Transfusion and Apheresis Science 52 (2015) 128–135

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Universal pooled plasma (Uniplas®) does not induce complement-mediated hemolysis of human red blood cells *in vitro*

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ARTICLE INFO

Article history:
Received 10 December 2012
Accepted 25 April 2013
Available online

Keywords:
Uniplas
Universal plasma
Soluble immune complexes
CIC
In vitro hemolysis
Functional assay

ABSTRACT

Background: Pooling of plasma of different blood groups before large scale manufacturing of Uniplas® results in the formation of low levels of soluble immune complexes (CIC). The aim of this study was to investigate the level and removal of CIC during Uniplas® manufacturing. In addition, an *in vitro* hemolysis assay should be developed and investigate if Uniplas® does induce complement-mediated hemolysis of human red blood cells (RBC).
Materials and methods: In-process samples from Uniplas® (universal plasma) and Octaplas(LG)® (blood group specific plasma) routine manufacturing batches were tested on CIC using commercially available ELISA test kits. In addition, CIC was produced by admixing heat-aggregated immunoglobulins or monoclonal anti-A/anti-B antibodies to plasma and removal of CIC was followed in studies of the Uniplas® manufacturing process under down-scale conditions. The extent of RBC lysis was investigated in plasma samples using the in-house hemolysis assay.
Results: Levels of CIC in Uniplas® are within the normal ranges for plasma and comparable to that found in Octaplas(LG)®. Down-scale experiments showed that both IgG/IgM-CIC levels are significantly removed on average by 40–50% during Uniplas® manufacturing. Uniplas® does not induce hemolysis of RBCs *in vitro*. Hemolysis occurs only after spiking with high titers of anti-A/anti-B antibodies and depends on the antibody specificity (i.e. titer) in the plasma sample.
Conclusion: The results of this study confirm the safety of Uniplas® regarding transfusion to patients of all ABO blood groups.

1. Introduction

ABO-incompatible blood transfusion and transfusion-related acute lung injury (TRALI) are the leading transfusion-related causes of death in transfusion therapy [1,2]. Transfusion of ABO-incompatible blood components may result in serious, often fatal hemolytic transfusion reactions. The severity of reaction is based on the antibody titer, administered volume and administered speed of plasma, as antibodies in the transfused plasma are rapidly diluted and neutralized by free A and B antigens, possible anti-idiotypic antibodies and numerous antigen bearing cells in the blood and tissue of the recipient [3]. The handling and storage of plasma of different ABO-types poses logistic problems and increases the risk of transfusing the wrong unit to a patient [2,4–6]. An ABO-universal blood supply, on the other hand, would simplify logistics, reduce safeguarding costs and eliminate transfusion errors caused by ABO incompatibility [7,8]. Therefore, the basic idea behind

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http://dx.doi.org/10.1016/j.transci.2013.04.039
1473-0502/

Uniplas® (Octapharma PPGmbH, Vienna, Austria) was to develop a universally applicable, pooled, solvent/detergent (S/D) treated plasma that can be used without taking into account the blood group of the recipient. By optimal mixing of plasma of different blood groups prior to S/D treatment (i.e. blood groups A, B and AB), potentially damaging ABO blood group specific anti-A and anti-B antibodies of both IgM- and IgG-types are neutralized by binding to free A and/or B substances and residual human RBCs.

The formation of soluble immune complexes (CIC) in plasma of healthy humans is a protective, ongoing and usually benign process of the *in vivo* immune system. Immune complexes are in general efficiently removed from the circulation in the normal host, on a current basis, through complement activation and subsequent removal by the mononuclear phagocyte system [9–11]. Immune complexes are only dangerous to patients when they are found in large amounts in plasma for a longer period of time, mostly in patients with severe autoimmune diseases [9,12,13]. Pooling of plasma from donors with different blood groups before large scale manufacturing of Uniplas® results in the formation of low levels of CIC. Several studies in the past demonstrated that Uniplas® shows the same high quality features as the blood group specific S/D plasma siblings Octaplas® (1st generation product) and OctaplasLG® (2nd generation product) [14–20]. In addition, Uniplas® was proven safe and efficacious for use in different clinical studies [21–25], and is used on a named patient basis in Ireland since 2002 with good results [26]. The aim of this study was to investigate the level and removal of CIC during Uniplas® manufacturing by using commercially available ELISA test kits. Furthermore, an additional assay based on biological determination and functional activity should be developed. Finally, it should be confirmed that Uniplas® does not induce complement-mediated hemolysis of human red blood cells (RBCs) *in vitro*.

2. Materials and methods

2.1. Materials

Uniplas® and Octaplas®/OctaplasLG® plasma pool and final container (FC) samples were collected from routine batches manufactured at Octapharma PPGmbH. The Uniplas® manufacturing process is an exact copy of the OctaplasLG® process, except of the optimal mixing of plasma prior to S/D treatment (i.e. universal plasma vs. blood group specific plasma). The only difference between Uniplas®/OctaplasLG® and Octaplas® is in the exposure time to S/D (i.e. 1–1.5 h vs. 4 h) and in the prion removal step (i.e. with vs. without prion removal step) [14].

The Seraclone® anti-A and anti-B reagents were from Biotest AG (Dreieich, Germany). Heat-aggregated human gamma-globulins (HAGG) were induced by incubation of purified IgG (i.e. Octagam® 5%, Octapharma PPGmbH) for 20 h at +63 °C. Remaining IgG monomers were separated by size-exclusion chromatography (SEC). Purified HAGG was eluted from the SEC column with phosphate buffer, pH 7.0.

2.2. Studies of the Uniplas® manufacturing process under down-scale conditions

In the first set of experiments (i.e. studies 1), Uniplas® or Octaplas® plasma pool samples of different blood groups were spiked with HAGG at a final concentration of 100, 500 or 1000 μg Eq/ml. In the follow-up studies (i.e. studies 2), soluble complexes consisting of blood group antigens and anti-A/anti-B antibodies (i.e. CIC of A/anti-A and/or B/anti-B) were produced by spiking of blood group A, B or AB plasma with Seraclone® anti-A and/or anti-B monoclonal antibodies, respectively. The final concentration of the anti-A/anti-B reagent in the plasma mixture was 10% (V/V). Subsequently, the plasma mixtures were incubated under gentle stirring for 90 min at +37 °C and overnight at +4 °C, which are the classical incubation times to form immune complexes *in vitro*. Finally, the Uniplas® manufacturing process under down-scale conditions was performed including the following process steps: 1.0 μm filtration, S/D virus inactivation, oil extraction, phase separation, clear filtration, solid phase extraction (i.e. C-18 chromatography), affinity ligand chromatography (i.e. prion removal step) and sterile filtration. For in-process samples collected during the down-scale experiments please refer to Fig. 1.

2.3. Determination of soluble immune complexes by ELISA assays

CIC-C1q and CIC-Raji ELISA test kits (Quidel, supplier Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria) were used for the determination of IgG–CIC levels in plasma. CIC-C1q is based on the binding of complement fixing CIC to immobilized human C1q purified protein (i.e. classical complement pathway), while the CIC-Raji ELISA uses Raji cell CR2 receptors to bind CIC containing fragments of the third complement factor (i.e. alternative complement pathway). Results are expressed as HAGG equivalents per ml (μg Eq/ml). Values less than 4 μg Eq/ml for the CIC-C1q ELISA and values less than or equal to 15 μg Eq/ml for the CIC-Raji ELISA are considered negative. The average CIC concentration in healthy subjects was 2.1 ± 1.9 μg Eq/ml ($n = 312$) and 5.0 ± 4.6 μg Eq/ml ($n = 51$) for the CIC-C1q and CIC-Raji ELISA, respectively. In addition, the IgM-CIC ELISA (DRG Instruments GmbH, Marburg, Germany) was used to quantify IgM-CIC complexes. IgM-CIC levels are assessed by measurement of the optical density (OD) at 450 nm. OD_{450nm} levels of 1.090–1.738 (mean 1.396) were found by screening of plasma of healthy donors (i.e. Standard Human Plasma, Siemens Healthcare Diagnostics GmbH, Vienna, Austria; and Normal Control Plasma, Instrumentation Laboratory GmbH, Vienna, Austria). OD_{450nm} level lower than 1.828 (i.e. 2× standard deviation) were regarded as negative results.

2.4. In vitro *hemolysis assay*

The *in vitro* RBC hemolysis (in house method Octapharma R&D) was estimated by determination of hemoglobin released into plasma (OD_{414nm}), following exposure of ABO antigens on the surface of RBCs to blood group specific antibodies from ABO-incompatible plasma. RBC were separated from fresh citrated whole blood of blood groups AB, A and