Is NO the King?

Pathophysiological Benefit with Uncertain Clinical Impact

(Editorial)

Fedor Šimko[1,2]

[1]*Department of Pathophysiology, School of Medicine, Comenius University, Bratislava, Slovak Republic,* [2]*3rd Clinic of Medicine, School of Medicine, Comenius University, Bratislava, Slovak Republic*

Running title: The role of NO in experimental and clinical settings

Address for correspondence: Prof. Fedor ŠIMKO, MD, PhD, Department of Pathophysiology, School of Medicine, Comenius University, Sasinkova 4, 81372 Bratislava, Slovak Republic, Tel.: +421-(0)2-59357276, Fax: +421-(0)2-59357601, E-mail: fedor.simko@fmed.uniba.sk

Abstract

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_1 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.

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 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 e NOS has many potentially protective effects within the cardiovascular

system and the shortage of NO may induce pathology. NO acts as a second messenger converting GTP to cGMP, which decreases intracellular calcium concentration in the muscle cells in a cascade of events, in this way stimulating 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 antigrowth potential with 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 counterregulatory 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 in the above mentioned disorders of the circulatory system NO bioavailability is reduced, encourages one to suppose 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 for this approach.

NO in myocardial ischemia treatment

Chronic ischemic heart disease is the oldest indication for NO-donors usage 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 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 long-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 extend the length of life. Antiplatelet medications, beta blockers, statins and angiotensin converting enzyme (ACE) inhibitors have the priority in preventing 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 ß-

blockers are contraindicated or in combination with ß-blockers when β-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 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 Trial) 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 vasodilatative 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 in acute heart failure NO-donors are routinely used for symptomatic treatment (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 stiffed 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 antigrowth activity on cardiomyocytes and 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 (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 for skeptics regarding NO delivery in cardiovascular disorders?

First, NO-donors started to be used in clinical setting decades ago, where 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*1*-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 protective drugs will be performed, and comparison with placebo will not be done for ethical reasons.

Second, investigation of NO production is difficult both in 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 poor in general in the clinical setting.

hird, our belief that 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 (Torok *et al.* 2007). One should be aware, however that the improvement of arterial relaxation is more a matter of balance between vasoconstrictors and vasodilators within the endothelial layer 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.

Conclusion

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.

Acknowledgement: The study was supported by the research grants VEGA 1/3429/06 and APVV 51-027404.

References

ABRAMS J: Mechanisms of action of the organic nitrates in the treatment of myocardial ischemia. *Am J Cardiol* **70**: 30B-42B, 1992.

ALBRECHT EWJA, STEGEMAN CA, HEERINGA P, HENNING RH, VAN GOOR H: Protective role of endothelial nitric oxide synthase. *J Pathol* **199**: 8-17, 2003.

ALBER HF, FRICK M, SUSSENBACHER A, DORLER J, DICHTL W, STOCKER EM, PACHINGER O, WEIDINGER F: Effect of atorvastatin on peripheral endothelial function and systemic inflammatory markers in patients with stable coronary artery disease. *Wien Med Wochenschr* **157**: 73-78, 2007.

ANDERSON T, BANF F: an update. *Can J Cardiol* **15**: 29B-30B, 1999.

CHENG JW: A review of isosorbide dinitrate and hydralazine in the management of heart failure in black patients, with a focus on a new fixed-dose combination. *Clin Ther* **28**: 666-678, 2006.

FOX K, GARCIA MA, ARDISSINO D, BUSZMAN P, CAMICI PG, CREA F, DALY C, DE BACKER G, HJEMDAHL P, LOPEZ-SENDON J, MARCO J, MORAIS J, PEPPER J, SECHTEM U, SIMOONS M, THYGESEN K, PRIORI SG, BLANC JJ, BUDAJ A, CAMM J, DEAN V, DECKERS J, DICKSTEIN K, LEKAKIS J, MC GREGOR K, METRA M, MORAIS J, OSTERSPEY A TAMARGO J, ZAMORANO JL, Task Force on the Management of Stable Angina Pectoris The European Society of Cardiology ESC Committee for Practice Guidelines (CPG). Guidelines

on the management of stable angina pectoris: executive summary: the Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. *Eur Heart J* **27**: 1341-1381, 2006.

FRANCIS GS, COHN JN, JOHNSON G, RECTOR TS, GOLDMAN S, SIMON A. Plasma norepinephrine, plasma renin activity, and congestive heart failure. Relations to survival and the effects of therapy in V-HeFT II. The V-HeFT VA Cooperative Studies Group. *Circulation* **87**: VI40-VI48, 1993.

GARG UC, HASSID A: Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* **83**: 1774-1777, 1989.

GEWALTIG MT, KOJDA G: Vasoprotection by nitric oxide: mechanisms and therapeutic potential. *Cardiovasc Res* **55**: 250-260, 2002.

HOLLENBERG SM: Vasodilators in acute heart failure. *Heart Fail Rev* **12**: 143-147, 2007.

KOLPAKOV V, GORDON D, KULIK TJ: Nitric-oxide generating compounds inhibit total protein and collagen synthesis in cultured vascular smooth muscle cells. *Circ Res* **76**:305-309, 1995.

LOEB HS, JOHNSON G, HENRICK A, SMITH R, WILSON J, CREMO R, COHN JN: Effect of enalapril, hydralazine plus isosorbide dinitrate, and prazosin on hospitalisation in patients with chronic congestive heart failure. The V-HeFT VA Cooperative Studies Group. *Circulation* **87**: VI78-87, 1993.

MANCINI GB, HENRY GC, MACAYA C, O´NEIL BJ, PUCILLO AL, CARERE RG, WARGOVICH TJ, MUDRA M, LÜSCHER TF, KLIBANER MI, HABER HE, UPRICHARD AC, PEPINE CJ, PITT B: Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing Endothelial Dysfunction) Study. *Circulation* **94**: 258-265, 1996.

MANCIA G, DEBACKER G, DOMINICZAK A, CÍFKOVÁ R, FAGARD R, GERMANO G, GRASSI G, HEAGERTY AM, KJELDSEN SE, LAURENT S, NARKIEWICZ K, RUILOPE L, RYNKIEWITZ A, SCHMIEDER RE, STRUIJKER BOUDIER HAJ, ZANCHETTI A. 2007 Guidelines for the management of arterial hypertension. The task for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* **28**: 1462-1536, 2007.

RAKHIT RD, MARBER MS: Nitric oxide: an emerging role in cardioprotection? *Heart* **86**: 368-372, 2001.

RITCHIE RH, SCHIEBINGER RJ, LAPOINTE MC, MARSH JD: Angiotensin II-induced hypertrophy of adult rat cardiomyocytes is blocked by nitric oxide. *Am J Physiol* **275**: H1370-H1374, 1998.

ROVELLI F, DE VITA C, FERUGLIO GA, LOTTO A, SELVINI A, TOGNONI G AND GISSI INVESTIGATORS: GISSI trial: early results and late follow-up. *J Am Coll Cardiol* **10**: 33B-39B, 1987.

SANDERS PW, CHEN PY, GASTON RS, WARNOCK DG: L-arginine and nitric oxide in the regulation of blood pressure. In: *Hypertension: Pathophysiology. Diagnosis and Management* LARAGH JH, BRENNER BM (eds). Raven Press, New York, 1995, pp 1097-1098.

ŠIMKO F, ŠIMKO J: The potential role of nitric oxide in the hypertrophic growth of the left ventricle. *Physiol Res* **49**: 37-46, 2000.

ŠIMKO F, MATÚŠKOVÁ J, ĽUPTÁK I, KRAJČÍROVIČOVÁ K, KUCHARSKÁ J, GVOZDJAKOVÁ A, BABÁL P, PECHÁŇOVÁ O: Effect of simvastatin on remodeling and aorta in L-NAME-induced hypertension. *Life Sci* **74**: 1211-1224, 2004.

SLEIGHT P: Vasodilators after myocardial infarction ISIS IV. *Am J Hypertens* **7**: 102S-105S, 1994.

SNOW V, BARRY P, FIHN SD, GIBBONS RJ, OWENS DK, WILLIAMS SV, MOTTUR-PILSON C, WEISS KB, FOR THE AMERICAN COLLEGE OF PHYSICIANS, AMERICAN COLLEGE OF CARDIOLOGY CHRONIC STABLE ANGINA PANEL: Primary care management of chronic stable angina and asymptomatic suspected or know coronary artery disease: A clinical practice guideline from the American College of Physicians. *Ann Intern Med* **141**: 562-567, 2004.

STOKES GS: Nitrates as adjunct hypertensive treatment. *Curr Hypertens Rep* **8**: 60-68, 2006.

STOKES GS, RYAN M: Can extended-release isosorbide mononitrate be used as adjunctive therapy for systolic hypertension? An open study

employing pulse-wave analysis to determine effects of antihypertensive therapy. *Am J Geriat Cardiol* **6**: 11-19, 1997.

STOKES GS, BUNE AJ, HUON N, BARIN ES: Long-term effectiveness of extended-release nitrate for the treatment of systolic hypertension. *Hypertension* **45**: 380-384, 2005.

SWEDBERG K, CLELAND J, DARGIE H, DREXLER H, FOLLATH F, KOMAJDA M, TAVAZZI L, SMISETH OA, GAVAZZI A, HAVERICH A, HOES A, JAARSMA, KOREWICKI J, LÉVY S, LINDE C, LOPEZ SENDON JL, NIEMINEN MS, PIÉRARD L, REMME WJ: Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005). The task force for the diagnosis and treatment of chronic heart failure of the European society of cardiology. *Eur Heart J* **26**: 1115-1140, 2005.

TAYLOR AL: The African American heart failure trial: a clinical trial update. *Am J Cardiol* **96**(7B):44-48, 2005.

TIKKANEN I, FYHRQUIST F: Nitric oxide in hypertension and renal diseases. *Ann Med* **27**: 353-357, 1995.

TÖRÖK J, MATÚŠKOVÁ J, PECHÁŇOVÁ O, KRAJČÍROVIČOVÁ K, ŠIMKO F: Comparison of the effect of simvastatin, spironolactone and L-arginine on endothelial function of aorta in hereditary hypertriglyceridemic rats. *Physiol Res* **56** (Suppl 2): S00-S00, 2007.