Endothelial dysfunction in hypertension: from bench to bedside

Stefano Taddei
Department of Clinical and Experimental Medicine
University of Pisa, Italy
Press Release: The 1998 Nobel Prize in Physiology or Medicine

The Nobel Assembly at Karolinska Institutet has today decided to award the Nobel Prize in Physiology or Medicine for 1998 jointly to

Robert F. Furchgott, Louis J. Ignarro and Ferid Murad

for their discoveries concerning "nitric oxide as a signalling molecule in the cardiovascular system"
Endothelial dysfunction in human hypertension
Endothelium-dependent relaxation in WKY and SHR
Genetic Hypertension
Forearm blood flow (plethysmography)

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

Forearm blood flow (plethysmography)

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Intra-arterial infusion

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

Taddei S et al. Hypertension 1993
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)

(45-60 mmHg)

Isobaric (pressure myograph)

Micromiography
Identification of sources of oxidative stress in small arteries of essential hypertensive patients

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

Virdis A et al, Hypertension 2013
Essential Hypertension

Endothelial cells

NAD(P)H oxidase → ↑ ROS → COX-2

COX-2 → AA → PGH₂

PGH₂ → PGI₂, PGE₂, PGD₂, TXA₂, PGF₂α

NO → PGI₂

Relaxation → Contraction

Vascular Smooth Muscle Cells
INHIBITION

AGGREGATION

Renin → Thrombin → ATG → AT-I

ACE → ET-1 → AT-II

Renin → ATG → AT-I

Thrombin → ATG → AT-I

AT-1 → AT1

Cyclooxygenase

Mitocondria

NADPH-oxidase

Xanthine-oxidase

Shear Stress

Ach → ADP → ET → ET_B → S1 → BK

PGI2 → EDHF

EDHFs

Mitocondria

cGMP

cGMP

PGI2

PGI2

K+

CONTRACTION

RELAXATION

Platelets

Endothelium

Smooth muscle cells
Effect of L-NMMA (to block NO-synthase) and ouabain (to block hyperpolarization) on response to bradykinin

![Graph showing the effect of L-NMMA and ouabain on FBT Δ% in Normotensive Subjects and Essential Hypertensive Patients. The graph compares the response to bradykinin at different concentrations (0.005, 0.015, 0.05 μG/100 ml/min) for Saline, L-NMMA, and Ouabain. * P<0.05.](image-url)
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

Normotensive subjects

Hypertensive patients

Taddei S et al, JACC 2006
The isoenzyme 2C of the cytochrome P450 epoxyxygenase (named CYP 2C) is a major source of EDHF.
Fibrinolitic properties of endothelial cells

Pro-fibrinolitic

Anti-fibrinolitic

CV risk factors
Hypertension
Diabetes
Smoking
e etc

Fibrinolysis

t-PA

plasminogen

plasmin

fibrin

FDP

PAI-1

Anthrax

Coagulation

platelets
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) x [F_BF x (1 - H_t)]

v_C = venous concentration
a_C = arterial concentration
F_BF = forearm blood flow
H_t = 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?).

![Graph showing t-PA release induced by Acetylcholine and Bradykinin in Normotensive subjects and Hypertensive patients.](image)

* 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?).

Giannarelli C et al. *Hypertension* 2007

Giannarelli C et al. *Circulation* 2009
Relaxation

Smooth muscle cells

Healthy Conditions

NO

CYP 2C9-derived EDHF

Endothelial cells

Bradykinin

t-PA release
Smooth muscle cells

Endothelial cells

Relaxation

Essential Hypertension

NO

CYP 2C9-derived EDHF

Bradykinin

t-PA release

Endothelial cells
Hypertension causes premature aging of endothelial function in humans

Taddei S et al, Circulation 1996

Bruno RM et al, Hypertension 2017

60mmHg
Clinical significance of endothelial dysfunction in hypertension: moving to non-invasive tests
Pathogenesis of atherosclerosis from endothelial dysfunction to clinical disease

endothelial dysfunction

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

PGI, EDHF, NO

plaque growth

remodeling/proliferation

clinical manifestations

acute coronary syndrome

ischemia / angina pectoris
Brachial Artery Flow Mediated Dilation (FMD)

**Endothelium-dependent stimulus**

↑ shear stress = post-ischemic flow (Reactive Hyperemia) to 5 min. ischemia

**Endothelium-dependent response**

↑ Diameter following Reactive Hyperemia

FMD Studio (www.quipu.eu)
Prediction of future cardiovascular outcomes by brachial artery FMD: a meta-analysis

Inaba Y et al. Int J Cardiovasc Imaging 2010
In the MESA study FMD did not improve cardiovascular risk assessment in intermediate-risk individuals.
FMD reproducibility in the MESA study: was it enough?

Reproducibility in the MESA study:
- CAC: intraobserver and interobserver agreement $k=0.90$ and 0.93
- IMT: coefficient of variation 7.07%
- FMD: intraclass coefficient 0.54

FMD standardization improves reproducibility

1 month CV: 12.9%

3 month CV: 18.3%


Gemignani V et al. UM&B 2007

Ghiadoni et al. J Hypertens 2012

Charakida M et al. Eur Heart J 2010

Charakida M et al. Eur Heart J 2013
FMD as a surrogate endpoint in clinical trials

Vascular effects and safety of dalcetrapib in patients with or at risk of coronary heart disease: the dal-VESSEL randomized clinical trial

Thomas F. Lüscher¹*, Stefano Taddei², Juan-Carlos Kaski³, J. Wouter Jukema⁴, David Kallend⁵, Thomas Münzel⁶, John J.P. Kastelein⁷, and John E. Deanfield⁸, on behalf of the dal-VESSEL Investigators

- 466 patients with target LDL-C levels received dalcetrapib (a CETP-inhibitor) 600 mg/day or placebo for 36 weeks on top of standard therapy (including statins).
- The primary outcome measures were the change from baseline of flow-mediated dilatation (%FMD) of the right brachial artery
No change in FMD, despite increased HDL levels

No benefit on CV events in the DAL-outcome study

Luscher TF et al, EHJ 2012
CV Risk Factors

- NO-Synthase
- Inflammation
- Oxidative Stress

- NO
- ·O₂⁻

Endothelial dysfunction

Cardiovascular events

Treatment
Aging, endothelial dysfunction and aerobic physical exercise

Taddei S et al. Circulation 2000
Different Effect of Antihypertensive Drugs on Conduit Artery Endothelial Function

FMD (%)

- **Normotensive subjects**: 7.1
- **Hypertensive patients (baseline)**: 5.2

**after 6 months treatment**

- **Perindopril**: 6.4*
- **Nifedipine**: 4.8
- **Amlodipine**: 5.1
- **Atenolol**: 5.7
- **Nebivolol**: 5.6
- **Telmisartan**: 5.6

*p < 0.01 vs other treatments

Ghiadoni L et al. Hypertension 2003
Ramipril dose-dependently increases nitric oxide availability in the radial artery of essential hypertension patients

Effect of pharmacological treatment on endothelial dysfunction

<table>
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<th>ACE-I</th>
<th>AT$_1$-Ant</th>
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<tr>
<td>bradikynin</td>
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</table>
Beyond hypertension: endothelial dysfunction is a key player in the greatest epidemics of XXI century:

- Obesity
- Dementia
Nitric oxide availability in obese patients and control subjects

Virdis A et al, JACC 2011
Role of ROS on endothelium-dependent relaxation in obese patients and control subjects

Virdis A et al, JACC 2011
Role of TNF-α on endothelium-dependent relaxation and NO availability in obese patients

Virdis A et al, JACC 2011
Endothelial nitric oxide: protector of an healthy mind

Katusic ZS and Austin SA. Eur Heart J. 2013
Cardiovascular risk factors are involved in Amyloid-beta deposition and Alzheimer’s dementia through two main mechanisms:
• Blood-brain barrier dysfunction
• Brain hypoperfusion

Endothelium-derived NO plays a role in both mechanisms
Cognitive-physical training in mild cognitive impairment: The Train the Brain – Mind the Vessel Study

- cross-sectional study (MCI vs nonMCI) + single center, parallel group interventional study (MCI-EE vs MCI-noEE)
- clinicaltrials.gov. identifier NCT 01725178
- Funding source: Fondazione Pisa

Randomized patients: N=121
(113 MCI + 8 mild AD)
≥65 and ≤89 years

Healthy individuals
(non MCI): N=45
≥65 and ≤89 years

Baseline evaluation

Month
-1
0
7

N=61 (58 MCI + 4 mild AD)
Standard treatment
Environmental enrichment (cognitive training 6 h/week, physical training 3 h/week)
Drop out (N=4)
N=57

N=60 MCI (55 MCI + 5 mild
Drop out (N=2)
N=58

The Train the Brain Consortium, Scientific reports 2017
Bruno RM et al, Hypertension 2017 in press
Cognitive-physical training improves cognitive function, cerebral blood flow and endothelial function.

Hippocampal and parahippocampal regions are crucial for memory and processing of non-verbal/spatial information.

The Train the Brain Consortium, Scientific reports 2017
Cognitive-physical training improves cognitive function, cerebral blood flow and endothelial function

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Bruno RM et al, Hypertension 2017 in press

The Train the Brain Consortium, Scientific reports 2017
Endothelial function is a determinant of parahippocampal blood flow in patients with mild cognitive impairment

Clinical determinants: none

Vascular determinants:
- FMD ($r=0.26$, $p=0.03$)

Multiple regression model:

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Bruno RM et al, oral presentation at Artery 2017
I need to thank a lot of people!!!
My mentor Prof. Paul M. Vanhoutte
My group
My family