CONCISE REPORT

Use of Laser-UV for Inactivation of Virus in Blood Products

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Inactivation of virus by UV radiation was examined as a potential method for sterilization of blood products. Samples of attenuated poliovirus, platelets and plasma were uniformly irradiated with a XeCl excimer laser that delivered 40 nsec pulses of UV at 308 nm (UVB). Intensities and exposure doses were varied from 0.11 to 1.40 MW/cm² and 0.51 to 56.0 J/cm², respectively.* In studies conducted with low intensity UVB (<0.17 MW/cm²), using exposure doses ≥10.8 J/cm², it was possible to inactivate poliovirus by 4 to 6 log₁₀. Platelets irradiated with doses ≤21.5 J/cm² exhibited minimal damage as assessed by aggregation activity and spontaneous release of serotonin. Examination of the coagulation activity of irradiated plasma indicated that exposure doses ≤21.5 J/cm² resulted in a 20% increase in prothrombin and partial thromboplastin times. The use of UVB at a higher intensity (1.4 MW/cm²) over a similar range of exposure doses did not enhance viral inactivation but did result in increased damage to platelet and plasma proteins. These results demonstrate that at 308 nm there exists a “window of efficacy” for exposure doses between 10.8 and 21.5 J/cm² and peak intensities ≤0.17 MW/cm² in which a hardy virus is significantly inactivated and platelets and plasma proteins are, by functional criteria, minimally affected. Increased viral inactivation cannot be accomplished with higher UV intensities and will require additional or alternate measures.

*Glossary of terms:

Photon: a quantum of light energy that is transferred to an absorbing molecule. For 308 nm, the energy of photons is equal to 92.8 kcal/mol.

Intensity (or sample irradiance): the power (or number of photons per second) of light per unit (incident on a sample). Expressed as megawatts (MW) per square centimeter; (eg, 1 MW/cm² = 6.2 photons at 308 nm incident on each square angstrom within 40 nsec).

Exposure dose (or energy density): the cumulative energy incident on a sample per unit area; the product of intensity and total exposure duration. Expressed as joules (J) per square centimeter (eg, 1 J = 1 watt for 1 sec). In this study, 0.5 to 50 J/cm² is equivalent to 78 to 7,800 photons at 308 nm per square angstrom.

A PART OF the effort to reduce transmission of viral disease by blood products, donors are routinely screened for hepatitis B surface antigen (HBsAg) and for antibody to human immunodeficiency virus (HIV). Although such screening has been successful in providing a safer blood supply, the potential extent of this approach is limited. Screening procedures are expensive, time-consuming and, since some are necessarily hypersensitive, they result in loss of false positive donors. Finally, not all donors infected with HBsAg or HIV are detected and there are no direct tests to screen for non-A, non-B hepatitis or for other known and emerging agents potentially transmissible by blood.

The early studies of Murray et al showed that doses of UV radiation that were sufficient to inactivate hepatitis virus caused unacceptable plasma protein denaturation. Subsequently, a variety of physical and chemical agents, alone and in combination, have been demonstrated to inactivate virus while preserving some degree of plasma protein function, but no similar demonstration has been offered for cellular blood components. Since the cells most often transfused are anucleate and nonreplicating, appropriate conditions of UV irradiation should favor photo-induced damage of viral nucleic acid with minimal effects on cellular proteins. In this report, we describe results of the treatment of attenuated poliovirus (chosen as a model of a hardy, RNA virus), platelets and plasma with pulsed UV radiation at 308 nm. The demonstration of a dose-dependent decrease in virus titers with radiation doses that only moderately affected platelet and plasma function indicates that UV irradiation is a potential method for reduction of the viral bioburden of transfusible blood products.

MATERIALS AND METHODS

A XeCl excimer laser (Hyperex 460, Lumonics Inc. Kanata [Ottawa], Ontario, Canada) was used as the source of radiation at a fixed wavelength of 308 nm (the UVB region of the spectrum). Pulses (40 nsec) were delivered at rates of one to 83 pulses per second with low (0.11 to 0.17 MW/cm²) and high (1.4 MW/cm²) intensities. Exposure doses (10 to 1,000 pulses) were varied from 0.51 to 56.0 J/cm². One-milliliter samples of material to be irradiated were placed in 5-mL polyethylene tubes (Falcon no. 2063, Sarstedt, Inc, Princeton, NJ) and rotated during exposure in the beam at a fixed distance from the source. The rotation of the sample partially smooths out the nonuniformity of the sample irradiance due to absorption within the plasma or buffer with albumin. The irradiance within the sample decreased 3.8-fold from the surface of the sample to the center (see Discussion).

Platelets obtained from whole blood were prepared as previously described 3 with the following modifications: platelet wash buffer, pH 7.40, contained 137 mmol/L NaCl, 2.7 mmol/L KCl, 0.5 mmol/L NaH₂PO₄, 12 mmol/L NaH₂CO₃, 1 mmol/L MgCl₂, 5.6 mmol/L glucose, and 50 g/L human albumin (ICN Immunobiologics, Lisle, IL). Prostaglandin E₁ (Sigma Chemical Co, St Louis), final concentration 1 μmol/L, was added to the platelet rich plasma and the first wash. The washed platelets were resuspended in wash buffer containing 0.5 mmol/L CaCl₂. One-million liters of the platelet

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serotonin following irradiation. Results are expressed as percent of the final platelet suspension added to described. Before platelet washing, platelet rich plasma (PRP) was obtained from Ortho Diagnostic Systems, Inc. Raritan, NJ. Control was suspension (400,000/μL) were subjected to UVB30 as described above.

Sabin’s attenuated poliovirus type 1 was suspended in platelet wash buffer containing 50 g/L human serum albumin and 1-mL samples subjected to UVB30 as described above. Poliovirus activity was determined by virus titration on Vero cell cultures grown in microtiter trays. Titters are expressed as infectious doses (50%) in 0.05 mL of experimental material.

Spontaneously released serotonin was assayed as previously described. Before platelet washing, platelet rich plasma (PRP) was incubated with 0.05 μCi/mL ³⁵S-serotonin (New England Nuclear, Boston, MA) for 30 minutes at 37°C. Imipramine (3.4 μmol/L) was added to the final platelet suspension to prevent re-uptake of serotonin following irradiation. Results are expressed as percent of releasable serotonin remaining in the platelets. The value for 100% releasable serotonin was determined by stimulating an aliquot of labeled platelets with 5 μmol/L A23187 calcium ionophore.

The aggregation response of washed platelets following UVB was measured on a dual channel aggregometer (Payton Associates, Inc, Buffalo). Platelet suspensions were incubated at 37°C for 30 minutes post irradiation, then stirred at 1,000 rpm for 30 seconds before the addition of 164 μg/mL calf skin collagen (Cooper Biomedical Inc, Malvern, PA). The optical density of the nonirradiated platelet suspension was used to set the baseline for all aggregation response experiments. This allowed monitoring of “baseline shift,” which reflected the formation of microaggregates in some irradiated samples before addition of collagen.

Plasma was prepared from citrated blood by centrifugation at 4,800 x g for ten minutes. One-milliliter samples were subjected to UVB3 as described above, then held on ice. Prothrombin times and partial thromboplastin times were determined on 0.1-mL samples using standard procedures. Prothrombin time reagents were obtained from Ortho Diagnostic Systems, Inc, Raritan, NJ. Control values ranged from 12.8 to 14.6 seconds. Activated partial thromboplastin time reagents were obtained from Organon Teknika Corp, Morris Plains, NJ. Control values ranged from 29.9 to 40.7 seconds.

RESULTS

The intensity and dose dependence of 308 nm irradiation on washed platelets is illustrated in Fig 1A. At the lower intensities (0.11 to 0.17 MW/cm²), the spontaneous release of ³⁵S-serotonin was minimal over the range of exposure doses applied (0.51 to 53.7 J/cm²). Platelets treated with a UVB3 dose of 2.15 J/cm² retained approximately 80% of the releasable serotonin compared with 85% in the nonirradiated control. However, increasing the laser intensity to 1.4 mW/cm² and delivering a similar range of exposure doses resulted in an increased dose-dependent release of serotonin. Platelets that received a UVB3 dose of 22.4 J/cm² at the higher intensity retained only 50.5% of the releasable serotonin.

Attenuated poliovirus in buffered medium (+5% albumin) was inactivated in a dose-dependent manner by 308 nm irradiation at doses ranging from 0.51 to 56 J/cm² (Fig 1B). In contrast to the UVB30 effect on platelets, inactivation of poliovirus was not dependent on laser intensity. Virus activity was decreased by 4 to 6 log₁₀ with low intensity (0.11 to 0.17 MW/cm²) 308 nm irradiation at doses of 10.8 to 22.4 J/cm². Results were similar with high intensity UVB30.

Platelets irradiated with the lower intensity aggregated in response to 164 μg/mL collagen (Fig 2) over an exposure dose range of 2.1 to 21.5 J/cm². At the highest UVB30 dose, the amplitude of aggregation was decreased to 50% of the control amplitude. A marked loss of aggregation activity was observed at lower UVB30 doses with higher peak intensity (1.4 MW/cm²).

A shift in baseline optical density was noted in platelets treated with energy densities ≥21.5 J/cm² at lower intensities and in samples treated with ≥5 J/cm² at the higher intensity. This reproducible shift reflected the presence of microaggregates, as confirmed by phase microscopy. The addition of 50 μmol/L prostaglandin I₂ or 2 mmol/L EDTA to the platelet suspension before irradiation did not prevent the formation of microaggregates. However, warming irradiated platelet samples to 37°C for one hour partially reversed the shift in baseline optical density (data not shown).
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Fig 2. Effect of UVB<sub>308</sub> on the platelet aggregation response to collagen (164 μg/mL). Values to the left of each curve indicate the UVB<sub>308</sub> dose (J/cm<sup>2</sup>) applied. Solid curves represent aggregation activity following low intensity (0.11 to 0.17 MW/cm<sup>2</sup>) UVB<sub>308</sub>. The dashed curve illustrates the platelet response to collagen following treatment with high intensity (1.4 MW/cm<sup>2</sup>) UVB<sub>308</sub> at a dose of 11.2 J/cm<sup>2</sup>.

The plasma prothrombin (PT) and activated partial thromboplastin (PTT) times were assayed as general indicators of radiation effects on plasma proteins. With low intensity UVB<sub>308</sub>, both tests showed dose-dependent increases on irradiation with energy densities ≥5 J/cm<sup>2</sup>. Plasma samples irradiated with approximately 10 J/cm<sup>2</sup> (n = 3) demonstrated an average increase of 9.6% over control values for PT and 7.4% for PTT. With a UVB<sub>308</sub> dose of 20 J/cm<sup>2</sup> (n = 3), the average increases were 19% and 17% for PT and PTT, respectively. At the same dose and a higher peak intensity (1.4 MW/cm<sup>2</sup>), PT and PTT were elevated 27% and 40%, respectively, above control values.

DISCUSSION

The results of this study demonstrate the inactivation of a hardy virus by pulsed UVB<sub>308</sub> with rigid control of radiation parameters and mode of delivery. In separate experiments with low intensity UVB<sub>308</sub>, at exposure doses of 10.8 and 21.5 J/cm<sup>2</sup>, poliovirus titers were decreased by 4 to 6 log<sub>10</sub>, while in vitro assays of platelets and plasma indicate persistence of adequate levels of function. Although the clinical hemostatic efficacy of irradiated platelets was not determined, the degree of modification of the in vitro functions measured is not unlike that seen in platelets stored for periods up to five days after collection. In our experiments, low intensity UVB<sub>308</sub> at energy densities ≥21.5 J/cm<sup>2</sup> caused the spontaneous release of only 5% to 10% of releasable serotonin from platelets. Although a diminished aggregation response to collagen with UVB<sub>308</sub> doses of 10.8 to 21.5 J/cm<sup>2</sup> was observed, the platelets responded to a single agonist, while stored platelets often exhibit only synergistic aggregation responses.

The excimer laser used in this study permitted very high intensities (up to 2 MW/cm<sup>2</sup>) at its single wavelength of 308 nm. If UV irradiation of stored blood products is to be practical, it must employ a wavelength that is not highly attenuated by the plastics used in the storage containers or by plasma proteins over a path length of approximately 1 cm. For wavelengths below 300 nm, these attenuations are very large, but diminish rapidly with increasing wavelength above 300 nm. On the other hand, nucleic acid photochemistry is dependent on absorption of the irradiation by the nitrogenous bases that also diminish rapidly with increasing wavelengths above 300 nm. Thus, the 308 nm of the XeCl excimer laser is near the optimum for treatment of plasma and platelets within their sterile storage containers. Based on our results, a sample with thickness of 1 cm (either a planar sheet, as in a blood bag, or a tube) could be treated effectively by uniform irradiation over its entire surface with 10 to 20 J/cm<sup>2</sup> at 308 nm at peak intensities below 0.2 MW/cm<sup>2</sup>. At 80 pulses per second, our laser could deliver these doses in as little as 25 seconds. The uniformity of sample irradiance could be increased either by mixing the sample during exposure or by reducing the sample thickness. The latter would reduce the volume treated at a given time.

The very high intensities of the excimer laser allow the possibility of exciting a nucleic acid molecule with two photons within the lifetime of an excited state, leading to new, more energetic photochemical products in addition to pyrimidine dimers produced by single photon absorption. These multiple photon processes in thymine molecules have been reported at intensities >0.8 MW/cm<sup>2</sup> using 10 nsec pulses at 266 nm. Such additional nucleic acid damage might be associated with significantly increased virucidal action.

At diminished excimer intensities (0.1 to 0.2 MW/cm<sup>2</sup>), our results are consistent with virucidal effects reported for much lower intensity UVB radiation, which causes single photon photochemical effects in nucleic acids. We saw no increase in virucidal efficacy by increasing the laser intensity approximately tenfold to 1.4 MW/cm<sup>2</sup> while maintaining the same cumulative dose (0.51 to 56 J/cm<sup>2</sup>). However, this same increase in intensity significantly increased damage to coagulation proteins and platelets, presumably by multiple photon excitation of tryptophan residues. Marked alteration of some platelet proteins at these higher intensities (dose ≥20 J/cm<sup>2</sup>) was observed as reduction of specific Ag<sup>+</sup>-staining bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (data not shown). Thus, at 308 nm we observed selective virucidal effects in plasma and platelets at intensities ≤0.17 MW/cm<sup>2</sup>, but at higher intensities this selectivity is diminished by a multiple photon mechanism of protein damage. Procedures that further selectively enhance nucleic acid damage may require use of photoactivating agents as well as modification of radiation wavelength and pulse rate.

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