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Effectiveness of a flow-based device using riboflavin photochemistry in damaging blood-borne viral nucleic acids

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ABSTRACT

Background: Effectiveness of a flow-based treatment device using riboflavin photochemistry was demonstrated by cytopathic effect method using indicator viruses. However, inactivation efficacy against real blood-borne viruses needs to be evaluated, especially at nucleic acid level.

Material and Methods: Special plasma samples with varying concentrations of blood-borne virus were selected using a strict blood selection procedure and were treated with device treatment (DT). Nucleic acid test (NAT) using polymerase chain reaction fluorescence method was used to detect virus copies.

Results: The NAT value of 4325 in plasma with high Hepatitis B Virus (HBV) concentrations decreased to 1330 with DT. After 100-fold dilution, the NAT value was below the NAT detection limits with DT compared with 23.0 that without DT. The NAT value of 61.9 in plasma with medium HBV concentrations decreased to 37.8 with DT, and after 10-fold dilution, the NAT value was below the NAT detection limits with DT compared with below 20 that without DT. The Ct values of plasma with low concentrations of blood-borne viruses were below the NAT detection limits with DT.

Conclusion: There was a dose effect with DT which was effective in blood-borne viruses damaging nucleic acids to a level below the NAT detection limits.

1. Introduction

Riboflavin based pathogen reduction has been reported as effective against viruses, bacteria or parasites in plasma, platelets and whole blood [1–4]. New studies have shown that treatment with the riboflavin photochemical method could reduce the infectious levels of some aboviruses such Ross River virus, Barmah Forest virus, Murray Valley encephalitis virus to prevent potential virus transmission by transfusion [5,6]. Previously, we developed a flow-based system for virus inactivation in plasma using the riboflavin photochemical method, which has been demonstrated abroad extensively, and our relative results were published [7]. The flow-based device was initially designed to be used for treating pooled plasma such as the universal virus-inactivated plasma for Chinese Han population which was developed by our group [8]. With respect to the virus inactivation effectiveness, a commonly used method involved the cytopathic effect (CPE) and 50% tissue culture infectious dose ($TCID_{50}$), requiring indictor viruses of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) and susceptible cells for relative indictor viruses. The effectiveness of the system for virus inactivation and proteins retention was validated; treatment at an ultraviolet (UV) dose of 3.6 J with a 60 μmol/L concentration of riboflavin was considered as the optimal procedure to inactivate indictor viruses above 4 logs [7].

However, indicator viruses used in previous studies failed to fully represent blood-borne viruses, the effectiveness of riboflavin photochemistry inactivating blood-borne viruses, such as HBV, HCV and HIV, that could be carried in plasma and be transmitted by blood transfusion, should been proved. The rationale of UV combing riboflavin method is to damage the nucleic acid to inactivate viral particles [9,10]. Therefore, besides the demonstration by CPE, it is necessary to evaluate the

Abbreviations: CPE, cytopathic effect; Ct, cycle threshold; DT, Device treatment; ELISA, enzyme-linked immunosorbent assay; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; NAT, nucleic acid test; PCR, Polymerase chain reaction; RPM, round per minutes; UV, ultraviolet

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level and relevance of nucleic acid damage in real blood-borne viruses caused by our device using nucleic acid test (NAT) which was most advanced methodology for blood screening test.

In addition, to fully improve blood transfusion safety, some blood centers and hospitals in China performed enzyme-linked immunosorbent assay (ELISA) and NAT dual blood screening tests [11–13]. Despite the advances, there remains residual risk that threatens the blood safety, especially the window period for virus infection [14,15]. Studies in five Chinese blood centers estimated that the current HIV residual risk is 0.00054% in whole blood donors [16,17]. The infectious risk from having viruses at an extreme low concentration or window period in plasma should be strongly considered. Therefore, this device also should be evaluated for whether it can be added as another processing step in assuring blood safety on the basis of the ELISA and NAT dual tests for blood screening in China.

To evaluate inactivation efficacy against blood-borne viruses at nucleic acid level using riboflavin photochemistry, in this study, plasma contaminated with HBV, a kind of blood-borne virus whose nucleic acid is DNA, was selected as the experimental subject and the commercial NAT was selected to detect the viral copies in plasma to evaluate the damage effect on nucleic acid [18]. Additionally, we also evaluate whether the coat proteins of virus had been changed or destroyed with treatment using this method. The commercial ELISA method was selected to detect the HBsAg on the surface of HBV to evaluate the damage effect on HBV surface proteins. Plasma with ELISA negative but NAT positive was finally selected to demonstrate the inactivation effectiveness against virus with a low concentration with device treatment (DT).

2. Materials and Methods

2.1. Plasma Source and Preparation

The whole blood units were from donors in the Center of PLA Clinical Blood Transfusion, Chinese PLA General Hospital. Plasma units were prepared from whole blood by 4000 ×*g* centrifugation at 22 °C for 10 min using a centrifuge (Biofuge Primo R, Heraeus,Waltham, MA). The separated plasma was stored at −30 °C, or cooler, as FFP. Before use, plasma was thawed in a shaking water bath (MultiTemp III, GE Healthcare Europe GmbH, Freiburg, Germany) at 37 °C.

2.2. Sample Selection

All plasma samples were detected by the routine procedure of blood screening tests, in China, including ELISA and NAT (Fig. 1). One sample extracted from a single unit of plasma was detected by blood screening tests, including the rate method for ALT (c501, Roche Molecular System, USA), ELISA for HBsAg (WanTai, Beijing and DiaSorin, UK), ELISA for anti-HCV (WanTai, Beijing and DiaSorin, UK), ELISA for anti-HIV (JinHao, Beijing and DiaSorin, UK), and ELISA for anti-TP (WanTai, Beijing and DiaSorin, UK). If one sample was both WanTai-HBsAg and DiaSorin-HBsAg positive, while it was negative for all other tests, the sample was preliminarily selected. Next, the sample was further evaluated for HBV-DNA by the individual NAT (COBAS, Roche Molecular System, USA). If the NAT result of plasma was positive and NAT value was between 50 IU/mL and 5000 IU/mL which were considered between positive and strong positive and most common in blood screening tests, the plasma unit was finally selected for experimental use and was set aside until the next virus inactivation treatment. A sample that was negative by all ELISA blood screening tests, while it was HBV-HCV-HIV (1 + 2) NAT positive was selected as “ELISA window period” plasma with a low level virus. The selection procedure for the experimental plasma was summarized (Fig. 2).

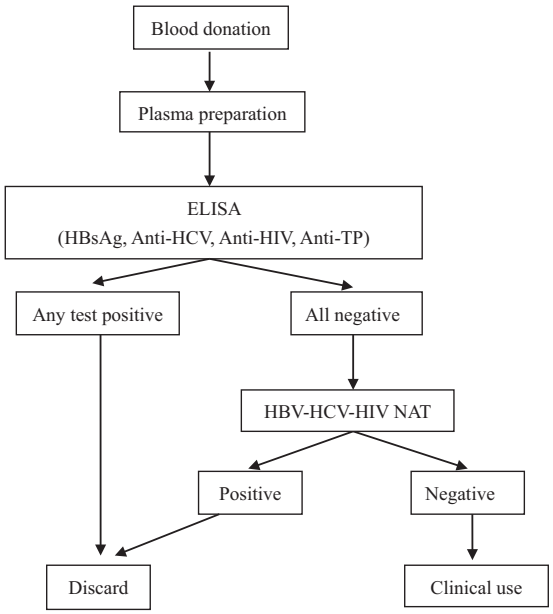

Fig. 1. Routine tests for plasma in the Center of PLA Clinical Blood Transfusion.

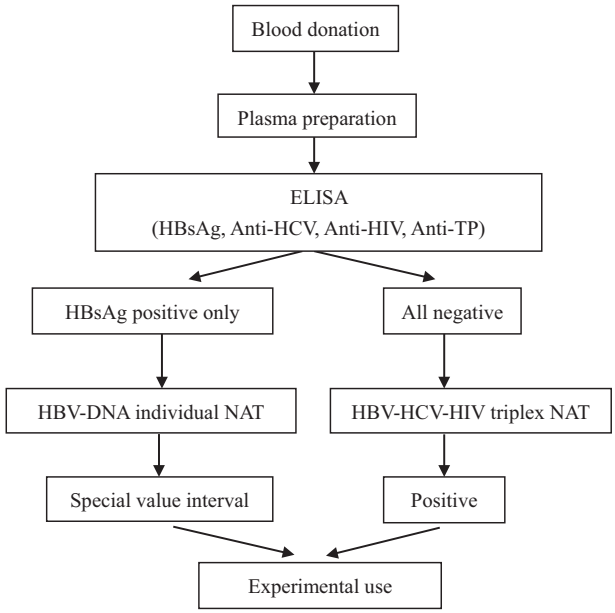

Fig. 2. Selection procedure for experimental plasma.

2.3. A Flow-Based Device

The flow-based device using riboflavin and broadband UV was assembled with two parallel rows of UVA and UVB lamps which were arranged alternately and an S-shape quartz sample-flow tube with a capacity of 20 mL. The interior structure of the device was displayed (Fig. 3). An S-shape quartz sample-flow tube can increase capacity and save space in the same field compared with a straight tube. The spectrum was measured by a spectrometer (iHR550, Horiba Jobin Yvon, Longjumeau, France) [7]. Before treatment, several units of plasma were pooled into a large container and riboflavin solution was subsequently added into plasma container to mix for 10 min. At the entrance of the device, there was a sterile connection with plasma container and a peristaltic pump which could be set at different parameters such as round per minutes (RPM) to send impulses to flow the plasma at different velocities. Plasma was exposed to illumination source to