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Original article

Efficient inactivation of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in human apheresis platelet concentrates with amotosalen and ultraviolet A light



Inactivation efficace du coronavirus-2 du syndrome respiratoire aigu sévère (SARS-CoV-2) dans des concentrés de plaquettes humaines d'aphérèse traités par amotosalen et rayons ultraviolets A

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ABSTRACT

Objectives. – The detection of SARS-CoV-2 RNA in blood and platelet concentrates from asymptomatic donors, and the detection of viral particles on the surface and inside platelets during *in vitro* experiments, raised concerns over the potential risk for transfusion-transmitted-infection (TTI). The objective of this study was to assess the efficacy of the amotosalen/UVA pathogen reduction technology for SARS-CoV-2 in human platelet concentrates to mitigate such potential risk.

Material and methods. – Five apheresis platelet units in 100% plasma were spiked with a clinical SARS-CoV-2 isolate followed by treatment with amotosalen/UVA (INTERCEPT Blood System), pre- and posttreatment samples were collected as well as untreated positive and negative controls. The infectious viral titer was assessed by plaque assay and the genomic titer by quantitative RT-PCR. To exclude the presence of infectious particles post-pathogen reduction treatment below the limit of detection, three consecutive rounds of passaging on permissive cell lines were conducted.

Results. – SARS-CoV-2 in platelet concentrates was inactivated with amotosalen/UVA below the limit of detection with a mean log reduction of $> 3.31 \pm 0.23$. During three consecutive rounds of passaging, no viral replication was detected. Pathogen reduction treatment also inhibited nucleic acid detection with a log reduction of $> 4.46 \pm 0.51$ PFU equivalents.

Conclusion. – SARS-CoV-2 was efficiently inactivated in platelet concentrates by amotosalen/UVA treatment. These results are in line with previous inactivation data for SARS-CoV-2 in plasma as well as MERS-CoV and SARS-CoV-1 in platelets and plasma, demonstrating efficient inactivation of human coronaviruses.

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R É S U M É

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But/Objectif. – La détection de l'ARN du SARS-CoV-2 dans le sang de donneurs asymptomatiques, et de particules virales à la surface et à l'intérieur des plaquettes, ont suscité des inquiétudes quant au risque potentiel de transmission transfusionnelle. L'objectif de cette étude était d'évaluer l'efficacité de la technique de réduction des agents pathogènes amotosalen/UVA pour le SARS-CoV-2 dans des concentrés de plaquettes humaines.

Matériel et méthodes. – Cinq unités de plaquettes d'aphérèse en plasma ont été inoculées avec un isolat clinique de SARS-CoV-2 puis traitées avec de l'amotosalen/UVA. Des échantillons ont été collectés avant et après traitement ainsi que des témoins positifs et négatifs non traités. Le titre viral infectieux a été évalué par une épreuve sur plaque et le titre génomique mesuré par RT-PCR quantitative. Pour exclure la présence de particules infectieuses après traitement en dessous de la limite de détection, trois passages sur des lignées cellulaires permissives ont été effectués.

Résultats. – Le SARS-CoV-2 dans les concentrés plaquettaires a été inactivé en dessous de la limite de détection avec une réduction logarithmique moyenne $> 3,31 \pm 0,23$. Aucune réplication virale n'a été détectée au cours de trois passages consécutifs. Le traitement de réduction des agents pathogènes a également inhibé la détection des acides nucléiques avec une réduction logarithmique $> 4,46 \pm 0,51$.

Conclusion. – Le SARS-CoV-2 a été efficacement inactivé dans les concentrés plaquettaires par traitement amotosalen/UVA. Ces résultats sont conformes aux données d'inactivation.

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1. Introduction

On 11th of March 2020 the WHO declared a pandemic of the respiratory COVID-19 disease caused by SARS-CoV-2, a beta-Coronavirus spreading rapidly by respiratory transmission throughout the globe. To date, more than 214 million infections have been confirmed and more than 4.4 million disease-related deaths were reported globally according to the WHO.

The detection of SARS-CoV-2 genomic RNA at low viral loads in serum and blood samples of symptomatic patients was reported, but only in a minority of approximately 10% of the samples (linked to disease severity) in a quantity often close to the limit of detection (CT values above 35) [1]. Low viral load SARS-CoV-2 genomic RNA was also detected in rare occasions in screening samples, post-donation information (PDI) samples and blood products from asymptomatic donors, as well as in platelet units, but infectious virus could not be isolated [2–4]. Interestingly, platelets of symptomatic patients were shown to be associated with viral RNA [5]. *In vitro* studies showed the attachment of viral particles to the surface of platelets, as well as the presence of viral particles inside platelets, but the meaning of these findings is still unclear [6]. The transmission of infectious SARS-CoV-2 through blood transfusion has not been reported yet, but even unlikely it cannot be excluded and is perceived as a theoretical risk [2–4].

The amotosalen/UVA pathogen reduction (PR) process uses a photochemical reaction to crosslink nucleic acids, resulting in the inhibition of cell and pathogen replication and transcription [7,8]. Efficient inactivation was shown for a broad variety of viruses and parasites [9]. A recent study showed an effective inactivation of many bacterial species with a breakpoint of $> 7 \log \text{ cfu/mL}$ [10] in human platelet concentrates, which is in line with former findings [11]. The treatment of platelet units also efficiently inactivates white blood cells, eliminating the need for gamma-irradiation [12,13]. The treated platelets have been shown clinically comparable to untreated platelets with respect to hemostasis [14] and component utilization [15].

To address potential concerns regarding the theoretical transmission of human coronaviruses by blood transfusion, and after the demonstration of efficient inactivation of MERS-CoV in plasma and platelets with amotosalen/UVA [16,17], we recently demonstrated the efficient inactivation of a local clinical SARS-CoV-2 isolate in human plasma with amotosalen/UVA [18]. In the current study, we

expanded that work assessing the pathogen inactivation efficacy of the amotosalen/UVA PR treatment for SARS-CoV-2 in human apheresis platelet concentrates in 100% plasma.

2. Materials and methods

2.1. Viral stock and culture

We used a clinical SARS-CoV-2 isolate (SARS-CoV-2/human/SAU/85791C/2020, gene bank accession number: MT630432) maintained in Vero E6 cells (ATCC# CRL-1586) in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS). Vero E6 cells and SARS-CoV-2/human/SAU/85791C/2020 were used in all experiments as described previously [18].

2.2. Preparation of the SARS-CoV-2 viral stock

The SARS-CoV-2 stock was prepared as described previously [18]. Briefly, 90–95% confluent Vero E6 cells were inoculated with a multiplicity of infection (MOI) of one and incubated at 37 °C with 5% CO₂ in a tissue culture incubator until 80%–90% of cells showed a cytopathic effect (CPE). Supernatant was then collected; cellular debris removed, and aliquots of the viral stock were subsequently stored at –80 °C. The infectious titer was determined by plaque assay.

2.3. Platelet preparation

Apheresis platelet units in 100% donor plasma (~380 mL volume, 4.6×10^{11} platelets per unit) were collected at the King Abdulaziz University Hospital (KAUH) Transfusion Services, Jeddah, Saudi Arabia, from voluntary donors with a Trima apheresis unit (Terumo BCT, Japan) as described previously [17]. The platelet units were stored at 20–22 °C under continuous agitation. All platelet units were screened for antibodies against HCV, HBsAg, HBc, HIV (1/2), HTLV (1/2) and Treponema as well as for HCV, HBV and HIV by NAT. All units were assessed for the presence of anti-SARS-CoV-2 neutralizing antibodies using an in-house micro-neutralization (MN) assay. The assay was conducted as previously described using a local SARS-CoV-2 clinical isolate (SARS-CoV-2/human/SAU/85791C/2020) (Genbank accession

number MT630432.1) [19]. MN titers of $\geq 1:20$ were considered positive.

2.4. SARS-CoV-2 inactivation

Platelet units were spiked with SARS-CoV-2 viral stock in a 1:100 dilution. The spiked units were subsequently treated with the INTERCEPT Blood System for Platelets using the Large Volume Processing Set and the INTERCEPT Illuminator INT-100 (Cerus Corporation, USA) according to the manufacturer's instructions. The platelet container was sterile connected to the processing set (a closed system), followed by mixing the platelet concentrate with 17.5 mL amotosalen solution (3 mM) in the illumination container. Subsequently the illumination container was exposed to 3 J/cm² UVA light (320–400 nm) under reciprocal shaking to induce the photochemical reaction (using the INTERCEPT illuminator). Amotosalen intercalates in nucleic acids and forms irreversible covalent bonds in the presence of UVA light, cross-linking nucleic acids, inhibiting replication and transcription. After illumination, the platelet concentrate was transferred to the compound absorption device (CAD) container followed by a 16 h to 20 h incubation step under gentle agitation in a platelet incubator to reduce residual amotosalen and photoproducts by non-specific binding to surface of a polystyrene matrix. After the CAD incubation step, the platelets were transferred to platelet storage containers. The following samples were collected for analysis: a negative control (the platelet units before spiking), a positive control (viral stock), a pretreatment sample (spiked unit post-inoculation) and a post-treatment sample (spiked unit post-PR treatment). All samples were stored at -80°C until testing.

2.5. SARS-CoV-2 passaging experiments

SARS-CoV-2 passaging experiments to detect low-abundant replication-competent particles in human apheresis platelet units in 100% plasma pre- and post-PR treatment were conducted as previously described [18] with minor modifications. Pre- and post-treatment samples were diluted in a 1:10 dilution in DMEM with 2% FBS, transferred to susceptible Vero E6 cells in duplicates, and incubated for 1 h at 37°C . Then, the inoculum was exchanged against 2 mL DMEM with 2% FBS and the cells were cultured for 3 days at 37°C in a tissue culture incubator. Supernatants were collected, diluted 1:10 with DMEM with 2% FBS and re-transferred to non-infected Vero E6 cells for two more passages. Supernatants were collected at day 3 of incubation of each passage for viral load determination.

2.6. SARS-CoV-2 plaque assay

Plaque assays were conducted as previously described [18] with a minor modification. Samples were serially diluted in DMEM with 2% FBS and 1 mL from each dilution was inoculated on susceptible confluent Vero E6 cells and incubated for 1 h at 37°C . The inoculum was subsequently removed and overlaid with DMEM with 0.8% agarose and incubated for 3 more days at 37°C in a tissue culture incubator. Cells were then stained with crystal violet for 4 h at 37°C . The viral infectious titer was expressed as plaque forming unit (PFU)/mL.

2.7. Quantitative detection of viral genomes

RNA was extracted from all samples directly from the platelet units with a QiAmp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Quantitative one-step dual-target RT-PCR was conducted with a RealStar SARS-CoV-2 RT-PCR

Kit 1.0 (Altona Diagnostics, Germany) detecting an E-gene (beta-Coronavirus specific) and a S-gene (SARS-CoV-2 specific) target as well as an internal control using a 7500 Fast Real-Time PCR System (Applied Biosystems, USA) according to the manufacturer's instructions. The decrease in viral load was expressed by comparing the cycle threshold (CT) values from each sample relative to the CT values of the pretreatment inoculated sample (with the S-gene primers). The SARS-CoV-2 titers were expressed as PFU equivalents per mL (PEq/mL) using a standard curve (standard: serial dilutions of the viral stock based on the PFU titer) and choosing dilutions of the original sample (10⁻¹ to 10⁻⁸) with CT values in the exponential phase. Each run included a positive viral template control and no-template negative control. Each sample was tested in duplicate, and the mean is reported as PEq/mL.

2.8. IRB approval

The study was approved by the Unit of Biomedical Ethics of the King Abdulaziz University Hospital (approval #285-20).

3. Results

3.1. Inactivation of infectious SARS-CoV-2 particles in human apheresis platelet units

The platelet units used for this experiment were tested negative for the presence of SARS-CoV-2 neutralizing antibodies using the MN assay. Five human apheresis platelet units (A-E) in 100% donor plasma were collected and spiked with SARS-CoV-2. The units were subsequently treated with amotosalen/UVA. The mean infectious titer in the pre-PR treatment samples was $3.31 \pm 0.23 \log_{10}$ PFU/mL (3.68–3.11 \log_{10} PFU/mL) (Table 1). The PR treatment resulted in a reduction of $> 3.31 \pm 0.23 \log_{10}$ PFU/mL, since no infectious virus was detected in the PR samples in the plaque assay (Table 1). Fig. 1 shows a representative plaque assay for the units tested in this study. Negative control samples and post-PR treatment samples showed no detectable replication-competent viral particles. The mean infectivity of the viral stock was $5.27 \pm 0.19 \log_{10}$ PFU/mL and post-spiking in a 1:100 dilution in the pre-PR treatment samples $3.31 \pm 0.23 \log_{10}$ PFU/mL, which is close to the expected post-spiking titer of $3.27 \log_{10}$ PFU/mL, confirming no unexpected loss of infectivity by dilution and the addition of amotosalen.

3.2. The impact of amotosalen and UVA light treatment on the viral genomic titer

For further confirmation of the results, the viral genomic titer was assessed for all collected samples. The median Ct value in the spiked samples pre-PR treatment was 20.1 (18.0–22.2) for the SARS-CoV-2 S-protein specific primers. The mean pre-PR treatment viral genomic titer was $4.46 \pm 0.51 \log_{10}$ PEq/mL (3.71–4.99 \log_{10} PEq/mL) (Table 2), approximately one level of magnitude higher as the infectious titer ($3.31 \pm 0.23 \log_{10}$ PFU/mL). Post-PR treatment, very low viral genome titers were detectable (Table 2). The internal control was always positive indicating no PCR inhibition, confirming that the decrease of the signal from the SARS-CoV-2 S-gene specific primers in the PR samples is due to a mean minimum inactivation of $4.46 \pm 0.51 \log_{10}$ PEq/mL.

3.3. Passaging of pathogen-reduced platelets to confirm complete inactivation of infectious SARS-CoV-2 particles

To exclude the possibility of any remaining replicating SARS-CoV-2 particles in the treated platelet units (Table 1), we inoculated the collected samples on Vero E6 cells and evaluated infectivity

Table 1
Reduction of infectious SARS-CoV-2 titers in human platelet units after amotosalen/UVA treatment.

Experiment	Viral infectivity titer, log ₁₀ PFU/mL				Log reduction
	Positive control	Negative control	Pretreatment sample ^a	Posttreatment sample	
A	5.34	ND	3.2	ND	> 3.2
B	5.53	ND	3.68	ND	> 3.68
C	5.19	ND	3.36	ND	> 3.36
D	5.02	ND	3.19	ND	> 3.19
E	5.27	ND	3.11	ND	> 3.11
Mean ± SD	5.27 ± 0.19	ND	3.31 ± 0.23	ND	> 3.31 ± 0.23

ND: not detected.

^a After addition of amotosalen.

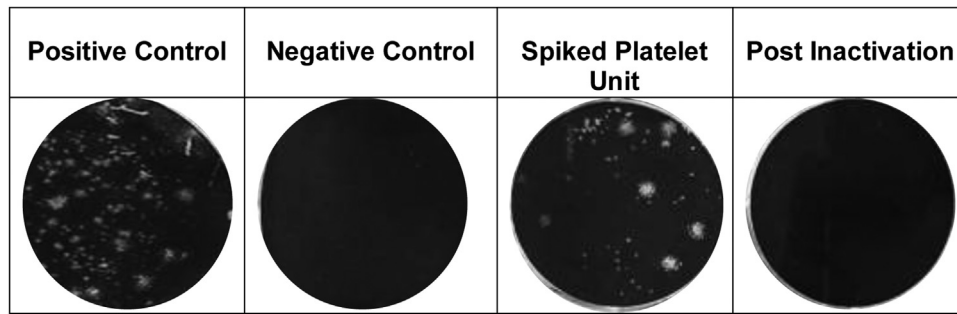


Fig. 1. Inactivation of SARS-CoV-2 in platelets by amotosalen and UVA treatment assessed by a plaque assay. Vero E6 cells were inoculated for 1 h with the following samples in a 1:10 dilution in DMEM: the SARS-CoV-2 viral stock (positive control), human platelets (negative control), platelets from a SARS-CoV-2 spiked pretreatment sample (spiked plasma unit) and amotosalen/UVA-treated, SARS-CoV-2 spikes platelets (post-inactivation). The cells were overlaid with agarose, incubated for three more days followed by crystal violet staining. Experiments were conducted in serial dilutions. Photographs (4×) are shown from one of five representative experiments.

Table 2
SARS-CoV-2 genomic load in platelets before and after amotosalen/UVA treatment^{a,b,c}.

Experiment	Positive control	Negative control	Pretreatment sample	Posttreatment sample
A	6.84	ND	4.35	0.10
B	6.35	ND	4.36	0.21
C	6.39	ND	3.71	0.15
D	6.30	ND	4.99	0.30
E	6.23	ND	4.87	0.04
Mean ± SD	6.02 ± 0.99	ND	4.46 ± 0.51	0.16 ± 0.1

ND: not detected.

^a Data are shown as log₁₀ PEQ/mL.

^b Titers were determined from the same samples used in Table 1.

^c Internal controls were positive for all tested samples with an average CT value of 24.2 showing less than 10% variation between samples.

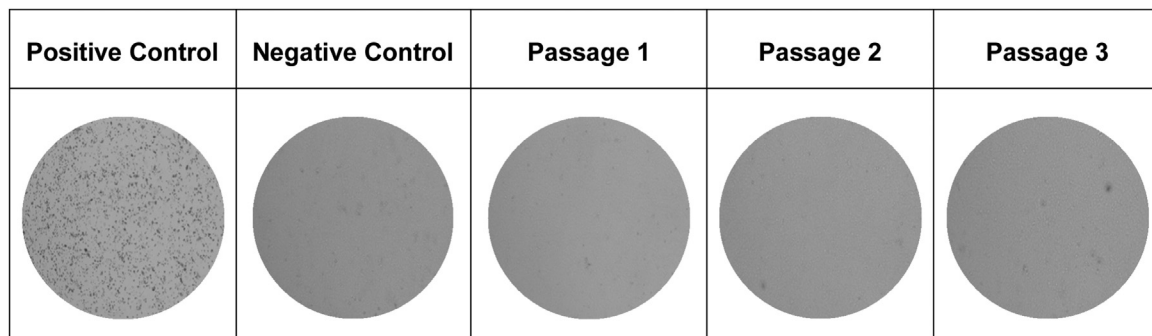


Fig. 2. Complete inactivation of replicative SARS-CoV-2 post-amotosalen/UVA treatment by passaging experiments. Vero E6 cells were inoculated for 1 h with the following samples in a 1:10 dilution in DMEM: plasma from a SARS-CoV-2 spiked pretreatment sample (positive control), human platelets (negative control) and amotosalen/UVA-treated, SARS-CoV-2 spikes platelets (passage 1–3) passaged for three consecutive passages. Both, the positive control and the pretreatment sample caused extensive CPE by day 3 post-inoculation in all three passages. Negative control and inactivated sample did not show any CPE in Vero E6 cells. Photographs (4×) are shown from one of five representative experiments on day 3 post-inoculation from each passage.

over three successive passages. While culture of all pre-PR treatment samples showed viral replication and complete CPE within 3 days post-inoculation comparable to the positive control, neither viral replication nor CPE was observed in cells inoculated with PR

samples similar to the negative controls (Fig. 2), even after 9 days of incubation in all three passages. For further confirmation, we determined the genomic viral load from supernatants collected from all passages inoculated with either pre-PR treatment or PR samples. As

Table 3
Results of passing experiments of SARS-CoV-2 in Vero E6 cells before and after inactivation of spiked platelets^{a,b}.

Experiment	Passage 1	Passage 2	Passage 3
A			
Pretreatment sample	6.3	6.2	5.9
Posttreatment sample	ND	ND	ND
B			
Pretreatment sample	6.1	5.9	5.8
Posttreatment sample	ND	ND	ND
C			
Pretreatment sample	5.6	5.3	5.0
Posttreatment sample	ND	ND	ND
D			
Pretreatment sample	6.4	6.0	5.7
Posttreatment sample	ND	ND	ND
E			
Pretreatment sample	5.9	5.6	5.3
Posttreatment sample	ND	ND	ND

ND: not detected.

^a Data are shown as log₁₀ PEq/mL.

^b Samples in Table 1 were used in this experiment. Samples were used at 1:10 dilution and titer was determined on day 3 post-inoculation.

shown in Table 3, passaging of pre-PR treatment samples showed viral replication as evident by CPE. On the other hand, viral genomes in cells inoculated with PR samples in culture supernatants were not detectable. Together, these data confirm the complete inactivation of SARS-CoV-2 in the tested platelet units and the absence of replication-competent virus post-PR treatment.

4. Discussion

When a new pathogen spreads rapidly, like during the current COVID-19 pandemic, there is likely not sufficient time to develop and implement blood screening assays to prevent collection and transfusion of contaminated blood products and protect patients from transfusion-transmitted infections while ensuring blood continuity. With universal PR technology, such risks could be mitigated proactively. The amotosalen/UVA PR technology has already shown potential during emerging arbovirus outbreaks including chikungunya virus, dengue virus and Zika virus [20]. However, the implementation of PR takes a certain time, to be prepared against newly emerging pathogens it should be already in place [20].

We showed complete inactivation of $>3.31 \pm 0.23$ log PFU/mL and $>4.64 \pm 0.5$ log PEq/mL SARS-CoV-2 in human apheresis platelets in 100% donor plasma. In the present study, the genomic titer was 10-fold higher compared to the infectious titer (Tables 1 and 2). No CPE and no genomic viral load were detectable after 3 consecutive rounds of passaging on Vero E6 cells, pointing towards complete inactivation. The reported genomic viral load in the blood of symptomatic patients and asymptomatic donors is relatively low, often close to the limit of detection [1–4], indicating sufficient inactivation efficacy of amotosalen/UVA to mitigate potential transfusion-transmission (Table 2). In an earlier study analyzing the capacity of amotosalen/UVA PR to inactivate SARS-CoV-1, Pinna et al. showed a mean log₁₀-reduction of >6.2 [21]. The maximum inactivation capacity which can be shown is dependent on the input titer in a given experiment and is usually several magnitudes lower in clinical isolates compared to tissue culture attenuated viral stock preparations. Therefore, it is needed to ensure experimentally that the entire viral inoculum is inactivated, underlining the importance of passaging experiments or, as is the case for bacteria, the inclusion of enrichment culture analyses.

A study evaluating the inactivation of SARS-CoV-2 using the Riboflavin/UVB PR technology reported a reduction capacity of $\geq 4.79 \pm 0.15$ log in human plasma [22], ≥ 4.35 log in human apheresis platelets in 100% plasma [23] and $\geq 3.3 \pm 0.26$ log in

whole blood [22]. This difference in results between the two technologies could be attributed to the different SARS-CoV-2 isolates translating into different maximum input titers reported in the studies. The inactivation efficacy of a photochemical PR system largely depends on the pathogen structure and genomic organization [24]. For nucleic acid targeting PR technologies using photoactive compounds and UV light illumination, the photoactive substance must penetrate the viral particle and reach the pathogen's genome, as well as the UV-light during illumination. That points towards a comparable sensitivity of taxonomically closely related pathogens to a PR-system due to their closely related morphology and genome structure. Considering previous data showing the efficient inactivation of MERS-CoV [16,17] and SARS-CoV-1 [21,25], coronavirus sensitivity to amotosalen/UVA PR is high likely. Amotosalen/UVA treatment of platelet concentrates and plasma units likely benefits coronaviruses TTI risk mitigation.

Disclosure of interest

M.P.M. is employee and Q.A. consultant of Cerus Europe B.V., the manufacturer of the INTERCEPT Blood System. All other authors declare that they have no competing interest.

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