Effect of storage time and additive solution on post-transfusion clearance and metabolic recovery

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1) Compare post-transfusion RBC clearance of fresh and stored RBCs and investigate whether glycolytic enzyme function, glycolysis, pentose phosphate pathway function and related metabolic pathways recover after transfusion of a 35 day stored RBC...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Other condition
Study type	Interventional

Summary

ID

NL-OMON47236

Source
ToetsingOnline

Brief title
Metabolic recovery of transfused RBCs

Condition

- Other condition

Synonym
blood transfusion, Erythrocyte transfusion

Health condition

Effectiviteit van rode bloedceltransfusie

Research involving
Human

Sponsors and support

Primary sponsor: Academisch Medisch Centrum
Source(s) of monetary or material Support: Sanquin Bloedvoorziening

Intervention

Keyword: Metabolomics, Red Blood Cell, Transfusion

Outcome measures

Primary outcome

1) Compare post-transfusion RBC clearance of fresh and stored RBCs and investigate whether glycolytic enzyme function, glycolysis, pentose phosphate pathway function and related metabolic pathways recover after transfusion of a 35 day stored RBC product in humans.

Secondary outcome

1) Glycolytic enzyme function, glycolysis, pentose phosphate pathway function and related metabolic pathways recovery after transfusion of stored RBCs compared to fresh RBCs.

2) Glycolytic enzyme function, glycolysis, pentose phosphate pathway function and related metabolic pathways function during storage of RBCs in PAGGGM or a similar alkaline additive solution compared to RBCs in SAGM.

3) Glycolytic enzyme function, glycolysis, pentose phosphate pathway function and related metabolic pathways recovery after transfusion of stored RBCs in PAGGGM or a similar alkaline additive solution compared to RBCs in SAGM.

Study description

Background summary

Red blood cell (RBCs) transfusions are frequently administered to patients that require increase in circulating blood oxygen delivery capacity to improve tissue oxygenation. In most countries shelf-life of RBCs has been maximized to 35 - 42 days as blood products undergo changes referred to as the *storage lesion* during aging. The *storage lesion* is characterized by RBC vesiculation, membrane loss and lysis, reduced glutathione, cellular levels of 2,3-diphosphoglycerate, adenosine triphosphate and nitric oxide, decreased membrane expression of CD47, and increased oxidation of cellular lipids and proteins. These processes induce storage dependent decrease in pH, increase of potassium and loss of hemoglobin. The *storage lesion* effects post-transfusion survival and function. Therefore, the United States Food and Drug Administration (FDA) requires submission of satisfactory in vitro biochemical and hematological characteristics and in vivo recovery data before approval of RBC systems. This includes tests of in vitro hemolysis, pH, glucose, lactate, white blood cell concentrations, RBC morphology and other parameters of RBC transfusion product quality. Manufacturers of RBCs are required to quantify 24-hour survival in healthy volunteers of their transfusion products. In at least 20 or more 24-hour recoveries on average at least 75% of RBCs have to survive to receive approval.

However, even though these products meet quality criteria in healthy volunteers, several studies have reported <75% survival of RBCs after 24 hours in patients. It is unclear which factors are implicated in clearance of these transfused RBCs. Age of RBCs may influence this process: as RBCs age they express enhanced levels of *eat me* signals, as for example phosphatidylserine (PS); in a murine model stored RBCs were found to adhere to the vasculature due to conformational changes of the RBC; red cell deformability of stored RBCs impedes microcirculation; red cell deformability diminishes in stored blood and a study investigating the relation between survival and storage time found reduced recovery with aged RBCs. This study reported a mean 24 hour post transfusion recovery of 86.4% in RBCs stored < 10 days. For RBCs stored 25-35 days the average survival after 24 hours was 73.5%.

Over the last decade an enormous impulse was given for research into the storage lesion of red cells. Observational studies showed an association between storage time of RBCs and mortality and morbidity in the recipients of these products. Recently, results of several large RCTs were published, which showed that standard stored RBCs (median 21 days) is not associated with higher mortality and morbidity compared to fresh stored RBCs (<8days). An important limitation of these studies is that previous studies showed an association between maximum stored RBCs and the onset of transfusion-related morbidity and mortality, but in the recent RCT studies the RBCs were stored for the standard storage time and not maximum storage time. Furthermore, it is known that the quality of RBCs products deteriorates during storage which is undesirable from both the blood bank and bedside perspective.

In the past decade, several alkaline, chloride-free storage solutions have been

developed, based on the original work of Meryman et al. but all with different compositions. In vitro there are some differences among the three solutions (AS-7, E-Sol and PAGGGM), with PAGGGM (Sanquin development) showing the lowest hemolysis after 35 days and the highest 2,3-DPG and ATP levels (results to be published). AS-7 (also known as SOL-X) is already approved for red cell storage in the US and both E-Sol and PAGGGM are available for research purposes. For both AS-7 and PAGGGM, metabolomics results are published, but so far, these findings are not confirmed with enzyme activity tests. Moreover, very little is known about the recovery of metabolism after transfusion of the stored red cells. Insight in the mechanism of the RBC storage lesion may result in development of improved storage conditions, which in turn results in improved product quality with decreased side-effects of transfusion, and/or the possibility to store products longer than the current standard.

RBC clearance and metabolic recovery after transfusion can be investigate with biotin labelling of RBCs. This method has proven to be safe in healthy volunteers study completed in the AMC. Biotin labelling facilitates infusion of several populations of RBCs by labelling them with separate concentrations of biotin.

With this study we will use a healthy volunteer transfusion model of biotin labelled RBCs to investigate the effect of 1) maximum storage time and 2) different RBC storage solutions on the clearance of transfused RBCs and metabolic recovery of transfused RBCs.

The advantages of such a model are the following;
1) an autologous transfusion model makes it possible to investigate the effect of storage time on the clearance of RBCs;
2) transfusion of both 2 days autologous stored RBCs and 35 days autologous stored RBCs in one volunteer enhances the power of the study as volunteers serve as their own control and eliminated inter-volunteer confounding;
3) biotin labelling makes detailed clearance studies possible and makes it possible to isolate transfused cells from the receiver*s circulation and subject them to additional investigations.

Study objective

1) Compare post-transfusion RBC clearance of fresh and stored RBCs and investigate whether glycolytic enzyme function, glycolysis, pentose phosphate pathway function and related metabolic pathways recover after transfusion of a 35 day stored RBC product in humans.
2) Investigate whether glycolysis (G6PD and other enzymes) and energy metabolism are better preserved during storage in PAGGGM or a similar alkaline additive solution compared to storage in SAGM.
3) Compare post-transfusion RBC clearance of RBCs stored in PAGGGM or a similar alkaline additive solution to RBCs stored in SAGM and investigate whether

post-transfusion RBC clearance, glycolytic enzymes function and metabolites of glycolysis, pentose phosphate pathway and related metabolic pathways recovery after transfusion of a 35 day stored RBC product in humans is improved in RBCs stored in PAGGGM or a similar alkaline additive solution compared to SAGM.

Study design

Study type: Open label randomized intervention trial.
Subjects: Healthy volunteers.

Methods:

Screening and RBC donation:
All subjects will be screened (medical history, physical examination, ECG, blood and urine examination) by the research physician of our hospital and must meet Sanquin Blood Bank Donor Criteria prior to involvement in the experiment (Screening Phase). Twenty healthy volunteers, aged 18-35, will be randomized to one of two groups and donate one unit of full blood 35 days before the study day at Sanquin Blood Bank. A second donation of 200 ml (miniature whole blood donation processed with a whole blood leukodepletion filter) will be completed 2 days before the study day. Before the study day volunteers screening and blood donation will result in collection of approximately 735 ml of whole blood in three sessions (screening, donation 35 days prior to the study day, dondation 2 days prior to the study day). This equals to 15% of circulating volume of 175 cm tall healthy males and is according to Sanquin Blood Bank standards. However, volunteers will receive this donated blood during the study day which results in a small netto loss of blood. Processing and storage of donated blood will be according to Sanquin Blood Bank (SBB) protocol in either SAGM or a new alkaline solution like PAGGGM. During storage blood products will be sampled weekly with a sterile coupler. These samples will be stored until further analysis.

Randomization groups:
Group 1a: Standard RBCs in SAGM - 2 days stored RBCs labelled with high density biotin, 35 days stored RBCs labelled with low density biotin
Group 1b: Standard RBCs in SAGM - 2 days stored RBCs labelled with low density biotin, 35 days stored RBCs labelled with high density biotin
Group 2a: RBCs stored in alkaline addative solution - 2 days stored RBCs labelled with high density biotin, 35 days stored RBCs labelled with low density biotin
Group 2b: RBCs stored in alkaline addative solution - 2 days stored RBCs labelled with low density biotin, 35 days stored RBCs labelled with high density biotin

Study day:
Prior to the study day autologous 2 days (*fresh*) and 35 days (*stored) RBCs, will be labelled with two different densities of biotin (Vitamin B8).25,26

Preparation will be done under sterile conditions. To exclude any effect of biotin label concentration half of each group will receive fresh RBCs labelled with a low biotin concentration and stored RBCs labelled with a high concentration. The other half will receive fresh RBCs labelled with a high concentration biotin and stored RBCs labelled with a low concentration of biotin. Cultures will be taken of labelled products to confirm sterile conditions. Detection of the biotinylated RBCs can then be performed in blood samples taken after transfusion, after staining with streptavidin-FITC by flow cytometry. Subsequently on the study day at the AMC healthy volunteers will first receive a indocyanine green infusion. This will be used to calculate the volunteers circulating volume according to previously published protocols and is required to detect clearance of transfused RBCs directly after infusion.29 After collection of samples for calculation of circulating volume, the volunteers will receive autologous transfusion of the full unit of fresh and stored biotin labelled RBCs (BioRBCs). Blood samples will be drawn from an indwelling venous canula prior to indocyanine green infusion, 5, 10 and 20 minutes after indocyanine green infusion, directly before transfusion and 10 minutes, 0.5, 1, 2, 4, 6, 8 and 24 hours and 2, 7, 30 and 90 days after transfusion. In the course of the study day, in total 135 ml blood will be drawn to obtain the required volume to calculate circulating volume and to detect and sort the two populations of transfused RBCs with flow cytometry. Metabolomics will be performed on the sorted populations and on samples of stored blood products, in combination with measurement of the activity of selected glycolytic enzymes, to investigate the effect of storage on enzyme-activity in the stored RBC and the effect of transfusion on the recovery of the stored cell.

Post study day follow-up
Twenty-four and forty-eight hours after transfusion volunteers will return to the AMC for an additional blood sample (12 ml). Seven days, 30 and 90 days after transfusion a venous blood sample (12 ml) will be collected to monitor RBC clearance and to detect development of biotin antibodies (AMC Visit 4 and 5). This data will be used to monitor antibody prevalence after exposure to biotin. Antibodies will be tested with a IgG gel card test (Ortho Clinical Diagnostics, MTS* Anti-IgG Card).

Intervention

Screening
All subjects, healthy volunteers aged 18-35 will be screened at the AMC and at Sanquin.
Investigation of the medical history, a physical examination, ECG and blood and urine examination will be performed to determine volunteer eligibility to participate in the study. Volunteers will be excluded if they have any abnormalities at the screening, if they use any medication on doctor*s prescription, if they have lost >500 ml blood in the past 3 months, regardless of the cause and if they participate in another randomized trial during the

course of our study. If the volunteers are found to have no exclusion criteria to participate in the study, they will be enrolled into the study.

Study
All included healthy volunteers (n=20) will donate 1 unit of whole blood at Sanquin Blood Bank which will be processed into 1 unit of RBCs (approximately 300ml) 35 days before the experiment. Processing and storage will be according to Sanquin Blood Bank protocol and products will be stored in either SAGM or an alkaline storage solution like PAGGGM, AS-7or E-Sol (to be selected). Every week, a sample will be collected from these products with a sterile coupler. Two days before the experiment healthy volunteers will donate a second smaller blood product (approximately 200 ml; miniature whole blood donation processed with a whole blood leukodepletion filter; validated Sanquin product) which will be processed into a small RBC concentrate. Both donations will be labelled with biotin, using two densities of biotin according to previously published protocols. To exclude any effect of biotin label concentration half of each group will receive fresh RBCs labelled with a low biotin concentration and stored RBCs labelled with a high concentration. The other half will receive fresh RBCs labelled with a high concentration biotin and stored RBCs labelled with a low concentration of biotin.

Before the study day volunteers screening and blood donation will result in collection of approximately 735 ml of whole blood in three sessions (screening, donation 35 days prior to the study day, dondation 2 days prior to the study day). This equals to 15% of circulating volume of 175 cm tall healthy males and is according to Sanquin Blood Bank standards. The collected RBCs will be used to produce an autologous transfusion product and volunteers will receive their own RBCs back at the study day. This results in a small netto loss of blood.

Two days after the second smaller donation subjects will be admitted to the medium care of the AMC where they first will receive indocyanine green to calculate circulating volume, followed by the autologous biotinylated RBCs stored for 2D and 35D. At the study day, blood samples will be drawn from an indwelling venous canula prior to indocyanine green infusion, 5, 10 and 20 min after infusion, prior to the transfusion and 10 minutes, 0.5, 1, 2, 4, 6 and 8 hours after transfusions. Volunteers will return to the AMC 24 hours after transfusion and 2, 7, 30 and 90 days later for follow-up samples. These samples will be used to detect and sort the two populations of transfused RBCs with flow cytometry.

Indocyanine green will be used to calculate circulating volume. This volume is required to be able to detect any immediate clearance of transfused BioRBCs direct after transfusion. This clearance cannot be detected without an estimation of circulating volume on forehand.29 The counts of BioRBCs in the circulation will be used to measure post-transfusion clearance. In cooperation with a laboratory in Denver Colorado, USA, with staff trained in metabolomics, the energy metabolism of all sorted BioRBC populations and samples of stored

blood products will be investigated. These analyses will be combined with direct enzymatic measurements (spectrophotometry and cytofluorometry) of selected enzymes in these samples, for example glucose-6-phosphate dehydrogenase (G6PD). Thus we will be able to investigate the effect of storage on enzyme-activity in the stored RBC and the effect of transfusion on the metabolic recovery of the stored cell.

During the experiment subjects will be monitored for heart rate and blood pressure by non-invasive continuous monitoring at the ICU.

One, two, seven, 30 and 90 days after transfusion a venous blood sample (12 ml) will be collected to monitor RBC clearance and to detect development of biotin antibodies. This data will be used to monitor antibody prevalence after exposure to biotin. Antibodies will be tested with a IgG gel card test (Ortho Clinical Diagnostics, MTS* Anti-IgG Card).

Study burden and risks

Benefits: none.
We recently showed that transfusion of one unit autologous aged biotin labelled Red Blood Cells (RBCs) (METC protocol 2012-299) is safe in healthy volunteers suffering endotoxemia. Transfusions will be prepared and transfused using the standard clinical protocols by Sanquin and our hospital. Our study requires donation of a standard full blood donation. We therefore feel that this study has a lower volunteer burden and volunteer risk than our previous study.

Prior to transfusion stored RBCs will be biotinylated to allow their identification with flow cytometry. In vivo and in vitro testing of biotinylated RBCs and PLTs showed that survival and recovery of RBCs was not affected by biotinylation. Although in a healthy volunteer study on biotinylated RBCs 1 out of 8 subjects developed a transient positive test for antibody to biotin, at 11 months post transfusion antibodies to biotin had disappeared. The survival of RBCs was not altered noticeably in this subject by the presence of this antibody; this is in line with previous reports. Three months after the study day a last blood sample will be drawn to detect development of biotin antibodies. Although the presence of absence of antibodies had no clinical relevance and repeated intravenous exposure to biotin did not produce adverse effects in previous studies, this data can be used to monitor antibody prevalence after exposure to biotin. In our recently completed study in healthy volunteers who received biotin-labelled RBCs, none of the volunteers developed biotin antibodies (unpublished data).

Volunteers will donate approximately 735 ml of whole blood in three sessions (screening, donation 35 days prior to the study day, dondation 2 days prior to the study day) before the study day. This equals to 15% of circulating volume of 175 cm tall healthy males which should not result in any adverse effects and is according to Sanquin Blood Bank standards. However, as these donations are

not standard care at Sanquin, the Sanquin Medical Ethical Board is also required to approve the study protocol. During the study day volunteers receive 1.5 autologous transfusion which negates any effect of whole blood collection before the study day.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

1) Healthy volunteer
2) Age * 18 years <35 years

Exclusion criteria

1) No informed consent
2) Any abnormal test result during the screening prior to inclusion of the study (medical history, physical examination, ECG, blood and urine examination)
3) History of drugs or alcohol abuse
4) Any present medication use on prescription
5) Smoking < 6 months
6) Blood loss of more 500 ml < 3 months, including blood donation
7) Previously transfused
8) Participation in any other intervention study during the course of this study
9) Allergy or hypersensitivity for iodine
10) Active thyroid disease

Study design

Design

Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Double blinded (masking used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	17-08-2017
Enrollment:	20
Type:	Actual

Ethics review

Approved WMO

Date:	31-01-2017

Application type:	First submission
Review commission:	METC Amsterdam UMC
Approved WMO Date:	04-07-2018
Application type:	Amendment
Review commission:	METC Amsterdam UMC

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

ID: 24366
Source: NTR
Title:

In other registers

Register	ID
CCMO	NL59816.018.16
OMON	NL-OMON24366