The regulatory role of coagulation factors on arterial function

Jérémie Lagrange

ARTERY18

AG Wenzel, Center for Thrombosis and Hemostasis, University Medical Center Mainz
Rudolph Virchow, 1846: Über die Verstopfung der Lungenarterie

- Hypercoagulability
- Low flow
- Vessel wall damage
Rudolph Virchow, 1846: Über die Verstopfung der Lungenarterie

- Hypercoagulability $\rightarrow$ blood makes too much thrombin
- Low flow $\rightarrow$ thrombin remains in force locally
- Vessel wall damage $\rightarrow$ blood is triggered to make thrombin

Common denominator: thrombin
THE THROMBOGENIC HYPOTHESIS AND ITS IMPLICATIONS

J. B. DUGUID, M.D.

From the Department of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne

The discovery that mural thrombi in arteries become incorporated in the intima and form fibrous thickenings has raised many new questions, some of which are at present occupying the attention of members of the Pathology Department.
The mechanism by which thrombin activates platelets and other cells is unknown, and, despite considerable effort by a number of laboratories, the functional receptor(s) that mediates thrombin signaling has not been identified.
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Molecular Cloning of a Functional Thrombin Receptor Reveals a Novel Proteolytic Mechanism of Receptor Activation

Thien-Khai H. Vu,* David T. Hung,**†† Virginia I. Wheaton,* and Shaun R. Coughlin*†
*Cardiovascular Research Institute
†Department of Medicine
‡Cancer Research Institute
University of California, San Francisco
San Francisco, California 94143–0524

Vu, Cell. 1991
Role of tissue factor in embryonic blood vessel development

Peter Carmeliet*, Nigel Mackman†, Lieve Moons*, Thomas Luther‡, Pierre Gressens§, Ilse Van Vlaenderen*, Hilde Demunck||, Michael Kasper‡, Georg Breier¶, Philippe Evrard§, Martin Müller‡, Werner Risau¶, Thomas Edgington† & Désiré Collen*

Tissue factor, a member of the cytokine-receptor superfamily and high-affinity receptor and cofactor for plasma factor VII/VIIa (ref. 1), is the primary cellular initiator of blood coagulation. It is involved in thrombosis and inflammation associated with sepsis, atherosclerosis and cancer², and can participate in other cellular processes including intracellular signalling³, metastasis⁴, tumour-associated angiogenesis⁵, and embryogenesis⁶. Here we report that inactivation of the tissue factor gene (TF) results in abnormal circulation from yolk sac to embryo beyond embryonic day 8.5, leading to embryo wasting and death. Vitelline vessels from null mice were deficient in smooth-muscle α-actin-expressing mesenchymal cells, which participate in organization of the vessel wall. This implies that tissue factor has a role in blood-vessel development.

Carmeliet, Nature. 1996
Introduction

Intramural thrombin

- Prothrombin (FII) and thrombin (FIIa) are present in the vascular wall.

Touat et al., Arterioscler Thromb Vasc Biol. 2008
Introduction

Intramural thrombin

• Prothrombin (FII) and thrombin (FIIa) are present in the vascular wall

• PAR-3 and -4 activation accelerates TF-induced thrombin generation on the surface of VSMCs

Vidwan et al., Arterioscler Thromb Vac Biol. 2010
Introduction

**Pulsatile stretch and coagulation factors**

1. Prothrombin and thrombin are present in the vascular wall
   - PAR-3 and -4 activation accelerates TF-induced thrombin generation on the surface of VSMCs

2. Tissue Factor Pathway Inhibitor (TFPI) increases *in vivo* and *in vitro* with pulsatile stretch

### Free TFPI (ng/mL) vs. PWV (m/s)

- **r = 0.366**
- **P < 0.0001**

### 10% cyclic stretch

- RNA
- cell TFPI
- TFPI
- f-TFPI

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Regnault et al., *Arterioscler Thromb Vac Biol*. 2011
Introduction

**Pulsatile stretch and coagulation factors**

1. **Prothrombin and thrombin are present in the vascular wall**
   - PAR-3 and -4 activation accelerates TF-induced thrombin generation on the surface of VSMCs

2. **Tissue Factor Pathway Inhibitor (TFPI) increases in vivo and in vitro with pulsatile stretch**
   - Cyclic stretch-induced thrombin generation is mediated by the integrin $\alpha_v\beta_3$ pathway

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**Mao et al., Cardiovasc Res. 2012**
Introduction

Aging
Inflammation

Stiffness
Pulsatility

proliferation
focal contact

thrombin

intramural
microthrombi

coagulation cascade

TF / TFPI

1

2

Hypertension
High pulsatility
Arterial stiffness

Hypercoagulability

Inflammation – endothelial dysfunction
Membrane spatial organisation
Pro- and anticoagulant factor synthesis

Vessel wall

Blood

Proliferation
Focal adhesion formation
Contractility
Cytokine production
Introduction

Altered VSCMs modulation?
- Inflammation – endothelial dysfunction
- Membrane spatial organisation
- Pro- and anticoagulant factor synthesis

Metabolic and aging modulation?
- Hypertension
- High pulsatility
- Arterial stiffness

Immune cell modulation?
- Hypercoagulability

Proliferation
- Focal adhesion formation
- Contractility
- Cytokine production

Raas?
- Thrombin

Vessel wall

Blood

Stiffness
Pulsatility

Aging
Inflammation

Proliferation
Focal contact

Thrombin
Intramural microthrombi

TF / TFPI

Coagulation cascade

2
To explore the role of coagulation factors on the regulation of vascular cells and function and in the development of vascular diseases
Models

Zucker rats

Spontaneously hypertensive rats (SHR) +/− FXI antisense oligonucleotide

Endothelial mineralocorticoid receptor (MR) mice

Angiotensin II infused animals

Altered VSCM modulation?

Inflammation – endothelial dysfunction
Membrane spatial organisation
Pro- and anticoagulant factor synthesis

Metabolic and aging modulation?

Hypertension
High pulsatility
Arterial stiffness

Hypercoagulability

thrombin

RAAS?

Proliferation
Focal adhesion formation
Contractility
Cytokine production

Immune cell modulation?
1: Metabolic syndrome, aging and vascular function

**Results**

MSZR rats display increased thrombin generation

Fibrin clot structure is altered in MSZR

Lean Zucker rat (LZR)

Metabolic Syndrome Zucker Rat (MSZR)

Results are means ± SEM of 7-11 rats per group; * $P<0.05$ vs same age LZR

Lagrange et al., Front Physiol. 2017
Results

1: Metabolic syndrome, aging and vascular function

Incremental elastic modulus-wall stress is altered with age

MetS increases gelatinolytic metalloproteinase activity in the aortic wall

Results are means ± SEM of 7-11 rats per group; * P<0.05 vs same age LZR

Lagrange et al., Front Physiol. 2017
Results

2: Tissular thrombin generation in hypertension

**SHR rats display an enlarged thrombosis in vivo**

**FeCl₃-induced thrombus formation in carotids**

10% FeCl₃ application for 3 minutes
n = 10 Wistar, 14 SHR

Thrombin generation on VSMCs

*Ait Aissa*, Lagrange* et al., ATVB. 2015
**Results**

2: Tissular thrombin generation in hypertension

PAR-1-dependent increased proliferation of SHR VSMCs

* P<0.05 versus SMCs from Wistar rats at the same age; † P<0.05 vs media alone

Results are means ± SEM, n=5 per group

Ait Aissa*, Lagrange* et al., ATVB. 2015
Results

3: Endothelial MR protects against thrombosis

**Intravital microscopy**

Robertson *et al.*, Thromb. res. 2009

**Blood flow (mL/min)**

- CT
- MR-EC

**Occlusion time (min)**

- CT
- MR-EC

* p < 0.05 versus control

Results are mean ± SEM

Lagrange *et al.*, FASEB. 2014
Results

3: Endothelial MR protects against thrombosis

Endothelial Protein C receptor (EPCR) is increased in MR-EC mice endothelium

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CT</th>
<th>MR-EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TM, ng/ml</td>
<td>2.1 ± 0.2</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>EPCR, ng/ml</td>
<td>13.5 ± 0.4</td>
<td>15.7 ± 0.8 *</td>
</tr>
<tr>
<td>ADAM17, ng/ml</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Plasma levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sEPCR, ng/ml</td>
<td>22 ± 5</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

* p < 0.05 versus control

Results are mean ± SEM

Lagrange et al., FASEB. 2014
Results

4: FXI implication in vascular dysfunction development

FXII impact on vascular relaxation

FXI impact on vascular relaxation

Results are mean ± SEM
4-12 animal per group

Inhibition of FXI synthesis attenuates blood pressure increase in response to angiotensin II infusion

Means ± SEM of 4-5 animal per group
*, p<0.05 vs. C57BL/6 and C57BL/6 +FXI ASO; #, p<0.05 vs. C57BL/6 +ATII.
Results

4: FXI implication in vascular dysfunction development

Inhibition of FXI synthesis attenuates blood pressure increase in response to angiotensin II infusion

Means ± SEM of 4-5 animal per group
*, p<0.05 vs. C57BL/6 and C57BL/6 +FXI ASO; #, p<0.05 vs. C57BL/6 +ATII.

Results

4: FXI implication in vascular dysfunction development

FXI is crucial for leukocytes adhesion and rolling

C57BL/6 + Angiotensin II

Means ± SEM of 3-5 animal per group *, p<0.05, **, p<0.01, ***, p<0.001 vs. control

Results

4: FXI implication in vascular dysfunction development

Thrombin generation is increased in hypertensive patients and FXI inhibition normalized thrombin generation parameters

controlled hypertension (control, n = 19), arterial hypertension grade I (HT°I; n = 16), and arterial hypertension grade II or higher (HT°II; n = 36)

Data are means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001

Summary

- VSMCs are responsible for the increase of thrombin generation within the vascular wall
- MR increases the EPCR
- FXI inhibition limits vascular inflammation induced by thrombin activation of platelets and immune cells
Summary

- VSMCs are responsible for the increase of thrombin generation within the vascular wall
- MR increases the EPCR
- FXI inhibition limits vascular inflammation induced by thrombin activation of platelets and immune cells

FXI inhibition to limit vascular diseases progression?
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Nexts steps

Implication of VWF and GPIbalpha
### Results

**Patient characteristics of all participants of the FACTO-RR study (1)**

<table>
<thead>
<tr>
<th></th>
<th>Controlled Hypertension</th>
<th>Uncontrolled Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypertension grade I</td>
<td>Hypertension grade II or &gt;II</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td><strong>Male (%)</strong></td>
<td>7 (36.8%)</td>
<td>10 (62.5%)</td>
</tr>
<tr>
<td><strong>Age [years]</strong></td>
<td>65.4±3.5</td>
<td>62.8±3.1</td>
</tr>
<tr>
<td><strong>Blood pressure [mmHg]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>122.6±2.5</td>
<td>138.8±1.2</td>
</tr>
<tr>
<td>Diastolic</td>
<td>70.8±1.7</td>
<td>85.6±1.6 *</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>88.1±1.5</td>
<td>103.3±1.2 *</td>
</tr>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td>72.5±2.1</td>
<td>75.7±3.6 *</td>
</tr>
<tr>
<td><strong>BMI [kg per m²]</strong></td>
<td>26.5±1.0</td>
<td>28.3±1.3</td>
</tr>
<tr>
<td><strong>Medication (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>6 (31.6%)</td>
<td>2 (12.5%)</td>
</tr>
<tr>
<td>AT₁ receptor blockers</td>
<td>5 (26.3%)</td>
<td>10 (62.5%) *</td>
</tr>
<tr>
<td>Ca²⁺ channel blockers</td>
<td>2 (10.5%)</td>
<td>4 (25.0%)</td>
</tr>
<tr>
<td>β blockers</td>
<td>13 (68.4%)</td>
<td>9 (56.3%)</td>
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<tr>
<td>thiazide diuretics</td>
<td>2 (10.5%)</td>
<td>8 (50.0%) *</td>
</tr>
<tr>
<td>loop diuretics</td>
<td>3 (15.8%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>aldosterone antagonists</td>
<td>5 (26.3%)</td>
<td>1 (6.3%)</td>
</tr>
<tr>
<td>renin inhibitors</td>
<td>1 (5.3%)</td>
<td>0</td>
</tr>
<tr>
<td>alpha blockers</td>
<td>1 (5.3%)</td>
<td>1 (6.3%)</td>
</tr>
<tr>
<td>statins</td>
<td>6 (31.6%)</td>
<td>7 (43.8%)</td>
</tr>
<tr>
<td>acetylsalicylic acid</td>
<td>8 (42.1%)</td>
<td>10 (62.5%)</td>
</tr>
<tr>
<td>anti diabetic drugs</td>
<td>4 (21.1%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>1 (5.3%)</td>
<td>2 (12.5%)</td>
</tr>
<tr>
<td>thyroid hormone</td>
<td>2 (10.5%)</td>
<td>4 (25%)</td>
</tr>
</tbody>
</table>

ACE, angiotensin converting enzyme; NSAIDs, non steroidal anti-inflammatory drugs; BMI, body mass index; MI, myocardial infarction; Kruskal-Wallis test with Dunn´s multiple comparison test or Fisher´s exact test were used. *, p<0.05 vs. Control. #, p<0.05 vs. Hypertension grade I.
## Results

Patient characteristics of all participants of the FACTO-RR study (2)

<table>
<thead>
<tr>
<th></th>
<th>Controlled Hypertension</th>
<th>Uncontrolled Hypertension</th>
<th>Hypertension grade I</th>
<th>Hypertension grade II or &gt;II</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>16</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>7 (36.8%)</td>
<td>10 (62.5%)</td>
<td>16 (44.4%)</td>
<td></td>
</tr>
<tr>
<td>Age [years]</td>
<td>65.4 ± 3.5</td>
<td>62.8 ± 3.1</td>
<td>65.3 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Platelet count [10³ per µl]</td>
<td>183.7 ± 19.1</td>
<td>228.4 ± 14.8</td>
<td>191.2 ± 12.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>Controlled Hypertension</th>
<th>Uncontrolled Hypertension</th>
<th>Hypertension grade I</th>
<th>Hypertension grade II or &gt;II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>8 (42.1)</td>
<td>3 (18.8%)</td>
<td>9 (25%)</td>
<td></td>
</tr>
<tr>
<td>Cigarette Smoking</td>
<td>1 (5.3%)</td>
<td>2 (12.5%)</td>
<td>2 (5.6%)</td>
<td></td>
</tr>
<tr>
<td>History of Smoking</td>
<td>5 (26.3%)</td>
<td>5 (31.3%)</td>
<td>9 (25%)</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>8 (42.1)</td>
<td>7 (43.8%)</td>
<td>11 (30.6%)</td>
<td></td>
</tr>
<tr>
<td>Obesity, BMI&gt;30kg/m²</td>
<td>5 (26.3%)</td>
<td>5 (31.3%)</td>
<td>11 (30.6%)</td>
<td></td>
</tr>
<tr>
<td>History of MI or CAD</td>
<td>12 (63.2)</td>
<td>6 (37.5%)</td>
<td>5 (13.9%) *</td>
<td></td>
</tr>
<tr>
<td>History of Stroke</td>
<td>1 (5.3%)</td>
<td>1 (6.3%)</td>
<td>2 (5.6%)</td>
<td></td>
</tr>
<tr>
<td>PAD</td>
<td>2 (10.5%)</td>
<td>3 (18.8%)</td>
<td>1 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>1 (5.3%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Family history of CVD</td>
<td>6 (31.6%)</td>
<td>8 (50%)</td>
<td>17 (47.2%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol abuse</td>
<td>8 (42.1%)</td>
<td>8 (50%)</td>
<td>13 (36.1%)</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>3 (15.8%)</td>
<td>2 (12.5%)</td>
<td>1 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>5 (26.3%)</td>
<td>0 *</td>
<td>4 (11.1%)</td>
<td></td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; PAD, peripheral artery disease; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease. Kruskal-Wallis test with Dunn’s multiple comparison test or Fisher’s exact test were used. *, p<0.05 vs. Control. #, p<0.05 vs. Hypertension grade I.
Vascular disease and hemostasis: example of hypertension

Introduction

In humans:

- Increases thrombotic risk due to atherosclerosis
- Increased platelet reactivity
- Increased FVII and D-dimers...
- ...but also antithrombin and protein C

Arikan et al., Thromb Hemost. 2005
Nadar et al., Am j Cardiol. 1994
Junker et al., J. Hypertens. 1998
Lip et al., J. Hypertens. 1998
Regnault et al., Arterioscler Thromb Vac Biol. 2011

In animal models:

- Increased TF, prothrombin, TAT, fibrinogen
- Decreased TM

Sawada et al., Clin. Exp. Hypertens. 2003
Corseaux et al., Mol. Med. 2002
Ait Aissa*, Lagrange* et al. Arterioscler Thromb Vac Biol. 2015
Results

3: FXI implication in hypertension development

FXI inhibition decreased blood pressure in 5/6 nephrectomized rats even after increased blood pressure

Data are presented as mean±SEM

n=4 (sham) and n=6 (Nx) animals/group;

# vs. sham
* vs. sham SCR ASO
$ vs. sham FXI ASO
Improved vascular reactivity and decreased inflammation in FXI ASO treated ATII infused rats

1-way ANOVA and Bonferroni`s Multiple Comparison test of maximal relaxation, n=5-9 animals/group; *, p<0.05 vs. Wistar, #, p<0.05 vs. Wistar +ATII
Data are presented as mean±SEM

PCR: Kruskal-Wallis and Dunn´s Multiple Comparison test, n=3-6 animals/group; *, p<0.05; **, p<0.01
Data are presented as mean±SEM
GPIba binding domain of vWF is involved in SHR parietal hypercoagulability

Aurintricarboxylic acid (ATA)
Results are means ± SEM of 4 experiments
* P<0.05 versus Wistar rats; † P<0.05 vs SHR before ATA
Summary

VSMCs migration and proliferation

ECs dysfunction

Increasing:
- Blood pressure
- Vascular inflammation
- Atherosclerosis

Shear stress

Blood

Monocytes