Vasoprotection By Nitric Oxide. Mechanisms, Physical Activity And Pharmacotherapeutic Potential

Prof. Dr. Georg Kojda
Institut für Pharmakologie und Klinische Pharmakologie, Universitätsklinikum Düsseldorf, Düsseldorf, Germany

The NO/cGMP Pathway

Cardiovascular Prevention

Physiologic

α

Pharmacologic

cGMP → Vasodilation etc.

soluble Guanylyl Cyclase

cGMP

K+-channels

Hyperpolarization

decreased Ca²⁺-entry

decreased intracellular Ca²⁺-concentration

PKGs

IRAG

Phospholamban

Inhibition of IP₃-mediated Ca²⁺-release

SERCA-activation

increased Ca²⁺-sequestration

Effects of NO in the vascular system
- vasodilatory
- antioxidative
- antiaggregatory
- antiproliferative
- antiadhesive
- antiapoptotic

**Cardiovascular Prevention**

**Unique Hemodynamic Profile Improves Quality of Live**

- Preload (left ventricular enddiastolic pressure)
- Coronary vascular resistance (vasomotor tone)
- Afforded (peripheral resistance)

therapeutic dosage

therapeutic activity (antilanginal)

primarily a side effect (hypotensive effect)

adopted from: Kojda G., Pharmacologie Toxikologie Systematisch, UNI-MED Verlag 2002

**Cardiovascular Prevention**

Vascular Bioactivation of Nitrates Determines Venous Pooling, The Positive Steal Effect and Moderate Tolerance

Vascular Cell

GTN
ISDN
ISMN

β
α

GTP
cGMP

CYP3A4, mtALDH

Illustration: Prof. Dr. H.-D. Höltje
Pharmaceutical Chemistry
University Düsseldorf

In both GISSI-3 and ISIS-4 more than 50% of the MI-patients in the placebo group received nitrates, most likely because they were considered as indispensable

Yusuf et al., 1988; Metaanalyse, 2041 Patients
GISSI-3, 1994; Mega-Trial, 18,895 Patients
ISIS-4, 1995; Mega-Trial, 58,050 Patients
Cardiovascular Prevention

**Improved Survival In Heart Failure**

1050 Afroamericans
NYHA III or IV

Initial: 37.5 mg Hydralazin plus 20 mg ISDN, 3x/day

Chronic: 225 mg Hydralazin plus 120 mg ISDN/Tag

Figure 1. Kaplan-Meier Estimates of Overall Survival.


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**Intermittent Administration To Avoid Tolerance**

(10 h Nitrate Pause each day is mandatory!)

Severe Nitrate Tolerance
(e.g. by continuous transdermal GTN)
- strong impairment of vasodilation by nitrates
- increased vascular oxidative stress

Moderate Nitrate Tolerance
(e.g. by eccentric high-dose ISMN)
- selective impairment of vasodilation by nitrates
- normal endothelial function
- lack of vascular oxidative stress

No Nitrate Tolerance
- normal vasodilation by nitrates
- normal endothelial function
- lack of vascular oxidative stress

Possibly Vasotoxic (very rare)
Decreased Vasodilation to Nitrates

Cardiovascular Prevention

**Moderate Nitrate Tolerance After 4 Months of High-Dose (200 mg/kg) Oral ISMN**

Decreased Vasodilation to ISMN

No Impairment of Endothelial Function


Cardiovascular Prevention

**No Increased Superoxide After 4 Months of High-Dose (200 mg/kg) Oral ISMN**

Pharmacological Activation of the NO/cGMP System is a Useful Approach to Treat CAD and Heart Failure!

Nitrates in clinical practice display a rapid onset of action, even with slow-release formulations.

Nitrates in combination therapy predominantly reduce preload, complement the hemodynamics of other CAD drugs.

Nitrates in cardiovascular emergencies are almost indispensable in CAD (MI) and heart failure.

Nitrates Safety are devoid of rare but life-threatening side effects such as angioedema (ACE-inhibitors, AT-1-blockers, calcium antagonists), do not change blood levels of lipids and glucose and do not interfere with the hepatic drug metabolism (statins).

ACE-Inhibitor-Induced Angioedema, which is mediated by bradykinin, can be viewed as a local endothelial overstimulation leading to vasodilation and capillary hyperpermeability to which endogenous NO contributes.

Incidence: 0.4 - 0.7 %, appr. 1 % lethal


The discovery of endothelium-dependent vasodilation uncovered an entirely new principle for signalling in the human organism.

Can We Actively Use Endogenous NO As A Remedy?

Shear stress (blood flow) + L-Citrulline

Phenylephrine, Acetylcholin (Bradykinin, Serotonin) → Vasoconstriction → Endothelium-dependent vasodilation

Mediators e.g. bradykinin, serotonin

L-Arginine

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Cardiovascular Prevention By Exercise

During our history, daily exercise has always been a constant but this has changed in modern times.

### History
- Homo habilis
- Homo erectus
- Homo sapiens
- Homo relaxus

### Modern Times

What is the evidence for beneficial effects of regularly physical activity?

**Cardiovascular Prevention By Exercise**

- **Overt Cardiovascular Disease**
  - Reduction Of Mortality by 30 %
    - (Metaanalysis, Circulation 1995;88:236-244)

- **Secondary Prevention**
  - Early CAD-Development
    - 20 min/day reduces CAD-Mortality by 29 %
  - Health status of older men
    - 3.2 km walking/day Reduces Overall Mortality by 50 %

- **Primary Prevention**
  - Health status of postmenopausal women
    - 2 km walking/day Reduces CAD-Risk by 30 %

**Cardiovascular Prevention By Exercise**

- **Why is Exercise Beneficial?**

**“MRFIT“**

- **O2- Utilisation ↑**
- **Collaterals**, **Heart rate**, **stroke volume ↑**
- **Insulinresistance ↓**
- **HDL ↑**
- **LDL ↓**
- **Blood pressure ↓**
**Vascular Adaptations To Exercise**

**Effect Of The NO-Donor DETA/NO On ecSOD Protein Expression**

- **Figure a:**
  - DETA-NO concentration (µM): 0, 1, 3, 6, 12, 24 (h)
  - Graph showing the effect of DETA-NO on ecSOD protein expression.

- **Figure b:**
  - DETA-NO concentration (µM): 0, 50, 100 (µM)
  - Graph showing the effect of DETA-NO on ecSOD protein expression.

**Structure of the Plasmid Constructed For Vascular-Specific Overexpression of eNOS**

- The plasmid contains:
  - Tie 2 Promotor: 2.1 kb
  - Enhancer: 19.75 kb
  - eNOS: 4.1 kb

**Overexpression of eNOS in different Colonies**

- Western blot analysis showing the relative eNOS expression in different colonies.
  - Colonies: C57BL/6, Colony 5, Colony 1, Colony 2
  - Relative eNOS expression: Colony 2 > Colony 1 > Colony 5

*Note: Suvorava, Oppermann, Kojda, unpublished*
Vascular Adaptations To Exercise

Reduction of Blood Pressure in eNOS++ Mice.

Suvorava, Oppermann, Kojda, unpublished

Reduction of Blood Pressure in eNOS++ is inhibited by the NOS-Inhibitor L-Nitroarginine (L-NA)

Suvorava, Oppermann, Kojda, unpublished

Overexpression Of eNOS In eNOS++ Drives Overexpression Of ecSOD

SOD3-Expression in eNOS++- Mice

Suvorava, Oppermann, Kojda, unpublished

Exercise Increases Vascular eNOS Expression. Does Exercise Increase ecSOD-Expression As Well?
Vascular Adaptations To Exercise

Exercise Increases ecSOD Expression NO-Dependently

Based on these observations it appears reasonable to assume that exercise training can be viewed as an effective antioxidant and antiatherogenic therapy.

Mechanisms of Vascular Adaptations To Exercise

Does permanent malfunction of one eNOS gene inhibit exercise-induced expression of vascular eNOS?
Does permanent malfunction of one eNOS gene inhibit exercise-induced expression of vascular eNOS?

Blood pressure and heart rate are unchanged

Mechanisms of Vascular Adaptations To Exercise

The loss of one eNOS Gene impairs the upregulation of eNOS expression induced by exercise

The loss of one eNOS Gene impairs the upregulation of eNOS function induced by exercise

Mechanisms of Vascular Adaptations To Exercise
Mechanisms of Vascular Adaptations To Exercise

Permanent Vascular Overexpression of Catalase


Permanent reduction of Hydrogen Peroxide Inhibits Exercise-Induced Expression of Vascular eNOS

Mechanisms of Vascular Adaptations To Exercise

Permanent Reduction of Vascular Hydrogen Peroxide Reduces Blood Pressure


Mechanisms of Vascular Adaptations To Exercise

Permanent Reduction of Vascular Hydrogen Peroxide Reduces Blood Pressure Independent of eNOS.


Mechanisms of Vascular Adaptations To Exercise

Permanent Reduction of Vascular Hydrogen Peroxide Reduces Blood Pressure: Inhibition by Aminotriazole.

Mechanisms of Vascular Adaptations To Exercise

Experimental sedentary lifestyle induced by singularization
Forced physical inactivity

Quantitation of regular physical activity in mice by skeletal muscle citrate synthase activity

Mechanisms of Vascular Adaptations To Exercise

Physical inactivity, the so-called sedentary lifestyle, may increase cardiovascular risk in young healthy individuals by inducing endothelial dysfunction.

Exercise training increases vascular NO-production and decreases vascular ROS-production.

The effects of exercise training on vascular eNOS expression are dependent on both eNOS genes.

The induction of vascular eNOS expression by exercise training is dependent on endogenous hydrogen peroxide formation.

Physical inactivity - the so-called sedentary lifestyle - rapidly causes reduced eNOS expression and endothelial dysfunction in young healthy individuals.
**Conclusions**

Exercise training can be viewed as an effective antioxidant and antiatherogenic therapy.

In cardiovascular disease patients exercise reduces the degree of endothelial dysfunction.

In young healthy individuals normal physical activity and/or moderate exercise might delay the development of cardiovascular disorders by maintaining normal endothelial function.

**Hypothesis For Vascular Adaptations To Exercise**

Short term peaks of vascular oxidative stress induced by exercise increase vascular antioxidative defense mechanisms, because gene expression lasts longer than exercise-induced vascular oxidative stress.

Increase of the vascular antioxidative capacity induced by an increased expression ratio of ROS-scavenging vs ROS-producing enzymes.

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**Co-Workers**

- Björn Hüsgen, MD
- Andreas Hacker, PharmD
- Thorsten Keller, MD
- Ute Labs, MD
- Nadine Lauer, PharmD
- Dorothea Marburg, MD
- Senta Müller, DMV
- Petra Nix, MD
- Verena Schmitz, PharmD
- Martina Weber, PharmD

**Cooperation-Partners**

- Eberhard Bassenge, MD, Freiburg
- Thoru Fukai, MD, Atlanta
- Rainer Haas, MD, Leipzig
- David Harrison, MD, Atlanta
- Henning Morawietz, PhD, Halle
- Just Müllenheim, MD, Düsseldorf
- Hans-Michael Piper, MD, Gießen
- Benedikt Preckel, MD, Düsseldorf
- Klaus-Dieter Schlüter, PhD, Gießen
- Volker Thämer, MD, Düsseldorf
- Heinz-Gerd Zimmer, MD, Leipzig