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Quantitative evaluation of plasma after methylene blue and white light treatment in four Chinese blood centers

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ABSTRACT

Background: Pathogen reduction technology is an important process in the blood safety system, including solvent/detergent treatment, filtration and methylene blue-photochemical technology (MB-PCT). To investigate the quality of MB-PCT-treated plasma, plasma samples from four Chinese blood centers were analyzed over 12 months of storage to determine the recovery of activities and levels of various plasma proteins.

Materials and methods: Ten plasma units each from the Suzhou, Yancheng, Chongqing and Shandong blood centers were divided into two aliquots. One was subjected to treatment with one of two methylene blue-photochemical technology instruments and the other was used as control. The treated and untreated sample pairs were stored at −30 °C. The recovery rates of coagulation factors, inhibitor proteins, total protein, immunoglobulins, and complement proteins were measured at different time points after storage.

Results: The mean recovery of most proteins exceeded 80% after MB treatment. The exceptions were factor XI and fibrinogen, of which only 71.3–74% and 69.0–92.3% were retained during storage. The two equipment types differed in terms of residual MB concentration in the plasma samples (0.18 μM and 0.29 μM, respectively). They had similar protein recovery rates at 0.5 month but differed at later time points. The four blood centers differed significantly with regard to factor II, V, VIII and fibrinogen activities. Only the samples from the Shandong blood center met the methylene blue treated fresh frozen plasma requirement. The major factor that influenced the quality of the MB-FFP samples was the time taken between blood collection and storage.

Discussion: Methylene blue treated plasma showed reduced coagulation factor (CF) activity and protein levels. The MB treatment-induced damage to the proteins was acceptable at the four Chinese blood centers, but the quality of the MB-treated plasma in general was not satisfactory. The main factor affecting plasma quality may be the duration of the collection and processing.

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1. Introduction

The safety of blood donation has been improved by developing and implementing a donor-screening strategy and by instituting specific tests during transfusion. However, if pathogens are not inactivated, there is still a risk of transfusion-transmitted infection [1] due to the window period (the period between the infection and detection of the infectious organism by the test) and the potential presence of hazardous emerging and reemerging pathogens, such as west Nile virus, human parvovirus B19, and *Trypanosoma cruzi*.

Pathogen reduction technology (PRT) inactivates viruses, bacteria, parasites, and white blood cells (WBCs), and thus is an important procedure in the blood safety system. It effectively compensates for testing and donor-screening defects and has been used to treat blood components and products for several decades now. Currently, there are many different pathogen-reduction methods. Solvent/detergent (S/D) is the most widely used method to treat pooled plasma and blood-derived products such as factor VIII [2]. Another common method is the methylene blue-photochemical technology (MB-PCT), which is used to treat individual plasma units. There are other methods [3,4] also have been used with both plasma and platelets. These methods have been licensed in different countries and there are also other methods being researched [5,6]. However, all of these methods suffer from the fact that they also damage essential proteins by different ratios in the blood while killing pathogens. The ultimate goal of PRT is to maintain the benefits of pathogen reduction while simultaneously preventing the loss of protein as much as possible. In practice, this balance is extremely difficult to obtain.

Methylene blue (MB) is a phenothiazine compound that can be activated by visible light and that generates reactive oxygen species, mostly singlet oxygen, through a Type II photodynamic reaction. These highly active molecules are responsible for its pathogen-inactivating properties. MB was first used clinically by Paul Ehrlich in the 20th century and has been employed as a virucidal agent for more than 60 years [7]. The original MB-treatment system was developed by the Institute Springe in Germany and its routine clinical use started in 1992. The system initially involved a freeze/thaw WBC-disruption step, after which a quantity of MB was added according to the plasma weight. MB-PCT has now been approved and is used in many countries, including the United Kingdom (UK), Germany, France, Italy, Spain, Brazil, and Australia. The MB-PCT instruments are produced by the Baxter and Macopharma companies [8]. These companies produced the Pathinact and Theraflex MB systems. The Theraflex system consisting of the Macotronic illumination machine, together with an appropriate disposable set which is a closed bag system for WBC-reduction filtration, illumination, and plasma storage. MB treatment efficiently inactivates lipid-enveloped viruses and is somewhat useful for some non-lipid-enveloped viruses such as human parvovirus B19 and adenovirus. However, it does not inactivate bacteria, protozoa and some non-lipid-enveloped viruses such as hepatitis A virus (HAV) [9,10].

Apart from inactivating the pathogen, the MB treatment also affects the functional activity of plasma proteins, including coagulation factors (CFs) and inhibitors. Previous study has shown that MB treatment clearly reduces the activity of CFs, especially VIII and fibrinogen, whose activities are decreased by up to 20–35% [11]. Given this loss of activity, the quality monitoring of MB-treated plasma is different from that of fresh-frozen plasma (FFP). The guidelines of the UK Blood Transfusion Services specify that methylene blue treated fresh frozen plasma (MB-FFP) must have at least 0.5 IU/ml of FVIII, whereas FFP must have at least 0.7 IU/ml of FVIII [12]. Apart from its documented effect on CF activity, the MB that is added to the plasma during treatment is also a potential mutagen. Therefore, it is desirable to remove MB. The process by which MB is removed varies in different countries. While Italy and Spain, etc., use the Theraflex Plasma system without the Blueflex filter which removes 90% of the MB since more than 10 years [13,14], other countries, including France, the UK, and Austria use the Blueflex filter to remove residual MB and its photoderivatives from the plasma at the end of the treatment [15,16].

In China, MB-PCT is currently the only method that is used to treat individual plasma units. This is supported by two main suppliers, namely, Shandongzhongbaokang medical devices Co., Ltd. (SMD) and Shanghai Transfusion Technology Co., Ltd. (SHT). These suppliers independently developed MB inactivation equipments with matching plasma membrane filtration, MB illumination and MB Depletion systems. MB is used at a concentration of 1 μM and the light intensity is 30,000–38,000 lx. However, there were no official standards for MB-treated plasma in China until the new quality requirements for whole blood and blood components were published in 2012 [17]. It specifies that the process for MB removal should be validated to give components with a MB concentration $\leqslant$0.30 μM and the activity of FVIII must $\geqslant$0.5 IU/ml for MB-FFP or the content of total protein $\geqslant$50 g/L for methylene blue treated frozen plasma (MB-FP). Before the publication of the guidelines, most blood centers produced MB-treated plasma reference with frozen plasma (FP). Since this standard is still new in China, there are only limited data regarding the quality of MB-treated plasma. These data derive from one-site study [18] that suffer from small sample sizes and differences in terms of regional medical resource availability, sufficiency of medical staff training, and the use of locally adapted standard operating procedures.

In the present study, the quality of MB-treated plasma in China was assessed by measuring the percentage of recovery of the activities and levels of various plasma proteins. To do so, paired MB-treated and untreated control samples were obtained from four major blood centers and stored for 12 months. The effect of the two different MB-treatment instruments on plasma quality was also assessed.

2. Material and methods

2.1. Collection and preparation of plasma samples

Ten plasma units were collected between January and July, 2010 from each of four Chinese blood centers, namely,