

# Is NO the King?

## Pathophysiological Benefit with Uncertain Clinical Impact

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### Summary

NO is the “hero” molecule of the last few decades. It is a ubiquitous and omnipotent radical with both hemodynamic and antiproliferative effects within the cardiovascular system. NO is an important counterregulatory factor for vasoconstrictors and growth promoting substances. Endothelial dysfunction with decreased NO production is related to many cardiovascular disorders, such as coronary artery disease, heart failure and hypertension. Despite the important role of NO within the circulation, there is only limited evidence in the form of large clinical trials that NO delivery can reduce cardiovascular morbidity and mortality. Thus, NO donors are not in the first line therapy in ischemic heart disease, heart failure or arterial hypertension and NO delivery is recommended only in particular clinical situations, when a well established treatment is contraindicated or has an insufficient effect. It is concluded that the insufficient NO production is the principal disorder in endothelial dysfunction, which is related to cardiovascular pathology with deteriorated prognosis, but the impact of therapeutically increased NO bioactivity on the morbidity and mortality is inferior to well established treatment with ACE-inhibitors, AT<sub>1</sub> receptor blockers, beta-blockers, statins and certain antihypertensive drugs. There is little doubt that NO is king in the circulation, but kings seldom decide the battles.

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### Key words

Nitric oxide • NO donor • Cardiovascular mortality • Clinical trial

### Physiological impact of nitric oxide

NO is the “hero” molecule of the last few decades. It is a ubiquitous and omnipotent radical with both hemodynamic and antiproliferative effects within the cardiovascular system. It is generated by nitric oxide synthase (NOS). Three isoforms of NOS have been identified, which can be divided into two functional

classes. The constitutive class involves calcium-dependent endothelial NOS (eNOS) and neuronal NOS (nNOS), which produce NO continuously in small – picomolar concentrations. In contrast, the inducible form of NOS (iNOS) is a high output enzyme producing NO in the nanomolar range only under the conditions of enzyme activation, mostly by inflammatory cytokines (Albrecht *et al.* 2003, Rakhit and Marber 2001). This quantitative

difference has an important impact. NO produced in small amounts may result in tissue protection and NO in high concentrations may be harmful (as seen in septic shock) (Rakhit and Marber 2001).

Endothelial dysfunction is an interesting phenomenon related to many cardiovascular disorders, such as coronary atherosclerosis with myocardial ischemia, arterial hypertension with potential left ventricular hypertrophy or to heart failure. Although the dysfunction of endothelium involves the whole number of alterations of the endothelial layer, including excessive production of vasoconstriction and procoagulation factors, production of inflammatory cytokines and growth factors or expression of cell surface adhesion molecules attracting leukocytes, the alteration of NO production is considered to be a decisive disorder in the pathology of the endothelium. NO produced by eNOS has many potentially protective effects within the cardiovascular system and the shortage of NO may induce pathological changes. NO acts as a second messenger converting GTP to cGMP, which decreases intracellular calcium concentration in smooth muscle cells in a cascade of events, which stimulate smooth muscle relaxation and vasodilatation (Sanders *et al.* 1995). As a result it exerts a hypotensive effect *via* peripheral artery dilation, thus decreasing afterload, and through reducing preload by dilatation of venous system (Šimko and Šimko 2000). Venodilatation and arterial relaxation result in a decrease of intraventricular pressure and LV chamber size, resulting in reduction of oxygen consumption. NO-donors also dilate epicardial coronary arteries and prevent epicardial and distal coronary artery vasoconstriction and enhance collateral flow (Abrams 1992). NO exerts its antigrowth potential by the inhibition of mitogenesis and proliferation (Garg and Hassid 1989), as well as of total protein and collagen synthesis in smooth muscle cell culture (Kolpakov *et al.* 1995) and cardiomyocyte growth (Ritchie *et al.* 1998). NO is an important counter-regulatory factor to vasoconstrictors and growth promoting substances, such as angiotensin II and endothelin (Tikannen and Fyhrquist 1995). Moreover, through the cGMP-activated protein kinase, NO decreases intracellular  $Ca^{2+}$  concentration and inhibits platelet aggregation (Gewaltig and Kojda 2002).

### **Potential therapeutic benefit of NO supplementation**

The attributes of the NO molecule and the fact that NO bioavailability is reduced in the above mentioned disorders of the circulatory system, suggests that

treatment with NO-donors (isosorbide mononitrate and dinitrate, nitroglycerin, molsidomin) or precursors of NO formation (L-arginine or L-citrulline) would have therapeutic benefits. Although this presumption has some support in the form of clinical trials, the evidence shows only a limited value of this approach.

#### *NO in myocardial ischemia treatment*

Chronic ischemic heart disease is the oldest indication for the use of NO-donors in cardiology. The theoretical background for the use of NO-donors in chronic myocardial ischemia is their ability to dilate the coronary arteries, thus improving the oxygen supply and to relieve the hemodynamic burden by preload and afterload reduction, thus attenuating oxygen demands. Moreover, the antithrombotic potential of NO may prevent thrombosis.

Although these drugs relieve angina and mitigate myocardial ischemia, the clinical data on the effect of chronic treatment with nitrates on the morbidity and mortality and the progression of coronary artery disease are lacking (Gewaltig and Kojda 2002). The megatrials GISSI-3 (Rovelli *et al.* 1987) and ISIS-4 (Sleight 1994) have shown that nitrates were safe when used routinely after myocardial infarction but without significant mortality reduction. The major aim of the current treatment of stable angina pectoris is to prevent myocardial infarction and death and thereby to extend the length of life. Antiplatelet medications, beta-blockers, statins and angiotensin converting enzyme (ACE) inhibitors have the priority in preventing the death. A second purpose of stable angina treatment is to reduce the symptoms and occurrence of ischemia. Nitroglycerin has an important place for the immediate relief of angina. Long acting nitrates are used only when  $\beta$ -blockers are contraindicated or in combination with  $\beta$ -blockers when  $\beta$ -blockade alone has an insufficient effect (Snow *et al.* 2004, Fox *et al.* 2006).

#### *NO in the treatment of chronic heart failure*

There are three large trials investigating the long-term effects of NO-donors in chronic heart failure. The pre- and afterload reducing effect of NO with improved hemodynamics and energy production within the heart lead to the hypothesis that NO-donors may be beneficial in this serious cardiovascular state. V-HeFTs (Vasodilator Heart Failure Trials) have shown that the combination of isosorbide dinitrate and hydralazine induced a significant decrease in mortality compared to prazosin or placebo (Loeb *et al.* 1993) although

vasodilators were inferior in comparison to enalapril (Francis *et al.* 1993). Similarly, the A-HeFT (African-American Heart Failure Trial) showed that the fixed-dose combination of isosorbide dinitrate and hydralazine in African-American patients with severe heart failure reduced the sum of morbidity and mortality compared to placebo (Taylor 2005, Cheng 2006). Moreover the SENIORS Study (Study of the Effects of Nebivolol Intervention on Outcomes and Rehospitalisation in Seniors Heart Failure) indicated that nebivolol reduced morbidity and mortality in patients older than 70 years with heart failure regardless of the initial ejection fraction. Nebivolol is a highly selective beta-1 blocker with vasodilative properties, which appear to be due to stimulation of NO release (Flather *et al.* 2005). Perhaps the beneficial effect of NO on left ventricular relaxation could contribute to the fact that nebivolol reduced mortality also in patients with preserved systolic function in the SENIORS study (Flather *et al.* 2005). However, no study has investigated the sole use of nitrates in chronic heart failure (Swedberg *et al.* 2005), although NO-donors are routinely used for symptomatic treatment in acute heart failure (Hollenberg 2007).

#### *Treatment of hypertension with NO-donors*

Systolic hypertension, an important risk factor for stroke and myocardial infarction, is often refractory to treatment with established antihypertensive drugs. The presence of a prominent reflection wave in the arterial pulse wave profile of patients with atherosclerotically stiffened conduit arteries suggests that nitrates, directly acting on the arterial wall, could produce endothelium-independent vasorelaxation with a reduction of reflection wave and pulse pressure (Stokes 2006). Moreover, NO, through its antiproliferation effect on fibrocytes, antagonizes the growth effect of angiotensin II-aldosterone system and seems to participate on the prevention or regression of left ventricular hypertrophy (Šimko and Šimko 2000). Isosorbide mononitrate and isosorbide dinitrate were shown to decrease systolic blood pressure in short-term trials of elderly patients, although long-term antihypertensive efficacy of NO-donors has much less support in trials (Stokes and Ryan 1997, Stokes *et al.* 2005). Moreover, no trial was performed to show whether NO-donors reduce cardiovascular events in hypertension. Thus, NO-donors are not recommended for routine use in hypertensive patients in the latest guidelines for blood pressure treatment (Mancia *et al.* 2007), but nitrates may

serve as adjunct antihypertensive therapy in isolated systolic hypertension.

### **What are the potential reasons to skeptics regarding NO delivery in cardiovascular disorders?**

First, NO-donors started to be used in clinical setting decades ago, when pharmacological approach was based more on experience and recommendations of medical authorities than on evidence based medicine. Meanwhile a number of drugs including ACE-inhibitors, AT<sub>1</sub>-receptor blockers, beta-blockers, statins and a number of antihypertensive drugs were proven to reduce cardiovascular morbidity and mortality in ischemic heart disease, heart failure, atherosclerosis and hypertension. Since highly effective treatment is accessible, it is difficult to expect that a trial comparing NO donors with well established cardioprotective drugs will be performed, and comparison with placebo will not be done for ethical reasons.

Second, investigation of NO production is difficult in both experiments and clinical settings. Acetylcholine-induced vessel relaxation, inorganic nitrates in the urine, tissue NOS activity, cGMP concentration and most frequently expression of eNOS and seldomly iNOS at mRNA or protein level have been used to characterize nitric oxide production (Šimko *et al.* 2004). The estimation of vasorelaxation in peripheral arteries seems to be most accessible, reproducible and reliable under clinical conditions; however, it is not routinely available. Thus, our knowledge on endothelial function in a particular patient is generally poor in the clinical setting.

Third, our belief that the improvement of endothelial function may improve prognosis in serious cardiovascular pathologies is associated with the finding that drugs reducing cardiovascular mortality and morbidity, especially blockers of renin-angiotensin-aldosterone system and statins, significantly improve endothelial dysfunction. It was shown that the ACE-inhibitor quinapril improved endothelial function and endothelium-dependent relaxation in coronary (Mancini *et al.* 1996) or peripheral arteries (Anderson *et al.* 1999) and statins improved endothelial function in patients with stable coronary artery disease (Alber *et al.* 2007) and in experimental hypertension in animals (Török *et al.* 2007). One should be aware, however that the improvement of arterial relaxation is more a matter of balance between

vasoconstrictors and vasodilators than of pure enhancement of NO production. Moreover, it is almost impossible to estimate the biological value of improved endothelial function in relation to other well established beneficial effects of cardiovascular drugs, such as their antiremodeling potential, antiarrhythmogenic properties, atherosclerotic plaque stabilizing effects or antihypertensive ability.

## Conclusions

With these facts in mind, many questions emerge: Does the deficit of NO play a substantial or only a minor role in ischemic heart disease, heart failure or essential hypertension? Is NO deficiency a reason or a consequence of these states? Is the amount of NO

produced or the stability of NO molecule against free radicals decisive in cardiovascular pathologies? Under which conditions may NO be harmful? The answers are not simple or unambiguous at the current level of knowledge.

Insufficient NO production is related to cardiovascular pathology with increased mortality, but the impact of therapeutically increased NO bioactivity on morbidity and mortality remains unclear. There is little doubt that NO is king in the circulation, but kings seldom decide the battles.

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