Iranian Journal of Virology 2013;7(1&2): 1-6
©2013, Iranian Society of Virology

Original Article

Induction of Nucleic Acid Damage in Viral Genomes using Riboflavin in Combination with UV Light

Mirshafiee H[1], Hosseini SM[1*], Sharifi Z[2], Latifi H[3], Yari F[2], Nikbakht H[3], Elikaei A[1]

1. Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran.
2. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
3. Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran.

Abstract

Background and Aims: Despite the screening of blood donors, blood transfusion represents an ideal port of entry for blood-borne infection. Blood-borne pathogen transmission has been a concern since the earliest days of transfusion. The blood product of platelet (PLT) concentrates is still faced with the risk of bacterial and viral contaminations. Pathogen inactivation technologies offer a proactive approach and the potential to further improve blood safety. Here we study the pathogen inactivating capacity of riboflavin with UV light treatment in platelet concentrates contaminated with enveloped and non-enveloped viruses.

Materials and Methods: The inactivation effects of riboflavin in combination with UV light was examined on Herpes simplex virus (HSV), Vesicular stomatitis virus (VSV) and Polio virus classified as enveloped and non-enveloped DNA and RNA viruses, respectively. After spiking viruses in PLT concentrate, treatment was undertaken with riboflavin (50 µM) and exposed to different doses of UV light. Residual viral infectivity was titrated using 50%Tissue Culture Infective Dose ($TCID_{50}$).

Results: Combination of Riboflavin and UV light treatment reduced the titer of HSV, VSV and Poliovirus with different doses of UV light. Log reduction of HSV with UV doses of 0.82, 1.63, 2.44 and 3.25 J/cm^2 was 0.5, 1.3, 3.5 and 3.8, respectively. Log reduction of VSV for 0.82, 1.63, 2.44 and 3.25 J/$cm^2$was 2.8, 3.8, 4.6 and 5.6, respectively. Also, log reduction of poliovirus in the same order for 0.82, 1.63, 2.44 and 3.25 J/$cm^2$was 1.7, 2.5, 2.7 and 3.1 respectively.

Conclusion: The final dose of UV (3.25 J/cm^2) resulted in a larger amount of viral inactivation for enveloped and non-enveloped viruses, in suspended PLT. This method offers a potential to be used for prevention of the majority of PLT transfusion-associated viral pathogens.

Keywords: Riboflavin; UV light; Platelet concentrate; Virus inactivation

Introduction

The Safety of blood supply has dramatically improved with implementation of careful donor selection procedures coupled with extensive laboratory testing. Improved screening and testing strategies considerably reduced the risk of known transfusion-transmissible viruses, e.g. HIV, HBV and HCV.

However, the residual risk of pathogens could not be eliminated because of i) screening testing exert only to known pathogens and not to other presently unknown infectious agents; ii)they are reactive to new transmissible diseases and thus some patients may be harmed

***Corresponding author:** Hosseini SM, Ph.D. Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran. Email: Ma_Hosseini@sbu.ac.ir

[Downloaded from journal.isv.org.ir on 2026-10-14]

[DOI: 10.21859/isv.7.1.2.1]

before preventive measures can be implemented; iii) they do not prevent bacterial contamination; iv) they do not prevent transmission of other known organisms such as malaria, babesia, Trypanosoma cruzi and cytomegalo virus, or unknown infectious agents (1). Among blood products, contaminated platelets (PLTs) have the potential to cause bacterial sepsis owing to their storage at room temperature.

To improve the safety of platelet and plasma transfusion, a number of different techniques based on nucleic acid-targeted photochemical treatment (PCT) have recently been described. One method utilizes the psoralen compound amotosalen (S-59) and long wavelength ultraviolet A (UVA) light which was developed to inactivate viruses, bacteria, and protozoa that may contaminate platelet and plasma components (2). Another method that has been used takes advantage of the properties of a naturally occurring vitamin supplement, riboflavin (vitamin B2). Riboflavin intercalates between the bases of DNA or RNA and upon illumination with UV (265 to 370nm), oxidizes the guanine in nucleic acids resulting in single-strand breaks. This damage is so extensive to the guanine bases that could cause to irreversible damage to the pathogen (3).

The aim of this study was examining the efficacy of UV light and riboflavin treatment for pathogen inactivation of enveloped and non-enveloped viruses in laboratory-scale with the prediction that its application could prevent potential infectious complications of PLT clinical use.

Methods

Preparation of platelet concentrates

Platelet concentrates were prepared by the platelet-rich plasma (PRP) method according to the standard protocol (4) in the Iranian Blood Transfusion Organization, Tehran.

Cell Culture and Virus Preparation

Vero cell line (NCBI Code: C101, Pasture Institute of Iran, Tehran) and HeLa cell line (NCBI Code: C115, Pasture Institute of Iran, Tehran) were used for culture and titration of model viruses. These cells were grown and maintained in RPMI media (Sigma-Aldrich Corp., St. Louis, MO, USA), supplemented with 5% FCS, 100 IU/mL Penicillin and 100 µg/ mL Streptomycin, at 37°C in 5% CO_2 incubator. Viruses which were evaluated represented DNA and RNA were included Herpes Simplex Virus (HSV) and Vesicular Stomatitis Virus (VSV) and Poliovirus. Then viruses were selected as models for the experiments.

Quantitative estimation of model virus titers after illumination

Quantitative estimation of model virus was carried out by microtiter plate method. The cell culture infectivity titer of model viruses was estimated by Reed and Muench method employing 10-fold serial dilution and four replicate cultures per dilution in cell lines to calculate the TCID50/ml based on viral CPE. The serial dilution of viral samples inoculated onto cell lines were started with undiluted (10^0) sample. The microplates were incubated at 37ºC with 5% carbon dioxide for a period of 5 days. The plates were examined daily for the appearance of cytopathic effect. The final results were recorded as positive for the presence of cytopathic effect and negative for the absence of cytopathic effect on day 5. The titer for HSV, VSV and Poliovirus before treatment, was 10^6, $10^{8.6}$, $10^{8.5}$ TCID50/ml, respectively (5-6).

Riboflavin preparation

500 µM of Riboflavin (RB) was dissolved in a 0.9% sodium chloride solution with pH 4-5 (3). It was sterilized using 0.22 µm filter (Millipore®).The solution was wrapped in an opaque foil pouch to protect it from light and was used freshly for each experiment. The solution was mixed with PLTs for further treatment; RB was diluted to the concentration (50µM) for pathogen reduction treatment.

Small-scale illumination

The final volume of 10±0.5 mL of platelet concentrate suspended in a mixture of virus and RB was illuminates in a petri dish (9 mm in diameter). This resulted in a fluid layer with a thickness of approximately 1 mm. The illumination device (Hamamatsu Lightning Cure LC5) was able to emit UV light at wavelength 200- 400 nm with the radiation

[Downloaded from journal.isv.org.ir on 2026-10-14]

[DOI: 10.21859/isv.7.1.2.1]

peak of 365 nm. Different doses of light were used to examine virus inactivation. Petri dishes containing platelet concentrate, model viruses and RB (50µM) were exposed to doses equal to 0.82, 1.63, 2.44 and 3.25 J/cm2. Also, 2 samples were used as a control with and without riboflavin, and no UV light encountered.

Statistical Analysis

Each experiment was performed three times, and the average and SD of the mean presented for each sample. Statistical analysis (general linear model analysis of variance [ANOVA]) was performed using GraphPad Prism® (Version 5.0) software for each experimental condition.

Results

Destruction of the monolayer cells (CPE) allows for easy monitoring and assessment of viral replication. The TCID50 method was used to calculate the concentration of a viral preparation at which 50% of the cells were infected. In this study, CPE caused by HSV, VSV and poliovirus were examined microscopically for presence of viral infection (Figure 1, 2 and 3).

In these experiments, the final concentration of RB was 50 µM and light intensity determined to be 0.01356W/cm^2 that the different doses were used (e.g. 3.25 J/cm^2 resulted from 240 sec exposure). One-way ANOVA followed by Tukey's post-hoc test showed that illumination in the absence of RB for 2.44 J/cm^2 could not decrease virus titration significantly, as well as RB without illumination ($p>0.05$). One-way ANOVA followed by Tukey's post-hoc test showed that inactivation effects on examined viruses were dependent on the combination of the riboflavin and UV light ($p<0.05$). Log

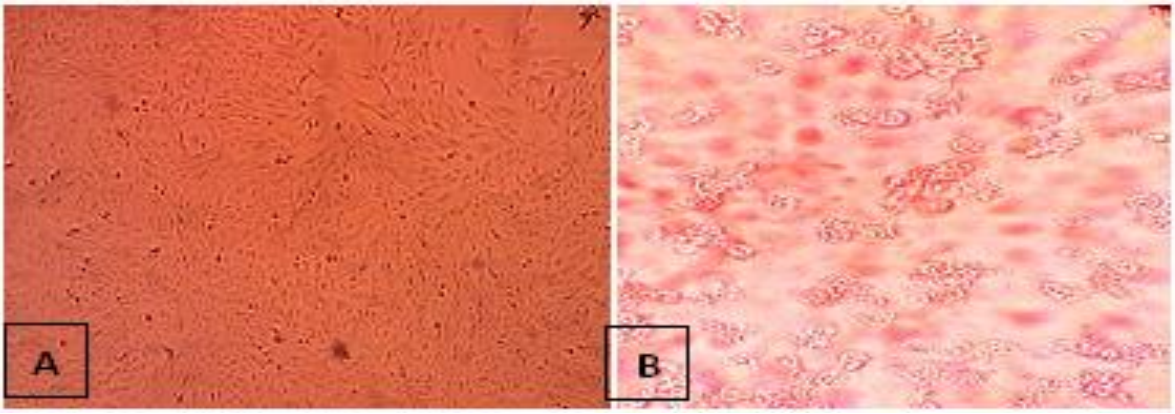

Fig. 1. A: Uninfected Vero cells. B: CPE due to HSV in Vero cells typically develops as enlarged, rounded cells.

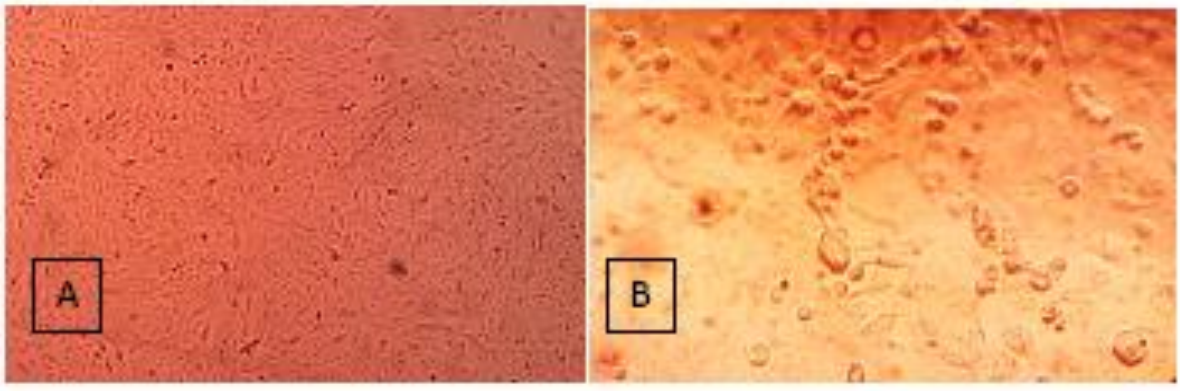

Fig. 2. A: Uninfected Vero cells. B: CPE of VSV in Vero cells consists of cells typically rounded to a pear shape. The nucleus is lying at the rim of the cell; a round eosinophilic mass occupies the center.

[Downloaded from journal.isv.org.ir on 2026-10-14]

[DOI: 10.21859/isv.7.1.2.1]

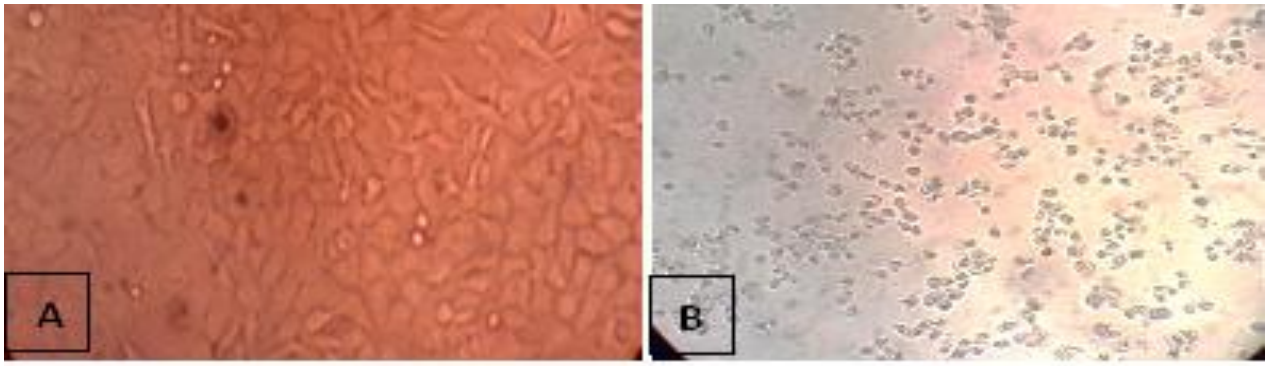

Fig. 3. A: Uninfected HeLa cells. B: CPE of poliovirus infection in HeLa cells is diffuse and consists of rounding of the cells, and shrinking of the nucleus, which is pushed to the edge of the cell.

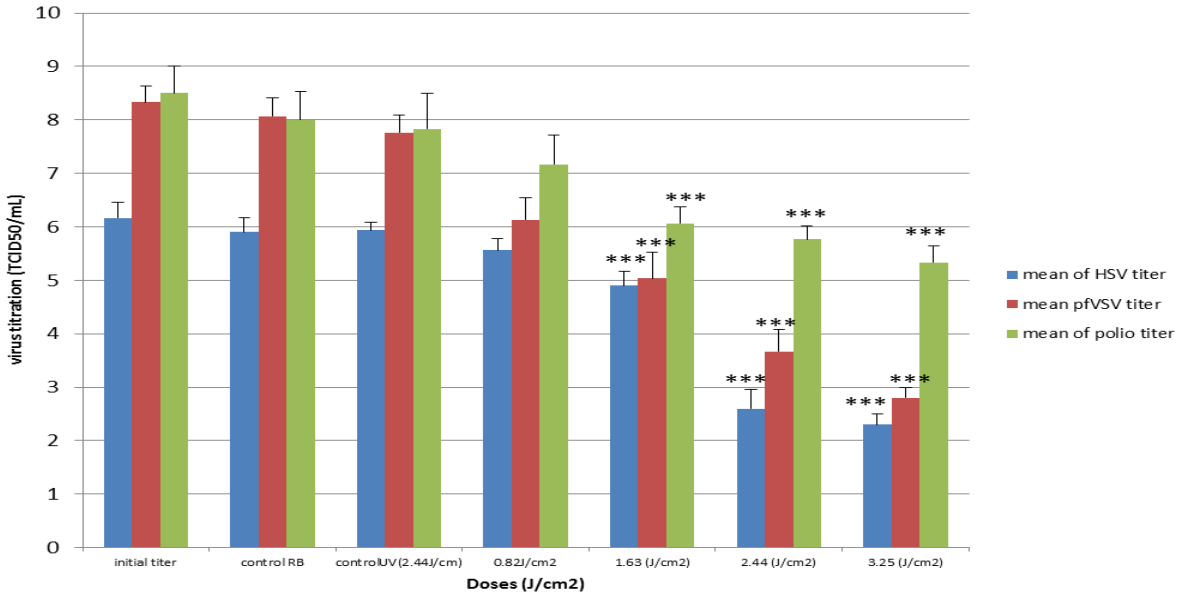

Fig. 4. Comparison of virus titration before and after illumination (illumination dose for control UV was 2.44 J/cm^2). ***: P<0.05 compared to initial titer.

reduction of HSV for 0.82, 1.63, 2.44 and 3.25 J/cm^2 was 0.5, 1.3, 3.5 and 3.8, respectively. Log reduction of VSV for 0.82, 1.63, 2.44 and 3.25 J/cm^2 was 2.8, 3.8, 4.6 and 5.6, respectively. Also, log reduction of poliovirus for 0.82, 1.63, 2.44 and 3.25 J/cm^2 was 1.7, 2.5, 2.7 and 3.1, respectively.

In comparison with non-enveloped virus (poliovirus), two-way ANOVA followed by Bonferroni post test showed that the enveloped viruses (HSV& VSV) were effectively inactivated by 2.44 & 3.25 J/cm^2. VSV (RNA virus model) was more sensitive to the proposed inactivation method than HSV.

Discussion

Blood product safety is a major priority of blood suppliers. Transmission of viruses or other infectious agents can have deleterious effects for transfused patients. To maximize the safety of blood products, various procedures are initiated starting from the moment a person is willing to donate blood (7). However, the blood supply remains under threat from known pathogens which are not assayed in conventional blood screening protocols, from low-titer viruses that escape detection during the window phase of infection, and from novel emerging transfusion-transmissible diseases (8).

[Downloaded from journal.isv.org.ir on 2026-10-14]

[DOI: 10.21859/isv.7.1.2.1]

Pathogen photochemical or dynamic procedures for inactivation of pathogens is a promising technique to improve blood safety of cellular blood products. The MirasolPRT ® pathogen inactivation system is capable of inactivating significant levels of pathogens and leukocytes and is thus expected to reduce the risk of disease transmission and adverse events while maintaining acceptable quality of the treated blood products. In this work, the effects of photochemical method on enveloped and non-enveloped viruses were studied.

Riboflavin, a naturally occurring essential nutrient, with UV light has been used as a pathogen-inactivating agent for platelets and plasma. As shown in table 2, the effect of riboflavin and UV light, each of which alone had no significant effects on virus titers but according to the absorption spectra of riboflavin and its activation process, significant reduction in virus titer is observed.

In as much as non-enveloped virus has remained a challenge which is more difficult to inactivate, results of this study showed this method for non-enveloped virus (poliovirus as a model) inactivation has more potential and efficient than Methylene blue/ light technique (9).

The results of this work also indicated that VSV was more sensitive than HSV as virus log reduction with 3.25 J/cm^2 was 5.4 and 3.4 for VSV and HSV, respectively. In agreement with Mirasol® system (*CaridianBCT Biotechnologies, Lakewood, Colorado, USA*) it was shown that the log reduction of VSV (model virus for ssRNA) was 6.3 whereas log reduction for Hepatitis B was Virus (HBV) and Cytomegalovirus (CMV) as model viruses for dsDNA was 2.5 and 2.1, (10-13). This confirms that ssRNA viruses are more sensitive to this method of pathogen inactivation. Methylene blue/ light is one of the pathogen inactivation methods for plasma and Plasma derivatives. This method compared to Riboflavin/ UVB had more effects on HSV with reduction of more than 4 log (9, 14).

The advantage of the this method, unlike other systems such as amatosalen and methylene blue developed to inactivate pathogens in blood products, there is no need for subsequently removal of RB and its metabolites from blood products, since it is a vitamin that already is present in the body of the recipient. In order to optimize this method, quality control parameters of platelet should be examined after exposure in subsequent experiments and attempt to be done to prepare the bags for irradiation.

In conclusion, results showed that RB and UV light are effective in inactivating model viruses including enveloped DNA- or RNA containing and nonenveloped viruses and, therefore, offers the potential to prospectively prevent the majority of PLT transfusion–associated viral diseases.

References

1. McCullough J. Pathogen inactivation of platelets. Transfusion Alternatives in Transfusion Medicine. 2006;2:121- 126.

2. Lin L, Cook DN, Wiesehahn GP. Photochemicalinactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion.1997;37:423-435.

3. Ruane PH, Edrich RA, Gampp D, KeilSh D, Leonard RL, Goodrich RP. Photochemical inactivation of selected viruses and bacteria inplatelet concentrates using riboflavin and light. Transfusion 2004; 44:877-85.

4. Saran RK. 2nd ed. New Delhi, India: WHO; 2003. Transfusion medicine technical manual.

5. Reed LJ, Muench H. A simple method ofestimating fifty per cent endpoints. Am J Epidemiol 1938; 27(3): 493-7.

6. Carter JB, Saunders VA. Virology: principlesand applications. West Sussex: John Wiley & Sons Ltd. 2007; p10-29.

7. Meulenbroek A.J. Van bloed tot geneesmiddel, gezond vertrouwen. Sanquin ed. Amsterdam: Artoos Nederland; 2002.

8. Dodd RY. Current risk for transfusion transmittedinfections. Curr Opin Hematol. 2007;14:671–676.

9. Elikaei A, Hosseini S.M, Sharifi Z, Latifi H, Nikbakht H, Mirshafiee H, Asadollahi A. Methylene Blue Based Device for Pathogen Reduction in Human Plasma. Iranian Journal of Pediatric Hematology and Oncology. 2013;3(3):97-102.

10. Goodrich RP, Edrich RA, Li J, Seghatchian J. The Mirasol™ PRT system for pathogen reduction

[Downloaded from journal.isv.org.ir on 2026-10-14]

[DOI: 10.21859/isv.7.1.2.1]

of platelets and plasma: an overview of current status and future trends. Transfus Apher Sci. 2006;35:5-17.
11.Mohr H, Ute G, Bayer A, Müller TH. Sterilization of plateletconcentrates at production scale by irradiation with short-wave ultraviolet light. Transfusion. 2009 doi: 10.1111/j.1537-2995. 2009.02228.x.
12.Ruane PH, Edrich R, Gampp D, Keil SD, Leonard RL,Goodrich RP. Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. Transfusion. 2004;44:877-85.
13.Marschner S, Goodrich R. Pathogen Reduction Technology Treatment of Platelets,Plasma and Whole Blood Using Riboflavin and UV Light. Transfus Med Hemother. 2011;38:8–18.
14.Seghatchian w, Struff G and ReichenbergS. Main properties of the THERAFLEX MB- plasma system for pathogen reduction. Transfusion Med Hemother. 2011;38:55-64.

[Downloaded from journal.isv.org.ir on 2026-10-14]

[DOI: 10.21859/isv.7.1.2.1]