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Research Article

The influence of cold storage on the characteristics of platelet concentrate

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Abstract

Introduction: Platelet concentrates (PC) are among the most needed blood components used to correct thrombocytopenia of various origins. The main problems in transfusion therapy are the risks of infectious complications associated with the limited shelf life of platelet concentrates at storage. The article presents the results of a comparative analysis of the quality parameters of apheresis platelet concentrate prepared on two additional solutions: experimental additional solution (AS_{exp}) containing sodium fumarate and SSP^+ solution.

Objective: To perform a comparative analysis of quality parameters, metabolic rate, hemostatic ability, and markers of activation and apoptosis of platelets (15 units) prepared on an experimental additional solution containing sodium fumarate and on an additional SSP^+ solution for prolonged storage periods (15 days) at a temperature of 4 °C ± 2 °C.

Materials and methods: We have studied the parameters of the thrombography, metabolism, hemostatic potential, markers of activation, and apoptosis for prolonged periods (15 days) of storage at a temperature of 4 °C ± 2 °C.

Results: The results of the study indicate that during prolonged cold storage (15 days), there is a slight decrease in the number of platelets, a decrease in the level of metabolism, and significant morphofunctional changes occur both when using SSP^+ and AS_{exp} additional solutions. At the same time, hemostatic activity remains at a fairly high level throughout the entire storage period. The results of the analysis for most of the studied parameters did not reveal significant differences between the groups. At the same time, the parameters mandatory for quality control and safety met the standard values by the end of the observation period.

Conclusion: Our data indicate the possibility of using the developed additional solution based on sodium fumarate for storing platelets at a temperature of +4 °C ± 2 °C for 15 days.

Abbreviations

AS: Additional Solution; AS_{exp}: Experimental Additional Solution for platelet storage with the addition of sodium fumarate; PC: Platelet Concentrate; fL: Femtoliter; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PCT: Plateletcrit; PLT: Platelets; SSP^+: Commercial Additional Solution for Platelet Storage

Introduction

Platelet Concentrates (PC) are among the most needed blood components used to correct thrombocytopenia of various origins. The main requirement for platelet concentrate is the preservation of platelet activity when they enter the vascular bed during transfusion. The quality of PC is determined by a number of factors, among which of particular importance is the medium for their storage – plasma or additional solution. The blood plasma of a donor is the natural environment for platelets, but it is also the best environment for bacteria, and in addition, contains donor antibodies that can harm the recipient of PC.

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The use of additional solutions as a storage medium allows for minimizing the frequency of adverse reactions caused by transfusion of PCs and using platelets that are not identical in the blood group, with a lower titer of hemagglutinins. It also simplifies the identification of bacteria in contaminated PCs, enables photochemical treatment to inactivate bacteria and other pathogens in PC using certain techniques, improves storage conditions, and increases the yield of activated platelets. Reducing the amount of plasma transfused with platelets also saves the plasma for further processing.

Platelet concentrate prepared in an apparatus using platelet storage additional solution typically contains about 30% native plasma and about 70% additional solution.

The main problems in transfusion therapy are the risks of infectious complications associated with the limited shelf life of platelet concentrates at storage. These circumstances complicate the management of stocks of PCs and create serious logistical problems. The production departments of the blood service are faced with the urgent task of increasing the shelf life of platelet concentrates. To address this problem, alternative storage methods are currently being explored, such as cryopreservation or cold storage at 4 °C ± 2 °C. The storage environment largely determines the state of platelets. Preparing a PC in additional solution (AS) offers many advantages [1,2]. At the same time, the composition of AS affects the quality parameters of platelets during storage, including when using the cold regime [3].

The creation and use of additional solutions with components included in their composition that optimizes the storage conditions of PCs by reducing activation and maintaining a sufficient level of metabolism and hemostatic potential is considered one of the possibilities for increasing the storage time of PC [4,5].

The question of the expediency of using substances in the AS composition that allows compensation for the lack of energy sources, membrane stabilizers, metabolism regulators, and other things in the storage environment is widely discussed [6].

It is well known that the energy supply of cells is one of the main factors of their viability. At the same time, all methods for preparing PCs include the removal of platelets from the bloodstream and their resuspension in a medium that does not contain oxygen carriers. This leads to a decrease in oxygen supply to cells. Storage of PCs in gas-permeable containers, which is an indispensable requirement, only reduces but does not eliminate the lack of oxygen. The resulting hypoxia can cause disturbances in oxidative metabolism in cells, followed by the development of energy deficiency. Platelets, like many other human cells, can generate ATP in two ways – anaerobically and aerobically. The balance between cytosolic and mitochondrial oxidation reactions depends not only on the physiological activity of platelets but also on their storage conditions. According to the available data, sodium fumarate, one of the Krebs cycle substrates, can support ATP synthesis both under anaerobic and aerobic conditions, exerting a favorable influence on the mitochondrial metabolism of the liver and myocardial cells under conditions of hypoxia [7,8].

The composition of the additional solution based on sodium fumarate was developed and patented in our Institution, which allows for a significant extend the shelf life of PC.

Objective

To perform a comparative analysis of quality parameters, metabolic rate, hemostatic ability, and markers of activation and apoptosis of platelets prepared on an experimental additional solution containing sodium fumarate and on an additional SSP⁺ solution for prolonged storage periods (15 days) at a temperature of 4 °C ± 2 °C.

Materials and methods

We used platelet concentrates prepared by apheresis using the Trima Accel device (Terumo BCT Inc., USA) from blood donors who signed an informed consent to the procedure. The selection and examination of donors were carried out in accordance with the requirements of the current regulatory documents. After the end of the operation of the apparatus, the blood component for the study was divided into two equal parts of 100–110 mL each. An additional storage solution (AS_{exp} or SSP⁺) was added to the containers with PC. The addition of AS was performed at the following proportion: 30% autologous plasma and 70% AS.

We have evaluated quality parameters, metabolic and hemostatic activity, and expression of activation and apoptosis markers of 15 PC units during cold storage. Containers with PCs were stored in a refrigerator for blood storage at +4 °C ± 2 °C. A comprehensive laboratory study was carried out on the day of harvesting, and on the 5th, 10^{th,} and 15th day of storage.

Statistical analysis was performed in Microsoft Excel with the Real-Statistics add-in (by Charles Zaiontz) using nonparametric statistical methods. Differences were considered statistically significant when the error probability was no more than 0.05 ($p < 0.05$).

Results and discussion

The content of platelets in the peripheral blood of donors was not less than 200 x 10^9/L. During storage, the following parameters were evaluated: platelet count (PLT) x 10^9/L, thrombocrit (PCT) in %, Mean Platelet Volume (MPV) in fL, and platelet distribution width (PDW) in %. The baseline content of platelets in PC was 916 (853-991.5) x 10^9/L for storage in AS_{exp} and 1023 (902.5-1113.5) x 10^9/L for storage in SSP⁺ additional solution.

An increase in PLT was noted in both groups by the 5th day of observation. The reason for this fact may be increased aggregation or agglutination of platelets on the day of harvesting, caused by cold exposure and/or components of the storage medium, which requires further study. At the same time, the number of platelets in the SSP⁺ additional solution tended to decrease by the 10th day of storage and significantly decreased by the end of the experiment ($p < 0.05$). The PLT

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parameter in the AS_{exp} remained stable at all follow-up periods. The decrease in the number of platelets by the end of the storage period was 11% in SSP^+ and 4% in AS_{exp}.

Thrombocrit changes were similar. PCT values increased by the 5th day of observation in both groups and the increase was statistically significant in AS_{exp} ($p < 0.05$), and then tended to decrease. By the end of the observation period, the change in PCT was 0% for AS_{exp} and 10% for SSP^+.

The average platelet volume increased in both groups with an increase in the storage period, and by the 5th day of observation, it was significant ($p < 0.05$) in the SSP^+ group. At this time, intergroup differences were revealed between the compared additional solutions. By the end of the observation period, there was a slight increase in MPV in both groups, which is one of the signs of platelet aging [9]. At the same time, the revealed changes in the size of platelets were within the physiological reference limits.

The platelet distribution width (PDW) was determined based on the distribution histogram and quantitatively reflected the heterogeneity of the cell population in terms of size. In both groups, an increase in PDW was observed at all periods of storage, which may indicate the appearance of both larger platelets and small fragments of destroyed cells.

The content of glucose in the studied samples on the day of preparation did not differ significantly and was 6.4 (6.2 - 6.7) mmol/L for the AS_{exp} and 6.7 (6.2 - 6.7) mmol/L for the SSP^+ ($p > 0.05$). Subsequently, this parameter intensively decreased in both groups throughout the entire storage period ($p < 0.01$). Starting from the 5th day of observation, intergroup differences were noted, and the level of glucose in SSP^+ remained higher until the end of the observation. By the end of storage, the glucose concentration decreased to 1.05 (0.1 - 2.58) mmol/L ($p < 0.01$) in AS_{exp}, and to 2.25 (0.8 – 4.05) in SSP^+. During the experiment, glucose consumption compared to the baseline amount was 83% in AS_{exp} and 67% in SSP^+.

The baseline content of lactate in AS_{exp} was 1.1 (0.9 - 0.35) mmol/L and did not differ significantly from this parameter in SSP^+: 0.9 (0.8-1.25) mmol/L. Intergroup differences were revealed on the 5th day of storage ($p < 0.05$). Subsequently, there was a significant ($p < 0.01$) increase in lactate concentration in both groups: 4.3 (4.1 -4.7) → 7.05 (5.47 - 8.8) → 10.4 (7.63 - 12.63) mmol/L in AS_{exp} and 3.7 (3.5 - 4.2) → 6.55 (5.28 - 7.3) → 9.8 (7.95-11.53) mmol/L in SSP^+. At the same time, there were no intergroup differences on the 10th and 15th days of observation. The absence of significant intergroup differences in lactate levels by 10 and 15 days of storage may be due to the depletion of the sodium fumarate content in the storage medium and the weakening of mitochondrial oxidation processes in platelets.

The rapid formation of lactate in combination with a more active glucose consumption suggests the predominance of anaerobic oxidation processes in platelets prepared in an additional solution. By the end of the experiment, the concentration of lactate in AS_{exp} increased by 713% and in SSP^+ it increased by 781%.

Metabolic changes occurring at different periods of storage of PCs are most fully characterized by the dynamics of pH values. In both groups, in PC samples from the beginning of observation to the 5th day of storage, the pH remained relatively stable, which indicates the absence of a deficiency of buffer bases. However, later on, with increasing lactate concentration in the storage medium, pH significantly decreased by the end of the observation period being 7.34 ± 0.01 → 7.33 ± 0.01 → 7.24 ± 0.03 → 7.12 ± 0.04 ($p < 0.01$) for AS_{exp}, and 7.30 ± 0.01 → 7.33 ± 0.02 → 7.23 ± 0.01 → 7.15 ± 0.04 ($p < 0.05$) for SSP^+. It should be noted that at all periods of storage, the pH did not fall below the acceptable values (pH ≥ 6.4), which indicates sufficient compensatory capacities of the buffer systems of both additional solutions.

Different storage conditions are known to change platelet metabolism, which can affect the pH of the medium and can lead to non-compliance with predetermined quality specifications [10]. At the same time, the obtained data on residual concentrations of glucose (1.0 ± 0.4 mmol/L in AS_{exp} and 1.8 ± 0.4 mmol/L in SSP^+) and lactate (11.0 ± 0.9 mmol/L in AS_{exp} and 10.3 ± 0.8 mmol/L in SSP^+) confirm the preservation of metabolic activity. Glycolysis during storage leads to glucose consumption and subsequent accumulation of lactic acid, which lowers the pH in the absence of sufficient buffer capacity. The amount of bicarbonate present in the plasma is enough to efficiently buffer the medium containing platelets and maintain the pH during the standard shelf life of PC. In addition, acetate present in SSP^+ may be involved in metabolism via oxidative phosphorylation and provide additional buffer capacity through hydrogen ion uptake [8].

It is known that blood coagulation is a cascade of reactions in the blood plasma, which results in the formation of a network of fibrin fibers and the transfer of blood from a liquid state to a jelly-like state [11]. Key coagulation reactions are membrane-dependent, being accelerated by many orders of magnitude in the presence of negatively charged platelet phospholipid membranes, to which blood clotting proteins bind.

Thromboelastography was used to assess the ability of PC to restore the hemostatic potential of the blood. The coagulation potential of PCs was evaluated by the following parameters: maximum thromboelastogram amplitude (MA) in millimeters, clot formation time (R) in minutes, and clot strength (G) in degrees/second.

The MA value did not decrease significantly during the entire storage time. The strength of clot G, calculated based on the maximum amplitude, also did not differ significantly in both groups ($p > 0.05$) and remained stable throughout the experiment. A significant increase in the clot formation time R, apparently, is associated with the destruction of plasma proteins during long-term storage at a temperature of 4 °C ± 2 °C: 12.4 (9.8 - 13.8) → 17.3 (13.95 - 19.0) minutes for AS_{exp} and 13.2 (10.55 - 14.7) → 16.9 (16.0 - 19.2) minutes for SSP^+.

The data obtained indicate that, during prolonged cold storage, platelets prepared in an additional solution can restore their hemostatic potential. If the recipient retains the

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sufficient activity of plasma hemostasis factors, transfusion of PC stored in a cold regime compensates for the absence of platelet membranes necessary for the implementation of the coagulation cascade.

Platelets are activated during the procedures of harvesting, processing, and storage. Their activation is associated with significant morphofunctional changes. A distinctive feature of this process is the degranulation of the internal granular structures of platelets, followed by the translocation of molecules previously localized inside the cells to the platelet surface. An example is a P-selectin molecule (CD62P), which is stored in platelet alpha granules. Determination of membrane P-selectin or its soluble form, sP-selectin, in supernatants of PCs, is used to monitor platelet activation [12,13]. It is known that when PC was stored for 21 days at 4°C, the CD62 activation marker remained virtually unchanged in all units studied [14]. It was also found that the proportion of platelets expressing activation markers CD62P and CD63 increased during storage at 22°C, while the expression of these markers during cryopreservation was significantly lower [15].

When platelets are activated, phosphatidylserine fragments, physiologically localized on the inner cytoplasmic surface of the plasma membrane, are externalized. Such membranes are targets for annexin V, which is currently recognized as the most reliable and available marker of apoptosis.

Increased expression of phosphatidylserine is associated with cellular senescence and apoptosis, and these processes undoubtedly reflect the increasing changes in platelets during storage, which, apparently, are accelerated by activation that occurs during cell harvesting. The binding of annexin V indicates the effect of phosphatidylserine, which leads to the formation of platelets with an increased procoagulant tendency [16]. It has been shown that the use of additional solutions during cold storage reduces the proportion of annexin V-positive platelets compared with storage in 100% plasma [17].

Our results on the evaluation of the expression of phosphatidylserine (determination by the binding of annexin V) and P-selectin (CD62P) revealed the absence of significant differences ($p > 0.05$) of these parameters in the compared groups at all studied storage periods. At the same time, with the increase in storage duration in both groups, an increase in these parameters was observed. Thus, the proportion of platelets expressing annexin V in the SSP^+ storage group increased by 3.36-and 3.48-fold on the 5^{th} and 15^{th} days, respectively, while on the 10^{th} day there was a 1.2-fold decrease. Changes in this parameter while using AS_{exp} were similar, but less pronounced: 1.36-, 1.06-and 1.17-fold increase on the 5^{th}, 10^{th} and 15^{th} days of storage, respectively.

The study of CD62P expression also showed similar trends: an increase in the proportion of $CD62P^+$ platelets: 1.67-, 1.44- and 2.14-fold in the SSP^+ solution on the 5^{th}, $10^{th,}$ and 15^{th} days of storage, respectively, and 1.01-, 1.16- and 1.54-fold in the AS_{exp} storage group on the 5^{th}, 10^{th} and 15^{th} days of storage, respectively. The relative content of $CD62P^+$ platelets reached statistical significance compared with the baseline values on the 15^{th} day in both groups, amounting to 11.90% at baseline and 25.52% on day 15 ($p < 0.05$) in the SSP^+ group, and 17.07% at baseline and 26.33% on day 15 ($p < 0.05$) in the AS_{exp} group.

Thus, prolonged cold storage of platelets in AS_{exp} results in less pronounced changes in their characteristics compared to platelets stored in SSP^+ solution.

Conclusion

The results of our study indicate that during prolonged cold storage (15 days), there is a slight decrease in the number of platelets, a decrease in the level of metabolism, and significant morphofunctional changes occur both when using SSP^+ and AS_{exp} additional solutions. At the same time, hemostatic activity remains at a fairly high level throughout the entire storage period.

The results of the analysis for most of the studied parameters did not reveal significant differences between the groups. At the same time, the parameters mandatory for quality control and safety met the standards by the end of the observation period.

Our data indicate the possibility of using the developed additional solution based on sodium fumarate for storing platelets at a temperature of +4 °C ± 2 °C for 15 days. This study highlights the need for a deeper understanding of the effect of the additional solution on platelet quality during cold storage.

It is reasonable to conduct further research to optimize the timing, storage temperature, and choice of additional solution in order to improve the efficiency and safety of transfusion therapy using platelet concentrates.

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