

DEGRADATION OF HUMAN HEPATITIS B VIRUS DNA IN PLATELET CONCENTRATES BY SHORT WAVE ULTRAVIOLET LIGHT AS REVEALED BY REAL-TIME PCR

J. Knüver-Hopf, H. Mohr and T.H. Müller

Blood Center of the German Red Cross Chapters of NSTOB
Institute Springe, Germany

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Conclusion

The present results demonstrate HBV nucleic acid degradation after UVC treatment. This suggests that HBV in platelet concentrates is sensitive to UVC light.

Introduction

Treatment of platelet concentrates (PC) using short wave ultraviolet light (UVC) has been shown to effectively inactivate several pathogens [1]. Inactivation of human hepatitis B virus (HBV) however could not be proven up to now, because there is no infectivity assay available for HBV. The target structures in UVC treatment of viruses is its nucleic acid. Inactivation of viruses therefore might be determined by PCR.

Material and Methods

PC in storage medium SSP+ (MacoPharma, Langen, Germany) containing approx. 30% plasma were prepared from pools of 5 buffy coats. From each PC 120 ml were transferred to ethylvinyl acetate bags from MacoPharma (Langen, Germany). In this study, treatment with UVC light was performed with different doses on a BS10-illumination device (GROEBEL, Ettlingen, Germany). Routinely UVC treatment was done with a dose of 0.4 J/cm². The QIAamp Blood Mini Kit (QIAGEN, Hilden, Germany) was used for isolation of DNA from all samples. For long-range real-time PCR [LR-PCR] the LightCycler was used (ROCHE, Mannheim, Germany). Primers and hybridization probes were synthesized by TIB MOLBIOL (Berlin, Germany). Primers were selected for the nucleotide positions 44 to 2187 of the circular HBV genome.

References

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Results and Discussion

From previous investigations it is known that PCR inhibition cannot be shown by a short-range HBV real-time PCR [2]. This is probably due to the short genome region analysed. This finding is in agreement with previous PCR studies with HIV-1 and parvovirus B19. In those studies we found a correlation between the analysed viral genome region and inhibition of PCR in virus-infected samples after photodynamic treatment [3-5]. In the present study the effect of UVC treatment on HBV-DNA was evaluated by the use of LR-PCR. An increased amplicon size of 1090 base pairs was a sufficient target to demonstrate the effect of UVC treatment. As seen in figure 1, the fluorescence curves of treated samples shifted towards higher cycle number values compared to untreated samples. As shown in figure 2, the PCR signals were lowered from 100% to 2% after treatment with 0.4 J/cm² of UVC, i.e. from approx. 10x10⁷/ml genome copies to 10x10⁵/ml.

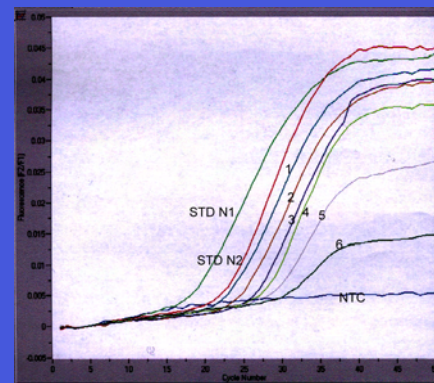


Figure 1: Amplification plot of LR-PCR before and after pathogen inactivation procedure with UVC of HBV in platelet concentrates **1)** untreated HBV sample; **2-6)** UVC- treated HBV sample; 0.1, 0.3, 0.4, 0.5 and 0.6 J/cm² **STD N1 and N2)** calibration standards; **NTC)** no template control

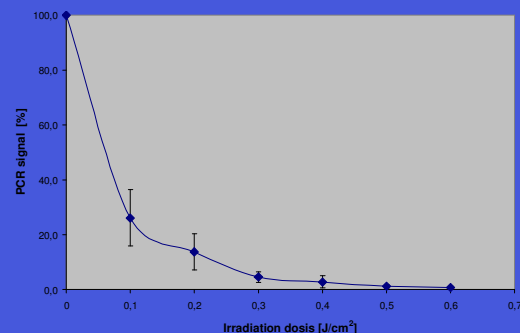


Figure 2: Kinetics of nucleic acid degradation of HBV in PC after UVC treatment detected by LR-PCR
N=6; +/-SD

THERAFLEX UV PLATELETS: NOTHING BUT UVC LIGHT AND STRONG AGITATION

H. Mohr¹, U. Gravemann¹, F. Tolksdorf², W.H. Walker², T.H. Müller¹

Purpose

Blood donations may not only be contaminated with viruses, e.g. HBV, HCV or HIV. In addition, they may contain bacteria. This is especially crucial for platelet concentrates (PCs), because they have to be stored at room temperature, at which bacteria can multiply to high levels [1-2].

Short-wave ultraviolet light (UVC, wavelength range: 200-280 nm) is germicidal, but low UV-permeability hampers its use for sterilizing PCs. A simple method was developed which overcomes this limitation.

Materials and Methods

Plasma-reduced PCs in storage medium SSP+ (volume approx. 350 mL, platelet concentration approx. 10⁹/mL, plasma content 30-35%) were prepared from pools of 5 buffy coats [3]. PC volume was approx. 350 mL. The PCs were spiked with approx. 10²-10⁶ CFU/mL of different bacteria species or up to 10⁷ TCID₅₀/mL of lipid-enveloped or nonenveloped viruses. Other PCs were spiked with 5x10⁶/mL peripheral blood mononuclear cells (PBMC). The PCs were filled into UV-transparent plastic bags and irradiated on a device (Fig.1), equipped with mercury vapour tubes emitting monochromatic UVC-light (wavelength: 254 nm). The device was equipped with an orbital agitator. Irradiation was from both sides of the bags. UVC doses applied were up to 0.6 J/cm² (approx. 90 sec). During treatment the PCs were strongly agitated. Bacteria or virus titers, PBMC viability and platelet parameters were determined before and after irradiation. Each experiment was repeated 3-6 times. Results are depicted as mean ± SD.

Results

Pathogen inactivation was enormously enhanced when the PCs were loosely placed on a quartz plate located between the two layers of UVC tubes of the irradiation device and, in addition, strongly agitated during irradiation (Fig. 2).

UVC-light at 0.3-0.4 J/cm² (irradiation time: approx. 1 min) reduced the titers of all bacteria tested by approx. 5-6 log₁₀ steps. PCs spiked with approx. 100 CFU/ml of bacteria were reproducibly sterilized (Tab.1). In one experiment with *B. cereus* the PC was sterile after 3 but unsterile after 6 days storage. This was probably due to spores of *B. cereus* that are more resistant to UVC than vegetative bacteria.

UVC sensitivity of the viruses tested was not so uniform (Table 1): The small single stranded RNA viruses VSV, Sindbis and WNV were completely inactivated at approx. 0.3-0.4 J/cm². Remarkably HIV-1 (also a small single-stranded RNA virus) was only moderately inactivated at UVC doses up to 0.6 J/cm².

The small nonenveloped DNA viruses PPV and EMCV proved to be very sensitive. Complete inactivation was achieved at 0.4-0.5 J/cm².

With the exception of HIV-1, SHV-1 was more resistant than the other viruses tested. This confirms that in general large double stranded DNA viruses are not as susceptible to UVC as smaller single stranded DNA or RNA viruses.

PBMC proved to be extremely sensitive to UVC irradiation: Complete inactivation was found at less than 0.1 J/cm² (Fig. 3)

PC properties remained almost unchanged at doses up to 0.6 J/cm². The storage stability of the treated PCs for up to 6 days after treatment (8 days after blood donation) was maintained (Table 2)

Conclusions

Irradiation with UVC under strong agitation may be used to sterilize platelet concentrates at a light dose that is not harmful to the products. The UVC dose required is 0.4 J/cm². Irradiation time is not more than approx. 1 min.

Parameter	Day 1 after irradiation				Day 6 after irradiation			
	Control	UVC dose (J/cm ²)			Control	UVC dose (J/cm ²)		
		0.4	0.5	0.6		0.4	0.5	0.6
Pts [x10 ⁹ /mL]	10.8 ± 0.6	10.2 ± 0.6	9.8 ± 0.6	9.1 ± 0.9	10.1 ± 0.8	9.8 ± 0.6	9.3 ± 0.8	9.3 ± 0.9
pH	7.10 ± 0.04	7.04 ± 0.05	7.09 ± 0.05	7.05 ± 0.04	7.27 ± 0.15	7.09 ± 0.06	7.11 ± 0.10	6.98 ± 0.07
Lactate [mmol/L]	7.7 ± 1.0	8.0 ± 0.5	7.7 ± 0.5	8.0 ± 0.7	12.7 ± 1.0	14.9 ± 1.0	14.6 ± 1.4	16.7 ± 1.4
Glucose [mg/dL]	122 ± 9	117 ± 7	117 ± 6	115 ± 7	62 ± 11	43 ± 8	44 ± 11	29 ± 10
Swirling	ok	ok	ok	ok	ok	ok	ok	ok
HSR [%]	69 ± 5	66 ± 2	61 ± 6	62 ± 4	68 ± 2	65 ± 2	62 ± 3	56 ± 5
Collagen-induced aggregation [%]	95 ± 4	90 ± 5	88 ± 3	87 ± 2	62 ± 9	69 ± 8	67 ± 2	69 ± 5
CD62 [%]	36 ± 1	46 ± 3	47 ± 2	49 ± 1	29 ± 1	45 ± 8	50 ± 10	57 ± 8
Annexin V [%]	5 ± 1	6 ± 3	7 ± 4	7 ± 4	9 ± 5	8 ± 2	10 ± 2	12 ± 3

Tab. 3: Treatment of PCs with different UVC doses. Influence on platelet parameters and on storage stability. n=6, mean + SD

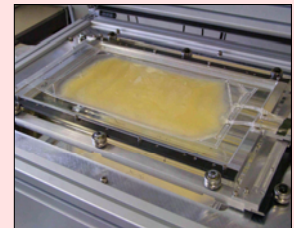


Fig. 1 : Irradiation device for UVC treatment of PCs

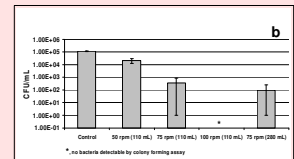
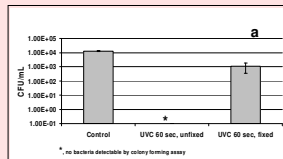


Fig. 2 : Inactivation of *St. epidermidis* in PC aliquots (110 or 280 mL) by irradiation with UV light: fixed vs. loosely placed irradiation bags (a); dependence of bacteria inactivation in loosely placed irradiation bags on the agitation speed (b). n=3, mean ± SD

Bacteria species	Characteristics	Gram stain	Number pf experiments	Spike (CFU/mL)	Bact/Alert result*	Remark
<i>B. cereus</i>	fac. anaerobic	pos	12	100-140	11 sterile 1 unsterile**	Spore former
<i>E. coli</i>	aerobic	neg	12	36-65	12 sterile	
<i>K. pneumoniae</i>	fac. anaerobic	neg	12	85-140	12 sterile	
<i>P. acnes</i>	anaerobic	neg	12	61-100	12 sterile	
<i>S. aureus</i>	fac. anaerobic	pos	22	60-110	22 sterile	
<i>S. epidermidis</i>	fac. anaerobic	pos	22	74-210	22 sterile	
<i>Str. pyogenes</i>	fac. anaerobic	pos	12	118-194	12 sterile	

*: Samples (2x10 mL each) were drawn after 3 and 6 days at 22°C
**: sterile after 3 days storage

Tab 1: Sterilization of PCs spiked with different bacteria species by irradiation with UVC (0.4 J/cm²)

Virus	Genome	Lipid Envelope	Model virus for	Log ₁₀ reduction factor
Vesicular stomatitis (VSV)	ss ⁺ RNA	X	-	≥ 6.41
Sindbis (Sindbis)	ss RNA	X	-	5.55
West Nile (WNV)	ss RNA	X	HCV	5.24
Human immunodeficiency (HIV-1)	ss RNA	X	-	1.36
Suid Herpes (SHV-1)	ds ⁺ DNA	X	HBV/CMV	3.57
Porcine Parvo (PPV)	ss DNA	-	Parvo B 19	≥ 6.42
Encephalomyocarditis (EMCV)	ss DNA	-	HAV	5.73

Tab 2: Inactivation factors of viruses by irradiation with UVC (0.4 J/cm²)

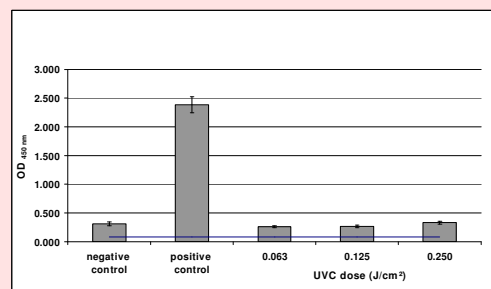


Fig. 3: Inactivation of T-lymphocytes in platelet concentrates by irradiation with UVC. Viability was assayed by mixed lymphocyte culture.

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¹ + Blood Center of the German Red Cross Chapters of NSTOB, Springe, Germany

² MacoPharma Int., Langen, Germany

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INACTIVATION OF SMALL NON-ENVELOPED VIRUSES IN PLASMA REDUCED PLATELET CONCENTRATES BY IRRADIATION WITH SHORT-WAVE ULTRAVIOLET LIGHT

H. Mohr¹, U. Gravemann¹, J. Knüver-Hopf¹, F. Tolksdorf², W.H. Walker², T.H. Müller¹

Aim

To investigate if small nonenveloped viruses in plasma reduced platelet concentrates are sensitive to irradiation with UVC light.

Introduction

Small nonenveloped viruses (e.g. parvovirus B19 and hepatitis A virus) are more resistant than lipid-enveloped viruses to most pathogen reduction procedures used for plasma or for cellular blood products (1-3). We have developed a procedure to decontaminate platelet concentrates (PCs) by irradiation with monochromatic short-wave ultraviolet light (wavelength: 254 nm). It is essential that the products are not fixed and at the same time strongly agitated during treatment (Fig. 1 and 2). We found that the procedure inactivates bacteria and lipid enveloped viruses. As the present data indicate, small nonenveloped viruses are also inactivated.

Materials and Methods

The virological investigations were conducted at NewLab BioQuality, Cologne, Germany. They were carried out according to CPMP/BWP guidelines 268/95 ("Note for guidance on virus validation studies") and 269/95 ("Note for guidance on plasma derived medicinal products"). The PCR investigations were performed in the own laboratory.

Plasma-reduced PCs (platelet concentration approx. 10⁹/mL, plasma content: approx. 30 %) were prepared from pools of 5 buffy coats. The storage medium used was SSP⁺ (MacoPharma). PCs (volume approx. 350 mL) in UV transparent polyolefine acetate bags (dimensions: 19x38 cm) were spiked with approx. 10⁷ up to 10⁸ TCID₅₀/mL of porcine parvovirus (PPV, strain ATCC CRL-6489 (NADL-2), a model for parvovirus B19) or echinocyclophor virus (EMCV, strain ATCC VR 129-B, a model for hepatitis A virus). The thickness of the PC layer was approx. 4-5 mm. Irradiation with UVC light was from both sides of the bags. The UVC dose applied was up to 0.6 J/cm² (irradiation time per 0.1 J/cm² was approx 15 sec). The PC-samples were loosely placed on a quartz plate located in the middle between two layers of mercury vapor tubes emitting monochromatic UVC light (wavelength: 254 nm). During irradiation they were intensively agitated using an orbital agitator. Agitation speed was approx. 100 rpm. Before and after irradiation virus titers (expressed as log₁₀ of tissue culture infectious doses (log₁₀TCID₅₀)) were determined.

The influence of UVC on the DNA of parvovirus B19 was investigated by long-range RT-PCR, using a LightCycler from Roche, Mannheim, Germany. The primer pair used spanned a region of 1028 bases (approx. one fifth of the genome of the virus). In these experiments PC aliquots of 110 mL were treated; dimensions of the irradiation bags were 12.5x14.5 cm.

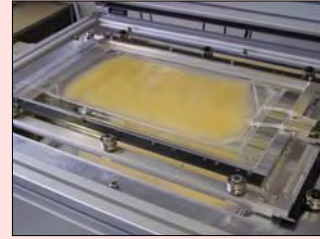


Fig. 1 and 2: Irradiation device for UVC treatment of PCs

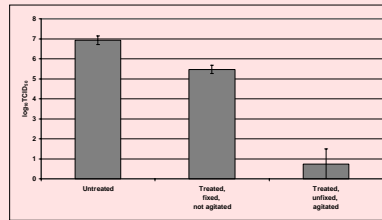


Fig. 2: Inactivation of vesicular stomatitis virus by irradiation with UVC (n=3). Comparison of two treatment modes: Fixed bags (changed between 2 quartz plates) vs. unfixed bags (loosely put on a quartz plate), both with agitation (100 rpm)

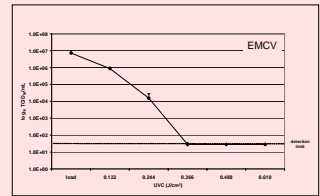
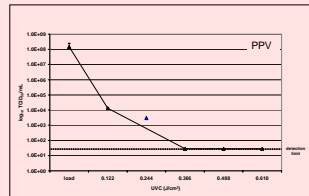


Fig. 3: Inactivation kinetics of PPV and EMCV in plasma-reduced PCs irradiated with UVC. (n= 2).

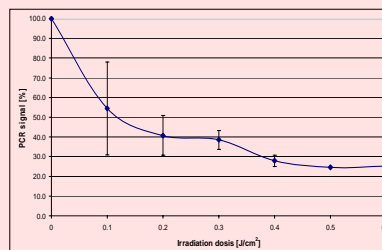


Fig. 4: Degradation of the DNA of parvovirus B19 spiked into plasma-reduced PCs by irradiation with UVC. (n= 3, mean ± SD).

Results

PPV as well as EMCV in plasma-reduced PCs were rapidly inactivated by irradiation with UVC light: no infective virus was detectable at doses higher than 0.366 J/cm² (Fig. 3). The log₁₀ reduction factors determined exceeded 6.4 and 5.5, respectively.

The PCR investigations revealed that the genome of parvovirus B19 was degraded by UVC treatment: at light doses between 0.4 and 0.6 J/cm² the PCR signal was reduced by approx. 75 % (Fig. 4). It remains to be established if this is indicative of complete inactivation of that virus.

At UVC doses up to 0.6 J/cm² platelet functions were only moderately influenced, and the storage stability of the treated products for up to 6 days after treatment (8 days after blood donation) was maintained (Table 1).

Conclusions

Irradiation with UVC light under strong agitation is a unique procedure to inactivate small nonenveloped viruses in PCs at conditions at which platelet functions and the storage stability of the PCs are not impaired.

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Parameter	Day 1 after irradiation				Day 4 after irradiation				Day 6 after irradiation			
	Control	UVC dose (J/cm ²)			Control	UVC dose (J/cm ²)			Control	UVC dose (J/cm ²)		
		0.4	0.5	0.6		0.4	0.5	0.6		0.4	0.5	0.6
Plt-concentration (x10 ⁹ /mL)	10.8 ± 0.6	10.2 ± 0.6	9.8 ± 0.6	9.1 ± 0.9	10.3 ± 0.8	10.1 ± 0.8	9.6 ± 0.8	9.5 ± 0.9	10.1 ± 0.8	9.8 ± 0.6	9.3 ± 0.8	9.3 ± 0.9
pH	7.10 ± 0.04	7.04 ± 0.05	7.09 ± 0.05	7.05 ± 0.04	7.21 ± 0.14	7.06 ± 0.04	7.10 ± 0.06	7.06 ± 0.20	7.27 ± 0.15	7.09 ± 0.06	7.11 ± 0.10	6.98 ± 0.07
Lactate (mmol/L)	7.7 ± 1.0	8.0 ± 0.5	7.7 ± 0.5	8.0 ± 0.7	10.7 ± 1.2	12.3 ± 1.3	11.7 ± 1.3	13.8 ± 2.0	12.7 ± 1.0	14.9 ± 1.0	14.6 ± 1.4	16.7 ± 1.4
Glucose (mg/dL)	122 ± 9	117 ± 7	117 ± 6	115 ± 7	86 ± 12	70 ± 10	75 ± 10	58 ± 15	62 ± 11	43 ± 8	44 ± 11	29 ± 10
Swirling	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok
Hypotonic shock response [%]	69 ± 5	66 ± 2	61 ± 6	62 ± 4	66 ± 3	67 ± 4	65 ± 3	61 ± 3	68 ± 2	65 ± 2	62 ± 3	56 ± 5
Spontaneous aggregation [%]	11 ± 2	14 ± 2	18 ± 3	18 ± 3	12 ± 2	15 ± 2	15 ± 1	17 ± 6	10 ± 2	16 ± 4	13 ± 2	14 ± 1
Collagen-induced aggregation [%] 100 µg/mL	95 ± 4	90 ± 5	88 ± 3	87 ± 2	77 ± 9	83 ± 10	80 ± 6	83 ± 5	62 ± 9	69 ± 8	67 ± 2	69 ± 5
Collagen-induced aggregation [%] 20 µg/mL	54 ± 13	77 ± 8	78 ± 6	74 ± 8	15 ± 7	35 ± 12	33 ± 9	39 ± 8	9 ± 1	16 ± 4	19 ± 1	22 ± 2
CD62 [V]	36 ± 1	46 ± 3	47 ± 2	49 ± 1	27 ± 3	36 ± 5	42 ± 6	48 ± 5	29 ± 1	45 ± 8	50 ± 10	57 ± 8
Annexin V [%]	5 ± 1	6 ± 3	7 ± 4	7 ± 4	6 ± 3	10 ± 5	9 ± 2	9 ± 2	9 ± 5	8 ± 2	10 ± 2	12 ± 3

Tab. 1: Irradiation of PCs with different UVC doses. Influence on platelet parameters and on storage stability. (n= 6, mean ± SD).

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