Inactivation of yellow fever virus with amotosalen and ultraviolet A light pathogen-reduction technology

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BACKGROUND: The reemergence of yellow fever virus (YFV) in Africa and Brazil, and massive vaccine campaigns triggered to contain the outbreaks, have raised concerns over blood transfusion safety and availability with increased risk of YFV transfusion-transmitted infections (TTIs) by native and vaccine-acquired YFV. Blood donor deferral for 2 to 4 weeks following live attenuated YFV vaccination, and deferral for travel to endemic/epidemic areas, may result in blood donor loss and impact platelet component (PC) stocks. This study investigated the efficacy of INTERCEPT Blood System pathogen reduction (PR) with use of amotosalen and ultraviolet A (UVA) light to inactivate high levels of YFV in PCs.

MATERIALS: Four units of apheresis platelets prepared in 35% plasma/65% platelet additive solution (PC-PAS) and 4 units of PC in 100% human plasma (PC-Plasma) were spiked with high infectious titers of YFV (YFV-17D vaccine strain). YFV-17D infectious titers were measured by plaque assay and expressed as plaque-forming units (PFU) before and after amotosalen/UVA treatment to determine log reduction.

RESULTS: The mean YFV-17D infectious titers in PC before inactivation were 5.5 ± 0.1 log PFU/mL in PC-PAS and 5.3 ± 0.1 log PFU/mL in PC-Plasma. No infectivity was detected immediately after amotosalen/UVA treatment.

CONCLUSION: The amotosalen/UVA PR system inactivated high titers of infectious YFV-17D in PC. This PR technology could reduce the risk of YFV TTI and help secure PC supplies in areas experiencing YFV outbreaks where massive vaccination campaigns are required.

Yellow fever virus (YFV) is the prototype member of the *Flavivirus* genus in the family Flaviviridae and is distinguished for being the first arthropod-borne human virus to be isolated. The virus is antigenically closely related to Zika virus (ZIKV), among other members of the Flaviviridae family indigenous to Africa.[1] Like the 70 related but distinct viruses belonging to this family, which includes dengue virus (DENV), West Nile virus (WNV) and ZIKV, YFV is a positive-sense, single-stranded RNA, enveloped virus. In humans and vertebrate hosts, the liver is the target organ, and hepatic dysfunction results in jaundice.[1,2] YFV infection ranges in severity from asymptomatic or mildly symptomatic forms to neurotropic and viscerotropic infection resulting in hemorrhagic syndromes and organ failure with substantial mortality.

The history of YFV is characterized by the recurrence, the severity, and the unpredictability of outbreaks. YFV may have originated in Africa and been introduced from West Africa into Western Europe from Spain to Ireland and the

ABBREVIATIONS: CHIKV = chikungunya virus; DENV = dengue virus; FDA = US Food and Drug Administration; PAS = platelet additive solution; PC = platelet component; PC-PAS = platelets prepared in 35% plasma/65% platelet additive solution; PC-Plasma = platelet component in 100% human plasma; PFU = plaque-forming units; PR = pathogen reduction; TTIs = transfusion-transmitted infections; UVA = ultraviolet A; WHO = World Health Organization; WNV = West Nile virus; YF = yellow fever; YFV = yellow fever virus; ZIKV = Zika virus.

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New World. Devastating outbreaks associated with high mortality and major economic losses were reported throughout the 19th century from Uruguay to Canada and in major eastern US cities. Since the last outbreak in the United States in 1905, YFV has been more endemic to Africa and Latin America.[3]

Traditionally, YFV is maintained via a jungle, or sylvatic, cycle through *Haemagogus* spp. mosquitoes in South America and by *Aedes* spp. in Africa. Sylvatic yellow fever (YF) cycles propagate the virus among monkeys with occasional transmission to humans, and are responsible for the majority of the human cases reported to the World Health Organization (WHO).[1] The risk for explosive urban YFV cycles becomes a concern when sylvatic YFV outbreaks occur near densely populated areas infested by *Aedes aegypti*.[4,5] In such areas, YF control can be obtained through mosquito control and through human immunization. Unfortunately, vector control programs, while successful in the past, have been discontinued, and mosquito populations returning to high levels are now responsible for recurring outbreaks with cocirculation of multiple arboviruses including DENV, chikungunya virus (CHIKV), ZIKV, and YFV.

While no antiviral therapy exists to treat YF disease, an efficient live attenuated YFV vaccine strain 17D (YFV-17D), developed in 1937, is being used for routine immunization in endemic areas and for travelers to endemic areas.[2] Reactive massive vaccine campaigns have been used to prevent urban YF cycles during the most recent 2015 Angola and 2016 to 2018 Brazil outbreaks.[4] YFV-17D live attenuated vaccine is associated with serious adverse events, such as vaccine-associated neurotropic or viscerotropic diseases, reported at a rate of 0.3-3.2 cases/100,000 doses with increasing risk factors in immunocompromised patients.[2]

Transfusion transmission of YFV-17D was documented for the first time in 2009 when 6 units of blood products collected from US active-duty trainees who had received the vaccine 4 days before donation were transfused to five patients, one of whom died.[6] This led to donor deferral recommendations by AABB, WHO, and Pan American Health Organization to defer recipients of YFV vaccine for more than 2 and up to 4 weeks. In areas with massive vaccine campaigns, platelet component (PC) shortages can be a concern.[7,8]

The US Food and Drug Administration (FDA)-approved amotosalen/ultraviolet A (UVA) pathogen-reduction (PR) technology for platelets and plasma used routinely in more than 30 countries has previously demonstrated robust inactivation of several arboviruses including flaviviruses and alphaviruses. This study investigated the efficacy of the INTERCEPT Blood System for platelets to inactivate high levels of YFV in PC prepared in 100% plasma (PC-Plasma) or 35% plasma/65% platelet additive solution (PC-PAS).

MATERIALS

Experimental design

Inactivation of YFV in PC by amotosalen/UVA light treatment with the INTERCEPT Blood System for platelets–small volume processing set was performed at Cerus Corporation according to the manufacturer's instructions. A total of 4 units of human apheresis platelets suspended in 35% human plasma/65% PC-PAS (InterSol, Fenwal) collected with the AMICUS (Fresenius-Kabi) platform (PC-PAS), and 4 units of platelets suspended in 100% human plasma collected on the Trima (Terumo BCT) platform (PC-Plasma) were tested. Samples of PCs for infectious virus quantitation by plaque assay were taken before and after amotosalen/UVA treatment to determine log inactivation values.

Blood collection

Each test unit was composed of a single PC collected from individual donors at Bonfils Blood Center who were screened for previous history of YFV vaccination or known exposure to the virus. The target volume and dose for platelet treatment of PC-PAS and PC-Plasma was 285 mL (range, 270-300 mL) with a target platelet dose of 3.6 to 4.4×10^{11}. PC volume and dose were adjusted, as necessary, to achieve these target conditions. Donor PCs were collected the day before each experiment and shipped to Cerus, where amotosalen/UVA treatment was performed.

Virus and cells

Individual PCs were inoculated with YFV-17D, obtained through the NIH Biodefense and Emerging Infectious Research Resources Repository, National Institute of Allergy and Infectious Diseases, National Institutes of Health (catalog no. NR-115). The vaccine virus is derived from the virulent Asibi strain of YFV prepared by serial in vivo and in vitro passage and differs from the virulent strain by 68 nucleotides.[9] The virus was propagated and titer determined on Vero76 African green monkey kidney cells (ATCC, catalog no. CRL-1587) grown at 37°C in an atmosphere of 5% CO_2 in 1X Eagle's Minimum Essential Medium outgrowth medium with phenol red, 1.5 g/L sodium bicarbonate, nonessential amino acids, L-glutamine, and sodium pyruvate (Corning, catalog no. 10-009-CV) and supplemented with 200 U/mL penicillin, 200 μg/mL streptomycin, and 2% fetal bovine serum.

INTERCEPT Blood System for platelets

Inactivation studies were performed as described by Laughhunn et al.[10] The INTERCEPT small volume processing set for platelets consists of the following sequentially integrated components: 15 mL of 3-mM amotosalen solution, a 1.0-L illumination container, a 1.0-L compound adsorption device container, an inline filter, and a 1.3-L final storage container. The compound adsorption device and storage

containers were not used in this study. For treatment, virus-contaminated PCs mixed with amotosalen solution in the illumination container were exposed to UVA light with the INTERCEPT Illuminator (Cerus Corp.). During illumination, containers undergo reciprocal shaking (approx. 72 cycles/minute) during illumination. The Illuminator delivers the UVA treatment dose to each platelet unit through a pair of opposing banks of fluorescent lamps.

Treatment process and sampling

In the present study, 4 units of PC-PAS and PC-Plasma each with a target volume of 285 mL (range, 270-300 mL) and a target platelet dose of 3.6 to 4.4×10^{11} were processed with amotosalen/UVA according to manufacturer's instructions. For virus spiking of the platelet units, 10 mL of PC were first removed from each unit via syringe using a Luer lock adaptor, and approximately 3.0 mL of YFV-17D stock was added to each unit to create an approximately 1:100 dilution. The tubing through which virus was added was then rinsed with the 10 mL of PC that had been removed, and the units were mixed end over end. Spiked PCs were allowed to flow by gravity through the amotosalen pouch and mix with amotosalen in the illumination container. A Luer lock adaptor was added to the illumination container, and a 3-mL sample was removed and stored frozen until virus titration of the PC pretreatment (Control). Following UVA illumination, a 30-mL sample of posttreatment PC was removed from each unit and frozen until virus titration (Test).

Virus plaque assay

For virus titration, the stock, pretreatment, and post-treatment samples were rapidly thawed in a 37°C water bath and diluted in 1X Eagle's Minimum Essential Medium with Earle's Salts supplemented with 5 U/mL heparin. Heparin was used to prevent fibrin clots, which are disruptive to the Vero76 cell culture monolayer. Before testing pretreatment and posttreatment study samples, validation studies were conducted to ensure that the dilutions chosen for testing of virus in PC-PAS or PC-Plasma were not cytotoxic or inhibitory to virus detection in the Vero76 cell plaque assay. Based on these studies, PC samples were tested at a minimum dilution of 1:2 in virus inoculation buffer.

Pretreatment samples were serially diluted 10-fold in virus inoculation buffer, and dilutions were plated into four wells (1.0 mL/well) of a six-well plate containing a confluent monolayer of Vero76 cells. Postillumination samples were tested in 20 wells at a 1:2 dilution (1.0 mL/well), and 10 wells at a 1:10 dilution (1.0 mL/well) representing approximately 3.7% of the volume of each treated platelet unit assuming an average treatment volume of 300 mL. The inoculated plates were incubated at 37°C with 5% CO_2 for approximately 1 hour. The inoculum was then removed, a 2-mL agarose overlay was added to each well, and the plates were returned to the incubator. Overlay was composed of equal volumes of 2X Eagle's Minimum Essential Medium with Earle's Salts, supplemented with fetal bovine serum to a final concentration of 2%, and 3.2% agarose (SeaPlaque, Lonza). After 8 to 9 days, the plates were removed from the incubator and stained with approximately 200 µL of 5 mg/mL methylthiazolyldiphenyl-tetrazolium bromide (Sigma-Aldrich) per well for plaque visualization. The plates were placed back into the incubator for approximately 60 minutes. After staining was complete, the plaques were counted macroscopically. Titers were reported in log plaque-forming units (PFU)/mL. When no detectable virus was identified in the post-treatment samples, the inactivation value was reported as the input (pretreatment) titer.

RESULTS

Treatment of YFV-17D in PC by amotosalen/UVA provided robust virus inactivation to below the limit of detection in both PC-Plasma and PC-PAS preparations (Table 1).

TABLE 1. Yellow fever virus (YVF) quantitation (expressed as log PFU/mL) in platelets prepared in 35% plasma/65% PAS or 100% plasma before and after treatment with amotosalen/UVA

Component	Unit	Virus Stock Titer	Sample	Titer	Inactivation	Mean inactivation
PC-PAS	1	7.42	Pretreatment	5.51	>5.51	>5.5 ± 0.10
			Posttreatment	ND		
	2	7.45	Pretreatment	5.63	>5.63	
			Posttreatment	ND		
	3	7.54	Pretreatment	5.54	>5.54	
			Posttreatment	ND		
	4	7.31	Pretreatment	5.40	>5.40	
			Posttreatment	ND		
PC-Plasma	1	7.52	Pretreatment	5.33	>5.33	>5.3 ± 0.10
			Posttreatment	ND		
	2	7.32	Pretreatment	5.20	>5.20	
			Posttreatment	ND		
	3	7.42	Pretreatment	5.34	>5.34	
			Posttreatment	ND		
	4	7.49	Pretreatment	5.38	>5.38	
			Posttreatment	ND		

ND = not detected/no plaques detected at any dilution tested.

Starting volumes ranged from 271.7 mL to 289.7 mL per unit with a dose of between 3.6×10^{11} and 4.4×10^{11} platelets. Stock YFV-17D used to spike PC units before treatment had a titer of approximately 7.4 log PFU/mL and was added at a 1:100 dilution to individual PC units to achieve a target final concentration of approximately 5.4 log PFU/mL. The mean and standard deviation of YFV-17D infectious titers in PC before treatment were assessed as an average of 5.5 ± 0.1 log PFU/mL for PC-PAS and 5.3 ± 0.1 log PFU/mL for PC-Plasma (Table 1). PC units were treated using a UVA light treatment and a final amotosalen concentration of 150 μM.

Samples from PC-PAS and PC-Plasma units removed immediately after amotosalen/UVA treatment did not contain detectable replicative virus. Virus titers in treated samples were below the limit of detection of the assay, therefore the log inactivation of YFV-17D in this study was equivalent to the mean input titer for PC-PAS and PC-Plasma units (Table 1), greater than 5.5 ± 0.1 log PFU/mL and greater than 5.3 ± 0.1 log PFU/mL, respectively.

DISCUSSION

The results of this study demonstrate that amotosalen/UVA treatment provided robust inactivation of YFV, one of several globally emerging mosquito-borne arboviruses that have been shown to be sensitive to amotosalen/UVA treatment. High levels of inactivation, namely, greater than 5.5 log PFU/mL in PC-PAS and greater than 5.3 log PFU/mL in PC-Plasma, are in range with those attained with amotosalen/UVA treatment of platelets and plasma containing CHIKV,[10,11] WNV,[12,13] DENV,[14,15] and ZIKV.[16,17]

While wild-type and vaccine strains of YFV differ by only 68 nucleotide differences, encoding 33 amino acid substitutions scattered throughout the genome,[18] the YFV-17D vaccine strain was used in this study for practical reasons, as it can be handled in a Biosafety Level 2 environment, while the wild-type YFV requires handling in a Biosafety Level 3 environment. Based on strong structural and genetic similarity, wild-type and vaccine strains of YFV may be similarly sensitive to amotosalen/UVA treatment.[18] This study investigated the efficacy of amotosalen/UVA treatment to inactivate high titers of YFV-17D in both PC-PAS and PC-Plasma as INTERCEPT Blood System is approved for PR of Amicus platelets resuspended in platelet additive solution (PAS) and Trima platelets in 100% plasma. In addition, while platelets are resuspended in PAS in Europe[19] and in PAS or in 100% plasma in North America,[20] in Latin America, which is most impacted by YFV outbreaks, platelet manufacturing is in 100% plasma, and this is the first demonstration of PR technology to inactivate YFV in PC-Plasma. A previous study by Faddy et al.[21] reported YFV-17D inactivation levels of greater than 4.77 log in plasma treated with the THERAFLEX MB-Plasma system and greater than 4.8 log in PC resuspended in 30% plasma/70% PAS treated with THERAFLEX UV-Platelets. We report here higher levels of inactivation using the INTERCEPT Blood System for platelets, which is currently used routinely in more than 30 countries and has been reported to be efficacious at inactivating other arboviruses that often cocirculate in impacted areas.

PR technology with high inactivation capacity is particularly important to safeguard the blood supply in areas impacted by emerging arboviruses. Not only such viruses can emerge in the human population months to years before surveillance detects them,[22–25] but most infections are commonly asymptomatic.[26,27] In 45% of the YF cases, symptoms may develop 3 to 6 days after infection. Wild-type YFV causes a pansystemic viral disease with viremia approximately 8 log GE/mL[28] and symptoms may develop after or concomitantly with peak viremia.[28] Therefore, asymptomatic or presymptomatic infected blood donors could present at the blood center and be allowed to donate blood while in the ramping phase of viremia. Assuming YFV is similar to that reported for other flaviviruses, which have a 2 to 3 log ratio of infectious particles to genome equivalent,[29,30] a PR system with inactivation capacity greater than 5 logs as measured by infectivity assays is preferable. While recipients of the YFV-17D live attenuated vaccine should be deferred, there is always a risk for donors to not disclose a recent vaccination. One dose of vaccine contains between 10^4 to 10^6 PFU of virus and half of vaccine recipients develop viremia peaking 5 days following immunization at 10^3 to 10^4 copies/mL (<200 PFU/mL).[30] As with all live attenuated vaccines, YFV-17D TTI could adversely impact patients undergoing immunosuppressive therapy,[31] who could be at increased risk for the development of vaccine-associated neurotropic or viscerotropic YF disease.[2,32,33]

Donor deferrals for a period of 2 to 4 weeks following vaccination negatively impact platelet availability in areas undergoing massive vaccine campaigns. PR could be considered to replace donor deferrals to maintain platelet availability. In several instances, during the CHIKV outbreaks in La Reunion and more recently in Rome, and during the ZIKV outbreak in Puerto Rico, the INTERCEPT Blood System was implemented as an alternative to deferrals[34,35] and blood screening.[36,37] The FDA mandated during the ZIKV outbreak that all blood collections on the island of Puerto Rico be either screened using a nucleic acid amplification testing (NAT) or treated using a licensed PR technology,[34] and allowed for PR to replace donor deferrals.[36] Following the same shift in paradigm, the FDA recently allowed for PR to replace deferrals and screening for *Babesia*[37] and granted variances to several blood centers willing to replace malaria deferrals with PR.[38]

While mosquito-borne disease outbreaks are unpredictable, areas with the potential to be severely impacted by arbovirus emergence including YFV, PR with use of amotosalen/UVA could be implemented proactively to mitigate the risk of TTI for all platelet recipients or for specific patient populations

who are severely immunosuppressed and cannot undergo YFV vaccination. PR could also be considered as an alternative to donor deferrals to maintain PC availability in remote and outbreak areas. Specifically, PR could be proactively implemented in areas where the potential for arbovirus spread is high, namely, areas infested with *Aedes* spp. mosquitoes.[39,40] PR could be considered in nonendemic regions as well, which are currently relying on donor deferrals, as these regions have experienced an increase in blood donor loss due to travel-associated deferrals over the past decade.[41]

PR with use of amotosalen/UVA could be considered in addition or as an alternative to deferrals to mitigate the risk associated with YFV and YFV-17D transmission through platelets while improving blood safety in general, reducing the risk of TTI associated with other viruses, parasites, and bacteria as well as transfusion-associated graft-versus-host disease.

CONFLICT OF INTEREST

YG, FSM, and MCL are employees of Cerus Corporation.

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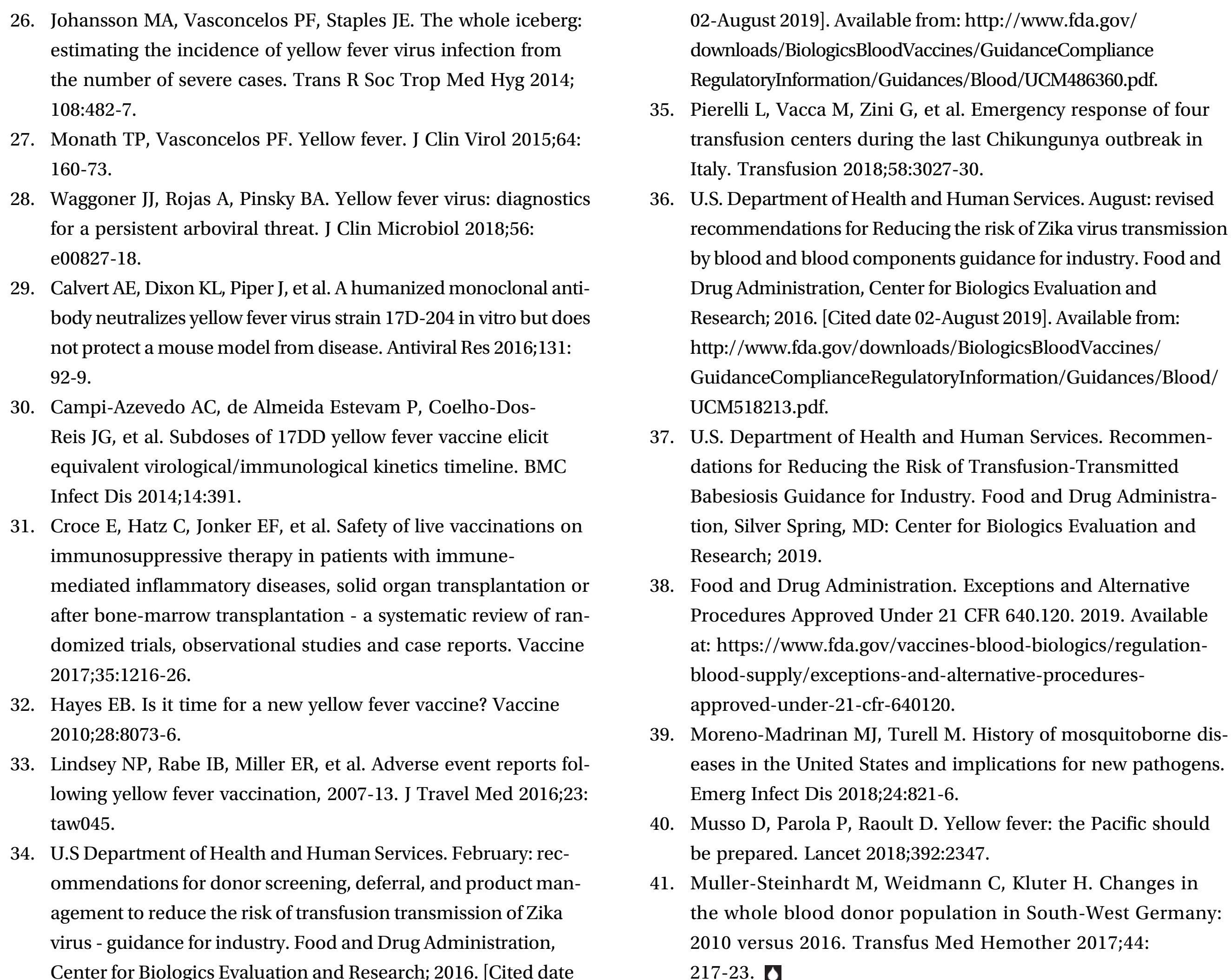

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