Endothelial dysfunction in hypertension: mechanisms and clinical significance

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1998 Nobel Prize winners

R.F. Furchgott
L.J. Ignarro
F. Murad
Endothelial Pathology

- **ENDOTHELIAL DYSFUNCTION**
  
  *reduced NO availability (vascular reactivity)*

- **ENDOTHELIAL ACTIVATION**

  *acquisition of fllogistic activity (plasma determination of adhesion molecules, ILs, CRP)*

- **ENDOTHELIAL INJURY**

  *anathomical disruption of endothelial cells (plasma determination of vWF, EPCs)*
Endothelial dysfunction in human hypertension
Endothelium-dependent relaxation in WKY and SHR

Lüscher TF & Vanhoutte PM, Hypertension 1986
Genetic Hypertension
Forearm blood flow (plethysmography)

- Data acquisition and analysis
- Cuff inflator
- Intra-arterial infusion
- Plethysmograph
- BP and HR monitoring

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Endothelium-dependent vasodilation in patients with essential hypertension or primary aldosteronism

Taddei S et al. Hypertension 1993
COX is responsible for endothelial dysfunction in patients with essential hypertensive but not in patients with primary aldosteronism.
Cyclooxygenase inhibition restores NO activity in essential hypertension

Taddei S et al. Hypertension 1997
Cyclooxygenase is a major source of oxidative stress in essential hypertension

COX and endothelial dysfunction in essential hypertensive patients: *unsolved questions*

- Which COX isoenzyme is the predominant isoform contributing to ROS generation in essential hypertension?

- Is COX the only recognized ROS source, in small resistance arteries from essential hypertensive patients?
Micromiography

3rd branch

Mesenteric artery (150 ~ 350 µm)

Peripheral resistance artery
(150 ~ 350 µm)

Gluteal subcutaneous or intrasurgery biopsy

Subcutaneous fat

Isometric (wire myograph)

Isobaric (pressure myograph)

(45-60 mmHg)
Patients with essential hypertension

Normotensive control subjects

\[ \text{Pressure micromyograph} \]

Laparoscopic surgical intervention
- Adrenalectomy (non-functioning mass >3.5 cm)
- Cholecystectomy (gallbladder stones)

Abdominal subcutaneous fat biopsy

Dissection

Bioptic sample

Endothelial function: Pressure Myograph

60mmHg

Small resistance arteries (lumen \( \varnothing \): 150 ~300 \( \mu \text{m} \))
Identification of sources of oxidative stress in small arteries of essential hypertensive patients

Virdis A et al, Hypertension 2013
Identification of sources of oxidative stress in small arteries of essential hypertensive patients

Essential hypertensive patients

Acetylcholine (10⁻¹⁰ mol/L)

Relaxation response (%)

saline
L-NAME
Apocynin (NADPH inhibitor)
Apocynin + L-NAME

Virdis A et al, Hypertension 2013
RT-PCR analysis of COX-1 and COX-2 expression in controls and hypertensive patients

Virdis A et al, Hypertension 2013
Immunostaining of COX-1 and COX-2 in controls and hypertensive patients
Essential Hypertension

Endothelial cells

NAD(P)H oxidase

\[ \text{COX-2} \]

\[ \text{ROS} \]

Vascular Smooth Muscle Cells

NAD(P)H oxidase

\[ \text{AA} \]

\[ \text{PGH}_2 \]

\[ \text{NO} \]

\[ \text{PGI}_2 \]

\[ \text{PGD}_2 \]

\[ \text{PGF}_{2\alpha} \]

\[ \text{PGE}_2 \]

\[ \text{TXA}_2 \]

RELAXATION

CONTRACTION

Vascular Smooth Muscle Cells
COX and endothelial dysfunction in essential hypertension

Future research

• Endothelial dysfunction is also present in secondary forms of hypertension, but it is not mediated by COX activity: thus this alteration is specific for essential hypertension and not related to high blood pressure values.

• COX-2 is an inducible enzyme: when and why is it activated in hypertensive patients?

• Selective COX-2 inhibitors increase CV risk: which role for COX-1?

• Could be vascular COX-2 a specific target for treatment?
ATG

Renin

AT-I

TGFβ1

Thr

AT-II

Ach

AA

Shear Stress

L-Arg

PGI₂

EDHF

Endothelium

Smooth muscle cells

Platelets

Contraction

Relaxation
Angiotensin II (active) leads to the activation of converting enzyme, producing EDHF and NO. Bradykinin also activates the converting enzyme, resulting in NO production. Peptide (inactive) does not lead to any significant enzyme activation. EDHF and NO, acting on the vascular smooth muscle cells, cause hyperpolarization and an increase in cGMP, leading to relaxation.
Effect of L-NMMA (to block NO-synthase) and ouabain (to block hyperpolarization) on response to bradykinin

**Normotensive Subjects**

- Saline
- L-NMMA
- Ouabain

FBT Δ%

* P<0.05

**Essential Hypertensive Patients**

- Control
- Vitamin C

FBT Δ%

* P<0.05

Taddei S et al. Circulation 1999
Mechanisms responsible for endothelium-dependent vasodilation in human hypertension

Normotensive Subjects

Essential Hypertensive Patients

Essential Hypertensive Patients
Effects of L-NMMA and sulfaphenazole on vasodilation to bradykinin

Taddei S et al, JACC 2006
The isoenzyme 2C of the cytochrome P450 epoxygenase (named CYP 2C) is a major source of EDHF

Busse R et al, Trends in Pharmacological Sciences, 2002
Fibrinolitic properties of endothelial cells

Pro-fibrinolitic

platelets

coaulation

Anti-fibrinolitic

Fibrinolysis

PAI-1

t-PA

CV risk factors
Hypertension
Diabetes
Smoking
etc

plasminogen

plasmin

fibrin

FDP
Determination of vascular balance

- Data acquisition and analysis
- Cuff inflator
- Intra-arterial infusion
- Arterial sample
- Deep venous sample
- BP and HR monitoring
- Plethysmograph

Intra-arterial infusion

Arterial sample

Deep venous sample

BP and HR monitoring

Plethysmograph

Data acquisition and analysis

Cuff inflator
Simultaneous blood sampling for the determination of venous-arterial differences

Venous value greater than arterial value

↓

RELEASE

Venous value lower than arterial value

↓

UPTAKE

Net balance = \((C_v - C_a) \times [FBF \times (1 - H_t)]\)

\(vC\) = venous concentration
\(aC\) = arterial concentration
\(FBF\) = forearm blood flow
\(Ht\) = hematocrit
Bradykinin, but not acetylcholine, can release t-PA in hypertensive patients with impaired NO availability by a mechanism involving a sulfaphenazol (SULFA)-sensitive pathway (EDHF?).

Acetylcholine induced t-PA release

![Graph showing t-PA release in normotensive subjects and hypertensive patients with and without L-NMMA.]

* p<0.01 vs baseline

Giannarelli C et al. Hypertension 2007
Bradykinin, but not acetylcholine, can release t-PA in hypertensive patients with impaired NO availability by a mechanism involving a sulfaphenazol (SULFA)-sensitive pathway (EDHF?).

**Acetylcholine induced t-PA release**

- Normotensive subjects
- Hypertensive patients

* p<0.01 vs baseline

**Bradykinin induced t-PA release**

- Normotensive subjects
- Hypertensive patients

* p<0.01 vs baseline

Giannarelli C et al. *Hypertension* 2007

Giannarelli C et al. *Circulation* 2009
Relaxation

Smooth muscle cells

Healthy Conditions

NO

CYP 2C9-derived EDHF

Bradykinin

Endothelial cells

t-PA release
Smooth muscle cells

Endothelial cells

Relaxation

NO

Essential Hypertension

CYP 2C9-derived EDHF

Bradykinin

t-PA release

Endothelial cells
Different mechanism of action of ACE-inhibitors and angiotensin receptor blockers

Bradykinin

ACE inhibitors

Inactive peptides

Vasodilation
Antiremodeling
Antioxidant
Antithrombotic

Angiotensin

Ang I

Ang II

AT$_1$

AT$_2$

Angiotensin receptor blockers

Dominiczak and Unger (eds) in Ang II-AT1- Receptor Antagonists. Steinkopff;1997
ACE inhibitors, but not ARBs, have an additional cardioprotective effect beyond BP lowering.
Effect of ACE-I and ARBs on total mortality in hypertensive patients

<table>
<thead>
<tr>
<th>Subgroup (number of trials)</th>
<th>All-Cause Mortality Pooled HR (95% CI)</th>
<th>P-value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (20)</td>
<td>0.96 (0.92 - 1.00)</td>
<td></td>
</tr>
<tr>
<td>Versus placebo (7)</td>
<td>0.95 (0.88 - 1.02)</td>
<td>0.435</td>
</tr>
<tr>
<td>Versus active treatment (13)</td>
<td>0.97 (0.93 - 1.01)</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor (7)</td>
<td>0.92 (0.85 - 0.99)</td>
<td>0.024</td>
</tr>
<tr>
<td>ARB (13)</td>
<td>0.99 (0.94 - 1.04)</td>
<td></td>
</tr>
<tr>
<td>&gt; 50% of DM / RI (3)</td>
<td>0.92 (0.82 - 1.03)</td>
<td>0.355</td>
</tr>
<tr>
<td>&lt;= 50% of DM / RI (17)</td>
<td>0.97 (0.93 - 1.01)</td>
<td></td>
</tr>
</tbody>
</table>

Van Vark LC et al. Eur Heart J 2012
Clinical significance of endothelial dysfunction in hypertension
Endothelial dysfunction and oxidative stress are associated to the major cardiovascular risk factors

<table>
<thead>
<tr>
<th>CV risk factors</th>
<th>Documented endothelial dysfunction</th>
<th>Presence of oxidative stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial History of CVD</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Age</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Menopause</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cigarette Smoking</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Effect of aging on endothelium-dependent vasodilation in the forearm microcirculation

Taddei S et al. Circulation 1995

Normotensive subjects

Essential hypertensive patients
Hypertension causes premature aging of endothelial function in humans

Taddei S et al, Circulation 1996
Vascular effects of principal endothelium-derived substances

Healthy conditions

- NO

ANTIATHEROSCLEROTIC EFFECT

CV risk factors

- EDCFs
  - (ET-1; A-II; TXA₂; PGH₂; ·O₂⁻)

PROATHEROSCLEROTIC EFFECT
Pathogenesis of atherosclerosis
defined as endothelial dysfunction to clinical disease

endothelial dysfunction

- ↓ stimuli-induced vasodilation (e.g., to shear stress)

plaque growth

- remodeling/proliferation

ischemia / angina pectoris

acute coronary syndrome

clinical manifestations
Cardiovascular risk has been calculated according to the IRA (Italian Rural Areas) Risk Chart [Menotti A. e Coll. Ital Heart J 2002]

<table>
<thead>
<tr>
<th>RISK</th>
<th>Mean ± DS (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORONARY</td>
<td>3.8 ± 8.7 (0.1 – 80.5)</td>
</tr>
<tr>
<td>CEREBROVASCULAR</td>
<td>1.0 ± 1.2 (0.1 – 7.1)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5.3 ± 8.8 (0.4 – 78.1)</td>
</tr>
</tbody>
</table>

(Number of events/100 individuals in 10 years)
Relationship between vasodilation to acetylcholine and total cardiovascular risk

\[ r = -0.48 \quad p < 0.001 \]

Slope change at around 8 events in 10 years/100 individuals

\[ r = -0.84 \quad P < 0.005 \]

Total Cardiovascular Risk
(Number of events/100 individuals in 10 years)
Receiver operating characteristic curves for FRS (AUC=0.74), brachial FMD (AUC=0.65), and FRS+FMD (AUC=0.74) to predict incident CVD events
Multivariant analysis of hazard ratio of present studies reporting association between coronary or peripheral endothelial function and cardiovascular events
Association of endothelial dysfunction with CV risk profile

- Demonstrated in untreated patients with CV risk factors

Endothelial dysfunction as independent prognostic marker of CV clinical events

- Demonstrated in high risk patients with CV disease and pharmacological treatment
Relationship between endothelial dysfunction and prognosis

- CV risk
- Events
- No events

Treatment
The determination of endothelial function might be a good marker of progression of disease (and possibly of the effectiveness of treatment)
Treatment of Endothelial Function and Prognosis

Improved brachial artery FMD

Persistent impaired brachial artery FMD

p < 0.0001

Modena MG et al. JACC 2002
CV Risk Factors

- Inflammation
- Oxidative Stress

NO-Synthase

NO

- \( \cdot O_2^- \)

Endothelial dysfunction

Cardiovascular events

Treatment
Aging, endothelial dysfunction and aerobic physical exercise

Taddei S et al. Circulation 2000
## Effect of pharmacological treatment on endothelial dysfunction

<table>
<thead>
<tr>
<th></th>
<th>ACE-I</th>
<th>AT(_1)-Ant</th>
<th>Ca-Ant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conduit arteries</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coronary</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>peripheral</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Subcutaneous</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>microcirculation</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Muscle microcirculation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetylcholine, metacholine</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>bradikynin</td>
<td>+</td>
<td>no data</td>
<td>+</td>
</tr>
</tbody>
</table>
THE GOOD GUY

NO

Vasodilation
↓ Platelet aggregation
↓ VSMC migration and proliferation
↓ Monocyte adhesion
↓ Adhesion molecules expression
↓ ET1

VASCULAR PROTECTION

THE BAD GUYS

ET-1; A-II; TXA₂; PGH₂; ·O₂⁻

Vasoconstriction
NO breakdown:
↑ Platelet aggregation
↑ VSMC migration and proliferation
↑ Monocyte adhesion
↑ Adhesion molecules expression

VASCULAR DAMAGE
I need to thank a lot of people!!!
My group
My mentor Prof. Paul M. Vanhoutte
My family
Experimental Model
