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Study on the immunological safety of universal plasma in the Chinese population in vitro

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ABSTRACT

Background: The prepared procedure for universal plasma in the Chinese population has been developed. However, the immunological safety with the level of antibodies, soluble immune complexes and complements is necessary to investigate.
Methods: The universal plasma was pooled at the optimal ratio of A:B:AB = 6:2.5:1.5 at 22 °C for 1 hour. The titer of IgM antibodies was detected by saline agglutination, and the titer of IgG antibodies was detected by a Polybrene test after IgM destroyed by 2-mereaptoethanol. The hemolysis extent of RBC was investigated by an extracorporal hemolysis test, and the concentration of free-hemoglobin was determined by the ortho-tolidine method. The levels of CIC-C1q, C3b and TCC (C5-9) were tested using an enzyme linked immunosorbent assay (ELISA).
Results: The titer of IgM and IgG in universal plasma was lower than 2 and 4, respectively. The hemolysis of the universal plasma with A, B and AB group RBCs was negative with values of 5.5, 6.8 and 5.7, respectively. The level of CIC-C1q and TCC (C5-9) in universal plasma was comparable to that in A or B type pooled plasma, but CIC-C1q was lower than that and TCC (C5-9) was higher than that in AB type pooled plasma. The level of complement C3b was comparable to that in A type pooled plasma, but lower than that in B type pooled plasma and higher than that in AB type pooled plasma.
Conclusion: The results of this study demonstrated that the immunological levels were within an acceptable range and confirmed the safety in vitro.

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1. Introduction

Plasma is a blood component product used for clinical therapy. Currently, fresh frozen plasma (FFP) and frozen plasma (FP) are commonly used as plasma products in China. The clinical indications of the plasma include coagulation factor disorders, thrombotic thrombocytopenic purpura (TTP), plasma exchange, disseminated intravascular hemolysis (DIC), and so on. Although plasma transfusion plays an important role in clinical therapy, it also could induce transfusion-related infectious diseases (i.e., hepatitis B, hepatitis C, AIDS, etc.) or a variety of adverse transfusion reactions (i.e., allergic reactions, transfusion related acute lung injury (TRALI) and immune suppression, etc.) [1]. There is no requirement for cross matching before plasma transfusion, and there is a risk that an ABO-incompatible blood transfusion may cause a fatal hemolytic transfusion reaction [2]. However, such incidents can be largely avoided by the use of universal plasma products.

Originally, universal plasma prepared by pooling several thousand liters of random plasma was extensively used by the Allied Forces during World War II and was then forbidden because of hepatitis virus infection and transmission [3,4]. However, in the 1990s, the solvent/detergent (S/D) was invented, and plasma treated by SD which was considered a safe way to inactivate viruses, allowing universal plasma to be widely used again [5–7]. Bioplasma FDP (National Bioproducts Institute, Pinetown, South Africa), which has been produced in South Africa since 1996, is pathogen-reduced ABO-universal plasma and is pooled with 200 liters of plasma in all ABO blood groups [4,8,9]. Uniplas® (Octapharma PPGmbH, Vienna, Austria) was developed in 1999 as a universally applicable pooled plasma treated by S/D, and it can be directly used without taking into account the blood group of recipients [10]. Uniplas® is prepared by selectively pooling 380 liters of plasma (approximately 70% A plasma, 20% B plasma and 10% AB plasma) to neutralize both the anti-A and anti-B antibodies

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in IgM- and IgG-types via soluble A and B antigens and residual human RBCs and possible anti-idiotypic antibodies in plasma [4,10–13]. The safety of Uniplas® was certified by confirming that the ABO blood-group specific antibody titer in Uniplas® was lower than 8 for anti-A and anti-B IgM antibodies and lower than 32 for anti-A and anti-B IgG antibodies and that the level of immune complexes was not above the normal range in fresh-frozen plasma, as monitored by the formation of the complement immune complexes (CIC) binding C1q (CIC-C1q) and C3 (CIC-C3), which do not induce complement-mediated hemolysis of human red blood cells in vitro [10,11]. Except for the levels of plasmin inhibitor and protein S, the levels of coagulation factors and inhibitors in Uniplas® are similar to those in Octaplas and Quarantine FFP [11,14]. The results of both in vitro tests and clinical studies indicated that Uniplas® was a safe product that could be administered irrespective of the ABO blood group of the patients [11,15–17].

Although the safety of Uniplas® is proven, it is not available in China and not suitable for the Chinese because the race and blood group distributions differ from those of the United States and Western Europe. Thus, our team studied the preparation of a new type of universal virus-activated plasma that was most suitable for the Chinese Han population. Studies found that the optimal ratio of pooled plasma was A:B:AB = 6:2.5:1.5, and the level of IgM was lower than that in the Uniplas® and the recommended level [4,13,15]. Specifically, our team used a novel pathogen-activated method, a combination of riboflavin and broadband ultraviolet (UV), and demonstrated the retention of proteins and inactivation of >4 logs of three model viruses and substantial inactivation of another model virus, Sindbis [18]. The production process of universal virus-activated plasma has been accomplished by our studies, but the safety needs to be evaluated in vitro and vivo.

In this study, it is worth further investigating the immunological safety of the new type of universal plasma for the Chinese population in vitro. Firstly, the titers of the anti-A and anti-B IgG antibodies need to be tested. Secondly, an extracorporal hemolysis test should be performed to explore the dose that does not induce complement-mediated hemolysis of human RBCs. Finally, the levels of CIC-C1q, C3b and TCC should be detected.

2. Materials and methods

2.1. Preparation of universal plasma

The FFP was obtained from healthy blood donors at the PLA General Hospital and was stored at −30 °C. We selected 10 specimens each of A, B and AB type FFP randomly and the pooled protocol was repeated to demonstrate in 10 times using different plasma. The FFP was thawed in a shaking water bath (MultiTemp III, GE Healthcare Europe GmbH, Freiburg, Germany) at 37 °C before pooling. A pooled plasma ratio of A:B:AB = 6:2.5:1.5, a reaction temperature of 22 °C and a reaction time of 1 h were considered as the optimal condition for the preparation of the new universal plasma [13].

2.2. Preparation of 2–4% RBC suspension

The RBCs were separated from fresh EDTA anticoagulated fresh whole blood of blood groups A, B and AB by 4000 r/min centrifugation at 22 °C for 10 min using a centrifuge (Baiyang China). The cell sediment was washed with 10 ml of normal saline 3 times. After the final washing step, the 2–4% RBC suspension was prepared.

2.3. Preparation of the complement solution

The complement solution was obtained from male healthy blood donors whose blood group was AB type, was stored at −80 °C and was thawed at 37 °C. The validity period of the complement solution was one month [19,20]. The determination of irregular antibodies screening was negative, and the concentration of C3 was more than 1.0 g/L.

2.4. Determination of antibody titer

The titer of IgM antibody was detected by saline agglutination, and the titer of IgG antibody was determined by a Polybrene test after IgM was destroyed by 2-mereaptoethanol.

2.5. Extracorporal hemolysis test

The ortho-tolidine method (Nanjing Jiancheng Bioengineering Institute, Jiang Su, China) was used for the determination of free-hemoglobin. In the experimental setup, 100 microL of 2–4% RBC suspension and 200 microL of universal plasma was mixed well, incubated at 37C for 30 min, subsequently incubated at 4C for 30 minutes, and then centrifuged at 100 × g at 22C for 30 s [19,20]. The supernatant was discarded after centrifugation. We added 200 μl of the complement solution to the mixture, and it was vortexed gently for 15 s, incubated at 38 °C for 6–8 h and centrifuged by 1000 × g centrifugation for 30 s. The AB type plasma was taken as a negative control sample, while osmotic lysis of RBCs in the water for injection (WFI) was defined as a positive control at 100% hemolysis. The extent of hemolysis induced by the plasma sample in vitro was calculated in percent using the following formula: Hemolysis[%] = $[(OD_{450nm\,(sample)} - OD_{450nm\,(blank)})/OD_{450nm\,(positive)}] \times 100$. Blank was normal saline. Test results showing OD450nm values lower than 2 × the value obtained for the mean of negative controls or hemolysis <7% were regarded as negative results [10].

2.6. Determination of soluble immune complexes and complements

The CIC-C1q (Dong Song Biotechnology, Beijing, China), C3b (BingQing Biological, Shanghai, China) and TCC (C5-9) (Abacebiology, Beijing, China) ELISA test kits were used for the determination of CIC-C1q, C3b and TCC (C5-9).

2.7. Statistics

The related experimental tests were each performed three times. The results are presented as the arithmetic mean ± standard deviation (SD). The negative results are represented by "−". T-test was used to analysis the difference and $p < 0.05$ represented significant difference.

3. Results

3.1. The titers of antibodies

The titer of IgM and IgG antibodies of the new universal plasma was ≤2 and ≤4, respectively (**Table 1**).

3.2. Complement-mediated hemolysis assay in vitro

In vitro test, the new universal plasma and the AB type plasma did not induce hemolysis with any group of RBCs, and in contrast, the O type plasma induced hemolysis with all groups of RBCs as a positive control (**Fig. 1**). The hemolysis (%) of the universal plasma with A group RBCs, B group RBCs and AB group RBCs were 5.5, 6.8 and 5.7, respectively, which were less than 7 (**Tables 2–3**).

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