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.

MATERIALS AND METHODS

As 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.1 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.2,4

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.

*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 nanoseconds [ns]).

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.

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serotonin following the final platelet suspension to described. Before platelet washing, platelet rich plasma (PRP) was applied on washed platelets. The value for 100% releasable serotonin remaining in the platelets. The amplitude of aggregation was decreased to 50% of the control amplitude. A marked loss of aggregation activity was observed at lower UVB308 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).
UV-INACTIVATION OF VIRUS IN BLOOD PRODUCTS

The excimer laser used in this study permitted very high intensities (up to 2 MW/cm²) 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/cm² at 308 nm at peak intensities below 0.2 MW/cm². 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/cm² 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/cm²), 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/cm² while maintaining the same cumulative dose (0.51 to 56 J/cm²). 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/cm²) was observed as reduction of specific Ag⁺-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/cm², 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.

REFERENCES

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