Visible 405 nm Violet-Blue Light Successfully Inactivates HIV-1 in Human Plasma

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Abstract

Background and Purpose: Threats from known and unknown transfusion transmitted infections (TTIs) are a major public health safety concern for ex vivo stored blood components, and the field is cautiously advancing towards developing robust pathogen inactivation (PI) treatments also known as Pathogen Reduction Technologies (PRTs) for whole blood safety. This study provides the first proof-of-concept that 405 nm violet-blue light successfully inactivates HIV-1 present in human plasma.
Methodology: Human plasma from six individual donations obtained from the National Institutes of Health Blood Bank (Bethesda, MD, USA). Both test plasma (HIV-1 spiked and treated with various doses of 405 nm light) and control plasma (HIV-1 spiked, but not treated with the light) samples were cultured with HIV-1 permissive H9 cell line for up to 21 days to estimate the viral titers. Quantitative HIV-1 p24 antigen (HIV-1 p24) levels reflective of HIV-1 titers were measured for each light dose to assess virus infectivity.
Results: Virus inactivation potential of the light and subsequent residual survival of HIV-1 in plasma were monitored using H9 cells. All plasma samples spiked with HIV-1 but not subjected to light treatment (i.e., controls) demonstrated a significant increase ($p < 0.001$) of virus titer from baseline of 1.4 log HIV-1 p24 pg/mL to a titer of 7 log HIV-1 p24 pg/mL by day 21. For plasma samples spiked with the virus and exposed to light doses ranging from 13.5 J/cm2 to 270 J/cm2, it was observed that while HIV-1 inactivation was variable, donor plasma sample with light doses up to 216 J/cm2, there was a consistent pattern of maximum inactivation of HIV-1 from ~7 log HIV-1 p24 pg/mL down to ≤ 2 log HIV-1 p24 pg/mL with 270 J/cm2 light dose, suggesting that 270 J/cm2 is the optimal light dose for HIV-1 inactivation. Our results demonstrate that a 405 nm light dose of 270 J/cm2 is capable of 4-5 log HIV-1 reduction in plasma under the conditions tested.
Conclusion: This study provides the first proof-of-concept that 405 nm violet-blue light successfully inactivates HIV-1 present in human plasma, thereby demonstrating its potential towards being an effective PRT for this blood component safety

Introduction

- Despite significant advances in ensuring the safety of the blood supply, there is continued risk of transfusion transmitted infections (TTIs) from emerging or re-emerging new infections with associated costs for implementation of new tests to detect emerging pathogens in donors.
- Tests used in blood establishments for pathogen detection are highly sensitive and target specific, but ineffective for detection of unknown pathogens.
- A promising alternative to these traditional blood screening methods is development of pathogen inactivation (PI) treatments aka Pathogen Reduction Technologies (PRTs) for whole blood safety.
- It is well established that the PI/PRTs around the world that are either currently approved in some countries or in experimental stage, all have demonstrated unintended consequences on the transfusion products' quality and efficacy, while effective against the pathogens; thus, the field didn't produce yet a balanced technology, and hence this is still an unmet need in the transfusion medicine.
- Our research focus has been in identifying and evaluating new approaches to pathogen reduction in stored human plasma that are safer relative to the existing solvent detergent or ultraviolet (UV) light-based technologies.
- Towards this goal, high intensity narrow spectrum (HINS) 405 nm violet blue light that falls within the visible light spectra was evaluated on human plasma spiked with high concentrations of HIV-1 to determine whether this enveloped virus can be inactivated in plasma.

Materials and Methods

Human Plasma and HIV-1 Virus

- Human platelet poor plasma (PPP) was prepared in the laboratory from six individual donations (n = 6) obtained from the National Institutes of Health Blood Bank (Bethesda, MD, USA).
- For HIV infectivity experiments, HIV-1 susceptible human H9 cell line (Cat No: ARP-87, derivative of HUT 78 cell line) and HIV-1 strain (MN) was obtained from the National Institutes of Health (NIH) AIDS Reagent Program (Germantown, MD, USA).

405 nm Violet-Blue Light Treatment of the Virus

- All experiments involving 405-nm light treatment of plasma were performed using a closable prototype system (US Patent Application no. 62/236, 706, 2015), which contained a light source composed of multiple narrow band 405 nm LED arrays (FWHM ~20 nm; LED Engin, CA, USA), with appropriate thermal management and powered by LED drivers (Mean Well, New Taipei City, Taiwan).
- .Figure 1 indicates Human plasma (1ml) spiked with HIV-1 clade B (final conc. 10ng/mL of p24) was either violet-blue light-treated (test) and untreated (control). At indicated time intervals both test and control plate wells plasma were removed, frozen -80°C. until.

Infectivity and Estimation of HIV-1 Infectivity in Plasma Samples

- Treated and untreated plasma samples thawed at RT for HIV-1 infectivity assay using H9 cells. Cells cultured up to 21 days to asses effect of light treatment.
- HIV-1 infectivity was quantitated from cell-free culture supernatant using an Alliance HIV-1 p24 Antigen ELISA Kit (Cat # NEK050B001KT, Perkin Elmer, Waltham, MA, USA) according to the manufacturer's instructions.

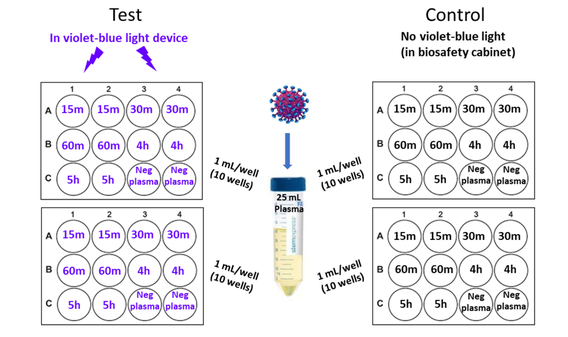

Figure 1. Virus inactivation study design

Statistical Analysis

- HIV-1 p24 values were log transformed and presented as mean ±SD. Statistical significance in viral titers were determined via the two-way ANOVA using GraphPad Prism 7·03 (GraphPad Software, Inc., San Diego, CA, USA). Statistical significance indicated by * p > 0·05 or *** where applicable.

Results and Discussion

1. To determine the optimal light dose that can achieve maximum inactivation of the virus.

- In this experiment HIV-1 spiked test plates were exposed to doses of 405 nm light ranging from 13.5 J/cm2 to 270 J/cm2 along with respective controls (no light treatment).
- HIV-1 spiked plasma without violet-blue light treatment (control, Fig.2A) incubated in a biosafety cabinet for 15 min to 5 hr, all have significant increase in viral infection (~7 log HIV-1 p24) by day 21 compared to day 0 ($p<0.001$).
- HIV-1 spiked plasma treated with violet-blue light (test, Fig 2B) for up to 5 hours, exhibited significant ($p<0.001$) reduction in the virus infectivity. Note that the light treatment for up to 4 hours did not inactivate HIV-1 and no significant (ns) differences in HIV-1 p24 levels was observed between 14 day or 21 days post infection.

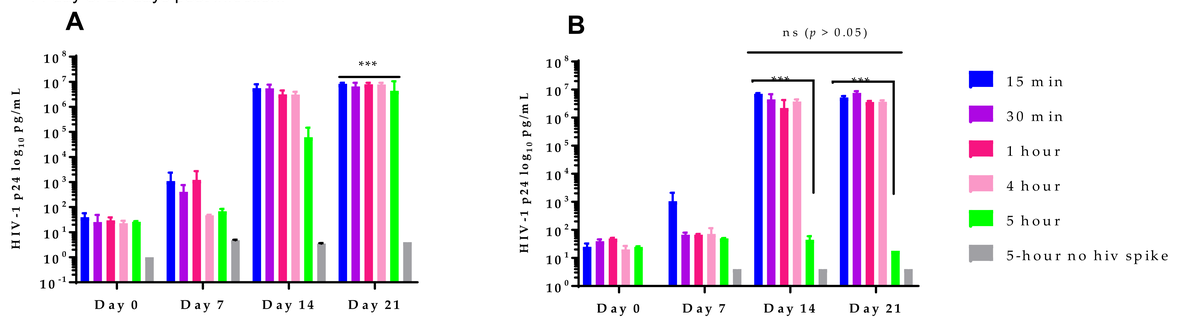

Figure 2. 405 nm light inactivates HIV-1. (A) HIV-1 spiked plasma without violet-blue light treatment (control), (B) HIV-1 spiked plasma treated with violet-blue light (test) for up to 5 h.

2. 405 nm light Inactivates HIV-1 in multiple plasma donors.

- HIV-1 spiked 5 donor plasma were either violet-blue light-treated (test) or untreated (control) and incubated for 30 min or 5h.
- All 5 plasma control samples (D1C to D5C) spiked with HIV-1 and incubated in a biosafety cabinet for 30 min or 5 hours have high HIV-1 titer (~6 log HIV-1 p24) at 14 days post infection.
- All 5 plasma test samples (D1T to D5T) exposed to violet-blue light for 30 min have high virus titer (~6 log HIV-1 p24) with no significant (ns, p>0.05) difference to control HIV-1 viral titers.
- However, plasma samples light-treated for 5 hours all have significant ($p<0.001$) reduction in infectivity HIV-1 (~2 log HIV-1 p24) compared to controls or, 30 min light treatment (Figure 3).

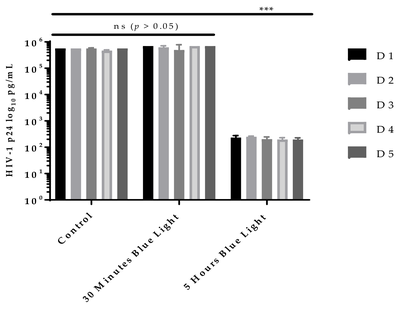

Figure 3. 405 nm light treated for 5 hours Inactivates HIV-1 in multiple plasma donors. .

Conclusion

The results obtained in this report demonstrate that HIV-1 can be successfully inactivated in plasma using 405 nm light at a dose of 270 J/cm^2 under the conditions reported here. This study on HIV-1 opens other possibilities for future evaluation of the potential of 405 nm light for inactivation of other important blood-borne viruses such as hepatitis and arboviruses relevant to transfusion safety.

Reference

1. Visible 405 nm Violet-Blue Light Successfully Inactivates HIV-1 in Human Plasma. Ragupathy V, Haleyurgirisetty M, Dahiya N, Stewart C, Anderson J, MacGregor S, Maclean M, Hewlett I, Atreya C. Pathogens. 2022 Jul 8;11(7):778.