Contemporary Issue

Frozen Red Blood Cells in Transfusion

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Abstract

Red blood cells (RBCs) can be cryopreserved with shelf life of 10 years. However, shelf life of deglycerolized RBCs in conventional open system is just 24 hours, resulting in sporadic use of Frozen RBC (FS-RBC). Recently Naval Blood Research Laboratory (NBRL) method using ACP 215 (ACP™ 215 Haemonetics Cell Processing System) has been introduced, where shelf life of deglycerolized RBC is 14 days. FS-RBC unit is prepared from single blood donation, which needs to be glycerolized and deglycerolized. NBRL method using ACP 215 in FS-RBC is described. Deglycerolized unit weighed between 325-350 gm with haemoglobin of 15-18 gm/dl and freeze- thaw- wash RBC recovery of 87%. Transfusion of deglycerolized RBC offered advantages such as elimination of need of crossmatching in emergent situations and reduction of transfusion reactions. FS-RBC by NBRL method using ACP 215 has advantages such as long shelf life, meeting unexpected high blood demand in mass casualties situations or availability of rare blood group requirement of individual patient. FS-RBC can be a potential candidate for Indian Armed Forces Blood programme for uninterrupted blood supply during peace and war.

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Key Words : Frozen red blood cell; Cryopreservation; Blood transfusion; Armed forces blood programme

Introduction

In 1950, it was first demonstrated that human red blood cells (RBC) could be cryo-preserved, thawed, washed free of cryoprecipitates and transfused with normal in vivo survival of 85-90% of the recovered cells [1]. Initially it was thought that the frozen RBC (FS-RBC) is panacea for all the problems associated with the liquid RBC in transfusion; such as - seasonal shortages, difficulty in meeting of high blood demand during unexpected major local or national catastrophes and enduring supply of rare blood group units [1]. High cost, difficulty in preparation and short shelf life of thawed FS-RBC were deterrents that dampened the enthusiasm for FS-RBC usages [1]. Advancement in technology during last few years wit the introduction of ACP™ 215 Haemonetics cell processing system (ACP 215) in preparation of FS-RBC has taken care of most of deterrents of FS-RBC [2,3]. In this background, there is need to re-look at FS-RBC as part of Frozen Blood Programme for Indian Armed Forces.

Cryo Preservation and Thawing of Frozen RBC

Cryoprotecting agent is essential to prevent the dehydration and mechanical trauma to RBC during freezing. Cryoprotecting agents are classified as penetrating and non-penetrating. Glycerol is a penetrating group of cryoprotecting agent [4,5]. The high concentration of glycerol in RBC prevents formation of ice crystals and consequent membrane damages [4]. Infusion of incompletely deglycerolized RBC has negligible effect except for shift in intracellular fluid volume [4]. Polyvinylpyrrolidone, hydroxyethyl starch (HES), polyethylene oxide are non penetrating cryoprotecting agents as they require high rates of cooling in liquid nitrogen at -196°C [6]. Non penetrating cryoprotecting agents protect cells by a process called 'vitrification', where they form glassy shell around the cell. HES is one of the promising cryoprotecting agent for cryopreservation of RBC, since its removal from thawed RBC prior to transfusion is not required [4-6].

For cryopreservation of RBC, glycerol is used in either 20% or 40% weight/volume (W/V) concentration; the methods are termed as low glycerol concentration (LGC) or high glycerol concentration (HGC) respectively [4,5]. HGC have advantages of initial slow uncontrolled freezing rate and storage of RBC below -65°C as against initial rapid controlled cooling and storage temperature below -120°C in LGC [4]. A study has reported higher haemolysis due to glycerolization in HGC as against better stability of deglycerolised RBC measured by haemolysis in HGC than LGC preserved RBC [7].

In cryopreservation of RBC, NBRL (Naval Blood Research Laboratory, Boston University School of Medicine) method [8] which is based on HGC using ACP 215 is adopted by United States (US) Armed Services Blood Programme (ASBP) [9].

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The salient features of the NBRL method are described below:

Equipments

ACP™ 215 Haemonetics cell processing system is an automated, functionally closed system for the glycerolization and deglycerolization of RBC that uses inline 0.22μ filters to deliver solutions, a disposable polycarbonate 275 or 325ml bowl with a diverter external seal for washing cells, integrally attached shaker and an optical system that measures haemoglobin concentration in waste solution during deglycerolization. ACP 215 uses disposable glycerolization or deglycerolization kits. The machine is easy to operate for both protocols [2,3]. The sterile connecting device (SCD) is used for attaching additional bags and compatible tubing to a blood bag without breaking sterile integrity of the system [4]. It is used for connecting RBC unit to glycerolization or deglycerolization kit in a closed system. Ultra low temperature (-80^0C) mechanical freezers are essential for cryopreservation of the FS-RBC with sensors for continuous monitoring of temperature [4].

NBRL Method

Red cell concentrates (haematocrit value 75 ± 5 v%) stored at 4°C for 3 to 6 days in any preservative is used for glycerolization. RBC unit is brought to room temperature and connected with glycerolization harness set loaded on ACP 215 with 6.2 M glycerolizing solution. Approximately 435 ml 6.2 M glycerol is added to the RBC in three aliquots, with short periods of equilibration between each addition, to achieve a final glycerol concentration of 40%. The unit is kept in cardboard box and frozen at -80°C with proper label [8]

US FDA (Food and Drug Administration) has licensed shelf life of 10 yrs for FS-RBC in 40%W/V glycerol when stored at -80°C [4,8]. However studies have shown acceptable in vitro quality of FS-RBC cryopreserved in 40%W/V glycerol at -80°C for 37 years of storage [10]. Thawing of FS-RBC involves its careful removal from the cardboard box, which is then wrapped in plastic bag and placed in water bath at 37°C for approximately 35 minutes.

The deglycerolization set is loaded to ACP 215. Hypertonic solution-12% sodium chloride solution, wash solution - 0.9% sodium chloride with 0.2 gm% glucose solution and preservative solution – Additive Solution-3 (AS 3) is connected to the set. The thawed RBC unit is docked to the deglycerolization set by using SCD. ACP 215 undertakes all sequences of deglycerolization in auto mode. Thawed red cells are diluted with hypertonic solution, followed by wash solution. The diluted red cells are transferred from the primary collection bag into the bowl and the washing procedure begins. RBCs are washed in five cycles. Finally 240 ml AS-3 is added to RBC and washed RBC are delivered in the blood collection bag for transfusion. Deglycerolization of unit takes approximately 55 minutes. The shelf life of deglycerolized RBC prepared by NBRL method in closed system, preserved in AS-3 and stored at 4°C is 14 days [4,8]. However, deglycerolized RBC prepared in open system has shelf life of just 24 hours [4,8].

RBCs stored in AS-1, AS-3, or AS-5 at 4°C for 42 days can be biochemically modified with pyruvate, inosine, phosphate, and adenine (rejuvenation). The rejuvenated RBC can also be cryopreserved with acceptable Freeze-Thaw-Wash (FTW) in vitro with acceptable in vivo RBC recovery [11].

USNS Mercy Experience

The author had the opportunity to serve onboard United States Naval Hospital Ship (USNS) Mercy (T-AH-19) [12] (Echelon III Medical Treatment Facility of US Navy) during 'Mercy Mission 2006' from 26 May -01 Sep 2006. The blood bank onboard is part of US- ASBP. She has storage capabilities of 3000 units of FS-RBC and 2500 units of liquid RBC. She maintains stock of 2850 FS-RBC units at all times [13]. She has six ACP 215 units, where single technician can deglycerolize 40 FS-RBC units in eight hours shift. Availability of FS-RBC played pivotal role in the mission, in which around 1000 major surgeries were performed. During the period 200 deglycerolized units were transfused to various patients including massive transfusion of three patients. Deglycerolized unit weigh between 325-350 gm with haemoglobin of 15-18 gm/dl. In addition to availability of RBC for entire mission, FS-RBC also offered advantages of transfusion of O Rh ± blood units without crossmatch test in emergent blood requirement and zero transfusion reaction including febrile non-haemolytic transfusion reaction due to removal of plasma and reduction of white blood cells (WBC) by washing protocol.

Discussion

The goal of FS-RBC is to make long term storage possible, which was fully realised in 1970s [14]. FS-RBC offers storage of RBC units for unexpected high blood demands during operations or calamities[1], for rare donor blood requirement [1,14], storage of special purpose blood such as cytomegalovirus free [14] and autologous blood particularly of patients with irregular antibodies[1]. In addition, FS-RBC has higher ATP and 2,3-DPG content close to fresh RBC resulting in superior oxygen carrying capacity [1]. Reduction of WBC and other biological active substances which contributes to immunomodulatory effects in recipients during washing of FS-RBC leads to significant reduction in transfusion reactions [2]. FS-RBC is screened for infectious markers in advance. FS-RBC gives option of use of donor retested RBC unit in which RBC unit is quarantined and subsequently released if negative on 'retest' for infectious markers after six months of donation [2]. FS-RBC can be important blood resources in situations such as epidemic/ pandemic or bio-terrorist attack. By affecting donor suitability, such biological attack may substantially limit the blood supply [15].

High cost, technically demanding method of glycerolization or deglycerolization and limited 24 hours post-deglycerolization shelf life of RBC were limitation of FS-RBC use [1,14]. The scenario has changed with introduction of glycerolization and deglycerolization by NBRL method with a significant reduction in processing time [2,3]. Further, deglycerolized RBC prepared by NBRL method has a shelf life of 14 days, resulting in reduction of wastage rate and cost per unit of blood [8].

Studies found acceptable in-vivo and in-vitro quality of deglycerolized RBC by NBRL method [2,3]. A study has demonstrated in-vitro FTW recovery of 87.0 ± 5% and mean haemolysis of 0.6± 0.2 % after storage at 4°C in AS-3 for 15 days by NBRL method [2]. Mean 24 hours post transfusion survival of 76 ± 6 % for RBC that have been stored at 4°C in AS-3 for 15 days after deglycerolization as against 72±5% for RBC stored at 4°C in AS-1 for 42 days has been reported [2].

US ASBP has implemented FS-RBC programme since last three decades and has adopted NSBL method. ASBP has stockpiled around 60000 FS-RBC units at different strategic locations [9]. FS-RBC offered advantages of eliminating need of crossmatch in emergency due to availability of universal blood group units, recruitment of donors during operation, readiness of blood product in theatre of combat before commencement of liquid RBC supply and stabilising supply during high-use period [9]. These are important logistic factors in war or mass casualties management.

Various countries have the Frozen Blood Program for rare blood groups [16,17]. In India, limited units of rare blood group RBC units have been cryopreserved at All India Institute of Medical Sciences. Nanu et al [17], reported improvement in utilization, reduction in wastage and supplementation of liquid stocks of Rh negative blood due to cryopreservation.

Currently various groups are pursuing research on artificial blood and various modalities for storage of RBC [18,19]. An oxygen carrier molecule as substitute for RBC transfusion is under research and may reshape the practice of transfusion medicine [18]. However such molecules may not replace need for blood donation in the foreseeable future [18]. Various groups are working on lypholisation as modality in storage of RBC. Lyophilised RBC is envisaged to offer many advantages such as weight, convenient transportation, room temperature preservation and ease of reconstitution [19]. However 'Lyophilised RBC' is in experimental stages and has long way for clinical use [19]. Thus FS-RBC is the only option in augmenting current liquid blood supply for Armed Forces Blood Programme.

Considering the disadvantages of liquid RBC and advantages of FS-RBC, there is need to examine the FS-RBC or Frozen Blood Programme for Indian Armed Forces. The Frozen Blood Programme will complement liquid RBC supply during mass casualties in war and peace. Frozen Blood Programme will help in uninterrupted supply of RBC and other plasma products for transfusion in forward surgical centres, technical support platoons, which will enable surgeons to conduct life and limb saving surgeries at forward locations.

To conclude, FS-RBC is an important blood product which can be stored for years and can be used for transfusion requirements of individual patients or mass casualties. NBRL method with ACP 215 is technically feasible for glycerolization and deglycerolization of RBC. The increase in shelf life of deglycerolized RBC to 14 days resulted in better inventory management and reduction in cost. Considering the advantages of FS-RBC, cryopreservation of FS-RBC by NBRL method is an important option for augmenting blood programme of Indian Armed Forces.

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Conflicts of Interest

None identified

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