ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Could stored blood transfusions (SBT) alter the mechanisms implied in wound healing, in burned patients?

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Abstract

Several years ago, researchers reported several complications produced after blood transfusions such as transfusion reactions, the transmission of a variety of infectious agents, etc. Recently, many authors appreciate that the stored blood transfusions (SBT) create an important damage for patients' life, because of oxygen metabolism disturbances, induced earlier, after three repeated transfusions and maintained longtime after. Our study proposed to note the consequences of SBT on wound healing, in burned patients, who were submitted to skin grafting and remained in hospital for a long period. We tried estimate the pathophysiological mechanisms implied in microcirculation's failure, microvascular systemic deficiency and death. *Results*: Critically patients receiving SBT repeated frequently (six times/monthly for skin grafting) have an oscillatory outcomes depending by the reactivity of their biological terrain, as reflected by a several parameters we have measured. *Conclusions*: SBT administered as a restrictive transfusions to the patients with hemoglobin values <8 g% and hematocrit <35% has good effects on wound healing evolution.

Keywords: body burned surface, skin grafts, stored blood, nitric oxide, oxidative stress.

Introduction

Many authors sustain the presence of several complications produced after stored blood transfusions (SBT), such as infections, immunosuppression, impairment of microcirculatory blood flow, 2,3-diphosphoglycerate deficiency physiological, and biochemical disturbances (hypocalcemia, hypercoagulopathy, hyperkalemia, and hypothermia). Several of these complications are due to the stored blood transfusions, especially of to the packed red blood cells (PRBC). Many of the studies reviewed are retrospective analyses, but the results of recent studies of outcomes in patients receiving SBT, indicate that critically patients have worse outcomes as reflected by mortality, infections, organ failure, and pulmonary complications [1]. Patients with more than 40% burned surface, have lower hemoglobin, because of loosing it: through necrotic tissues, vascular fragility of new vessels, prelevation of skin for autologous grafts, bleedings, from the degranulated tissues, also it is reported, that total blood loss per patient could be of 750 mL one day. It is possible, also, that erythrocytes production to decrease, after SBT, because of medullo-production inhibition. Loses of blood and inability of production of red cells, generate anemia. Many authors sustain the necessity of blood transfusion only for this kind of disease. To the wound healing at burned patients who have need of autologous skin grafts, the quality of healing is in relationship to the level of plasma protein and O_2 transporters. Plasma proteins decrease in burned patients, because of plasmarrhagia, skin prelevation and the nutrition status (usually hyponutrition or denutrition). Another objective of BT is those to expand intravascular volume to assure the adequacy in oxygen-carrying capacity or oxygen delivery to tissues [2, 3].

Recently, many published articles assess the safety of restrictive transfusion strategies, (only to resolve the anemia) because of many difficulties produced by the great volume of stored blood administrated to all patients [4]. The effect of storage on PRBC includes decreased levels of 2,3-diphosphoglycerate, means the increasing in oxygen affinity to hemoglobin and a decrease in its ability to offload oxygen to tissues [5]. This pathophysiological mechanism, determines morphological changes in host erythrocytes and other cells, namely: increased fragility, deformability, decreased viability, as well as the release of a number of substances resulting in systemic responses as fever, and cellular injury, at a distance, associated to regional and global blood flow hypoperfusion and organ dysfunction [6, 7]. Blood transfusion with PRBC stored for a long period of time, influence the host erythrocytes, which

determine a poorer oxygen delivery. Evidence also suggests that the transfusion of older blood (stored >14 days) is an independent risk factor for the development of multiple organ failure.

Purpose

In this status of knowledge about blood transfusion, we proposed in our study:

- To identify the oxidative stress as a risk factor inducing the wound healing alterations;
- To evidentiate the parameters, sensible modified after blood transfusion (markers of oxidative stress);
- To elaborate a therapeutic scheme, to prevent the bad effects of oxidative stress, and reversed it to a favorable activity on the wound healing process.

Patients and Methods

We realized our study upon healthy, young-volunteers subjects, comparatively, with surgical patients with burned body surface (BBS), from Plastic and Reconstructive Surgery Department, from the Emergency County Hospital of Craiova. The study was approved by Ethics Committee. We observed: 75 patients, aged 40 ± 10-year-old, submitted to a mild anesthetic surgical stress, for autologous grafts application, divided into three groups: group A, 25 cases, control, healthy patients having burns at 10±7% body surface, non-receiving BST; group B, 25 cases, with 40±5%; group C, 25 cases, 45±5% BSB, known to ingest alcohol, chronically and with a denutrition status. We consider this last group to create the group of critically ill patients. For these groups we calculated SOFA (sequential organic failure) score. From each group we have determined the values of leukocytes, thrombocytes, coagulability index, total antioxidant response (TAR), or total antioxidant capacity of plasma (TAOP) and nitric oxide level at three different moment: T1 (after first transfusion), T2 (second transfusion at 7–10 days) and T3 (after third transfusion) and we observed macro and microscopic evolution of wound. We obtained the results for our biochemical blood proves from the Laboratory of Oxidative Stress at the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca. We obtained biopsies from the region between burned and healthy tissues for morphological studies and blood from the venous effluent of a vein appropriate to the burned zone. Patients from all groups received: crystalloidal solutions, colloidal solutions and for group B and C, as a restrictive transfusion (hemoglobin <7.5 g%), only to restore the hematological values of hemoglobin, these ones creating the manifestations for T2 and T3 periods.

Techniques

We noted clinical parameters, calculated SOFA scores, and determined the values of hemoglobin, hematocrit, leukocytes and coagulability of plasma, at Hospital Laboratory and Blood Transfusions Centre in Craiova.

We used Oczan Erel technique for obtaining all values for oxidative stress evaluation (TAOP, free radicals, oxidative stress index).

Summary for technique description

Chemicals: ferrous ammonium sulfate, *o*-dianisidine dihydrochloride (3,3'-dimethoxybenzidine), vitamin C (L(+) ascorbic acid), bilirubin, uric acid, reduced glutathione (GSH), (F)-catechin, 5,5'-dithiobis-(2-nitro-benzoic acid) (DTNB), ethylenediamino-tetraacetic acid (EDTA), 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), potassium persulfate, glucose, ribose, saccharose and sodium citrate were purchased from Sigma Co. and Merck Co. The water soluble analogue of vitamin E (Trolox; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Sigma-Aldrich Chemical Co. All chemicals were ultra pure grade and type I reagent grade deionized water was used. Venous blood samples obtained were collected into tubes (and heparinized tubes) and serum (and plasma) was separated from cells by centrifugation at 1500 g for 10 minutes. Serum (and plasma) samples were run immediately or stored at 80jC. Antioxidants: stock solutions (1.0 mM) of ascorbic acid, glutathione and (F)-catechin were separately prepared in saline solution (0.9% NaCl). Uric acid and solid bilirubin were dissolved in 10 mM NaOH solution. Trolox was dissolved in phosphate buffer (10 mM, pH 7.4).

Apparatus: a Cecil 3000 spectrophotometer with a temperature controlled cuvette holder (Cecil), and an Aeroset automated analyzer (Abbott). Assay principle of the method: a standardized solution of Fe^{2+}-*o*-dianisidine complex reacts with a standardized solution of hydrogen peroxide by a Fenton-type reaction, producing OH_. These potent ROS oxidize the reduced colorless *o*-dianisidine molecules to yellow-brown colored dianisidyl radicals at low pH. The oxidation reactions progress among dianisidyl radicals and further oxidation reactions develop. The color formation is increased with further oxidation reactions. Antioxidants in the sample suppress the oxidation reactions and color formation. This reaction can be monitored by spectro-photometry. Assay calibration: the suppression of the color formation is calibrated with Trolox, which is widely used as a traditional standard for TAR measure-ment assays, so the results in this assay are expressed as in terms of millimolar Trolox equivalent per liter.

Technique for NO determination

We used a simple spectrophotometric method for simultaneous detection of nitrite by means of technique and apparatus from the Oxidative Stress Laboratory, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca.

Results

Clinical manifestations

Thirty-one percent of transfused patients from group C developed wound-healing disturbances *vs.* 18% of the group B ($p<0.05$). Allogeneic blood transfusion was the only predictor for development of minor wound-healing disturbances. Duration of hospitalization was prolonged in transfused patients (31.3 *vs.* 10.8 days) and could be predicted by four significant variables: requirement for blood transfusion (adds 4.5±0.5 days), presence of

wound-healing disturbances (adds 3.3±0.5 days), and duration of surgery (adds 1.4±0.1 days/10 minutes). These data suggested that allogeneic blood transfusions were associated with an increased incidence of wound-healing disturbances and that prevention of allogeneic blood transfusion may be relevant in limiting the duration of admission after skin grafting.

Perioperative blood loss was a major problem in plastic surgery. Allogeneic blood transfusion was the standard approach to treat potentially detrimental decreases in hemoglobin (Hb) concentration. However, allogeneic blood transfusion was associated with various adverse events, including febrile reactions induced by leukoagglutinins. Moreover, allogeneic blood transfusion has had an immunomodulatory effect, which is thought to increase the frequency of post-operative disturbances.

In this prospective observational study, we found that allogeneic blood transfusion is associated with prolonged hospital admission. We found that this prolonged admission is not a straightforward consequence of an increased postoperative infection rate. Deep infections (which have frequent morbidity) occurred at similar rates in both transfused and non-transfused patients. Other infections tended to be increased in the transfused group, but this did not reach statistical significance. The requirement for blood transfusion was not the only difference between the two study major groups (non-transfused and transfused), and some of these other significant factors may also have been responsible in part for the increased incidence of wound disturbances. However, many of the factors that showed significant differences between transfused and non-transfused patients can be logically linked to red cell loss and, thereby, to transfusion requirement. For example, male sex, greater height and weight, and a higher preoperative Hb (>8 mmol/L) are all associated with a larger red cell mass, and these patients will therefore tolerate more red cell loss before transfusion triggers are reached. Similarly, a longer operation and larger blood loss are logically correlated with transfusion incidence. However, allogeneic blood transfusion was the sole significant predictor of the development of wound-healing disturbances, and together these two factors were the main predictors of prolonged hospitalization. No significant influence on wound-healing disturbance and hospitalization was found, either by univariate or multivariate analysis, of age, sex, height, weight, operation duration, blood loss.

Staying in hospital because postoperative infections are relatively compared with those because of allogeneic blood transfusion was not yet established. It is possibly, other factors to be responsible for the prolonged hospital stay. A host of other factors (age, length of surgery, use of implants, and so on) might influence the incidence of postoperative infections as well, and, in addition, the end-point of frank wound infection may not be appropriate. Less severe types of wound-healing disturbances could occur after transfusion and lead to subsequent complications or prolonged hospitalization.

To analyze the effects of other factors, such as sex, height, weight, and preoperative Hb, more formally, we performed univariate and multivariate analysis across these factors and determined which of the variables best predicts the development of postoperative wound-healing disturbances.

At all the time points, we recorded vital signs, any signs of infection, and other relevant variables. Comparing the incidence of wound disturbances between groups, we found a clear difference: 31% of the transfused group developed a wound disturbance versus 18% of the non-transfused group ($p<0.05$).

The requirement for blood transfusion was not the only difference between the two study groups, and some of these other significant factors may also have been responsible in part for the increased incidence of wound disturbances. However, many of the factors that showed significant differences between transfused and non-transfused patients can be logically linked to red cell loss and, thereby, to transfusion requirement. For example, male sex, greater height and weight, and a higher preoperative Hb (>8 g%) are all associated with a larger red cell mass, and these patients will therefore tolerate more red cell loss before transfusion triggers are reached. Similarly, a longer operation and larger blood loss are logically correlated with transfusion incidence. However, allogeneic blood transfusion was the sole significant predictor of the development of wound-healing disturbances, and together these two factors were the main predictors of prolonged hospitalization. No significant influence on wound-healing disturbance and hospitalization was found, either by univariate or multivariate analysis, of age, sex, height, weight, operation duration, blood loss, or the use of gentamycin cement.

An alternative explanation might be that allogeneic blood transfusion induces a small but significant delay in wound healing. We observed that the duration of stay in hospital could be predicted by four significant variables requirement for blood transfusion, presence of wound disturbances, duration of operation, and patient age.

About the patients' reactivity, we observed three kinds of general reactions:

- Group A: discreet hypersimpaticotony, immediately after local surgical care, during 4–6 hours, and complete resolution of wound, after 3–5 days;
- Group B: pain to the region where it was applied the graft skin, agitation, inapetence (24–49 hours), local minor infections in T2;
- Group C: ARDS (8% cases), wound non-healing and death (3% cases) at T3.

Morphological aspects

Cellular edema, neutrophilic infiltration and tissular necrosis were the most important clinical signs in T1 (group A) and T2–T3 (group C) (Figures 1 and 2).

Wound-healing disturbance was defined by erythematous inflammation of >1 cm, wound fluid discharge, purulent suture, wound dehiscence, blister, or any degree of wound necrosis. Moreover, certain events were recorded at any moment during the follow-up period: postoperative infections, withdrawal from the study (including the reason for withdrawal), and death (including cause of death).

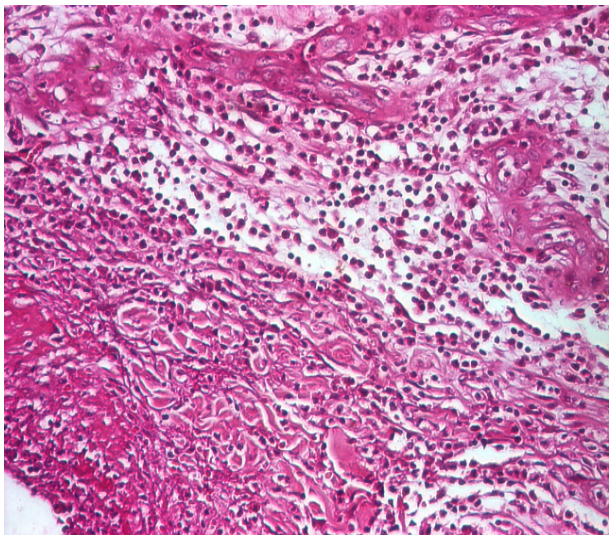

Figure 1 – ***The aspect of interstitial infiltration with neutrophilic granulocytes, in sample obtained from the group A, in T1 for the first skin grafting.***

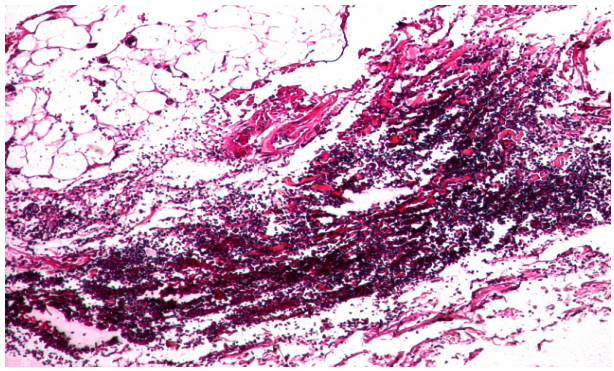

Figure 2 – ***The aspect of necrosis from wound sample obtained from C group, in T2 period, after blood transfusion, for the second skin grafting.***

Biochemical aspects

We obtained the results representing a TAOP level and NO values in B and C groups at T2 and T3 (Table 1, Figure 3).

Table 1 – ***NO values vs. ROS [mmol/L], p<0.05***

	T1		T2		T3	
	ROS	***NO***	***ROS***	***NO***	***ROS***	***NO***
Group A (Control)	0.011±0.02	4.76±0.75	–	–	–	–
Group B	0.096±0.24	4.41± 0.22	0.157±0.75	0.22±0.13	0.237±0.54	**1.68±0.23**
Group C	0.235±0.43	3.14±0.10	0.298±0.60	0.345±0.45	0.321±0.52	1.04±0.06

The values of ROS are increased progressively from T1 to T3, inducing an excessive consumption of endogenous antioxidants. The production of NO decreases and explains the appearance of the vasoconstriction and intravascular coagulation, unfavorable events, associated to oxidative stress, especially in patients with bad evolution (group C).

Coagulability

After the first period of transfusions (T1), 70% of cases, from B and C groups, developed a hyper-coagulability, because of hypersimpaticotony and hemo-concentration. We observed also, great fluctuations of thrombocytes count, in group C, independently associated to TAOP values which were different, in group C, in T1 (first 4–12 hours, post-transfusional) and C (T2, T3) (Figure 4).

The total antioxidant capacity of group B was increased. For group C, increasing of free radical reactions explained the decreasing of the plasma anti-oxidant capacity.

NO values

Statistically analysis, presented in Figures 5–7 is largely presented in the section of discussion.

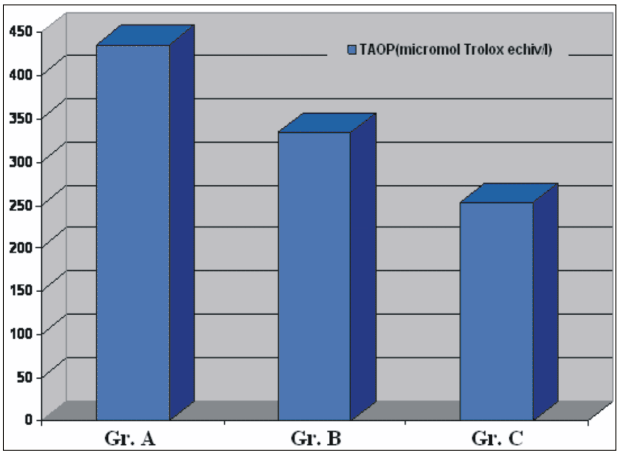

Figure 3 – ***TAOP values in the three groups (A, B and C) evidentiating the reducing of antioxidant potential of plasma.***

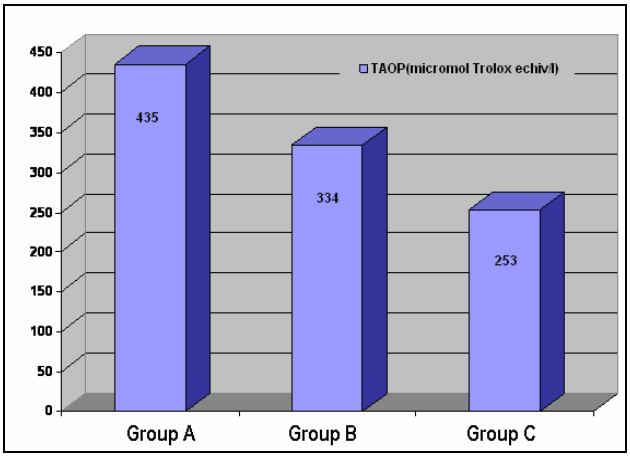

Figure 4 – ***TOAP values at T3 every individually: groups A, B and C (a substantial decreasing for the group C), p<0.05.***

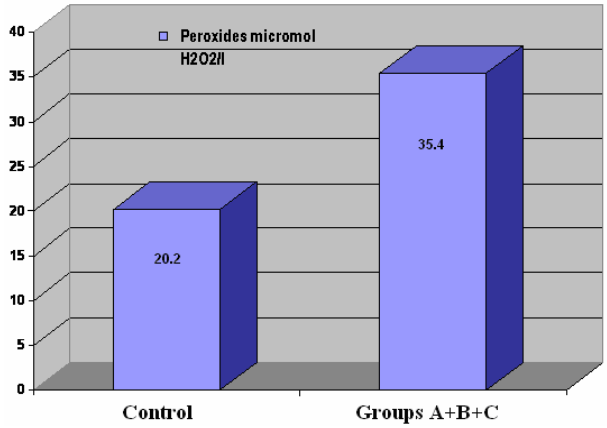

Figure 5 – ***The values of peroxides in micromoles H_2O_2. Control 20.2±2.6 and groups of studying at T3 35.4±6.4, p<0.001. Reflect the tendency and also the installation of oxidative stress.***

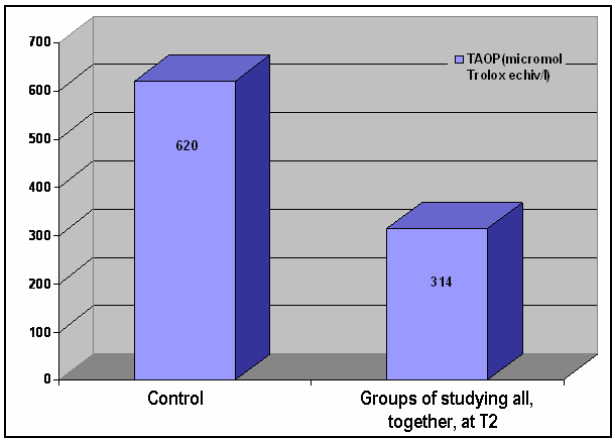

Figure 6 – ***Values of TAOP (determined for control comparative with the values of the groups of studying, at T2) (micromoles Trolox echiv./L): control 620±98.3; groups A, B and C, 314±57.1, p<0.027.***

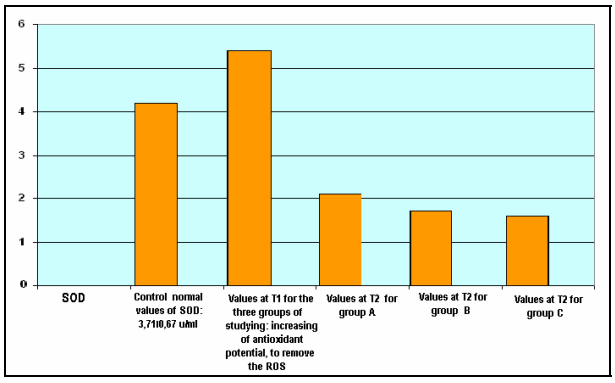

Figure 7 – ***The values of SOD (endogenous anti-oxidant). Decreasing progressively of SOD from group A to C reflects an indirect decreasing of TAOP, because of oxidative stress installation, (as a compensatory mechanism).***

Discussion

NO is a very important mediator of inflammation, known as a factor released from endothelial cells [8]. It causes vasodilatation by relaxing vascular smooth muscle, because of his activity is recognized as endothelium-derived relaxing factor (EDRF). NO is a soluble gas produced by endothelial cells, macrophages and specific neurons in the brain and acts in a paracrine manner, on target cells through induction of cyclic guanosine monophosphate (GMP), which, in turn, initiates a series of intracellular events leading to a response, such as the relaxation of vascular smooth muscle cells. *In vivo*, NO acts only on cells in close proximity to where it is produced. Its localized activity accounts for the specificity of its actions. NO complexes with thiol groups on proteins, form more stable adducts (S-nitroso-proteins) that have been implicated in some of NO actions. NO plays an important role in vascular function during inflammatory responses, namely: vaso-dilatation, reduce platelets aggregation, inhibit several features of mast cell-induced inflammation, and serves as a regulator of leukocytes recruitment. Blocking NO production under normal conditions promotes leukocytes rolling and adhesion in post-capillary venules, and delivery of exogenous NO reduces leukocytes recruit-ment in acute inflammatory processes [9, 10]. Over-production of NO from iNOS is an endogenous compensatory mechanism that reduces leukocyte recruitment in inflammatory responses.

Abnormalities in endothelial production of NO is involved in the pathogenesis of septic shock. NO also acts in the host's response to infection [8]. Evidence supporting the biologic importance of NO-related anti-microbial activity includes the following: antimicrobial activity: interactions between NO and reactive oxygen species, leading to the formation of multiple anti-microbial metabolites (e.g., peroxynitrite [OONO-], S-nitroso-thiols [RSNO], and nitrogen dioxide [NO_2]), each with distinct stability, compartmentalization, and reactivity, but shares the ability to damage microbial DNA protein and lipids. Production of NO is increased during host defense and is decreased in oxidative injuries and lesions. In local oxidative injury, activation of the endothelial cells initiates three types of activities, because of the releasing of substances, such as:

- vasomotor: endothelin ET-1, prostacyclin (PGI2), and nitrite oxide (NO);
- anti-thrombotic, anticoagulant effects exerted by thrombogenous substances (ADP, 5-HTP) because of platelets degradation;
- anti-adhesive properties.

The early reactivity phase in T1: 4–12 hours after transfusion, consisted in ROS increasing and decreasing of TAOP, contributes to develop the conditions for wound healing. The mechanisms accepted to explain this disturbances are the following: frequently and repeated transfusions, are associated with free radicals production: OH_ *via* Fenton reaction and/or reaction catalyzed by ferric ion (Haber–Weiss reaction). ROS oxidize different kind of proteins, creating a progressive cascade of free radicals (O_2 and nitrogen) activation. The accumulation of these radicals decreases strongly the antioxidant capacity of plasma, which together activates the intracellular contraction force that breaks the cytoplasm and cellular membranes. It is induced the disappearance of bridges between cells. These phenomena explain the cellular dysfunction or death and non-healing of wound. Water entering into the cells

(cellular swelling) is the most important factor of tissues disorganization and cellular destructure [11].

Marik PE and Sibbald WJ consider that the cells destroyed by the oxidative stress produce the occlusion of microcirculation, increasing the index of coagulability and producing the extension of ischemia [12].

The losing of reduced glutathione (GSH) and glutathione peroxidase (GSH-PX) in stocked red cells modify the integrity of cell membranes of host cells from mobile and fix tissues. Also, host leukocytes are activated by extracellular signals represented by defectuous and destroyed cells [13] which could activate principal leukocytes' functions adhesivity, chemotaxia, exocytosis of secretory granules, production of superoxide anion, cause of peroxynitrite appearance, an anion extremely toxic [14].

Bioreactive substances accumulated during the period of blood stocking are: cytokines (proinflammatory) and lipids [15]. Plasmatic supernatant released by red cells have effect on D11b, CD16 expression stimulating the intensifying of oxidative stress [16].

Conclusions

The much more units of transfused blood packed red cells are administrated the risk of multiorganic dysfunctions could generate a bad evolution and death.

For burned patients it is necessary to select the adequate strategy for indication of blood transfusion, namely, when the loss of blood is manifested by decreasing of the hemoglobin level (during surgical degranulation and skin prelevation).

Burned patients with hyponutrition and decreased plasma protein level, have need of fresh plasma, but units of blood offers the delivery of O_2 to the grafted regions.

NO has a good effect in normotensive and normoperfused patients, assuring to red cells to enter into peripheral tissues, because of peripheral vasodilatation.

NO overproduction installed in the same time, with oxidative stress, decrease strongly TAOP, and this moment represents the point from which surgeon could elaborate a bad prognosis (coincident with peroxinitrite overproduction).

TAOP values are predictive, and for that it is considered one of the golden standard tests, similar to tissues' biopsy in morphopathology.

For oxidative stress induced by red blood packed transfusions, the prophylactic treatment and tissular restauration, means to administrate exogenous antioxidants, every day, during the entire period of blood administration.

It is saying that in surgical activity, based on practice, theoretical considerations, based on isolated experiences must be avoided, if these ones are contradictory with the best results obtained. Our study sustain that knowing very well biological terrain and having a very good anesthetic-surgical team, blood administration is followed by very good results. It is true, that in our centers, there are insufficient units of stocked blood, so our results underline the necessity of blood transfusions, to the patients who lose more than 700 mL, once, and have the hemoglobin level decreased at 8 g%.

References

[1] Gould S, Cimino MJ, Gerber DR, *Packed red blood cell transfusion in the intensive care unit: limitations and consequences*, Am J Crit Care, 2007, 16(1):39–48; quiz 49.

[2] Dumaswala UJ, Wilson MJ, José T, Daleke DL, *Glutamine- and phosphate-containing hypotonic storage media better maintain erythrocyte membrane physical properties*, Blood, 1996, 88(2):697–704.

[3] Dumaswala UJ, Wilson MJ, José T, Daleke DL, *Effect of a glycerol-containing hypotonic medium on erythrocyte phospholipid asymmetry and aminophospholipid transport during storage*, Biochim Biophys Acta, 1997, 1330(2):265–273.

[4] Rael LT, Bar-Or R, Ambruso DR, Mains CW, Slone DS, Craun ML, Bar-Or D, *The effect of storage on the accumulation of oxidative biomarkers in donated packed red blood cells*, J Trauma, 2009, 66(1):76–81.

[5] Lall RN, Loomis W, Melbostad H, Hoyt DB, Lane T, Coimbra R, *Phosphodiesterase inhibition attenuates stored blood-induced neutrophil activation: a novel adjunct to blood transfusion*, J Am Coll Surg, 2006, 202(1):10–17.

[6] Jacobi KE, Wanke C, Jacobi A, Weisbach V, Hemmerling TM, *Determination of eicosanoid and cytokine production in salvaged blood, stored red blood cell concentrates, and whole blood*, J Clin Anesth, 2000, 12(2):94–99.

[7] Seghatchian J, *Platelet, storage lesion: an update on the impact of various leukoreduction processes on the biological response modifiers*, Transfus Apher Sci, 2006, 34(1):125–130.

[8] Cotran RS, Kumar V, Collins T, *Robbins pathologic basis of disease*, 6th edition, W.B. Saunders Company, Philadelphia, 1999, 50–88.

[9] Cardo LJ, Wilder D, Salata J, *Neutrophil priming, caused by cell membranes and microvesicles in packed red blood cell units, is abrogated by leukocyte depletion at collection*, Transfus Apher Sci, 2008, 38(2):117–125.

[10] Deree J, Lall R, Melbostad H, Grant M, Hoyt DB, Coimbra R, *Neutrophil degranulation and the effects of phosphodiesterase inhibition*, J Surg Res, 2006, 133(1):22–28.

[11] Cardo LJ, Hmel P, Wilder D, *Stored packed red blood cells contain a procoagulant phospholipid reducible by leukodepletion filters and washing,* Transfus Apher Sci, 2008, 38(2):141–147.

[12] Marik PE, Sibbald WJ, *Effect of stored-blood transfusion on oxygen delivery in patients with sepsis*, JAMA, 1993, 269(23):3024–3029.

[13] Dalpiaz A, Spisani S, Biondi C, Fabbri E, Nalli M, Ferretti ME, *Studies on human neutrophil biological functions by means of formylpeptide receptor agonists and antagonists*, Curr Drug Targets Immune Endocr Metab Disord, 2003, 3(1):33–42.

[14] Dumaswala UJ, Wilson MJ, Wu YL, Wykle J, Zhuo L, Douglass LM, Daleke DL, *Glutathione loading prevents free radical injury in red blood cells after storage*, Free Radic Res, 2000, 33(5):517–529.

[15] Shaiegan M, Pourfatollah AA, Namiri M, Babaee GR. *Generation of IL-8 and TNF-alpha in platelet concentrates during storage*, Arch Iran Med, 2006, 9(1):61–64.

[16] Chin-Yee I, Keeney M, Krueger L, Dietz G, Moses G. *Supernatant from stored red cells activates neutrophils*, Transfus Med, 1998, 8(1):49–56.

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Received: January 5th, 2011 *Accepted: May 5th, 2011*