

# Nitrates: new insights into old drugs

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**ABSTRACT:** Nitric oxide (NO), a highly unstable gas with a half-life of a few seconds, is the most important mediator produced by endothelial cells and has several important protective functions concerning the control of vascular tone, thrombogenesis, fibrinolysis, cell proliferation, inflammation and endothelial permeability, which are crucial for the maintenance of an anti-inflammatory, anti-atherogenic and anti-thrombotic state. The exogenous administration of NO with nitrates has been proposed as possible NO exogenous sources in endothelial dysfunction therapy and coronary artery disease because of their anti-atherosclerotic effect, through a reduction in oxidative stress, on low-density lipoprotein (LDL) oxidation, chemotactic molecules and growth factors, which mediate leukocytal adhesion and proliferation of vascular smooth cells. Moreover, nitrates show a vasodilator effect on the venous, arterial and coronary region with a consequent reduction in preload, afterload and an improvement in myocardial energetic metabolism. Due to these favorable effects nitrates are indicated not only in angina pectoris, acute myocardial infarction and pulmonary hypertension or hypertension crisis, but also in systolic and diastolic heart failure, in the prevention of ischemic and reperfusion damage after IMA or PCI and in the prevention of post-infarct remodeling. Moreover, recent data concerning the protective effects of nitrates on endothelial function, suggest their possible use in the prevention of endothelial damage and atherogenesis. (*Heart International* 2007; 3: 69-77)

**KEY WORDS:** Nitrates, Nitric oxide, Effects, Clinical indications

## INTRODUCTION

Organic nitrates that have been used for the treatment of the ischemic cardiopathy for over 100 yrs, are still the most commonly prescribed and used drugs in ischemic patients. The rationale for the use of these drugs is their vasodilatation effect on the venous, arterial and coronary district, confirmed by several studies and by years of clinical practice (1, 2). These favorable assumptions have been confirmed by expert knowledge of the role of endogenous nitric oxide (NO) in cardiovascular homeostasis, both from a physiologic and a physiopathologic point of view.

## ENDOGENOUS NITRIC OXIDE: BIOCHEMICAL ASPECTS

NO, a highly unstable gas with a half-life of a few seconds is synthesized from L-arginine by the oxidation of the guanidine-nitrogen terminal, a reaction catalyzed by the family of NO synthase (NOS). NOS is a dimeric hemoprotein, which has reductase and oxygenase catalytic domains and recognition sites for nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN). Catalytic activity of NOS requires other cofactors as calcium, calmodulin, heme and the tetrahydrobiopterin (BH4) (3). Studies have demonstrated two constitutive calmodulin

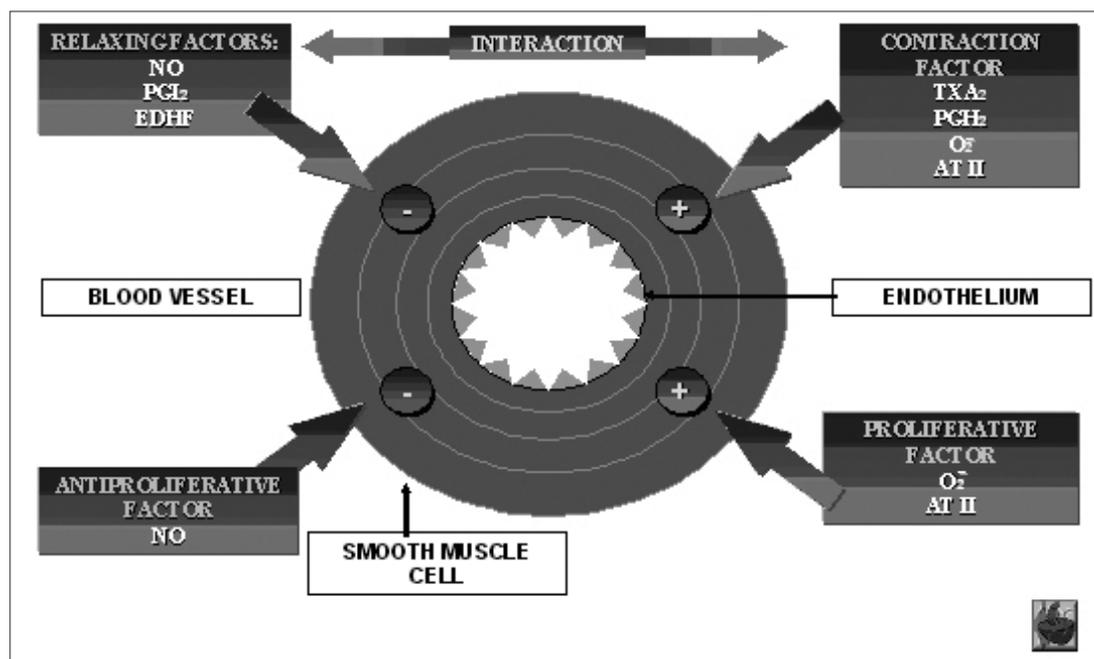


Fig. 1 - Vasoactive mediators released by the endothelium.

dependent NOS (cNOS): one is in the endothelium (eNOS), the other is set in the central and peripheral neurones, human skeletal muscle, B cells of pancreatic isles, the uterus and the stomach. Both cNOS types are stimulated by the intracellular calcium rise and calmodulin link and produce a small amount of NO over a few minutes.

The main stimulus producing NO is shear forces generated by blood circulation (4). Shear stress determines a tonic NO release, synchronous with pulsated blood flow, which is essential to maintain the basal vascular tone. Furthermore, phasic NO production is stimulated by neurotransmitters, hormones and substances derived from platelets and from the coagulation system such as acetylcholine, catecholamines, histamine, vasopressin, P substance, bradykinin, thrombin, ADP and serotonin (5, 6).

A second NOS isoform has also been found, the inducible NOS (iNOS), which is calcium calmodulin independent and can generate large NO quantities over several days (7, 8), until substrate or cofactor depletion is verified (9). iNOS expression is stimulated by bacterial endotoxin (lipopolysaccharides) and pro-inflammatory cytokines (IL-1, TNF- $\alpha$ , INF- $\gamma$ ): NO directly contributes to eliminate pathogenic agents and induces vasodilatation supporting an immune response. Endothelial cells and inflammatory cells produce superoxide anion (O<sub>2</sub><sup>-</sup>),

which reacts with NO, producing peroxynitrite (OONO<sup>-</sup>), a potent oxidizing and bactericidal agent (10).

Endothelium relaxation due to NO involves the synthesis of cyclic 3'5' guanosine monophosphate (cGMP) in smooth muscle cells (SMC), via the soluble enzyme guanylyl cyclase, with a consequent reduction in cytosolic free calcium and in myosin light chain phosphorylation (11, 12).

#### PHYSIOLOGIC ROLE OF ENDOGENOUS NITRIC OXIDE

The endothelium, once considered a simple covering of vessels, is an "endocrine organ" that, through the production of several mediators, plays a primary role in homeostasis and cardiovascular physiopathology. The modulation of vascular tone and the maintenance of the physiological structure of the vessels' wall depend on the balance between the production of vasodilator and antiproliferative factors (NO, PGL<sub>2</sub>, EDHF) and substances acting inversely (endothelin, angiotensin, O<sub>2</sub><sup>-</sup>) (Fig. 1).

NO develops several important protective functions on vascular tone control, thrombogenesis, fibrinolysis, cells proliferation, inflammation and endothelial permeability (3), which are crucial for the maintenance of an anti-inflammatory, anti-atherogenic, anti-thrombotic state.

Experimental evidence confirms the primary role of NO in the maintenance of the basal vascular tone and in the vasodilatation induced by physical and chemical factors. Some experiments *in vitro* on coronary artery sections have proved that L-arginina analogous (L-NMMA) administration, inhibiting basal NO production, induces vasoconstriction and inhibits acetylcholine vasodilator action (12, 13).

In addition *in vivo*, intravenous (i.v.) administration of L-NMMA determines an increase in the systemic pressure both in animals and in humans, with no significant pressure modification in the venous district. Studies in healthy subjects have proved direct vasoconstriction and the inhibition of endothelium mediated relaxation, induced by acetylcholine and bradykinin, in the brachial artery during L-NMMA infusion (12).

NO is an important antiproliferative factor and is crucial in the maintenance of the vascular structure. It was observed that the mechanical removal of the endothelium through a balloon catheter causes an intimal hyperplasia, mediated by growth factors released from platelets and monocytes (14).

Experiments *in vivo*, in which the eNOS gene was transferred into vascular walls damaged by a PCI in the carotid artery, showed not only a recovery of NO production and endothelium mediated relaxation, but even an inhibition of the neointimal production. Therefore, endothelial dysfunction, with decreased NO production is an initial step in the atherosclerotic process, and it contributes to post-PCI restenosis (15, 16).

The antiatherogenic effect of NO is performed through several mechanisms, well documented by studies *in vitro* in endothelial and SMC cultures; NO reduced LDL oxidation (through free radical scavenging), chemotaxis and the adhesion of monocytes to endothelial cells and SMC proliferation (14-18).

Most interventions attempting to improve endothelial dysfunction have targeted one or more risk factors that can cause endothelial damage (low NO availability): hypertension, hypercholesterolemia, diabetes mellitus, cigarette smoking, sedentary lifestyle and the menopause (19-23). In these conditions, endothelial dysfunction is due to an increased NO inactivation rather than a reduced synthesis (24). Superoxide anions reacting with NO produce  $OONO^-$  that oxidizes lipids, protein and non-protein SH groups and damages membrane cells. However, superoxide anions also reduce

NO bioavailability necessary for the maintenance of homeostasis.

In addition, NO has an antiaggregant and antithrombotic effect. NO release towards the vessel lumen inhibits platelet adhesion to the endothelium and avoids thrombus formation (25-27); platelet adhesion, through serotonin and ADP release, induces a local NO production with vasodilatation, useful in microaggregate removal. Therefore, endothelial dysfunction and reduced NO bioavailability, induce vasoconstriction and thrombosis, and, consequently, ischemic events.

## EFFECTS OF NITRATE ADMINISTRATION

Organic nitrates, such as NO group donors, have been proposed as possible NO exogenous sources in endothelial dysfunction therapy and coronary artery disease. Nitrates act even on atheromatous vessels, where NO cannot be generated by damaged endothelial cells, inducing the same endogenous molecular effects (Fig. 2).

In addition, evidence refers to an increase in endogenous NO during treatment with nitrate derivatives. In patients with heart failure (HF), Scharzw et al (28) described an increase in vasodilatation acetylcholine mediated, during nitroglycerin infusion at hemodynamic inactive doses, therefore, suggesting a possible favorable interaction of exogenous NO with the endogenous NO production by the endothelium.

### *Effects on endothelium*

Exogenous NO administration with nitrates, such as endogenous NO, has a strong anti atherosclerotic effect. This is due to a reduction in oxidative stress, LDL oxidation, chemotactic molecules and growth factors that mediate leukocytal adhesion and proliferation of vascular smooth cells. As proved by studies on endothelial cells *in vitro*, the administration of NO donors reduces the chemotaxis of monocytes induced by the increase of chemotactic factors (VCAM-1, MCP-1), after lipopolysaccharide stimulation (18).

It was observed that the administration of nitrate derivatives can inhibit the proliferation of vascular smooth cells as endogenous NO. Studies on vascular SMCs of human coronaries confirmed this antiproliferative effect:

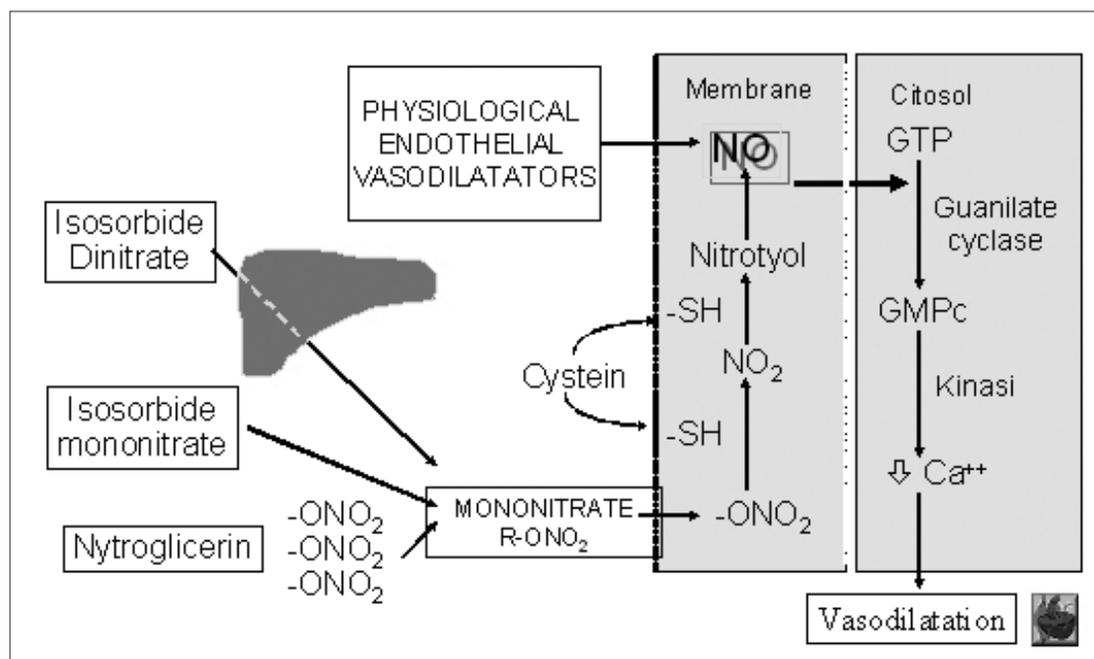


Fig. 2 - Mechanism of action of nitrates.

the incubation with increasing concentration of NO donors causes a progressive reduction in cell proliferation, evidenced by a reduction in the incorporation of titrated thymidine, a cell mitosis marker (17).

This seems particularly relevant for the maintenance of the normal vascular structure, as well as for limiting damage in atherosclerosis and for the prevention of neointimal formation, after the revascularization procedure through PCI. In addition, experimental studies on animals genetically susceptible to atherosclerosis, subjected to a high lipidic diet, proved the protective effect of the administration of high-dose nitrates on the development of atheromatous plaques, probably through an antioxidative mechanism (29).

NO administration might contribute to a major stabilization of the atheromatous plaque, supporting the formation of a thicker fibrous cap. Experimental studies on cells in vitro showed an increased production of collagen type I and III in vascular SMCs, induced by NO donors (14).

In the 1990s, several studies were performed to evaluate the effect of nitrate infusion on platelet aggregation. After 45 min of nitroglycerin infusion a 50% reduction was observed in platelet aggregation in response to ADP and thrombin. Fifteen minutes after the infusion, the platelets response was normal. The studies reported above documented a significant and reversible effect

of nitroglycerin at therapeutic doses on platelet function (25-27). This evidence explains the importance of these drugs for therapy and the prevention of acute coronary syndromes. Murry et al, first described the phenomenon of early ischemic preconditioning, demonstrating how short ischemic periods induced by short close/open cycles of a coronary vessel, can reduce the extent of myocardial infarction secondary to occlusion of the same vessel, probably due to the protective effect of endogenous NO release (30). Other studies on animals with ischemic damage confirmed that the administration of L-arginine and the consistent increase in the NOS reduce reperfusion myocardial damage.

Even the administration of NO donors has similar protective effects: several experimental studies have shown that early treatment with nitroglycerin, 24 hr before myocardial ischemia, significantly reduced the extent of the infarcted area (30).

This evidence has also been confirmed by studies on ischemic patients undergoing PCI. Leeser et al, confirmed the myocardial protective effect of ischemic preconditioning induced by nitrates on 66 patients randomized to receive an endovenous infusion of nitroglycerin or saline solution before PCI (31). Patients treated with nitroglycerin showed less frequently a significant modification of the ST segment and myocardial contractility in comparison with the placebo group.

### *Hemodynamic and anti-ischemic effects*

Nitrates can induce a maximal vasodilatation in capacitance venous vessels, even with a low drug concentration, while their effect in the arterial and arteriolar district is dose-related (2).

The action on the venous system is responsible for a reduction in preload, ventricular volumes and filling pressures, and consequently in myocardial oxygen consumption. The improvement in ventricular relaxation and compliance cause an additional reduction in the telediastolic left ventricular pressure. Furthermore, nitrates improve diastolic function through an increase in myocardial perfusion, especially in patients with diastolic dysfunction due to sub-endocardial ischemia.

In patients with severe left ventricle dilatation and high right atrial pressure, nitrates can improve ventricular filling through the reduction of atrial, coronary sinus, and intramyocardial venous and interstitial pressures and a reduction in right ventricle volume (32).

Furthermore, nitrates induce vasodilatation in the arterial district with an increase in aortic and arterial vascular compliance and a reduction in afterload. Several studies conducted in chronic heart failure (CHF) patients, demonstrated a significant increase in aortic distensibility and a reduction in systemic vascular resistance (with a consequent increase in peripheral blood flow), after i.v. nitrate infusion (33). The result is an improvement in myocardial performance, particularly in patients with left ventricular systolic dysfunction, related to a reduction in left ventricular filling pressures (preload) and to a low increase in cardiac output, caused by a reduction in systemic vascular resistances, in aortic impedance and degree of mitral regurgitation (afterload reduction).

Nitrates also improve myocardial energetic metabolism either increasing coronary flow (due to an increase in myocardial perfusion related to a direct vasodilation, to a reduction in ventricular pressure higher than diastolic aortic pressure and an increase in diastolic perfusion time) or either decreasing myocardial O<sub>2</sub> consumption (due to a reduction in the diastolic stress wall, mediated by a reduction in end-diastolic ventricular volume and pressure). These effects optimize subendocardial perfusion and oxygenation in ischemic and HF.

Finally, acute and chronic nitrate administration improves exercise tolerance, systemic and coronary blood flow, either in ischemic or in CHF patients (33).

### INDICATIONS

Favorable effects induced by nitrates on the arterial, venous and coronary districts and on myocardial performance explain several clinical indications. In addition to common indications, such as angina pectoris, acute myocardial infarction, pulmonary hypertension and hypertension crisis, nitrates can also be used with beneficial effects in systolic and diastolic HF, in the prevention of ischemic and reperfusion damage after IMA or PCI and in the prevention of post-infarctual remodeling. Moreover, the latest data concerning the protective role of NO and endothelial dysfunction extend the use of nitrates to the prevention of endothelial damage.

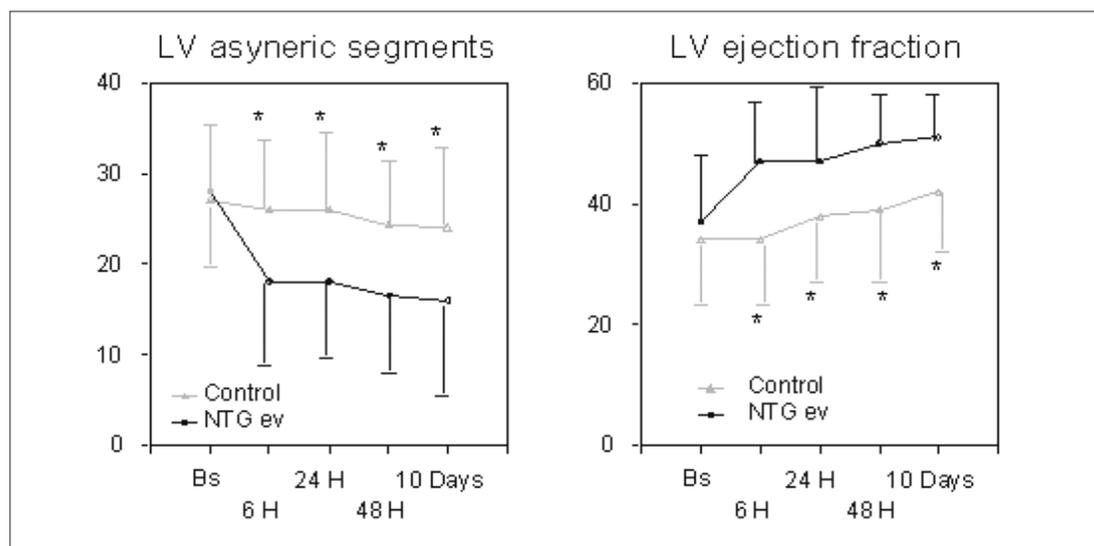
#### *Angina pectoris*

Sublingual uptake of short-action nitrates (for example, nitroglycerin, isosorbide dinitrate), due to coronary vasodilatation effects, is indicated for the treatment and prevention of angina in ischemic patients.

Guidelines on stable angina show that long-action nitrates (for example, transdermic and oral formulation) are indicated, in association with beta-blockers and calcium-antagonists, for the prevention of ischemic events, for the improvement of exercise tolerance and for the reduction in ST depression degree during exercise tests (34). Nitrate tolerance in chronic administration can be resolved by use of minimal effective dose and correct "therapeutics windows".

#### *Acute coronary syndrome*

Intravenous nitrates are the most common drugs in the treatment of acute myocardial infarction and unstable angina. In the first phases of acute coronary syndrome (ACS), nitrates have anti-ischemic effects and reduce the extension of the necrosis area, through their ability to increase coronary blood flow, vasodilating stenotic vessels and opening collateral circles (35), and to limit reperfusion damage (30, 31, 36). In myocardial ischemia nitrates increase the blood flow in stenotic vessel areas and improve blood flow redistribution from the epicardial to the endocardial (35). These effects on the myocardial O<sub>2</sub> need-demand balance, along with antiaggregant effects and protection towards myocardial stunning are very useful in all clinical manifestations of ischemic cardiopathy (stable and unstable angina,



**Fig. 3** - Effects of i.v. administration of nitroglycerin on left ventricular remodeling after infarction (42).

Prinzmetal angina, AMI) (37, 38).

Moreover, early nitrate administration in patients with an acute myocardial infarction reduces the expansion and the thinning of asynergical segments, to limit non-infarctuated myocardial hypertrophy and progressive ventricular dilatation (post-infarctual remodeling). These effects are due both to infarct size limitation and to the reduction in ventricular afterload and wall stress (39-42). The remodeling prevention or limitation reduces post-infarctual complications (HF, cardiogenic shock, aneurysmatic evolution) (Fig. 3).

In addition, nitrates prevent reflow damage. Positive prognostic effects of several clinical studies, performed in the pre-thrombotic era on small case reports, have not been confirmed by more recent trials. Among these ISIS-4 and GISSI-3 demonstrated a significant reduction in events and mortality, with nitrates and ACE-inhibitor association in comparison with ACE-inhibitor alone, particularly in high risk patients and in patients with CHF, angina and hypertension (43-46).

In AMI, the stenotic coronary reopening can prevent or limit necrosis, but reflow can also increase the damaged areas (reflow myocardial insult). After myocardial ischemia, reflow determines an increased production of oxygen free radicals through several endothelial oxidative enzymes, which leads to an increase in calcium and in phospholipase C endothelial permeability. These events induce the expression of several molecules such as piastriinic factors, adhesive molecules, thromboxane, leukotriene and pro-inflammatory factors. Neutrophil

infiltration in reflow tissue produces superoxide anions and necrosis. Endogen NO reduction seems to play a role in reflow damage. Endothelial relaxation and  $O_2^-$  reduction after L-arginina administration was observed. A deficit in L-arginina after myocardial ischemia increases the production of superoxide anions mediated by e-NOS; this process, associated to  $O_2^-$  degradation, is supposed to reduce endogen NO bioavailability (40). The use of NO donors can limit reflow damage and reduce the number of neutrophils in reflowed myocardial tissue: endogen NO would be unable to prevent ischemic damage; and therefore, esogenous NO administration could be a therapeutic option.

### Systolic and diastolic heart failure

ESC guidelines indicate the use of nitrates in patients with systolic and diastolic HF, because of their beneficial effect on preload, afterload, ventricular relaxing, ischemia prevention and mitral regurgitation (47).

Chronic nitrate therapy is useful in post-infarctual cardiomyopathy and also in HF secondary to recurrent myocardial ischemia (EPA, angina, dyspnea). Another therapeutic target is reducing symptoms in patients with diastolic HF, through a reduction in ventricular filling pressure without significant effects on cardiac output.

Another indication is systolic HF (II-III NYHA class) in the case of ACE-inhibitor intolerance or inefficiency. The Vasodilator Heart Failure Trial (V-HeFT I and V-HeFT II) demonstrated that hydralazine-isosorbide dinitrate asso-

ciation is a good alternative to ACE-inhibitors. This trial demonstrated major efficacy of ACE-inhibitors on survival, while nitrate-hydralazine association is more effective in improving exercise tolerance (48).

A following analysis of the same trial demonstrated that hydralazine-isosorbide dinitrate is more effective in black patients who are known to have poor response to ACE-inhibitors due to less endogen NO bioavailability and less SRA activation (49-51).

Conversely, a recent trial in African-American HF patients showed that hydralazine-nitrates associated to conventional therapy have a beneficial survival effect. In this study, there was a significant reduction in primary endpoint (total mortality + first HF hospitalization and quality of life score) in treated patients vs. placebo group: total mortality reduction was 43% ( $p=0.01$ ), HF hospitalization reduction was 33% ( $p<0.001$ ) and improvement in quality of life score ( $p=0.02$ ) (52) (Fig. 4).

This study suggests that hydralazine-nitrates association can reduce HF progression and improve survival in black patients, in contrast with the results of previous multicentric trials performed in heterogeneous populations where different genetic and environmental factors may influence the progression of HF and therapy response.

## CONCLUSIONS

Currently, nitrates are commonly used in clinical practice because of their antiatherosclerotic, antithrombotic and anti-ischemic properties. These substances have been used in angina pectoris, acute myocardial infarction and hypertensive crisis therapy for their beneficial coronary and hemodynamic effects, for over 100 yrs. Recently due to better knowledge about their pleiotropic effects, nitrates show an increase in their clinical indications, such as systolic and diastolic HF, prevention of reflow damage either in acute myocardial infarction or during PCI.

Furthermore, nitrates' protective effects on endothelial function suggest their possible application in the prevention of endothelial damage and atherogenesis.

Nitrate tolerance expressed by a reduction in positive effects and the onset of adverse events can be resolved by use of retard formulations able to ensure a slow and progressive increase in the blood concentration of the drug. The administration of high doses of IS-5-MN

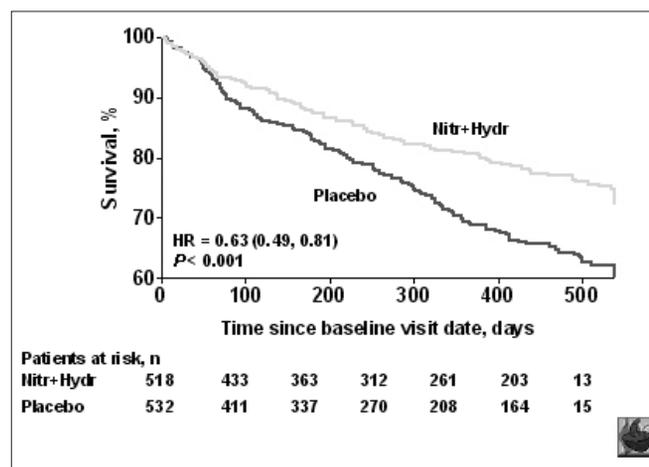


Fig. 4 - African-American heart failure trial: primary endpoint reduction (mortality + HF hospitalization) (52).

induces an antiplatelet effect also in cases of tolerance, resulting in protection of patients at high risk for ischemic events (53). Recent studies demonstrated that the organic nitrates with low potency (dinitrates and mononitrates), which develop little or no tolerance, are probably less bioactivated by ALDH-2, compared with trinitrates and tetranitrates (54, 55). In Italy there are different retard formulations of IS-5-MN, and a higher dose of this mononitrate is available in 80-mg slow release capsules.

There are still important unanswered questions. One of the most important one is related to the effects on cardiovascular morbidity and mortality with the chronic administration of nitrates, and whether these effects can be influenced by the association with ACE-inhibitors, beta-blockers and anti-oxidative drugs.

The successful results on HF hospitalization and mortality, derived from a recent trial in black patients, suggests the possible use of this treatment in specific subgroups of patients. Further genetic and molecular studies could supply important information about pharmacological optimization and therapy individualization.

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