

Pathogen Inactivation Plays an Irreplaceable Role in Safeguarding the Safety of Blood Transfusion

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Introduction

In the field of blood transfusion, blood safety is still a huge concern. In addition to strengthen donor screening, pathogen inactivation (PI) is one of the most important steps to ensure the blood safety. Pathogen inactivation is a technology that uses a variety of physical, chemical, or photochemical methods to remove or inactivate the blood-borne pathogens, such as viruses, bacteria, and parasites in blood components or products. These PI methods include but not limit to solvent/detergent (S/D), nanofiltration and photochemical inactivation such as using methylene blue (MB), psoralens, or riboflavin.

PI Treatments for Blood Products

Blood products include albumin, clotting/coagulation factors and globulin (Ig), etc. Several methods have been applied to inactivate the potential pathogens in these products. As a physical PI method, heat treatment has a long history for virus inactivation. Pasteurization (wet heat treatment), dry heat treatment and vapour treatment are commonly used as heat treatment [1,2]. It has been reported that Pasteurization by heating protein to 60°C in solution typically leads to 6 Log viral reduction and 6-8% recovery of clotting factors [3]. In order to preserve protein integrity, some additional stabilizers are also added. These stabilizers can be physically removed by fractionation (precipitation or chromatography) or filtration after pathogen inactivation. This method has advantages in inactivating enveloped viruses and some non-enveloped viruses (eg. HAV and B19V) [4]. Currently, physical heat treatment has been adapted to terminal dry heating of lyophilized products in the container, which is performed at 100°C for 30-120 min to inactivate both lipid enveloped and non-enveloped viruses [4,5].

Solvent/Detergent (S/D), as a chemical method, inactivates products through membrane-disruption. As such, S/D treatment is only effective for lipid-enveloped viruses [6]. The typical S/D treatment condition includes organic solvents (0.3% TNBP) combined with a nonionic detergent [1% Tween 80 (≥ 6 hr) or 1% Triton X-100 (≥ 4 hr)] at 24°C [4]; Following treatment, S/D compounds are removed by chromatography or oil extraction. S/D process is usually used at industrial scale. Octaplas, as a mature commercial product based on S/D inactivation, has already been widely used in Europe. Though non-enveloped viruses like Hepatitis A virus (HAV) or Parvovirus B19 are resistant to S/D inactivation, the risk of infection has been greatly reduced because of the presence of neutralization antibodies in the initial product pools [5,6].

The removing ability of nanofiltration depends on size of pathogens and membranes of nanometric pore size (typically 15–40 nm). Due to the size of most viruses are between 20–200 nm, nanofiltration can remove a wide range of both enveloped and non-enveloped viruses with 4–6 logs reduction of the viral load, preserving 90–95% recovery of protein activity. Prions are also reported to be removed [5,7]. A recent study by Caballero et al. demonstrated that nanofiltration can eliminate 99.99% of viruses (e.g., PRV; HIV-1; WNV; HAV), confirming the robustness and consistency of nanofiltration in virus removal [8].

PI Treatments for Blood Components (Plasma and Platelet)

Nowadays, research on PI technology for blood components (plasma and platelet) has made a great progress. Several inactivation methods including MB, Psoralens and Riboflavin can be chosen. These methods are targeting viral nucleic acids (NA) through photochemical inactivation. Methylene Blue (MB) is a phenothiazinium dye with a natural affinity for NA. Once exposed to visible light (620–670 nm), MB can release reactive oxygen species (mostly singlet oxygen) by photodynamic reaction to induce the guanine-specific cleavage of viral NA, resulting in virus inactivation. MB is effective in inactivating enveloped virus. However, due to poor penetration through plasma membranes, MB is unable to inactivate intracellular pathogens, leukocytes, non-enveloped virus, bacteria, platelet and red blood cells (RBCs) [9,10]. Although some allergic adverse events have been occasionally reported, MB has been used to inactivate single plasma units in 18 countries with minimal toxicity, confirming the long-term safety of MB-treated plasma [5].

Amotosalen (S-59), known as photosensitizers, is a synthetic psoralen obtained from numerous plants. Pathogens in plasma and platelets can be inactivated by Amotosalen upon exposure to ultraviolet A (UVA) light (320–400 nm). Through a three-step of intercalation, monoadduct and cross-links (diadducts) chemical reactions between single- or double-stranded DNA or RNA, UVA-light-mediated amotosalen inhibits nucleic acids replication, transcription and repair mechanisms as well. Due to hemoglobin absorbs the UVA light, Amotosalen/UVA based PI cannot be used for inactivation of RBCs [9,11]. Intercept® plasma (Cerus, Concord, CA), as a successfully developed commercial product of Amotosalen/UVA, has been used for nearly a decade in more than 20 countries. This technology has been proved to be effective to inactivate a broad spectrum of viruses, bacteria and parasites as well as leukocytes contained in the blood products and considered to be safe without any unusual adverse effects or toxicity events. It has received CE Class III mark approval in Europe and approval for use in the USA in 2006 and 2014, respectively [11,12].

Riboflavin (vitamin B2)-based compounds work through UVB light (265–370 nm) illumination. This photodynamic reaction generates

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singlet oxygen, which is responsible for photo-oxidation of guanine bases and results in strand breaks and fragmentation, thus damaging nucleic acids (DNA or RNA) irreversibly [9]. Riboflavin, the so-called "GRAS" (Generally Regarded as Safe) products by the FDA, due to its wide existence in a variety of natural foods and human blood, neither riboflavin nor its metabolic products need to be removed after this PI treatment. Many studies have demonstrated the effectiveness of this technology in inactivating a wide range of pathogens (bacteria, viruses and protozoa ect.). Mirasol® (Terumo BCT, USA), based on Riboflavin/UVB PI technology, has been used in a number of blood centers in many countries and received CE Class II b mark approval for platelets inactivation in October 2007 and plasma in August 2008. As for the Theraflex UV-Platelets system, it cannot be used routinely because further clinical evaluation is needed [11,13].

PI Treatments for Whole Blood and RBCs

Pathogen inactivation methods for Whole Blood and RBCs are still under research. Both S-303 (FRALE) and PEN110 (INACTINE) treatments have been halted due to immune response against neoantigens [10]. However, what deserves expecting is that Cerus has developed a 2nd generation S-303. Phase III clinical trial in the USA is currently recruiting participants (NCT01740531). Mirasol (Terumo BCT, USA), a system successfully applied for platelets and plasma inactivation, is now being developed for whole blood. A Phase III clinical trial for evaluating the efficacy of the Mirasol-treated system for whole blood has been completed. Allain et al. demonstrated the efficacy and safety of this technology in reducing the incidence of transfusion-transmitted malaria in epidemic areas [14]. All of these studies offer possibility that we may achieve great breakthroughs of PI treatment for the whole blood and RBCs in the near future.

Future Perspective

The efficacy of PI treatment maybe different due to various methods, and non-enveloped virus is particularly difficult to inactivate. Though every step of the blood supply chain including collection, processing and storage is strictly enforced, zero-risk in transfusion medicine is still non-existent. Recently, it has been reported that viral metagenomics combined with HTS (High Throughput Sequencing) seems to be a promising approach for the identification as well as global surveillance

of new or unknown viruses that may affect the blood safety [15]. Along with the development of modern technology, PI will surely play an irreplaceable role in safeguarding the safety of blood transfusion.

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