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Original Article

The effect of Methylene Blue in combination with red visible light on model viruses inactivation and coagulation factors in fresh frozen plasma

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Abstract

Background and Aims: Fresh Frozen Plasma (FFP) is one of blood components. The risk of transmission of viruses from blood components regardless selection of blood donors and screening donated blood still remains. There are several methods for viral inactivation. In this study methylene blue(MB) photo inactivation process was used for inactivating viruses

Materials and Methods: In this study Methylene Blue(MB) was used in final concentration of 1µM. Infected Fresh Frozen Plasma (FFP) illuminated by 143Pieces (PCs) of 1 w red Light Emitting Diodes (LEDs) from two side for 10,15 and 30 minutes and shacked 30 cycle in minutes. the central wavelength of these LED is 627 nm with 20 nm Full Width at Half Maximum (FWHM).Herpes simplex virus-1(HSV-1) and vesicular stomatitis virus(VSV) were used as model viruses to evaluated illumination effects on viral inactivation. level of fresh frozen plasma (FFP) coagulation factors such as fibrinogen, FV, FVIII, protein C, antitrombin measured pre and post illumination.

Results: Initial HSV-1 and VSV titer were calculated to be 107 and 106.5 TCID50/ml, respectively .The level of viral inactivation was expressed as log-reduction. Titer reduction of HSV for 10, 15 and 30 minutes irradiation with shaking was > 6, ≥ 7 and ≥ 7 log, respectively. the ratio of coagulation factors activity remaining unchanged after pathogen inactivation with MB calculated. illumination had a major effects on the mean levels of fibrinogen and FVIII. Significant differences between level of factors before and after illumination were evaluated with a t test for paired samples. No significant differences were seen in the FFP coagulation factors before and after illumination.($P>0.05$)

Conclusions: As results show the optimum time for viral inactivation were adjusted to be 15 minutes. Due to the reduction of virus titer at various times, agitation with illumination is effective.

Keywords: methylene –blue, light, virus inactivation, shacking.

Introduction

Viral contamination of biological products is of concern to public health officials. Pathogen inactivation raise the safety margin for blood products by inactivation pathogen that remained undetected during screening due to window periods on test error(1).It also provides as proactive approach to inactivation emerging and as-yet-unidentified pathogen before they enter the blood supply chain and before screening test have been performed.

Methylene Blue in combination visible light is one of method for inactivation of pathogen in

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fresh frozen plasma has been developed in clinical for 15 years(1,2).This system was shown to inactive a broad range of different DNA and RNA viruses in plasma.
FFP is used to treat congenital coagulation factor deficiencies, for which specific concentrates are not accessible but Methylene Blue pathogen inactivation process appear to result in loss of coagulation factor leading to additional transfusion of plasma.(2)
In this study we systemically investigated the inactivation HSV-1 model for HBV by methylene blue plus visible light treatment while agitate to assess the capacity of this procedure to prevent transfusion mediated HSV transmission. Also level of selected coagulation factors of plasma such as factor V, VIII, antitrombin activity ,protein C and fibrinogen before and after photoinactivatin evaluated.

Methods

Fresh Frozen Plasma(FFP). Fresh frozen plasma units of O positive blood group got(collected) from Iranian Blood Transfusion Center. This units were free from(were lacked of) HBV,HCV and HIV .All unit of plasma were stored at -70°C.
Device and Illumination. Each plasma samples was illuminated by 143 Pieces (PCs) of 1 w red Light Emitting Diodes (LEDs) from two side. These LEDs emit light at central wavelength of 627 nm with 20 nm Full Width at Half Maximum (FWHM). The distance of the middle of the bag from LED arrays, considered to be 4.5cm . This device was designed for light (LED, including the distance and placement of blood bags per LED) with the help of simulation software Wolfram Mathematica® considering the intensity profile LED sand right number of used LEDs. Light emission to the plasma bag was optimized and uniformed.

Cell culture. the efficacy of Methylene blue procedure for HSV-1 and VSV inactivation were evaluated using cell-culture-derived herpes simplex virus model virus for HBV and VSV model for enveloped viruses. Both viruses are able to infect selected cell line, including Vero cell line. Vero cell was propagated in Dulbecco›s Modified Eagles medium(DMEM), supplemented with 10% fetal bovin serum and penicillin and streptomycin were used in final concentration of 1% then cell culture incubated at 37°C in a humid atmosphere of 5% CO2.
Virus amplification and titration. Vero cell line cultured in sterile 96 well plate .To reach confluent cell monolayer cultured plates were infected with HSV-1 and incubated for 1 h at 37°c to allow attachment. cell were washed with phosphate -buffered salin(PBS) then DMEM with fetal bovin was added an incubated for 5 day at 37 °C. Cytopathic Effects(CPE) of virus investigated every day and virus titer are expressed as log TCID50 and were calculated according to the method Reed and Munch. Similar work has been done for measure titer of VSV.
MB/Light treatment. Fresh frozen plasma unit were MB/light treated. Methylene Blue and HSV were added to plasma.(1cc virus to 10cc plasma).the final concentration of MB is approximately 1µmol/l. Plasma transferred to illumination bag and air was removed from the plasma unit before illumination. Infected samples illuminated from two side for 10,15and 30 minutes and shacked 30 cycle in minutes. Before and after treatment a sample taken from the bag to evaluate titer of viruses.
control groups. plasma without virus spiking and plasma plus virus without treatment were used as negative control and positive control respectively . To detect of Methylene blue affection on pathogen inactivation one sample of plasma via MB without illumination assessed. Also light without MB was used to measure affection of light solely.
plasma coagulation factors. level of some proteins in plasma such as factorV(TriniClot

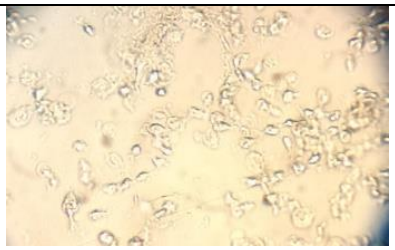
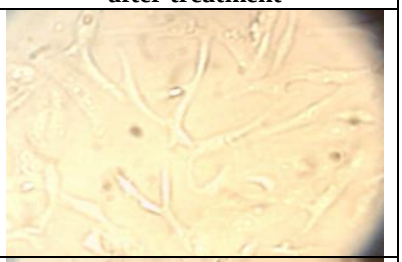
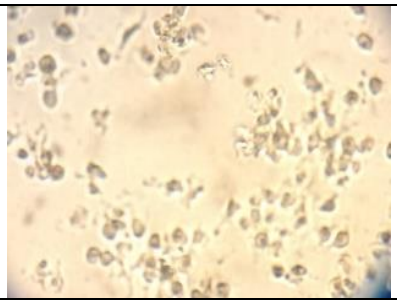
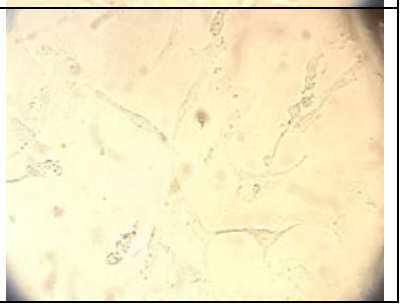
Viruses Model	Infected sample before treatment	Infected sample on Vero cell line after treatment
HSV-1		
VSV		

Fig. 1. Cytopatic Effects of model viruses pre and post treatment

TM factor V T1505)(Tcoag),factor VIII(TriniClot TM factor VIII T 1507), antitrombin activity(STA-STACHOROM AT III REF 00596)(Stago),protein C(STA-STACOL PROTEIN C REF 00747)(Stago) and fibrinogen(TriniCloTM fibrinogen kit T 1301)(Tcoag) were measured pre and post illumination.

Statistics. SPSS 15.00 and t test for paired samples were employed for data analysis.

Results

HSV-1 and VSV titer was calculated to be 107 and 106.5 TCID50/ml, respectively .The level of viral inactivation was expressed as log-reduction. Log reduction of HSV-1 for 10, 15 and 30 minutes irradiation with shaking was≥ 6.92 , ≥7.00 and ≥7.00 log respectively.VSV virus inactivated by 10,15 and 30 minutes illumination. No Cytopathic Effects(CPE) observed after mentioned time.

The ratio of coagulation factor activity remaining after pathogen inactivation with MB appraised. Illumination had a major affects on the mean levels of fibrinogen and FVIII. Significant differences between level of coagulation factors pre and post illumination were evaluated and no significant differences were seen in the factor before and after illumination.(P>0.05)

As results show, shaking during illuminating is the effective factor on viral inactivation. The best time for viral inactivation was 15 minutes. Due to the reduction of virus titer at various times, agitation with illumination was more effective.

Table 1:inactivation model viruses using different illumination time

Model virus(mean titer)	Log TCID50 HSV titer (mean)	Log reduction factor	Log TCID50 VSV titer(mean)	Log reduction factor
Exposure time(minutes)				
Initial titer	107		106.5	
10 min		≥6.90		≥6.00
15 min		≥7.00		≥6.00
30 min		≥7.00		≥6.00

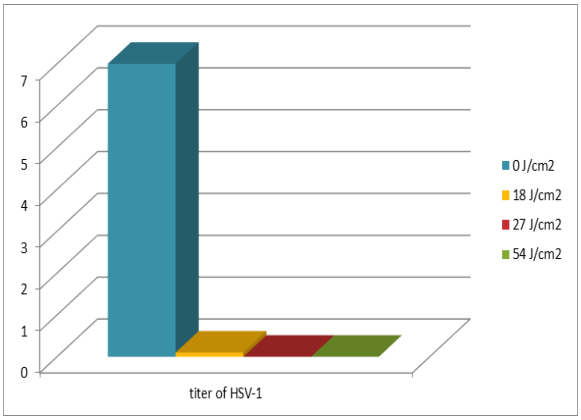


Fig. 2. Titre of HSV-1 pre and post treatment

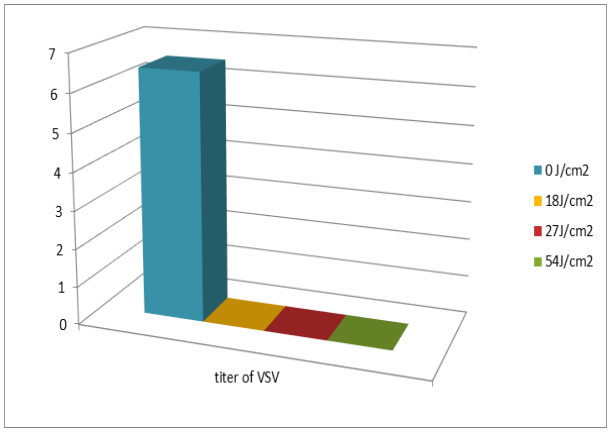


Fig. 3. Titer of HSV-1 pre and post treatment

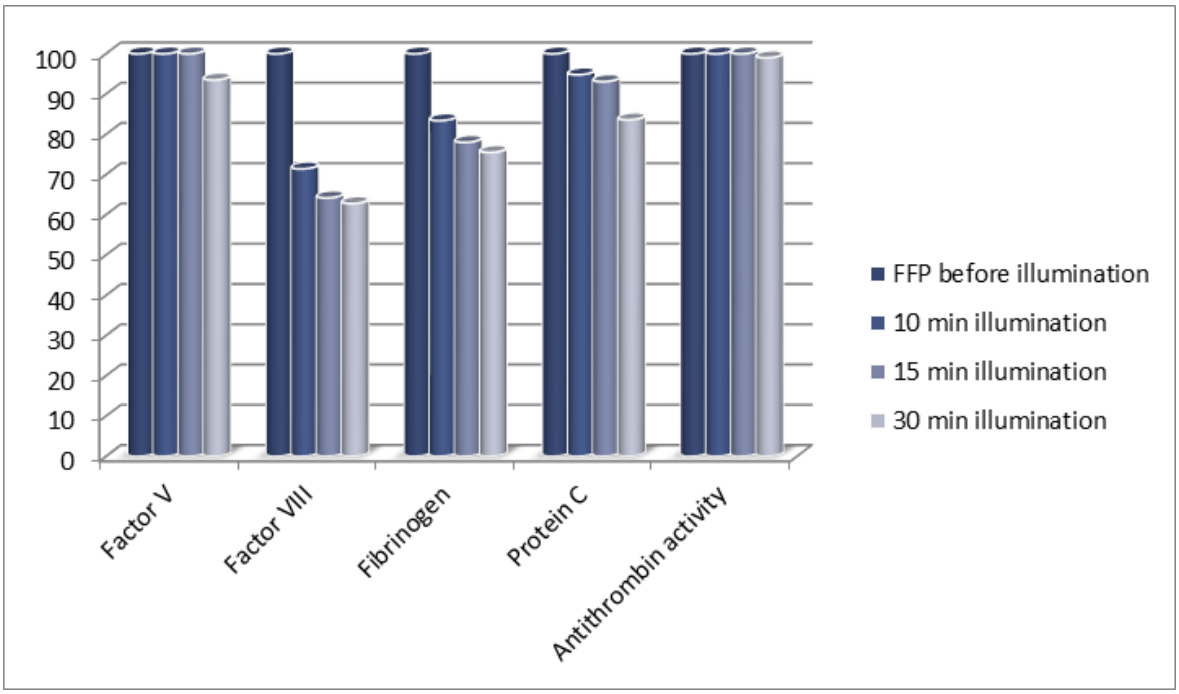


Fig. 4. Level of plasma coagulation factors pre and post illumination

Discussion

In present study the inactivation effects of MB/visible light on viruses investigated. Methylene Blue has long been known when it was used to treat malaria(2.13). It used at low doses to treat methemoglobinemia(3,). Methylene Blue can intercalate into DNA or combine with the outer helix, depending on the Mg 2+ ionic strength and concentration(3). Platelets ,plasma and red blood cells do not require genomic DNA to be viable therefore MB target nucleic acid of pathogen to interrupt their function. beyond pathogen›s genetic damage, protein and membrane molecules can be incurred s leading to reduction blood product quality.(3)

In this study, attempted achieve a proper balance between pathogen inactivation and the product quality by MB/visible light method. Also effect of shaking on time exposure investigated.

WHO recommendation show that in the current procedure, individual plasma units are

Table 2:Effect of photoinactivation on coagulation factors in FFP

Plasma coagulation factors	Reference Rang	FFP before illumination	10 min illumination	15 min illumination	30 min illumination	Preserve after 10min illumination (%)	Preserve after 15 min illumination (%)	Preserve after 30min illumination (%)
Factor V	60-130	62	65	69	58	100	100	93.5
Factor VIII	60-150	70	50	45	44	71.4	64.2	62.8
Fibrinogen	200-400	241	201	188	182	83.4	78.00	75.5
Protein C	70-130	116	110	108	97	94.8	93.1	83.6
Antitrombin activity	80-120	109	110	110	108	100	100	99

treated with 1µM methylene blue and white fluorescent light for 1h at 45000lux or
with low-pressure sodium lamps at 200 Joules/cm2 for 20 minutes(19). Later, work on other
systems (Baxter Pathinact and Maco Pharma Theraflex systems) was performed on fresh plasma (18). Optimum time irradiation of the newest version of the illumination device (Macotronic B; MacoPharma) is 12-15 min for 2 single unit of plasma. This system use visible light(630 nm, 180 J/cm 2) and Methylene Blue in 1µm final concentration.(12)
In this study optimum light dose 54 J/cm 2 calculated .The best time for illumination with the same light dose without shaking to inactive virus was 45 minutes.(4).This procedure with shaking reduce this time to 15 minutes .

conclusion

Available data demonstrate that MB/visible light shaking base device is effective on viral inactivation and show quality of coagulation indication factors of FFP during 15 minutes irradiation are better preserved.

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