Blood outgrowth endothelial cell - mediated coagulation

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The aim of this research is to improve the understanding of the coagulation system through a detailed characterization and quantification of biosynthesis and expression of several coagulation proteins in BOECs. The data obtained in the study will be...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Coagulopathies and bleeding diatheses (excl thrombocytopenic)
Study type	Observational non invasive

Summary

ID

NL-OMON35931

Source
ToetsingOnline

Brief title
BOEC-COAG

Condition

- Coagulopathies and bleeding diatheses (excl thrombocytopenic)
- Blood and lymphatic system disorders congenital
- Embolism and thrombosis

Synonym
Thrombosis; Blood clot in vein

Research involving
Human

Sponsors and support

Primary sponsor: Leids Universitair Medisch Centrum
Source(s) of monetary or material Support: Technologiestichting STW

Intervention

Keyword: Blood coagulation, Blood outgrowth endothelial cells, Protein C pathway, Thrombosis

Outcome measures

Primary outcome

The study will result in fundamental biochemical findings that have no direct

clinical significance. Given the nature of the experiments we do not anticipate

to make discoveries that directly relate to the volunteers.

Secondary outcome

Not applicable.

Study description

Background summary

Summary

The risk of developing a bleeding or thrombosis is still difficult to determine with the current understanding of the coagulation system. The aim of the current fundamental research proposal is to improve the understanding the coagulation system through a detailed characterization of endothelial progenitor cells, the so called "blood outgrowth endothelial cells (BOECs), which circulate in small quantities in the blood [1]. Improved information about the specific role of endothelium-mediated mechanisms in the coagulation system will help to identify new parameters that will allow for a better and more accurate assessment of individual risk tendencies of developing a bleeding diathesis or thrombosis.

The endothelial cell surface plays an important role in blood clotting. However, in regular clotting assays the contribution of the endothelium is excluded given that its function cannot be directly assessed in blood. In the Einthoven Laboratory for Experimental Vascular Medicine, we have the expertise to isolate and culture BOECs according to established procedures, resulting in fully functional endothelial cells [2]. In this study, BOECs will be isolated from the blood of healthy volunteers. In the subsequently generated endothelial cell lines, we will characterize and quantify the biosynthesis and expression

of several endothelial proteins that play an important role in blood coagulation. The results of this study will be incorporated into a mathematical model of the coagulation system, with the aim of using this improved model to indentify parameters that allow for assessing the risk of developing a bleeding or thrombotic complication.

Background

Estimated risk of bleeding/thrombosis is complicated to assess

The coagulation system comprises a complex set of responses that protect the body against bleeding and thrombosis. The latter is examplified by venous thrombosis, which is the most frequent encountered complication during hospitalization, and can be prevented provided the correct treatment protocol is used [3]. Unfortunately, there are no clotting tests available that are able to determine which patients are at increased risk of developing thrombosis. Venous thrombosis is treated with so-called anticoagulants, of which the effectiveness is tested using the International Normalized Ratio (INR). Although this method usually prevents bleeding due to a high dosage of anticoagulants [4], medication-related bleeding can still occur, even if the measurements stay within the normal INR range. For example, one study demonstrated that a severe (organ) bleeding occurred annually in 3% of patients treated with oral anticoagulants regulated by the Dutch anticoagulation clinic [5]. Clearly, there is a high need for new assays that determine the bleeding and thrombosis risk in each individual. An improved mathematical model of the coagulation system could serve as a tool to identify the parameters that can be assessed in these assays.

Role of endothelial cells in blood

Since endothelial cells line the vessel wall and contain many membrane-bound proteins, the contribution of these cells to coagulation is not directly measurable in blood and/or plasma. However, these cells and their components play an important role in the coagulation process and their actions should therefore be taken into account in order to make reliable predictions about coagulation. Endothelial cells are involved in coagulation through various mechanisms, such as through the production of von Willebrand factor (VWF). When the endothelium is activated, VWF is released into the bloodstream, where it contributes to the adhesion and aggregation of platelets. VWF also functions as a carrier protein for coagulation factor VIII; both of these functions are important for the procoagulant effect of VWF [6]. Other endothelial-specific coagulation proteins take part in the protein C system [7]. Activation of this system occurs upon the interaction of thrombin with thrombomodulin (TM), a membrane protein found on endothelial cells. The so formed complex converts protein C to activated protein C (APC) that, together with its cofactor protein S, inactivates the clotting factors Va and VIIIa, thereby downregulating coagulation. APC can also bind to the endothelial protein C receptor (EPCR), another membrane protein that is anchored to the endothelium. This complex is

capable of activating protease activated receptor 1 (PAR-1), an endothelial transmembrane protein, thereby initiating a cytoprotective effect on the endothelium [8]. Recently, it was discovered that the enzyme protein disulfide isomerase (PDI) is released when the endothelium adopts a prothrombotic phenotype. PDI converts encrypted tissue factor to activated tissue factor and as such contributes to blood coagulation [9].

The above mentioned mechanisms indicate that the endothelium plays an important role in the clotting cascade through various mechanisms. However, a detailed characterization of their direct contribution to clotting is lacking thus far.
This study aims to characterize these endothelial-mediated mechanisms and their relationship to the coagulation system by characterizing and quantifying the biosynthesis and expression of various proteins in BOECs. This will be performed under normal conditions and under conditions that may cause thrombosis in vivo (such as low oxygen, low nutrients, low pH, and in the presence of inflammatory mediators). The knowledge gained will provide unique insight into the contribution of endothelial cell-specific components to normal coagulation and thrombosis.

Mathematical model
All information obtained in this study will be imported in a new mathematical model of coagulation. By including the endothelial cell-mediated mechanisms into this model we anticipate to be able to better describe and predict the coagulation response. This simulation model will provide the basis for developing an optimal combination of coagulation assays to determine the hemostatic balance and the risk of developing a bleeding diathesis or thrombosis in individual patients.

Referenties (1)
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clinical outcome. A retrospective study of 2300 consecutive patients with venous thromboembolism. Br J Haematol. 2005 Feb;128(4):513-9
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Study objective

The aim of this research is to improve the understanding of the coagulation system through a detailed characterization and quantification of biosynthesis and expression of several coagulation proteins in BOECs. The data obtained in the study will be included in a mathematical model describing the entire coagulation system, with the ultimate goal of identifying parameters that predict the risk of developing a bleeding or thrombotic tendency in individual patients.

The specific research objectives are:
1. The quantitative and qualitative determination of the transcription and expression of endothelial specific blood components, including VWF, TM, EPCR, PAR-1, PDI, under various conditions:
a. under normal physiological conditions,
b. under conditions that in vivo may cause thrombosis (such as low oxygen, low nutrients, low pH and in the presence of inflammatory mediators).
2. To determine the immediate effect of endothelial factors on blood coagulation by including BOECs in existing clotting assays such the thrombin generation assay [10.11].

Referenties (2)
10. Hemker HC, Al Dieri R, De Smedt E, Béguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. Thromb Haemost. 2006 Nov;96(5):553-61
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Study design

Selecting subjects
For this study blood, will be collected from 50-100 healthy adults. Prospective subjects will be informed about this study by advertisement. The study will result in fundamental biochemical findings that have no direct clinical significance. Given the nature of the experiments we do not anticipate to make discoveries that directly relate to the study subjects. Therefore, no feedback on the data obtained will be communicated to the volunteers. Since the isolation of BOECs from blood is not always successful, the subjects will be asked whether they agree upon blood collection at later time points as well (up to three times total).

Implementation and blood collection
The volunteers will be informed about the research through advertisements. If interested in participating, the subjects will be informed about the study both in writing and by phone. Subsequently, they will be requested to come to the LUMC. After giving a written consent to participate in the study, 50-100 ml citrated blood will be sampled.

Coding of the blood samples
The personal data of the subjects will only be available to the responsible researcher. The blood samples will be encrypted, and the code to link the samples to the subjects is only available to the responible researcher, not to other employees.

Processing of blood samples
The BOECs will be isolated and cultured in the Einthoven Laboratory for Experimental Vascular Medicine. Subsequently, the generated endothelial cell lines will be stored in liquid nitrogen for future experiments. The cells will be preserved for ten years. Furthermore, DNA will be isolated from the cells to confirm the identity of some endothelial components. The mRNA levels of some proteins will be determined, as well as their expression levels. The BOECs will be further characterized under normal and prothrombotic conditions and included in existing coagulation assays.

Ethical aspects

Burden for the subjects
The subjects have no direct interest in the research. The study will provide more insight into the role of endothelial cells in blood, as this information will be used to create a new mathematical model of coagulation. The burden on the subjects is minimal, basically just a single blood sample, although in some cases a second or third blood sample might be requested. Subjects receive a reimbursement for travel costs and a ¤ 25 gift certificate as thanks for participating. The risk for the participants is negligible because it involves a single blood withdrawal only.

Informed consent
Participants in the research will be informed about the study in advance, both verbally and in writing. Subjects will be enrolled after they have given their permission. All participants can contact Dr. W.M. Lijfering, Tel: 071 526 5639, to obtain further information. For independent advice, participants can contact Dr. F.J.M. van der Meer, Tel: 071 526 3901.

Privacy
The blood samples will be coded as previously described and only the responsible researcher, Dr. M.H.A. Bos, has access to the key linking the code to personal data. The subject will not receive feedback of the research findings.

Study burden and risks

The burden on the subjects is minimal: basically just a single blood sample, although in some cases a second or third blood sample might be requested. The risk for the participants is negligible because it involves blood sampling only. A bruise may occur due to blood withdrawal, which can be prevented by firmly pressuring the puncture.

Contacts

Public
Leids Universitair Medisch Centrum

Albinusdreef 2
2333 ZA
NL
Scientific
Leids Universitair Medisch Centrum

Albinusdreef 2
2333 ZA
NL

Trial sites

Listed location countries

Netherlands

Eligibility criteria

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

Inclusion criteria

Healthy volunteers of 18 years and older.

Exclusion criteria

Being non-healthy.

Study design

Design

Study type: Observational non invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Other

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	05-07-2011
Enrollment:	100
Type:	Actual

Ethics review

Approved WMO

Date:	16-06-2011
Application type:	First submission

Review commission: METC Leids Universitair Medisch Centrum (Leiden)

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	NL36368.058.11