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Vitamin C as a modulator of oxidative stress in erythrocytes of stored blood

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ABSTRACT

Aim: To determine the effects of Vitamin C (VC-ascorbic acid) as an additive on erythrocytes of stored blood. **Background:** Oxidative stress (OS) plays a major role in the formation of storage lesion of erythrocytes. Antioxidants, such as VC could be beneficial in combating oxidative damage during storage. **Materials and methods:** Blood obtained from male *Wistar* rats was stored at 4 °C in anticoagulant solution citrate-phosphate-dextrose-adenine solution. Blood samples were divided into 3 groups – (i) Controls, (ii) VC 10 (VC at a concentration of 10 mM), (iii) VC 30 (VC at a concentration of 30 mM). Markers of OS in erythrocytes such as – hemoglobin, superoxides, antioxidant enzymes (superoxide dismutase; SOD, catalase and glutathione peroxidase), hemolysis, lipid peroxidation products (conjugate dienes and malondialdehyde), protein oxidation products and ascorbic acid were determined on days 0, 10 and 15 of storage. **Results:** Addition of ascorbic acid to the storage solution contributed to the protection of erythrocytes from oxidative damage. Ascorbic acid at a concentration of 30 mM decreased SOD levels and increased protein sulfhydryls (P-SH) levels on day 15 showing that higher concentration supplemented the inherent antioxidant defense system of erythrocytes during blood storage. **Conclusion:** VC proved to be effective in combating OS during blood storage. However, further exploration of antioxidants as additives and the erythrocyte storage lesion would result in better management of blood storage.

Introduction

Blood transfusion is an acute intervention, implemented to solve life- and health-threatening conditions on a short-term basis, and in general its long term effects tend to be of secondary importance [1]. Red blood cell (RBC) transfusion is a key element of modern medical care. The ability to store RBCs for reasonable times clearly improves their availability and lowers their cost [2]. Whole blood can be stored up to a period of 35 days in anticoagulant solution CPDA-1 (citrate, phosphate, dextrose and adenine) while erythrocytes (RBCs) possess a shelf life of 42 days at 4 °C in SAGM (saline, adenine, glucose and mannitol) solution [2].

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During storage, RBCs undergo structural and functional changes that may reduce function and viability after transfusion [3]. Changes accompanying the storage of RBCs are known as "storage lesion". During storage of blood and its components, in various additive solutions, it has been found that oxidative stress (OS), influences their functioning, efficacy and shelf life. OS is the fundamental phenomenon leading to the formation of storage lesion in erythrocytes [4–6]. Protein oxidation and lipid peroxidation (LPO) also occur, in turn causing vesiculation and loss of deformability [7]. OS plays an important role in the formation of storage lesions in blood and its components and there exists an inherent antioxidant system in blood to combat OS. However, during storage, there is a reduction in blood antioxidant capacity, leading to increased susceptibility to OS. Hence, there is a possibility of utilizing antioxidants to aid in protecting the erythrocytes against oxidative damage during storage.

Vitamin C (VC) or ascorbic acid is found in erythrocytes and can scavenge a wide variety of free radicals directly in the aqueous phase. VC reduces superoxide and lipid peroxyl radicals, a synergistic agent for Vitamin E [8–10] and protects membrane [11]. Ascorbic acid serves as both an antioxidant and a prooxidant. As a prooxidant, it generates cofactors of activated oxygen radicals during the promotion of LPO, in presence of Fe^{3+} and Cu^{2+} ions [12]. Studies on the effects of ascorbic acid on erythrocytes during storage have focused on biochemical parameters, hemolysis and fragility [13–19].

Rat models provide a platform for rapid experimentation, in turn forming a basis for further studies on human models. A comparative study between rat and human erythrocytes revealed that rat erythrocytes when stored for 1 week in CPDA-1 solution, develop a storage lesion equivalent to that of a lesion formed in human erythrocytes stored for 4 weeks [20]. Our previous work has elucidated that reactive oxygen species are maximum on days 10 and 15 of storage [21] and hence, our current study focuses on the storage lesion on days 0, 10 and 15 of storage. Therefore this study aims to evaluate the effects of VC on erythrocytes of stored rat blood through the following 3 aspects – (i) oxidant levels of erythrocytes in terms of hemolysis, LPO and protein oxidation products, (ii) antioxidant enzymes and (iii) VC as an additive in storage solution.

Materials and methods

Animal care and maintenance was in accordance with ethical committee regulations.

Chemicals

Hemoglobin (Hb) reagent was obtained from Coral Clinical Systems, Goa, India. Thiobarbituric acid (TBA), Epinephrine, Glutathione reductase (GR), Glutathione (GSH) and Bovine Serum Albumin (BSA) were purchased from Sigma–Aldrich Chemicals [St. Louis, MO, USA]. All other chemicals used were of reagent grade and organic solvents were of spectral grade.

Blood sampling

Animals were lightly anaesthetized with ether and restrained in dorsal recumbancy as described earlier [6]. In brief, a syringe needle was inserted just below the xyphoid cartilage and slightly to the left of midline. Four to five milliliter of blood was carefully aspirated from the heart into 5 mL plastic collecting tubes with anticoagulant solution, citrate-phosphate-dextrose-adenine-1 (CPDA-1).

Experimental design

Blood was drawn from *Wistar* rats (4 months old) and stored at 4 °C in anticoagulant solution, CPDA-1. Blood samples were divided into 3 groups – (i) Controls, (ii) VC 10 (samples with VC as additive at a concentration of 10 mM), (iii) VC 30 (samples with VC as additive at a concentration of 30 mM). Erythrocytes were isolated from stored blood and the biomarkers of OS were studied.

Erythrocyte separation

Erythrocytes were isolated by centrifugation for 20 min at 1000 × *g* at 4 °C, plasma and buffy coat were removed using a micropipette. Cell pellet was washed 3 times and suspended in an equal volume of isotonic phosphate buffer [22]. This constituted the erythrocyte suspension.

Hb estimation

Hb was measured using Hemocor-D Kit [Coral Clinical Systems, Goa, India], which utilizes Cyanomethemoglobin method [23]. Whole blood was incubated with Hb reagent for 3 min at room temperature and absorbance was measured colorimetrically at 540 nm. Hb concentration was represented in terms of g/dL.

Superoxide

Superoxide generated was determined by the method of Olas and Wachowisz [24]. Cytochrome c (160 μM concentration) was added to equal volume of sample and incubated at 37 °C for 5–15 min. Samples were then centrifuged at 3500 rpm for 5 min. Absorbance of the supernatant was measured at 550 nm.

Antioxidant enzymes

Superoxide dismutase [SOD, EC 1.15.1.1]
SOD was measured by the method of Misra and Fridovich [25]. Hemolysate was added to carbonate buffer [0.05 M]. Epinephrine was added to the mixture and measured [ELICO, Model SL 159, India] at 480 nm. SOD activity is expressed as the amount of enzyme that inhibits oxidation of epinephrine by 50%.

Catalase [CAT, EC 1.11.1.6]
CAT was determined by the method of Aebi [26]. Briefly, hemolysate with absolute alcohol was incubated at 0 °C. An aliquot was taken up with 6.6 mM hydrogen peroxide (H_2O_2)

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