Inactivation of vesicular stomatitis virus by photosensitization following incubation with a pyrene-fatty acid

S. Abou Rabia[1], E. Fibach[2], M. Kotler[3] and S. Gatt[1]

Departments of [1]Membrane Biochemistry and Neurochemistry, [2]Hematology and [3]Molecular Genetics, Hebrew University N Hadassah School of Medicine, Jerusalem, Israel

Received 4 June 1990; revised version received 14 July 1990

Vesicular stomatitis virus (VSV) was incubated with pyrene dodecanoic acid (P12), a fluorescent derivative of a medium-chain length fatty acid, and subjected to irradiation with a long wavelength ultra-violet light source at 366 nm (UVA). This inactivated the virus, resulting in a drastic decrease of its infectivity. The virus inactivation was proportional to the concentration of the pyrene-fatty acid, the length of exposure of the virus to P12 and the irradiation dose.

1. INTRODUCTION

Incubation of cells with various dyes, followed by illumination with a suitable light source results in a photosensitization and consequent cell death. The first photosensitizing agent, acridine orange, was discovered at the turn of the century [1] and this was followed by compounds such as porphyrins, merocyanins and phthalocyanins [2-5].

Some of these compounds were also employed for photosensitizing and inactivating viruses [6-10].

In previous experiments we have used lipid-bound pyrene as a photosensitizer [11,12]. Thus, administration of the fluorescent fatty acid derivative, pyrene dodecanoic acid (P12), into hemopoietic cells [13-15] rendered them sensitive to long wavelength ultraviolet light at 366 nm (UVA). Since leukemic cells took up and metabolized the sensitizing compound to a greater extent than their normal counterparts, this procedure could be utilized for selective killing of leukemic cells [16] and might serve for purging of bone marrow derived from patient in remission prior to autologous transplantation. In other experiments, P12-linked sphingomyelin was administered into skin fibroblasts derived from normal individuals and patients with Niemann-Pick disease. Irradiation of the cells with UVA permitted a selective killing of the lipidotic cells (manuscript submitted for publication).

In the present study we have tested the effect of P12-mediated photosensitization on inactivation of an enveloped virus. The results indicated that incubation of vesicular stomatitis virus (VSV) with P12 followed by irradiation with UVA resulted in a loss of infectivity of the virus.

Correspondence address: S. Gatt, Departments of Membrane Biochemistry and Neurochemistry, Hebrew University, Hadassah School of Medicine, Jerusalem, Israel

Abbreviations: VSV, vesicular stomatitis virus; P12, 12-(1-pyrene)-dodecanoic acid; PFU, plaque forming unit; UVA, long wavelength ultraviolet light

2. MATERIALS AND METHODS

Pyrene dodecanoic acid (P12) was purchased from Molecular Probes (Junction City, OR, USA).

BSC-1 cells, derived from monkey kidney were cultured in Dulbecco modified essential medium (DMEM, Gibco Labs., Grand Island, NY) supplemented with 5% fetal calf serum (FCS, Kibbutz Beth Haemek, Israel).

Vesicular stomatitis virus (VSV) was multiplied by infecting BSC-1 cells, the culture medium was harvested, clarified by centrifuging for 10 min at 10 000 × g and stored at −70°C. The titer of VSV was determined either by the standard plaque assay using 50 mm Nunc dishes, or by end-point dilution in a 96-well plate [17]. In either case, BSC-1 confluent monolayers were infected with serial decimal dilutions of the virus and, following overnight incubation, stained with Gentian Violet. In the former assay the number of plaques were counted, while in the latter wells were scored and the last viral dilution that caused cell death was determined. For studying photosensitization by the pyrene fatty acid, a 1 mM solution of P12 in dimethylsulfoxide was used as stock solution. The required quantity was diluted 1:50 in DMEM-5% FCS, and 50 μl of this solution was mixed with 50 μl virus in DMEM-5% FCS and after the indicated incubation time irradiated. The virus was irradiated for various intervals using 2 × 15 tubes emitting at long-wave UVA bands from 320 to 380 nm with a peak at 366 nm at an incident radiation intensity of 5 mW/cm^2 (both lamps and radiometer from Vilber-Lourmat, Marne-le-Vallees, France).

3. RESULTS

Irradiation of vesicular stomatitis virus, which had been incubated with P12, at 366 nm (UVA) resulted in a photosensitization-induced decrease in the titer of the virus.

Fig. 1 demonstrates the three basic parameters which

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

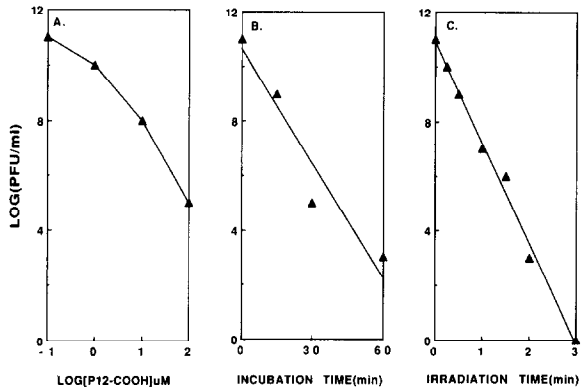

Fig. 1. Effect of P12 concentration, incubation and irradiation times on inactivation of vesicular stomatitis virus. (A) VSV (50 μl) in medium plus 5% FCS was incubated for 1 h with increasing concentrations of P12, diluted in 50 μl phosphate-buffered saline and irradiated with UVA for 30 s. The titer is expressed as the logarithm of plaque forming units (PFU) per ml. Control virus, that was incubated in the absence of P12 prior to irradiation, had a titer of 10^{11}. (B) VSV (50 μl) in medium supplemented with 5% FCS was incubated with 10 μM P12 in 50 μl of the same medium and irradiated with UVA for 2 min. (C) VSV (50 μl) in medium plus 5% FCS was incubated for 1 h with 10 μM P12 in medium plus 5% FCS and irradiated with UVA for various intervals.

controlled the decrease of the titer of VSV, namely: the concentration of P12, the length of incubation of the virus with P12, and the irradiation dose.

Fig. 1A, which describes the effect of increasing concentrations of P12, demonstrated that at 100 μM P12 a 0.5-min irradiation decreased the titer by 6 logs. Fig. 1B, which shows the effect of the length of incubation with P12 (10 μM) indicated that after 1 h incubation, UVA irradiation for 2 min decreased the titer by about 8 logs. Incubation of virus with P12 without irradiation or alternatively irradiation of VSV which has not been incubated with P12 had no effect on the titer of the virus.

The effect of the irradiation dose could be modified by changing the distance of the UVA source from the plate (i.e: the UVA-light flux; data not shown), or the length of irradiation. Fig. 1C demonstrates the latter, indicating that by increasing the UVA-dose a practically complete inactivation of the virus was achieved.

4. DISCUSSION

Incubating P12 with cultured eukaryotic cells resulted in a penetration of the acid into the plasma membrane of the cells, subsequent translocation and incorporation into their neutral- and phospholipids [13–15]. For studying photosensitization of a virus by P12, we selected an enveloped virus (VSV), expecting that the pyrene fatty acid would penetrate into the envelope and, via its hydrophobic portion (i.e. the pyrene nucleus and methylene groups), remain embedded in it. Though we have no direct evidence that this occurs, the expectation is supported by the increased sensitivity to irradiation with increasing concentrations of P12 or length of incubation of the latter with the virus. Very similar results were obtained in our studies of killing of hemopoietic cells by incubation with P12 and irradiation at 366 nm [11,12,16].

Following incubation of VSV with P12, illumination for 2–3 min with a light source at 366 nm, was sufficient to cause a nearly total loss of the infectivity of the virus. The titer decreased with increasing concentrations of P12 and the length of incubation of the virus with P12 as well as the irradiation dose.

The data show that the pyrene fatty acid employed, and most probably other pyrene lipids which penetrate into the envelope, e.g. P12-sulfatide, [18], can be used for photosensitizing and inactivating enveloped viruses. It would be of interest to define whether this procedure could be useful for sterilization of biological materials, i.e., blood products, that may be contaminated by such viruses and whether photoinactivated viruses can preserve their antigenicity, thus permitting their use as vaccines.

REFERENCES

[1] Raab, O. (1900) Z. Biol. 39, 524–546.
[2] Kelly, J.F. and Snell, M.E. (1976) J. Urol. 115, 150–151.
[3] Ben Hur, E. and Rosenthal, I. (1984) Photochem. Photobiol. 43, 615–619.
[4] Spikes, J.D. (1986) Photochem. Photobiol. 43, 691–699.
[5] Sieber, F., Stuart, R.K., Rowley, D., Sharkis, S.J. and Sensenbrenner, L. (1987) Leuk. Res. 11, 43–49.
[6] Wallis, C. and Melnick, J.L. (1965) Photochem. Photobiol. 4, 159–170.
[7] Sieber, F., Krueger, G.J., O'Brien, J.M., Schober, S.L., Sensenbrenner, L.L. and Sharkis, S.J. (1989) Blood 73, 345–350.
[8] Matthews, J.L., Meuman, J.T., Sogandares-Bernal, F., Judy, M.M., Skiles, H., Levenson, J.E., Marengo-Rowe, A.H. and Chanh, T.C. (1988) Transfusion 28, 81–83.
[9] Perlin, M., Mao, J.E., Otis, E.R. Shipkowitz, N.L. and Duff, R.B. (1987) Antiviral Res. 7, 43–51.
[10] Pompei, R., Cisani, G., Foddis, G. and Marcialis, M.A. (1989) Microbios. 58, 101–111.
[11] Fibach, E., Morand, E. and Gatt, S. (1986) J. Cell Sci. 85, 149–159.
[12] Fibach, E., Rachmilewitz, E.A. and Gatt, S. (1989) Leuk. Res. 13, 1099–1104.
[13] Morand, O., Fibach, E., Dagan, A. and Gatt, S. (1982) Biochim. Biophys. Acta 711, 539–550.
[14] Morand, O., Fibach, E. and Gatt, S. (1982) Biochim. Biophys. Acta 613, 143–150.
[15] Morand, O., Fibach, E., Livni, N. and Gatt, S. (1984) Biochim. Biophys. Acta 793, 95–104.
[16] Fibach, E., Gatt, S. and Rachmilewitz, E.A. (1990) Exp. Hematol. 18, 89–93.
[17] Luria, S.E., Darnell, J.E. Jr., Baltimore, D., Campbell, A. In General Virology, 3rd edn, Wiley, New York, pp. 21–32.
[18] Rousseau, A., Kotler, M., Gatt, S. FEBS Lett. (1990) 261, 271–273.