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Virus inactivation by high intensity broad spectrum pulsed light

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Abstract

The inactivation of a range of enveloped and non-enveloped viruses by treatment with high intensity broad spectrum pulsed light (PureBright®) has been investigated. In phosphate buffered saline, a dose of 1.0 J/cm^2 was sufficient to effectively inactivate, i.e. $>4.8-{>}7.2$ log of all the viruses tested, i.e. Sindbis, HSV-1, vaccinia, polio-1, EMC, HAV, CPV, BPV and SV40. However, in the presence of protein, i.e. 5% v/v foetal-calf serum (0.2% w/v protein), virus inactivation was less effective. At a dose of 2.0 J/cm^2, virus inactivation was $5.0-{>}6.4$ log, however, HSV-1 (3.8 log), BPV (2.4 log) and SV40 (2.9 log) were all relatively resistant. This virus inactivation procedure may have application for increasing the safety of therapeutic biological products.

Keywords: PureBright®; Broad spectrum pulsed light; Ultraviolet light; Virus inactivation

1. Introduction

The ability of ultraviolet (UV) light to inactivate cellular microorganisms and viruses is well known (Shechmeister, 1983; Kallenbach et al., 1989), however, such systems have only found limited practical application in the pharmaceutical industry. Recently a novel technique based on this approach has been developed, i.e. PureBright® which, in contrast to conventional UV light, uses high intensity broad spectrum white light delivered in short bursts (Cover, 1999, 2000; Dunn et al., 1998). The wavelengths covered are about 200–1100 nm, i.e. essentially similar to that of sunlight but with the inclusion of greater amounts at wavelengths below 300 nm. Each flash is of a very short duration, i.e. 300 μS but has an intensity of at least 1000 times that of conventional UV light. PureBright® treatment has been shown to be effective for the inactivation of bacteria in pharmaceutical products, e.g. water, saline and glucose, medical devices, packaging, surfaces and drinking water (Cover, 2000; Dunn et al., 1998; Furukawa et al., 1999). These properties may give this system potential advantages for virus inactivation over those previously described (Chin et al., 1995; Hart et al., 1993; McLean et al., 1999). However, although its effect on bacteria and other cellular microorganisms has been extensively studied, much less is know about the capacity of this method to inactivate viruses.

In the present study, the inactivation of a range of viruses by varying doses of high intensity broad spectrum white light, has been investigated using a small-scale PureBright® laboratory system. A number of resistant non-enveloped viruses have been included as such agents have proved particularly difficult to remove or inactivate in therapeutic biological products (Committee for Proprietary Medicinal Products, 2001; Roberts, 1996). In order to investigate the effect of the presence of protein on virus inactivation, studies were conducted in both the absence and presence of foetal-calf serum (FCS).

2. Methods

A range of enveloped, i.e. Sindbis, herpes simplex virus type 1 (HSV-1) and non-enveloped viruses, i.e. encephalomyocarditis (EMC), polio virus type 1, hepatitis A (HAV), bovine parvovirus (BPV) and canine parvovirus (CPV), were used. Virus was diluted (1 in 50) in Dulbecco's phosphate buffered saline (PBS) without

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calcium and magnesium, PBS with 5%v/v FCS, or Iscoves Modified Dulbecco's medium (IMDM) with 5%v/v FCS. The protein concentration of the serum containing solutions was determined by the Biorad dye binding method using BSA as standard.

Samples to be treated were placed in small plastic sample dishes to give a depth of ca. 5 mm. The samples were then treated using a PureBright® Anti-Pathogen Research (APR) bench-top system (PurePulse® Technologies, San Diego, CA, USA). The intensity (fluence) was adjusted, by varying the distance of the sample from the Xenon gas lamp and adjusting the iris width, to give standard exposure levels of 0.25, 0.5, 1.0, 1.5, 2.0 and 2.4 J/cm^2 for each flash. The total fluence received by each sample was calculated by multiplying the fluence per flash by the number of flashes.

Virus infectivity before and after treatment was determined by plaque assay on BHK-21 cells (Sindbis, EMC), vero cells (polio-1, vaccinia, HSV-1), BSC-1 cells (HAV), MDBK cells (BPV) or A72 cells (CPV). In addition to assaying volumes of 0.5 ml on individual wells (3.8 cm^2) of 12-well cell-culture dishes, the sensitivity of the assay was increased by assaying 7 ml volumes on one or more petri dishes (58 cm^2). Virus titre was calculated from sample dilution, assay volume and plaque number. Where virus was undetectable, the titre was calculated assuming 1 plaque was present in the total volume assayed and expressed as a 'less than' value. Virus inactivation values were calculated by subtracting log virus titre after treatment from that determined for the untreated control.

3. Results

In an initial experiment the inactivation of CPV, a virus known to be highly resistant to many physico-chemical inactivation methods, was tested (Table 1). Inactivation was evaluated in several different types of solutions and was tested over a wide range of total fluence, i.e. up to 19.2 J/cm^2. In PBS alone, PureBright® treatment readily inactivated CPV at relatively low doses, i.e. 0.25 J/cm^2. However, when 5%v/v FCS was present, i.e. a protein concentration of 0.2%w/v, a dose of about 1.0 J/cm^2 was required. CPV was even more resistant to inactivation in complete cell culture medium, i.e. IMDM with 5%v/v FCS and a dose of 2.4 J/cm^2 was required for effective virus inactivation. This inhibition of virus inactivation was presumably caused by light absorption by the phenol red in the medium. Due to this effect, cell-culture medium was not evaluated further.

In subsequent experiments, the inactivation of a wider range of viruses was tested in both PBS and PBS with 5%v/v FCS (Table 2, Fig. 1). In PBS alone, there was a continuous range of sensitivity to inactivation for the different viruses tested. A total fluence of 1 J/cm^2 was usually required for effective virus inactivation, i.e. >5 log. However, some viruses were somewhat more resistant, i.e. SV40 $>$ BPV $>$ HSV-1, than the others. In the presence of 5%v/v FCS (Table 2, Fig. 1), virus inactivation was substantially lower for all the viruses. A total fluence of 2.0 J/cm^2 was usually required for effective virus inactivation, i.e. >4 log. Even under these conditions, BPV and SV40 were again significantly more resistant. The inactivation of several different picornaviruses was tested in the present study, i.e. HAV, EMC and polio-1. Inactivation ranged from e.g. 3.1 to 3.8 log (0.25 J/cm^2) or 3.2 to 4.1 log (1.0 J/cm^2) in the absence or presence of protein, respectively. Susceptibility to inactivation was thus very similar in this highly related group of viruses.

4. Discussion

While bacterial inactivation has been extensively evaluated using the PureBright system, studies on virus inactivation have been more limited. The manufacturers of the device have demonstrated the inactivation of 4 log of simian rotavirus, polio-1, bacteriophages MS-2 and PRD-1 in water, using a flow-through system at a total fluence of 0.25 J/cm^2 (Cover, 1999). The likely mechanism involved in inactivation is, as for conventional UV-irradiation, via an effect on the nucleic acid resulting in the formation of thymidine dimers (DNA) or uracil dimers (RNA) (Shechmeister, 1983; Kallenbach et al.,

Table 1
Inactivation of CPV in PBS and cell-culture medium by PureBright treatment

Conditions[a]	Log inactivation					
	Total fluence (J/cm^2)[b]					
	0.25	1.0	2.4	4.8	9.6	19.2
PBS	5.7	>6.2	>6.2	>6.2	>6.2	>6.2
PBS+5%v/v FCS	2.5	>6.3	>6.3	>6.3	>6.3	>6.3
IMDM+5%v/v FCS	1.4	3.1	5.3	>6.7	>6.7	>6.7

[a] Phosphate buffered saline (PBS) or Iscoves Modified Dulbecco's medium (IMDM), with or without foetal-calf serum (FCS).
[b] All treatments used one pulse except for 4.8 (two pulses), 9.6 (four pulses), and 19.2 (eight pulses).

Table 2
Inactivation of viruses by PureBright treatment

Virus	Log inactivation							
	PBS				PBS+5%v/v FCS			
	Total fluence (J/cm^2)[a]							
	0.25	0.5	1.0	2.0[a]	0.25	0.5	1.0	2.0[a]
Sindbis	2.7	4.8	7.2	> 7.2	1.1	2.1	3.1	5.0
HSV-1	1.8	3.2	> 4.8	> 4.8	0.9	1.2	2.2	3.8
Vaccinia	1.7	4.1	> 5.1	> 5.1	1.3	3.2	> 4.7	> 5.7
Polio-1	3.7	5.8	> 6.7	> 6.7	1.0	1.9	3.2	5.5
EMC	3.1	> 5.9	> 5.9	> 5.9	1.2	2.0	3.6	> 6.1
HAV	3.8	> 5.7	> 5.7	> 5.7	1.3	2.1	4.1	> 5.6
CPV	6.0	> 6.5	> 6.5	> 6.5	2.6	4.6	> 6.4	> 6.4
BPV	1.4	2.3	4.3	> 6.6	0.7	1.0	1.5	2.4
SV40	2.0	2.8	3.7	6.2	1.4	1.7	2.4	2.9

[a] All treatments used one pulse except the 2.0 J/cm^2 treatment which used two pulses of 1.0 J/cm^2.

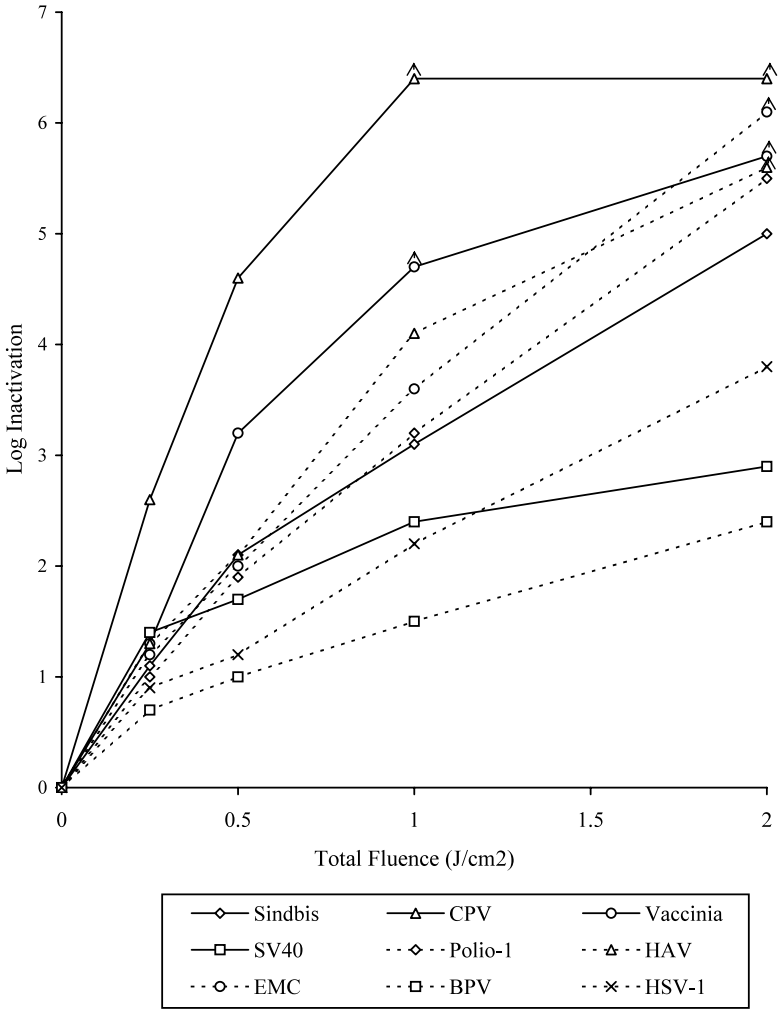

Fig. 1. Inactivation of viruses by PureBright treatment in the presence of protein. Greater than values are indicated by ∧. Data from Table 2. Viruses were diluted in PBS with 5%v/v FCS.

1989). Proteins on the other hand are generally more resistant to the effects of UV-irradiation.

In the present study, the inactivation of a range of animal viruses has been evaluated both in the presence and absence of protein. The viruses were chosen because they are commonly used as relevant or model viruses to evaluate the effectiveness of virus inactivation methods in plasma and other biological products (Committee for Proprietary Medicinal Products, 1996). PureBright treatment proved to be effective for virus inactivation in all cases. However, susceptibility to treatment varied between viruses, with some being significantly more resistant than others. Based on studies using PBS with FCS as a model system, it was found that the presence of protein substantially inhibited virus inactivation. Under these conditions, a total fluence of 2.0 J/cm^2 was required for the effective inactivation, i.e. ≥ 5.0 log of most of the viruses tested. However, even under these conditions, some viruses (BPV, SV40) were only partially inactivated, i.e. 2.4–2.9 log.

From a theoretical point of view it would seem likely that viruses with a large genome would be the most susceptible to inactivation because of their larger target size. In addition, that viruses with ds genomes (DNA or RNA) would be more resistant than those with ss genomes due to repair being possible using the undamaged strand as a template. Indeed there is some experimental evidence that this is indeed the case (Kallenbach et al., 1989). However, such a clear correlation was not seen in this limited study (Table 3). Based on the limited data available, there was some evidence for an inverse relationship between genome size and susceptibility to inactivation for the viruses with double standard genomes. However, the susceptibility of HSV-1 and vaccinia, both with large genomes of similar size, were very different in the presence of serum. The genome sizes of the single stranded virus tested were too similar to allow the relationship between genome size and susceptibility to inactivation to be assessed. All were essentially similar with regard to their susceptibility to inactivation, although BPV was clearly much more resistant than expected when compared with a related virus of identical genome size, i.e. CPV. Based on SV40 alone, viruses with a double stranded genome were more resistant to inactivation compared with those with a single stranded genome. Taken together, these findings suggest that other factors such as the proportion of the genome that encodes essential genes, genome packaging, capsid structure and presence of an envelope, may also influence inactivation.

Table 3
Relationship between virus nucleic and sensitivity to PureBright inactivation

Virus	Nucleic acid		Dose (J/cm^2)[a]	
	Type	Molecular weight (log)	PBS	PBS + 5%v/v FCS
Sindbis	ssRNA	6.6	0.4	1.5
Polio-1	ssRNA	6.4	0.3	1.3
EMC	ssRNA	6.4	0.3	1.1
HAV	ssRNA	6.4	0.3	1.0
CPV	ssRNA	6.2	0.2	0.4
BPV	ssDNA	6.2	0.9	3.7
Vaccinia	dsDNA	8.1	0.5	0.8
HSV-1	dsDNA	8.0	0.8	2.3
SV40	dsDNA	6.5	1.1	4.4

[a] Dose required to inactivate 4 log of virus. Where necessary the dose has been estimated by extrapolation.

While in the case of the three picornaviruses tested, i.e. polio-1, EMC and HAV, virus inactivation by PureBright treatment was very similar, this was not so for the two parvoviruses used, i.e. CPV and BPV. The reason for the much greater resistance of BPV compared with CPV is not clear. However, BPV, in contrast to CPV, was found to be highly aggregated as shown by filtration studies using filters with a nominal pore-size of about 35 nm (data not shown). Thus it cannot be ruled out that the degree of virus aggregation can also effect virus susceptibility to inactivation by PureBright treatment.

The range of viruses that could be inactivated by PureBright treatment included several that are known to have a generally higher resistance to at least some physicochemical inactivation methods, i.e. vaccinia, the parvoviruses CPV and BPV, SV40 and HAV. All of these, apart from BPV and SV40, were relatively susceptible to inactivation by PureBright treatment. Among these, parvoviruses such as human parvovirus B19 and minute virus of mice, are viruses of concern in plasma and recombinant products, respectively. Also, HAV has been transmitted by some factor VIII products. Thus PureBright treatment may have a potential application for virus inactivation in therapeutic biological products such as those derived from genetically engineered cells or human plasma. However, possible effects on the protein products themselves will have to be carefully investigated and the process carefully controlled in a manufacturing setting.

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