electrodes was indicated at all times by a meter mounted on the front panel of the instrument. Current output was adjustable between 0 and 12 μA. An electronic timer circuit was added to the current source to allow the duration of an experiment to be set precisely, ensuring the accurate repeatability of experiments. Cycle time ranged between 0 and 999 s.

All inactivation studies were performed in the timed current source apparatus. A constant light source was provided by an incandescent tensor lamp maintained at a fixed distance of 20 cm from the cell. Various amounts of MB dye were added to 100-ml samples of virus. The dye-virus mixture was then placed in the acrylic cell. For

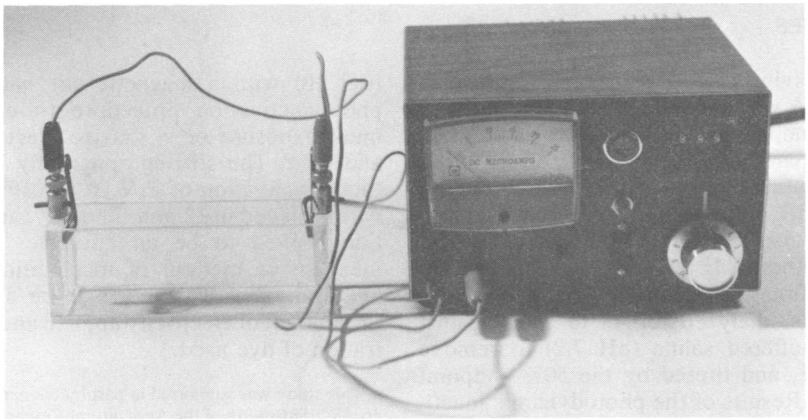

FIG. 1. The timed current source apparatus used to inactivate PrV. The transparent acrylic cell on the left held the virus-dye mixture; the current source instrument can be seen on the right.

TABLE 1. Results of photoinactivation of PrV with various amounts of MB dye and electrical current

Trial	MB dye concn (M)	Electrical current (μA)	Time (min)[a]	Original virus titer $(TCID)_{50}$	Results	
					Titer $(TCID)_{50}$	% Survival
1	10^{-5}	12	8	4×10^7	8.0×10^2	0.010
			12		8.0×10^2	0.010
			16.5		8.0×10^1	0.001
2	10^{-5}	0	8	4×10^7	1.0×10^4	0.125
			12		1.3×10^4	0.158
			16.5		8.0×10^2	0.010
3	2×10^{-5}	12	8	1.1×10^6	1.4×10^3	0.131
			12		1.2×10^3	0.112
			16.5		$<5.2 \times 10^1$[b]	<0.005[b]
4	2×10^{-5}	0	8	1.1×10^6	8.0×10^3	0.741
			12		8.0×10^2	0.074
			16.5		5.3×10^2	0.048
5	10^{-4}	12	8	1.2×10^5	$<5.2 \times 10^1$[b]	<0.043[b]
			12		0	0
			16.5		0	0
6	10^{-4}	0	8	1.2×10^5	7.2×10^2	0.600
			12		8.0×10^2	0.667
			16.5		1.1×10^3	0.869
7	0	12	8	1.3×10^6	4.7×10^5	35.778
			12		1.4×10^5	10.498
			16.5		6.3×10^5	48.523
8	10^{-4}	12	8	1.3×10^6	8.0×10^1	0.006
			12		5.2×10^1	0.004
			16.5		$<5.2 \times 10^1$[b]	<0.004[b]
9	10^{-4}	0	8	1.3×10^6	1.4×10^2	0.011
			12		8.8×10^1	0.007
			16.5		1.3×10^2	0.009
10	10^{-4}	12	8	1.7×10^6	$<5.2 \times 10^1$[b]	<0.003[b]
			12		0	0
			16.5		0	0

[a] Length of time virus or virus-dye mixture was exposed to electrical current, light, or both.

[b] Cytopathic effect was evident in less than 50% of the undiluted wells. Therefore, by the Reed-Muench method of calculating 50% endpoint titers (5), a more precise titer could not be obtained.

studies involving electrical inactivation, a current of 12 μA was applied for various lengths of time. In some trials, no electricity was applied, so that comparisons could be made between the viral inactivating efficiency of MB and light and of MB, light, and electricity in combination. Trials were also conducted with electricity and light but without MB. During the inactivation process, samples of virus were removed at various intervals, dialyzed for 48 to 72 h against phosphate-buffered saline (pH 7.2) to remove the MB dye, and titered by the 50% endpoint method (5). Results of the photodynamic inactivation trials are summarized in Table 1.

Trials conducted to compare the inactivating efficiency of MB_s, light, and electricity in combination and of MB and light showed that in all cases the addition of electrical current reduced the viral titer further than MB and light (Table 1, trials 1 and 2, 3 and 4, 5 and 6, and 8 and 9). It is also evident from the data in Table 1 that more virus was inactivated as more coulombs (i.e., amperes × seconds) were applied.

In trial 7, the effect of electricity and light (no MB) on PrV was investigated. The virus titer was reduced from 1.3×10^6 to 6.3×10^5 tissue culture infective doses ($TCID_{50}$) per ml (approximately 52%). In a control trial, the dialysis procedure alone was shown to reduce the virus titer approximately 50% (data not shown). Obviously, electricity and light had a negligible effect on the virus.

With the apparatus set at the maximum current for the maximum length of time, medium titered virus (10^5 $TCID_{50}$/ml) could be totally inactivated (Table 1, trial 5) whereas higher-titered PrV (10^6 $TCID_{50}$/ml) could not (Table 1, trial 8). The procedure was repeated (Table 1, trial 10) with a magnetic stir bar during the photoinactivation procedure to ensure maximum exposure of virions to electrical current and light. The stirring apparently helped since total inactivation of PrV (1.7×10^6 $TCID_{50}$/ml) was achieved in 12 min. Photodynamic inactivation proved to be an effective, simple, and inexpensive method of inactivating PrV. The antiviral effect was shown to be dependent on the amount of electricity applied and the concentration of dye used.

This study was supported in part by cooperative agreement no. 58-5198B-9-818 of the Agricultural Research Service, U.S. Department of Agriculture, and was published as paper no. 9183 of the Agricultural Experiment Station, Purdue University.

LITERATURE CITED

1. **Herzberg, K.** 1931. Vaccinevirus-versuche. Klin. Wochenschr. **10**:1626–1627.
2. **Melnick, J. L., and C. Wallis.** 1975. Photodynamic inactivation of herpesvirus. Perspect. Virol. **9**:297–314.
3. **Melnick, J. L., and C. Wallis.** 1977. Photodynamic inactivation of herpes simplex virus: a status report. Ann. N.Y. Acad. Sci. **284**:171–181.
4. **Raab, O. Z.** 1900. Ueber die Wirkung fluorescirender Stoffe auf Infusorien. Z. Biol. **39**:524–546.
5. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27**:493–497.
6. **Sun, I. L., D. P. Gustafson, and G. Scherba.** 1978. Comparison of pseudorabies virus inactivated by bromo-ethyleneimine, ^{60}Co irradiation, and acridine dye in immune assay systems. J. Clin. Microbiol. **8**:604–611.
7. **Swartz, M. R., L. E. Schnipper, A. A. Lewin, and C. S. Crumpacker.** 1979. Inactivation of herpes simplex virus with methylene blue, light and electricity. Proc. Soc. Exp. Biol. Med. **161**:204–209.
8. **Wallis, C., and J. L. Melnick.** 1964. Irreversible photosensitization of viruses. Virology **23**:520–527.
9. **Wallis, C., and J. L. Melnick.** 1965. Photodynamic inactivations of animal viruses: a review. Photochem. Photobiol. **4**:159–170.