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EFFECT OF UV-IRRADIATION ON ROTAVIRUS

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Summary. – The effect of UV-irradiation on SAll rotavirus infectivity was followed. The time course of infectivity inactivation in general showed an one-hit pattern. Two basic effects of UV-irradiation on virus particles were investigated: the phenomenon of RNA-protein linkages and the formation of uracil dimers. To determine the number of uridine dimers, ^{3}H-uridine labelled purified rotavirus was exposed to UV-irradiation, subsequently the RNA was extracted and analysed by ascending paper chromatography. Formation of photodimers was found to be an important mechanism of rotavirus inactivation at conventional UV-irradiation; the RNA-protein linkages were registered at high irradiation dosis only.

Key words: UV-irradiation; rotavirus; inactivation; uracil dimers; RNA-protein linkages

Introduction

UV-irradiation is one of the important environmental factors which inactivates viruses under natural conditions. It is used for inactivation of virus preparations as well as to study the structure of viral particles. The mechanisms of action of UV-irradiation on viral particles are known in general. The basic photoeffects are cleavage of polynucleotide chains, formation of nucleic-protein covalent linkages, as well as nucleotide dimers and other photolysis products. Any of these photo-induced damages may be lethal for the virus. The contribution of each of the above outlined mechanisms to the UV-inactivating effect is variable and may be different for different viruses. Thus, in the case of UV-inactivation of picornaviruses the formation of uracil dimers is considered to be the major factor responsible for inactivation of viral infectivity (Miller and Plagemann, 1974; Smirnov *et al.*, 1983). Formation of RNA-protein linkages plays a significant role in inactivation of orthomyxoviruses (Kolodkina *et al.*, 1981; Kaverin *et al.*, 1987). As for double-stranded RNA viruses, data are avai-

lable only concerning the course of reovirus infectivity inactivation (McClain and Spendlove, 1966) and the morphological changes of rotaviruses following high-dose irradiation (Meng *et al.*, 1987). Nothing is known about the molecular effects of irradiation (linkage or dimer formation). The present work provides information indicating the possible role of uridine dimer formation in UV-inactivation of SAll rotavirus and the lack of formation of RNA-protein linkages in the case of irradiation at doses ensuring infectivity inactivation.

Materials and Methods

Virus. SAll rotavirus was propagated on continuous culture of green monkey kidney cells (RAMT) in Eagle's MEM medium supplemented with 1 μg/ml trypsin. Before inoculation the virus was inactivated by incubation with trypsin (10 μg/ml, 1 hr, 37 ^{0}C).

Infectivity titration. An immunocytochemical assay was used to determine the amount of SAll infectious rotavirus (Amitina *et al.*, 1988). Virus-inoculated RAMT cells grown in flat bottom polystyren plates were incubated at 37 ^{0}C for 24 hr, subsequently fixed in cooled 85 % acetone, then treated with specific antirotavirus rabbit serum, peroxidase-labelled antibodies to rabbit IgG (commercial preparation obtained from Leningrad Research Institute of Vaccines and Sera) and the peroxidase substrate solution (3-amino-9-ethylcarbasol, „Sigma"). The reaction was stopped by adding distilled water. Using a light microscope the number of stained foci per well was counted. The virus titre was expressed as the number of focus-forming units (FFU) per 1 ml.

Production and purification of ^{3}H-uridine-labelled SA11 rotavirus. Monolayer culture of RAMT cells grown in rollers was inoculated with trypsin pre-activated SAll rotavirus (10 - 20 FFU/cell). Following 1 hr-adsorption at 37 ^{0}C the cells were washed, exposed to ^{3}H-uridine (50 MBq/ml) in Eagle's-MEM medium supplemented with trypsin (1 μg/ml) and incubated at 37 ^{0}C for 24 hr. The virus was purified by the method of Berds (1982). The procedure included low speed centrifugation, Freon 113 („Serva") treatment, sedimentation through sucrose layer and equailibrium centrifugation in CsCl. Fractions from the 1.36 g/cm^3 buoyant density region (density of double-shelled viral particles) were collected and dialysed against STE buffer (Tris-HCl 0.01 mol/l, pH 7.5; NaCl 0.1 mol/1; EDTA 0.001 mol/1).

UV-irradiation. Prior to irradiation the viral suspension was ultrasonicated (22 KHz, 6 x 30 c), irradiated for the given time interval and then kept in darkness. As a source of UV-irradiation, BUF-15 bactericide lamp was used, with 80 % of irradiation at 254 nm wave length (the rest within the visible region of the spectrum); 1-ml specimen was irradiated in a quartz cuvette, 1 cm^2 in cross-section, placed in an icy bath at a distance of 10 or 2 cm from the irradiation source. The irradiation dose was evaluated according to the intensity of light flow by uridine actinometry (Colvert and Pitts, 1968).

RNA extraction. Purified ^{3}H-uridine labelled virus was suspended in STE buffer. Sodium dodecylsulphate (SDS) in the final concentration of 1 % was added to the viral suspension and the latter was incubated for 20 min at room temperature. Thereafter, 2-ß-mercaptoethanol (ME) was added to the mixture in the final concentration of 0.5 %. Virion RNA was extracted in cold for 20 min. An equal volume of deproteinizing mixture was added to the material; it contained STE-buffer saturated phenol (4:1), chloroform and isoamyl alcohol in the ratio of 49:49:2, respectively. The phenol and water phases were separated by centrifugation at 2000 rev/min for 15 min using a Janetzki K-70 centrifuge. The same procedure was used for repeated extraction.

Ascending paper chromatography. To determine the UV-induced pyrimidine dimers, ascending paper chromatography was made as described by Miller and Plagemann (1974). ^{3}H-uridine labelled virus preparations were exposed to UV-irradiation, mixed with 200 μg bovine serum albumin and 1 ml 1mol/l perchloric acid and incubated at 4 ^{0}C for 30 min. The precipitate was sedimented by low speed centrifugation at 2000 rev/min for 10 min at 4 ^{0}C and exposed to acid

hydrolysis in 88 % formic acid at 175 ^{0}C for 2 hr. Hydrolysates were analysed using ascending chromatography on Whatman No. 1 paper at room temperature for 18 hr. The buffer system contained 2-isopropanol, concentrated ammonium hydrate and 0.1 mol/l boric acid, in the ratio of 7:1:2, respectively. Air-dried chromatograms were cut into 1 cm segments in the direction of ascending buffer migration, placed in scintillation fluid and total radioactivity was measured in a liquid scintillation counter Tricarb („Packard", U.S.A.).

Results and Discussion

The inactivation of SAll rotavirus infectivity occurred in one-hit pattern within the dose range which decreased infectivity by 1.0 – 1.5 log. Thereafter, usually a short plateau occurred and then inactivation followed at the initial rate (Fig. 1). The type of this curve indicates the admixture of the component inactivated in a two-hit-pattern (it may be diploid virions or aggregates consi-

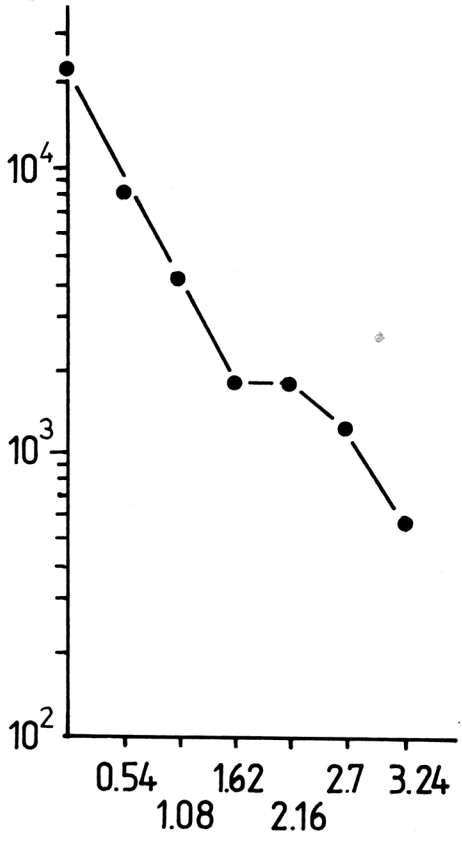

Fig. 1
UV-irradiation-induced inactivation of SAll rotavirus
Abscissa: UV doses (erg/mm^2 x 10^3); ordinate: rotavirus infectivity (in $\log_{10}$ FFU).

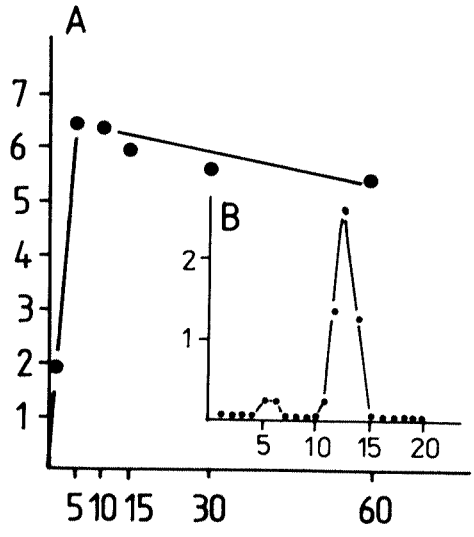

Fig. 2
The effect of UV-irradiation on the formation of uracil dimers in rotavirus RNA
A - suspension of ^{3}H-uridine labelled rotavirus was exposed to UV-irradiation and analysed (see "Materials and Methods"). Ordinate: ^{3}H-uracil dimers (% of the total ^{3}H-label); abscissa: time of UV-irradiation (min).
B - chromatographic distribution of hydrolysate of ^{3}H-rotavirus exposed to UV-irradiation in the dose of 4.91×10^4 erg/mm^2. Ordinate: ^{3}H-label (cpm x 10^3); abscissa: numbers of chromatogram fractions.

sting of two infectious viral particles). However, the dominating amount of infectious particles (over 90 %) consisted of single virions inactivated in a one-hit-pattern.

To determine the number of uridine dimers, the purified virus labelled with ^{3}H-uridine was exposed to UV-irradiation, subsequently the virion RNA was extracted, hydrolysed and analysed by ascending paper chromatography. Nonirradiated specimens revealed one peak in the position occupied by uracil. In irradiated specimens (with increasing doses of UV-irradiation) a second peak became apparent, which location on the chromatogram was typical for uracil dimers (fractions 5,6, Fig 2B). The number of dimers rose with increasing doses of irradiation, later on it reached a plateau and remained constant (Fig. 2A).

To determine the covalent RNA-protein linkages at UV-irradiation, phenol detergent method without pronase treatment was used to extract RNA from ^{3}H-uridine labelled purified virus after irradiation (Raghow and Kingsbury, 1979). In the case of RNA-protein linking, the RNA should be transferred from aqueous to phenol phase. Fig. 3 presents the data on RNA extraction from viral preparations exposed to UV-irradiation in varying doses, and its distribution in the two-phase system. The curve depicting decreasing RNA amount in the aqueous phase (the formation of RNA-protein covalent linakges) is markedly different from that of uracil dimer formation. At 5.89×10^5 erg/mm^2 dose of UV-irradiation only 55 % of viral RNA is covalently linked with protein, whereas the highest number of dimers has formed already at the dose of 4.91×10^4 erg/mm^2.

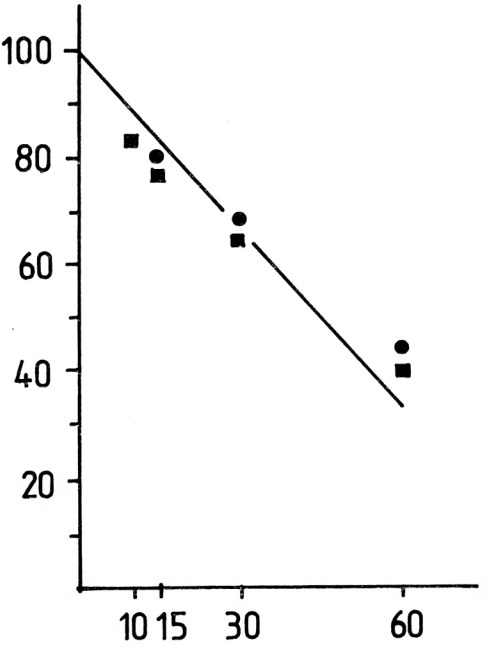

Fig. 3
RNA extraction from ^{3}H-uridine labelled rotavirus exposed to UV-irradiation
Ordinate: per cent of ^{3}H-RNA in aqueous phase; abscissa: time of UV-irradiation (min). Squares and circles represent the data of 2 experiments.

The course of UV-induced rotavirus infectivity inactivation described in this report closely resembles that registered in previous studies (McClain and Spendlove, 1966) in agreement with the classification of both genera to one family. They contain segmented double-stranded RNA of similar size and packing, thus the target of UV-inactivation should be similar for both. No measurement of molecular effects induced by UV-irradiation of viruses from that family have been done previously. Analysis of the relationship between the formation of uridine dimers and/or of RNA-protein linkages on one hand and UV-irradiation on the other sheds some light on the possible mechanism of rotavirus inactivation following conventional (low intensity) UV-irradiation. The dose inducing one inactivation event in the genome (37 % survival) is 700 erg/mm^2 (Fig. 1). The dose causing dimerization of 2 % ^{3}H-uridine is 9.82 x 10^3 erg/mm^2 (Fig. 2). Both values coincide with those for picornaviruses (Miller and Plagemann, 1974); on the basis of these values for Mengo virus the number of dimers per 1 inactivation event was determined to be 1.7.

Thus, based on the calculations reported by Miller and Plagemann (1974) it seems likely that formation of photodimers is probably the major mechanism of rotavirus inactivation at UV-irradiation. On the contrary, RNA-protein linkages arise only at high doses of irradiation. The number of linkages per 1 inactivating event is calculated to be about 0.001. In other words, by the time when the first RNA-protein linkage appeared the viral genome has already been inactivated due to previous formation of the uracil dimers.

Previously we demonstrated the significant role of covalent RNA-protein linkages in the case of influenza virus UV-induced inactivation, and suggested that contribution of different molecular phenomena in the inactivating effect of UV-irradiation varies for different viruses (Kolodkina *et al.*, 1981, Kaverin *et al.*, 1987). The data presented above indicate that RNA-protein linkages of rotaviruses in contrast to inactivation of orthomyxoviruses do not have a significant role in the inactivation, and, vice versa, the formation of photodimers appears to be an important factor of inactivation of rotaviruses. A similar finding was described previously with picornaviruses (Miller and Plagemann, 1974). However, it should be pointed out that factors limiting the appearance of RNA-protein linkages may be different for picorna- and rotaviruses. In picornaviruses with single-stranded RNA the limitation is either purely topological, i. e. it is accounted for by a small number of contact sites of RNA with a protein capsid, or, which is more likely, it is determined by the pattern of the interaction of protein subunits with the RNA (e. g. the predominant contact with sugarphosphate backbone but not with nitrogen bases). The limitation for rotaviruses may be the result of nitrogen bases coupled in RNA duplex which prevents their participation in RNA-protein interactions.

Although these results suggest the active role of photodimerization of nucleotides in UV-induced rotavirus inactivation, the significant contribution of other effects, i. e. of RNA strand cleavage should not be ruled out. Further studies are required to elucidate the role of these effects.

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