Development of a *two hit* in vivo autologous transfusion model in healthy volunteers

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to develop a *two hit* in vivo autologous RBCs transfusion model in healthy male volunteers.

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

Summary

ID

NL-OMON39642

Source
ToetsingOnline

Brief title
Development of a Transfusion Model in Healthy Volunteers

Condition

- Other condition

Synonym
acute hypoxia after blood transfusion

Health condition

transfusie gerelateerde longschade

Research involving
Human

Sponsors and support

Primary sponsor: Academisch Medisch Centrum
Source(s) of monetary or material Support: Beursaanvraag ZonMW

Intervention

Keyword: healthy volunteers, storage, TRALI, transfusion

Outcome measures

Primary outcome

A significant increase (p<0.05, ANOVA multiple comparison test) in total protein leakage in the BAL-fluid of the subjects receiving a *first hit* of LPS and one unit of RBCs stored for 35 days compared to controls.

Secondary outcome

1. A significant increase (p<0.05, ANOVA multiple comparison test) in inflammation and coagulation markers in the BAL-fluid and plasma samples of the subjects receiving a *first hit* of LPS and one unit of RBCs stored for 35 days compared to controls.

2. Identification of potential bio-markers for diagnosing TRALI

3. Identification of phenotypic changes of the transfused red blood cells, their interaction with other cell types in the receiver*s circulation and their potential contribution to the development of lung injury.

Study description

Background summary

Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related morbidity and mortality. The incidence is high and reported up to 8% in specific patient groups. TRALI is thought to be a *two hit* event.

The first event is the underlying condition, often sepsis or an infection, of the patient resulting in priming of neutrophils. The second event is the transfusion of a blood product, after which either antibodies present in the blood product or pro-inflammatory mediators present in stored cell-containing blood products (e.g. Red Blood Cells (RBCs)) or aged erythrocytes and/or platelets themselves activate the primed neutrophils, resulting in pulmonary edema. Opposed to the traditional view that TRALI has a good prognosis, evidence is accumulating that TRALI has a significant impact on morbidity and outcome, at least in specific patient groups such as critically ill patients.
The association of transfusion with adverse outcome resulted in blood product and donor management strategies aimed at decreasing the risk of acquiring TRALI. Plasma products originating from female donors and specifically multiparous donors have up to 40% presence of antibodies directed against leukocyte antigens and are associated with the onset of TRALI. From this point of view the Netherlands and the UK have started from 2006 and 2003 respectively a male only plasma donation policy. Excluding female donors for plasma donation seems to have reduced, but not prevented the occurrence of TRALI. Additional research is needed to determine whether the use of fresh (at present RBCs are stored up to 35 days in The Netherlands) cell-containing blood products may be an additional measure to reduce TRALI.
We recently demonstrated in an in vivo animal model that the supernatant of stored RBCs induce mild lung injury in the presence of a *first hit* of lipopolysaccharide (LPS). Other pre-clinical studies showed that transfused aged erythrocytes themselves induce mild lung injury by loss of the Duffy antigen and by induction of tissue inflammation by acute tissue iron deposition. However, clinical studies on the impact of transfusion of aged red blood cells on respiratory complications have yielded conflicting results. In cardiothoracic surgery patients, respiratory insufficiency and mortality was lower in patients that had received blood stored for less then 14 days compared with patients that had received blood stored for more then 14 days (7.4% vs. 11.0%, $P<0.001$). However, similar studies did not confirm these findings.
The discrepancy between results from pre-clinical and clinical studies on the effect of storage time of cell-containing blood products and the onset of mild lung injury calls for a randomized trial. Although recent studies showed a relative high incidence of TRALI, still the numbers are low and the presence or absence of a *first hit* is hard to measure in the clinical setting which in total makes it difficult to perform a clinical randomized trial. For this reason we propose to develop a *two hit* in vivo autologous RBCs transfusion model, i.e. a mild TRALI model in healthy volunteers. When the model is developed it has to confirm the hypothesis that stored RBCs products induce mild lung injury in the presence of a *first hit* in the human setting (i.e. a mild form of TRALI). 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 onset of lung injury as the effect of anti-body mediated TRALI is excluded; 2) the *first hit* is standardized; 3) this model will help us to investigate pathways involved in onset of stored blood induced lung injury and may enable us to test preventive or therapeutic measurements aimed

at improving storage conditions. The latter aspect of the model will become very important in the near future and results from these studies may prevent impeding a continuous reliable blood supply when a policy of fresh blood only is proposed.

We propose the development of a *two hit* in vivo autologous RBCs transfusion model, i.e. a mild TRALI model in healthy volunteers to confirm that stored RBCs are associated with the onset of mild lung injury in the presence of a *first hit* of LPS in the human situation.

Study objective

to develop a *two hit* in vivo autologous RBCs transfusion model in healthy male volunteers.

Study design

Subjects:
Healthy male volunteers.

Study groups:
Group 1 (n=6): *First hit* Lipopolysaccharide (LPS) 2ng/kg + *Second hit* Saline
Group 2 (n=6): *First hit* LPS + *Second hit* Fresh Red Blood Cells (RBCs) (2 day storage)
Group 3 (n=6): *First hit* LPS + *Second hit* Stored RBCs (35 day storage)

Methods:
All subjects will be screened (medical history, physical examination, ECG, blood examination, spirometry, DLCO, chest x-ray) by the research physician of our hospital and of Sanquin Blood Bank prior to involvement in the experiment. All included healthy volunteers (n=18) will donate 1 unit of whole blood at Sanquin Blood Bank which will be processed into 1 unit of RBCs (approximately 300ml). Processing and storage will be according to Sanquin Blood Bank protocol. Prior to transfusion stored RBCs will be biotinylated (Vitamin B8) to allow their identification with flow cytometry. In short stored RBCs will be labelled with Sulfo-NHS biotine of Pierce (6-20µg/ml).
Subsequently on the study day healthy volunteers receive a *first hit* of either E. coli lipopolysaccharide (LPS) 2 ng/kg i.v. (n=18). Two hours after the *first hit* they receive an autologous transfusion of 1 unit of fresh (2 day storage) biotinylated RBCs or an autologous transfusion of 1 unit aged biotinylated (35 days of storage) RBCs or an equivalent volume of saline 0.9% infusion. The transfusion itself will be performed in one hour. During the experiment subjects will be monitored for blood pressure and arterial oxygenation using an indwelling arterial line. Blood samples will be drawn from an indwelling artery line prior to the *first hit*, prior to the transfusion,

directly after transfusion, 0,5, 1, 2, 4 and 6 hours after transfusion. At the same time points exhaled air will be collected for E-Nose measurement. Furthermore, 6 hours after transfusion spirometry and DLCO measurement will be repeated. A chest x-ray and a directed broncho-alveolar lavage (BAL) will be performed 6 hours after transfusion. The BAL will be performed by an experienced pulmonologist according to the Dutch pulmonologist guidelines (NVALT Guidelines, 2004). In the BAL-fluid and plasma samples markers of inflammation, neutrophil activation and coagulation activation are measured to confirm whether we have developed a model of TRALI. Three months after the study day a venous sample of 4 ml will be collected to measure prevalence of biotin antibodies.

Intervention

Group 1: *First hit* Lipopolysaccharide (LPS) 2ng/kg + *Second hit* Saline
Group 2: *First hit* LPS + *Second hit* Fresh Red Blood Cells (RBCs) (2 day storage)
Group 3: *First hit* LPS + *Second hit* Stored RBCs (35 day storage)

Study burden and risks

Nature and extent of the burden and risks associated with participation.

De blood donation:
1. The donation of the blood transfusion will take place at the Sanquin Blood Bank. The blood donation can be accompanied by some pain and the chance of a bruise. The blood transfusions are processed by Sanquin according to their standard protocol for clinical blood products.

The blood products:
1. Blood transfusions originate from the test person himself and will not bare risk of for example virus transmission. The possibility exists that the transfusion will induce mild transient lung injury. The test person might perceive this as as mild stuffiness.
2. During storage of the blood product samples from the blood product will be taken to measure the storage related changes in the blood product. The sampling finds place under sterile conditions.
3. Prior to transfusion stored RBCs will be biotinylated (Vitamin B8) to allow their identification according to previously published protocols.6,28 In short stored RBCs will be labelled with Sulfo-NHS biotine of Pierce (6-20µg/ml). Preparation will be done under sterile conditions. Cultures will be taken of transfused products to confirm sterile conditions. Three months after the study day a venous blood sample will be collected to detect development of biotine antibodies. This data will be used to investigate antibody prevalence after exposure to biotin. The presence of absence of antibodies has no clinical relevance and repeated intravenous exposure to biotin does not produce adverse

effects.5,7

The experiment:
1. The test person will undergo two times a chest X-ray. The radiation exposure is considered to be minor.

2. During the experiment an artery line will be inserted in one of arteries of the arms of the test person. The placing of the artery line is performed by an experienced anesthesiologist. There is a small chance this procedure results in bruises or blood clot formation in the blood vessel. During the last years no major complications occurred with this procedure in our center.

3. The administration of endotoxin can lead to mild flu-like symptoms, which can include a slight increase in body temperature, muscle pain and/or fatigue.

4. The bronchial lavage (lung rinse) is performed by an experienced pulmonologist. The main source of discomfort during this procedure is a dry cough or mouth. However, these complaints are suppressed by the lidocaine spray. In addition, there may be onset of fever within 24 hours.

5. Blood sampling during the experiment will be from the artery line and will not result in additional punctures. The total amount of blood that is sampled during the experiment is 135 ml. The 135 ml sampling is next to the 500 ml of blood donation. However, depending on the experimental group the test person is in, the majority of the 500ml donation will be returned during the experiment by the blood transfusion. The human body can easily handle these changes in blood volume. It is not allowed to donate blood or participate in another study within the three months prior to this study or during this study.

6. Participation in this study may result in exclusion of future blood donation at Sanquin since the test person after this study is registered as a person who has received a blood transfusion.

7. The pulmonary function measurement and the collection of exhaled air is estimated as no burden or risk for the healthy volunteers.

Risks assessment:
1. Infusion of E. coli LPS with a dose of 4 ng/kg has previously been proven to be safe in healthy adult volunteers in our institution. We will use a lower dose of 2 ng/kg which will induce neutrophil priming but will not result in a SIRS reaction. 2. The use of an autologous transfusion human volunteer model has also been proven to be safe. 3. Furthermore transfusions will be prepared and transfused using the standard clinical protocols by Sanquin and our hospital. The combination of these two models is expected only to cause mild temporary side effects because of the following reasons: 1. Pre-clinical studies show only mild lung injury after a *first hit* of LPS 2mg/kg and a *second hit* of transfusion of stored blood products. 2. In the present model a

1000 fold lower dose of LPS (2ng/kg) will be used compared to the pre-clinical model which we assume will make the model less severe.
2. Studies in healthy human volunteers showed that biotinylated (Vitamin B8) RBCs can be safely administered without any side-effects. Although in a healthy volunteer study 1 out of 8 subjects developed a transient positive test for antibody to biotinylated RBCs, at 11 months post transfusion antibodies to biotinylated RBCs had disappeared. Biotine labeling has no effect on RBCs survival. Thus, biotinylation of RBCs is considered safe.

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 male 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, spirometry, chest x-ray).
3. History of drugs abuse
4. Any present medication use on prescription
5. Smoking < 6 months
6. History of blood donation < 3 months
7. Previously transfused
8. Participation in any other medical study < 3 months
9. Participated in previous volunteer studies using LPS

Study design

Design

Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Open (masking not used)
Control:	Placebo
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	13-02-2014
Enrollment:	18
Type:	Actual

Ethics review

Approved WMO

Date:	07-01-2013
Application type:	First submission

Review commission:	METC Amsterdam UMC
Approved WMO Date:	12-03-2014
Application type:	Amendment
Review commission:	METC Amsterdam UMC
Approved WMO Date:	15-07-2014
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

No registrations found.

In other registers

Register	ID
CCMO	NL42360.018.12