Blood Outgrowth Endothelial Cells (BOECs) and Megakaryocytes (MKs) for in vitro studies of hemostatic and secretory function

Gepubliceerd: 24-09-2020 Laatst bijgewerkt: 18-08-2022

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Ethische beoordeling	Positief advies
Status	Werving gestart
Type aandoening	-
Onderzoekstype	Interventie onderzoek

Samenvatting

ID

NL-OMON24571

Bron
Nationaal Trial Register

Verkorte titel
2020-BOEC-MK

Aandoening

Bleeding disorders

Ondersteuning

Primaire sponsor: Erasmus MC
Overige ondersteuning: NWA.1160.18.038 & LSBR bk-18729 - 1707

Onderzoeksproduct en/of interventie

Uitkomstmaten

Primaire uitkomstmaten

1. To establish optimized approaches for the isolation and characterization of BOECs, CD34+-derived MKs, iPSC- ECs and iPSC-MKs.
2. Dissect the cellular mechanisms that control endothelial and platelet (secretory) function, thereby understanding the pathophysiology of bleeding disorders and the genetic control of VWF secretion using BOECs, platelets, CD34+-derived MKs, iPSC- ECs and iPSC-MKs.
3. To develop in vitro methods to introduce or correct (disease-causing) mutations in regulators
of endothelial or platelet function in BOECs or iPSC-derived ECs/MK using CRISPR-mediated genome engineering.

Toelichting onderzoek

Achtergrond van het onderzoek

Rationale:
Hemostasis is critically dependent on Von Willebrand factor (VWF), a multimeric adhesive plasma protein that is crucial for mediating platelet adhesion to sites of vascular damage and that acts as a chaperone for coagulation factor VIII (FVIII) in plasma. The bulk of plasma VWF is synthesized by endothelial cells (ECs) where it is stored in and released from Weibel-Palade bodies (WPBs). Platelets also store and secrete VWF from alpha-granules after activation, which contributes to thrombus formation. The molecular mechanisms that control secretion of VWF from ECs and platelets are poorly understood. Abnormalities in the (secretory) function of platelets and endothelial cells can lead to bleeding, such as observed in platelet storage pool disorders (SPD), Von Willebrand disease (VWD) or in individuals with “low VWF”.

Problem definition: In ~30% of type 1 VWD patients and individuals with low VWF (defined as VWF levels <50 IU/dL) no VWF mutations are found. Another problem is that in around 50%
of bleeding patients referred to tertiary centers the underlying mechanisms that cause clinically
relevant bleeding problems cannot yet be identified, so-called bleeding of unknown cause (BUC). Desmopressin is administered therapeutically to improve platelet function and raise VWF in plasma by inducing WPB exocytosis from endothelial cells. In ~25% of patients desmopressin fails to trigger (sufficient) release of VWF/FVIII for reasons largely unknown.

Research hypothesis: The main hypothesis of this study is that defects in components of the secretory machinery of VWF are causative for reduced VWF levels and lack of desmopressin response. Also, defects in this machinery may be causative in patients with BUC. To identify new determinants of VWF levels and vascular health we will study secretory mechanisms in

ECs and platelets of individuals with abnormalities in hemostatic and/or secretory function. For
this we will establish ex vivo (patient-derived) cellular model systems of endothelial and platelet
secretion using blood outgrowth endothelial cells (BOECs), platelets, CD34+-derived megakaryocytes and induced pluripotent stem cell (iPSC)-derived endothelial cells (iPSC-ECs) and megakaryocytes (iPSC-MKs).

Doel van het onderzoek

The main hypothesis of this study is that defects in components of the
secretory machinery of VWF are causative for reduced VWF levels and lack of desmopressin response. Also, defects in this machinery may be causative in patients with BUC. To identify new determinants of VWF levels and vascular health we will study secretory mechanisms in ECs and platelets of individuals with abnormalities in hemostatic and/or secretory function. For
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secretion using blood outgrowth endothelial cells (BOECs), platelets, CD34+-derived megakaryocytes and induced pluripotent stem cell (iPSC)-derived endothelial cells (iPSC-ECs) and megakaryocytes (iPSC-MKs).

Onderzoeksopzet

A maximum of seventy milliliters of blood will be drawn for the isolation of PBMCs, platelets, plasma and DNA. Several parameters will be measured in plasma to characterize the hemostatic and angiogenic profile. BOECs, iPSC-ECs/MKs and CD34+-derived primary MKs will be characterized in vitro. Protein expression profiles will be determined by whole proteome mass spectrometry. DNA will be genotyped for SNPs and mutations in VWF and VWF associated genes.

Onderzoeksproduct en/of interventie

The intervention in this study is venous blood sampling. A maximum of seventy milliliters of blood will be drawn for the isolation of PBMCs,
platelets, plasma and DNA.

Contactpersonen

Publiek

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Wetenschappelijk

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Deelname eisen

Belangrijkste voorwaarden om deel te mogen nemen (Inclusiecriteria)

Patients
□□ Prior diagnosis of VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.
□□ For patients ≥16 years old; written informed consent
□□ For patients 12-15 years old; written informed consent from both the patient and their parent(s)/legal guardian(s)
□□ For patients <12 years old; written informed consent from their parent(s)/legal guardian(s)

Family members
□□ Age 18 years or older
□□ Family member of a patient with VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.
□□ Family members may or may not be affected.

Healthy controls
□□ Age 18 years or older
□□ Not previously diagnosed with VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.

Belangrijkste redenen om niet deel te kunnen nemen (Exclusiecriteria)

A potential subject who meets any of the following criteria will be excluded from participation in this study:
□□ Unable to give written informed consent.

□□ Use of medication that can compromise platelet function or hemostasis.

Onderzoeksopzet

Opzet

Type:	Interventie onderzoek
Onderzoeksmodel:	Parallel
Toewijzing:	N.v.t. / één studie arm
Blindering:	Open / niet geblindeerd
Controle:	N.v.t. / onbekend

Deelname

Nederland

Status:	Werving gestart
(Verwachte) startdatum:	11-08-2020
Aantal proefpersonen:	300
Type:	Verwachte startdatum

Voornemen beschikbaar stellen Individuele Patiënten Data (IPD)

Wordt de data na het onderzoek gedeeld: Nog niet bepaald

Ethische beoordeling

Positief advies

Datum:	24-09-2020
Soort:	Eerste indiening

Registraties

Opgevolgd door onderstaande (mogelijk meer actuele) registratie

Geen registraties gevonden.

Andere (mogelijk minder actuele) registraties in dit register

Geen registraties gevonden.

In overige registers

Register	ID
NTR-new	NL8923
Ander register	METC Erasmus MC : MEC-2020-0214

Resultaten