ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1976, p. 176-178
Vol. 10, No. 1

Printed in U.S.A.

Photoinactivation of Venezuelan Equine Encephalitis Virus Mediated by Tetracyclines

JOSE ESPARZA,* CARMEN I. PIÑA, AND ERNESTO NOVO

Instituto Venezolano de Investigaciones Científicas, Centro de Microbiología y Biología Celular, Apartado 1827, Caracas, Venezuela

Received for publication 1 March 1976

Demethylchlortetracycline and, to a lesser extent, chlortetracycline were found to mediate the in vitro photoinactivation of Venezuelan equine encephalitis virus.

Viruses can be rendered sensitive to visible light when treated with dyes such as acridine orange, proflavine, neutral red, and others (11). Dyes bind to the virus nucleic acid, acting as light absorbers for photochemical reactions in which molecular oxygen takes part, resulting in the inactivation of nucleic acid infectivity (6, 9). Phenomena of photosensitization have been described in a variety of biological systems, including drug-induced photosensitivity in humans (7). Treatment with the antibiotic demethylchlortetracycline (DMCT) results in a high frequency of sun-induced reactions, manifested as exaggerated sunburn-type lesions, which disappear soon after discontinuation of the drug (3, 8). Phototoxicity of DMCT has also been demonstrated using tissue culture techniques (5). The ability of DMCT and other tetracyclines to induce photosensitization in Venezuelan equine encephalitis virus is described in this report.

Vero cells, maintained in disposable tissue culture flasks (Falcon Plastics, Oxnard, Calif.), were inoculated with Venezuelan equine encephalitis virus, using a multiplicity of infection of 0.1 plaque-forming unit/cell. The virus used was the TC-83 strain, subjected in our laboratory to several passages in different cell lines. After a 1-h absorption at 37 C, infected cultures were washed once with phosphate-buffered saline. Cells were maintained in Eagle medium with a double concentration of vitamins and amino acids, 2% lactalbumin hydrolysate, 0.66% yeast extract, 0.16% sodium bicarbonate, 1.4% ovalbumin (fraction V), 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2.5 μg of amphotericin B per ml (2). In some experiments, DMCT (no. 48151, lot 338; a gift of Cyanamid de Venezuela, Caracas) was added to the maintainance medium to a final concentration of 1, 10, 50, or 100 μg/ml. After the addition of the drug, all handling of the cultures was done in the dark.

Infected cultures were incubated at 37 C through 72 h postinfection and harvested by one cycle of freezing and thawing. Suspensions were centrifuged at 2,000 rpm for 15 min, and 1-ml aliquots of supernatant fluid were dispensed into glass test tubes. Samples were exposed 10 cm from three "daylight-type" fluorescent lighting tubes (15 W each), giving a total of 5,000 lx of illumination. After light exposure for 30, 60, or 90 min, samples were immediately assayed in Vero cells by a plaque formation method using an agarose-containing, serum-free overlay (2).

Concentrations of DMCT up to 50 μg/ml present in the maintainance medium were nontoxic for the cells and resulted in normal virus yields. A concentration of 100 μg/ml induced a cytopathic effect in uninfected cultures and resulted in a 10-fold decrease in the yield of infectious virus. When virus grown in the presence of 1 to 10 μg of DMCT per ml was exposed to light, no significant inactivation of infectivity was observed. However, virus grown in the presence of 50 or 100 μg of DMCT per ml was readily inactivated by visible light (Fig. 1A). After 90 min of light exposure, a drop in the infectivity titer of 4 to 6 logs was observed when using 50 and 100 μg of DMCT per ml, respectively. Virus grown in the absence of DMCT expressed a slight decrease in titer, probably due to an intrinsic photosensitivity of the virus or to photosensitizing agents present in tissue culture medium (1). Control suspensions, containing virus grown in the presence of DMCT but not exposed to light, were not inactivated.

Virus grown in the absence of DMCT and mixed with the drug immediately prior to light exposure was also found to become photosensitive (Fig. 1B), being inactivated at a rate comparable to that observed with virus grown in the presence of the drug.

The ability of other tetracyclines to induce photosensitization was studied by mixing the

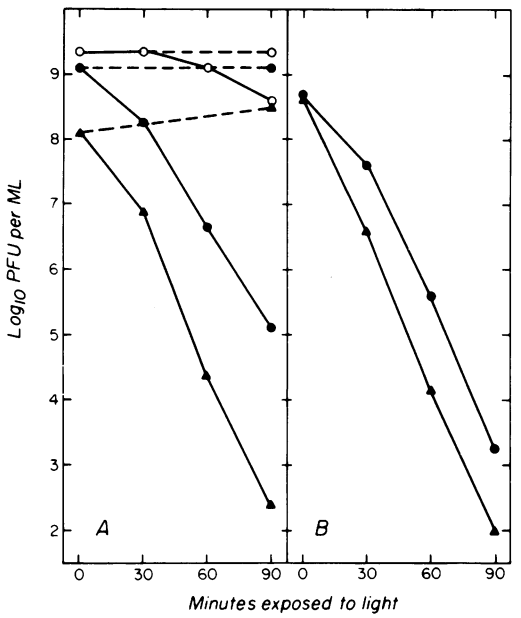

FIG. 1. *Photosensitization of Venezuelan equine encephalitis (VEE) virus by DMCT. (A) Photoinactivation of VEE virus grown in the presence of DMCT. (B) Photoinactivation of VEE virus grown in the absence of DMCT and mixed with the drug immediately prior to light exposure. Symbols: ○, no DMCT; ●, 50 μg of DMCT per ml; ▲, 100 μg of DMCT per ml. Solid lines represent samples exposed to light; broken lines represent samples kept in the dark.*

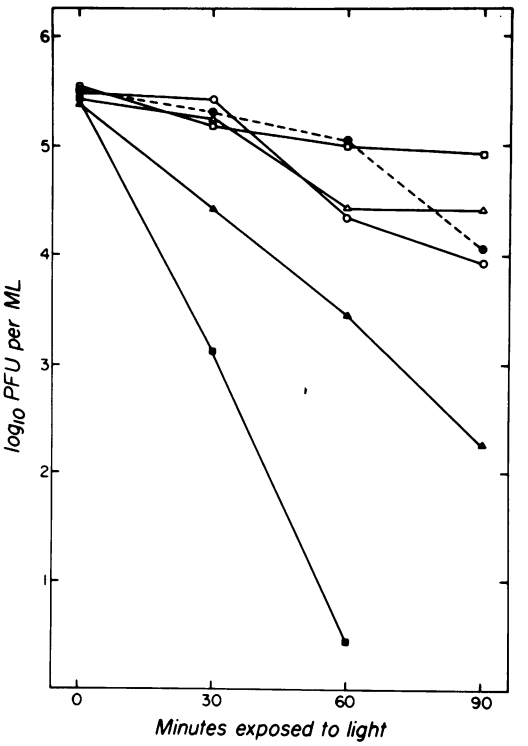

FIG. 2. *Photosensitization of Venezuelan equine encephalitis virus by different tetracyclines. Symbols: ●, control; □, oxytetracycline; △, tetracycline hydrochloride; ○, tetracycline crystalline; ▲, chlortetracycline; ■, DMCT.*

virus with the drugs at a final concentration of 100 μg/ml and exposing to light as described. Besides DMCT, only chlortetracycline was found to mediate virus photoinactivation, but at a slower rate than DMCT (Fig. 2).

The experiments described indicated that DMCT and, to a lesser extent, chlortetracycline act as sensitizer agents in the in vitro photoinactivation of Venezuelan equine encephalitis virus. The mechanism(s) of photoinactivation of Venezuelan equine encephalitis virus by tetracyclines is not known at present. The site of action of the drug remains to be established: the ability of DMCT and chlortetracycline to induce photosensitization when virus and drug are mixed in vitro seems to indicate that the possible site(s) of action could be exposed in the viral envelope or, alternatively, that the drug could penetrate through the envelope and capsid to bind an active site in the virus ribonucleic acid.

In this regard, it is interesting to note that it has recently been reported that chlortetracycline sensitizes tobacco mosaic virus ribonucleic acid to near- and middle-ultraviolet light (10).

The demonstration of tetracycline-induced virus photosensitization suggests that this biologically simple system could be used in the study of the mechanisms of drug-induced phototoxicity. It should be pointed out that photoreactive dyes are now being used in clinical medicine for the treatment of surface viral infections (4).

LITERATURE CITED

1. **Appleyard, G.** 1967. The photosensitivity of Semliki Forest and other viruses. J. Gen. Virol. 1:143-152.
2. **Bergold, G. H., and R. Mazzali.** 1968. Plaque formation by arboviruses. J. Gen. Virol. 2:273-284.
3. **Blank, H., S. I. Cullen, and P. M. Catalano.** 1968. Photosensitivity studies with Demethylchlortetracycline and Doxycycline. Arch. Dermatol. 97:1-2.
4. **Felber, T. D., E. B. Smith, J. M. Knox, C. Wallis, and J. L. Melnick.** 1973. Photodynamic inactivation of Herpes simplex. Report of a clinical trial. J. Am. Med. Assoc. 223:289-292.
5. **Freeman, R. G., W. Murtishaw, and J. M. Knox.** 1970. Tissue culture techniques in the study of cell photobiology and phototoxicity. J. Invest. Dermatol. 54:164-169.
6. **Fukunaga, T., N. Ishikawa, A. Igarashi, and K. Fukai.** 1967. Photodynamic inactivation of Japanese Encephalitis virus. Biken J. 10:1-9.
7. **Harber, L. C., and R. L. Baer.** 1972. Pathogenic mechanisms of drug-induced photosensitivity. J. Invest. Dermatol. 58:327-342.

8. **Harber, L. C., T. A. Tromovitch, and R. L. Baer.** 1961. Studies on photosensitivity due to Demethylchlortetracycline. J. Invest. Dermatol. 37:189–193.
9. **Hiatt, C. W., E. Kaufman, J. J. Helprin, and S. Baron.** 1960. Inactivation of viruses by photodynamic action of Toluidine blue. J. Immunol. 84:480–484.
10. **Murphy, T. M.** 1975. Inactivation of Tobacco Mosaic virus ribonucleic acid by near- and middle-ultraviolet light: sensitization by sulfanilamide and chlortetracycline. Biochem. Biophys. Res. Commun. 65:1108–1114.
11. **Wallis, C., and J. L. Melnick.** 1965. Photodynamic inactivation of animal viruses: a review. Photochem. Photobiol. 4:159–170.