

Methods of pathogen inactivation in whole blood and red blood cells: current state of knowledge

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Abstract

Although pathogen reduction technology was implemented for platelet concentrates and plasma, the risk of pathogen transmission has not been completely eliminated as no inactivation procedures were implemented for red blood cells and whole blood. Research was therefore focused on developing methods for effective pathogen inactivation in red blood cell components. Attempts were made to apply either chemical compounds (porphyrins and Sylsense compounds) or photosensitizers such as methylene blue (Theraflex MB Plasma System) and amotosalen hydrochloride (Intercept System) already in use for pathogen inactivation in plasma. None proved effective for pathogen inactivation in red blood cells.

Approval was recently given to pathogen inactivation methods based on S-303 compound (for red blood cells) and with riboflavin (for whole blood). Clinical trials are ongoing. Pilot studies have shown that packed red blood cells subjected to pathogen inactivation with S-303 demonstrated slight loss of red blood cells, decrease in hemoglobin concentration, significantly lower lactate concentration, and lower pH. Pathogen inactivated whole blood stored at room temperature for up to seven days showed slight hemolysis (within the normal range).

This paper presents several pilot clinical trials with pathogen inactivated red blood cells or whole blood. It focuses primarily on the recovery of red blood cells in the recipient's organism and on hemoglobin concentration.

Key words: pathogen inactivation, red blood cells, clinical trials

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Introduction

Since the end of the 20th century, many blood establishments (BEs) worldwide have begun the implementation of pathogen inactivation methods for blood components intended for clinical use. These methods were based on the experience gained from the pathogen inactivation methods developed for plasma fractionation. In the 1980s, the solvent/detergent (SD) method appeared which reduced the risk of enveloped virus transmission with blood products. In the 1990s, the method was modified and applied also to plasma intended for clinical use [1].

In 2000, Macopharma developed the Theraflex MB-Plasma system based on methylene blue and visible light for pathogen inactivation in plasma. Amotosalen hydrochloride and ultraviolet A (UVA) were used in the Intercept system, initially developed for pathogen inactivation in platelet concentrates (2002) and in plasma (2006). In 2007, the Mirasol[®]PRT system was developed, which was based on riboflavin and UV and intended for pathogen inactivation in platelet concentrates (PC), and a year later in plasma (Table I) [2–4].

Although pathogen reduction technology (PRT) was implemented for PC and plasma, the risk of pathogen

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Table I. Systems of pathogen inactivation in blood components

System	OctaplasLG®	Theraflex MB Plasma	Intercept	Mirasol PRT
Company	Octapharma	Macopharma	Cerus	Terumo BCT
Type of blood component	Plasma from apheresis or WB	Plasma from apheresis or WB	Plasma from WB (pooled) or from apheresis, PC in plasma or PAS (InterSol, SSP*)	Plasma from apheresis or from WB PC in plasma or PAS (SSP*)
Chemical compounds/ photosensitizer	Tri (n-butyl) phosphate (TNBP) and 1% Octoxinol 9	Methylene blue	Amotosalen hydrochloride (S-59)	Riboflavin (vit. B ₂)
Chemical compounds/ photoproducts	Residual amounts of chemical compounds	Azur A, B, C; tionona	Dimers S-59	Lumichrome, lumiflavin 2-ketoflavin, 4-ketoflavin, mononucleotide flavins, formylomethyloflavin
Inactivation conditions	4 h incubation at 30 °C	Visible light (590 or 630 nm) 180 J/cm ²	UVA (320–400 nm) 3 J/cm ²	UV (280–400 nm) 6.24 J/cm ²
Additional steps	Removal of chemical compounds (extraction in vegetable oil, ion exchange chromatography or gel filtration and sterile filtration (0.22 μm))	Leukoreduction (Plasmaflex) Removal of photosensitizer and photoproducts (Bluflex)	Removal of photosensitizer and photoproducts (CAD)	Not applicable

PRT – pathogen reduction technology; WB – whole blood; PC – platelet concentrates; PAS – platelet additive solution; UVA – ultraviolet A; CAD – compound adsorption device

transmission was not completely eliminated as there were no inactivation procedures for red blood cells (RBC) and whole blood (WB). Research studies were therefore focused on developing methods that would effectively inactivate pathogens in red blood cell components, but also maintain an acceptable quality level during storage for longer than the 5–7 day limit for platelets.

First attempts to develop pathogen inactivation methods for red blood cells and whole blood

The first trials to develop methods of pathogen inactivation in RBC and WB were based on reactions involving light and photosensitizers. One of the first groups of compounds used as photosensitizers were porphyrins. During trials, it turned out that most porphyrins are amphiphilic which leads to their aggregation in cell membranes. They are therefore effective only for inactivation of enveloped viruses. Hematoporphyrin and dihematoporphyrin derivatives are examples of such compounds effective for inactivation of various enveloped viruses, but inactive against non-enveloped viruses. Benzoporphyrin, on the other hand, is a photosensitizer with a high affinity for lipoproteins. The compound inactivates vesicular stomatitis virus (VSV) and human immunodeficiency virus (HIV) – both free and bound

– with limited damage to red blood cells [5, 6]. Research on these compounds has been halted due to the emergence of new photosensitizers of more promising characteristics. The Dutch developed cationic compounds called “Sylsense compounds”, and their effectiveness for the inactivation of red blood cell components has been confirmed in pilot studies. These photosensitizers are activated by visible light (>600 nm) and effectively inactivate pathogens such as enveloped viruses [HIV, VSV and bovine viral diarrhoea virus (BVDV), hepatitis C virus (HCV) and West Nile virus] and Gram positive and Gram negative bacteria. Qualitative studies of RBCs subjected to pathogen inactivation with Sylsense compounds demonstrated that the mean degree of haemolysis after five weeks of storage slightly exceeded 1%. All other parameters, adenosine triphosphate (ATP) concentration included, were comparable to those for control RBCs [7]. Methylene blue (phenothiazine dye), effective for pathogen inactivation in plasma with the Theraflex MB Plasma system could not be applied to PCs or RBCs due to high protein and lipoprotein binding affinity; envelope membranes of viruses and nucleic acids contain protein and lipoproteins. Moreover, the hydrophilic nature of methylene blue impeded penetration into cells so the compound could not be used for inactivation of intracellular pathogens. Another promising compound for pathogen inactivation in RBC was hydrophobic silicone found effective *in vitro*

for inactivation of the so-called model enveloped viruses. No *in vitro* detrimental effect on the quality of RBCs was confirmed but their survival time was significantly reduced. Research studies were therefore discontinued.

Under consideration was also the inactivation method based on a photochemical reaction with amotosalen hydrochloride and UVA radiation (320–400 nm). It could not however be applied to RBCs because hemoglobin absorbs UVA radiation. During pilot studies on development of PRT for RBCs several classic photosensitizers were tested. Despite the promising efficacy of these methods, studies have been halted due to increased haemolysis, significant oxidative damage, significant ATP reduction during storage, or unacceptable toxicity profiles [8].

Promising results were obtained *in vitro* with the light-independent alkylating compound PEN110 (Inactine). Positive outcome after transfusing healthy individuals with autologous RBCs pathogen inactivated with PEN110 led to initiation of clinical trials with sickle cell anemia patients and patients after cardiac surgery. The studies were stopped when anti-PEN100 antibodies were detected in some patients transfused with pathogen inactivated RBCs [9, 10].

Modern methods of pathogen inactivation in RBCs and whole blood

Method of pathogen inactivation in RBCs

The method is based on the reaction in which the S-303 compound cross-links with nucleic acids through a di-alkylating group. When added to RBCs the S-303 compound rapidly penetrates virus envelopes and integrates into host DNA. S-303 also reacts with other nucleophilic compounds of RBCs such as phosphates and proteins. To minimize these non-specific reactions, particularly the reactions with proteins, glutathione is added which is a natural antioxidant present in most cells. Following the first clinical reports on the formation of antibodies directed against inactivated RBC in patients with chronic anemia, the S-303 method was modified to include neutral pH glutathione in the reaction mixture, instead of unbuffered glutathione [11].

RBCs subjected to pathogen inactivation with S-303 demonstrate a slight loss of red blood cells, a decrease in hemoglobin concentration, significantly lower lactate concentration and a lower pH during storage. No differences in blood count and CD47 antigen expression on the surface of red blood cells were determined in either pathogen inactivated or non-inactivated RBCs [12, 13].

Pathogen inactivation in whole blood

The Terumo BCT company developed an option for pathogen inactivation in whole blood (with riboflavin and UV) which could replace the systems used for inactivation of individual blood components. This would significantly reduce the

workload and implementation cost since one unit of whole blood provides 2 or 3 blood components. The procedure still requires multi center studies to document the minimum acceptable effect of PRT on WB quality but also on the quality of blood components obtained from fractionation of inactivated WB [14].

Reddy and Marschner [15] evaluated the quality of pathogen inactivated WB stored at room temperature for up to 7 days to find slight haemolysis (but within normal). No significant differences in adhesion and aggregation were observed when the functions of platelets obtained from inactivated WB stored for 7 days and blood cells from the control group were compared. For fresh frozen plasma (FFP) obtained from inactivated WB, stored for up to 28 days, protein recovery was not significantly reduced while mean concentrations of fibrinogen, factors V, VIIIc and XI were even higher than for plasma inactivated with the Mirasol®PRT system. In RBC obtained from inactivated WB, haemolysis increased (on average <1% on the 35th storage day) while sodium and potassium levels decreased as compared to standard RBC. The system is currently under validation in several transfusion centers (in Ghana among others), where transfusion of Mirasol inactivated WB prevents the spread of malaria (28% of donations was infected with malaria parasites) [15–19].

Clinical trials

Clinical trials on pathogen inactivated RBC and PCs have been conducted for over 10 years. Table II presents data from 3 evaluation studies on transfusion of autologous RBCs and 4 clinical studies on RBCs inactivated with the Intercept system (2nd generation) evaluating the outcome of transfusions of autologous RBC obtained from WB inactivated with the Mirasol system.

In the studies of Cancelas et al. RBCs pathogen inactivated with the Intercept system were stored 35 days prior to transfusion and RBCs obtained from WB were subjected to pathogen inactivation with Mirasol and stored for 21 days before transfusion. In general, the PRT-RBC recovery 24 hours after transfusion was lower than for reference RBCs although the mean values were in line with United States Food and Drug Administration (US FDA) guidelines (minimum 75%). No safety issues for autologous RBC recipients were reported [20, 21].

Allain et al. [22] presented the protocol from the AIMS study, which stressed the possibility of preventing malaria spread through transfusion of Mirasol-inactivated WB to acute anemia patients in the endemic region of Sub-Saharan Africa. The AIMS study reported statistically significant reduction in malaria transmission in recipients of WB units inactivated with Mirasol system and stored for 3–4-days [22]. Brixner et al. reported no significant differences between the use of conventional RBCs and RBC inactivated

Table II. Autologous transfusion studies in healthy subjects and clinical trials in adults and children using second generation Intercept RBC-PRT and Mirasol WB-PRT

Reference	PRT	Type of study (location)	Subjects (n) and condition	Outcomes (mean ± SD)
Cancelas et al. [19]	I	Autologous RBC crossover study (USA)	27 healthy subjects	24-h RBC recovery: • 88.0 ±8.5% (T) • 90.1 ±6.9% (C) Mean RBC survival: • 74.6 days (T) • 88.3 days (C)
Cancelas et al. [20]	I	Autologous RBC crossover study (EU and USA)	42 healthy subjects	24-h post-transfusion recovery: • 83.2 ±5.2% (T) • 84.9 ±5.9% (C) Mean RBC survival: • 62.8 days (T) • 75.1 days (C)
Cancelas et al. (IM-PROVE II) [21]	M	Autologous RBC crossover study (USA)	24 healthy subjects	24-h post-transfusion recovery: • 82.5 ±3.9% (T) • 91.7 ±6.8% (C) Mean RBC survival: • 60.5 days (T) • 81.6 days (C)
Allain et al. (AIMS) [22]	M	Allogeneic WB transfusion, RCT (Ghana)	227 anemic patients	Incidence of TTM in 65 non-parasitemic patients exposed to parasitemic blood: • 1/28 =4% (T) • 8/37 =22% (C)
Brixner et al. (2018) (STARS)	I	Allogeneic RBC <i>in vitro</i> study and RCT (EU)	51 cardiovascular surgery recipients of 148 RBC units	Hb content in RBC (g): • 53.6 ±5.6 (T) • 56.3 ±6.0 (C) Clinical safety (T vs. C) – comparable renal and hepatic insufficiency
Trakhtman et al. (2019)	M	Allogeneic RBC <i>in vitro</i> study and RCT (Russia)	70 paediatric onco-hematology patients	Post-transfusion Hb (g/L): • 100.0 ±8.30 (T) • 101.6 ±7.57 (C)
Aydinok et al. (2019) (SPARC)	I	Allogeneic RBC crossover RCT (EU)	86 thalassemic patients	Transfused Hb (g/kg/day): • 0.113 ±0.04 (T) • 0.111 ±0.04 (C) No antibodies to S-303 RBC

RBC – red blood cells; PRT – pathogen reduction technology; WB – whole blood; SD – standard deviation; I – Intercept; USA – United States of America; T – treatment; C – control; EU – European Union; M – Mirasol; RCT – randomized controlled trial; TTM – transfusion transmitted malaria; Hb – haemoglobin concentration

with the Intercept system in patients subjected to cardiovascular procedures within the framework of the STARS RCT program. RBCs were transfused after an average storage period of 18.9 days. No antibodies to S-303 RBC were detected [23]. In a study involving pediatric onco-hematology patients transfused with RBCs from WB pathogen inactivated with the Mirasol system, the storage time was limited to 14 days. With longer storage time, increased hemolysis was reported. No antibody formation was observed [24]. Similar hemoglobin consumption was reported in the SPARC RCT study with multiple RBC recipients with thalassemia. During two separate periods, pediatric and adult patients were transfused a total of 1,024 Intercept and 1008 control RBCs stored for approximately 9 days prior to transfusion. No antibodies to S-303 RBC were detected [25].

It should be emphasized that the clinical trials with RBC-PRT and WB-PRT published so far were conducted in small groups of hematological patients with anemia who required rapid or repeated RBC support or patients subjected to cardiac surgery. Further research is required to assess the safety and efficacy in other clinical settings. Up-to-date information on new, larger clinical trials in different settings is available in international clinical trial registries. Additional studies are currently underway to expand knowledge on the safety and efficacy of RBC inactivated with the Intercept system and WB inactivated with the Mirasol system. "Study to evaluate the efficacy & safety of the INTERCEPT blood system for RBCs in complex cardiac surgery patients (ReCePI)" began in March 2018 and the program is expected to terminate on June 30, 2021. The aim of the study is to evaluate the efficacy and safety of transfusions of RBC subjected to pathogen inactivation with the Intercept system in patients undergoing complex cardiac surgery. The main outcome of the ReCePI project includes the assessment of renal failure, adverse events and emerging antibodies. "The efficacy and safety of WB-derived RBCs inactivated with Mirasol system and transfused to patients requiring multiple transfusions" are assessed within the framework of the PRAISE project (NCT03329404).

Author's contributions

EL – sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments

involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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