Nitric oxide mechanisms of nebivolol

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Abstract: β-blockers are among the most widely used drugs in the prevention and treatment of cardiovascular disease, although they are associated with increased peripheral resistance. Third-generation β-blockers avoid this adverse effect by inducing vasodilation through different mechanisms. In particular, nebivolol, a highly selective blocker of β1-adrenergic receptors, is the only β-blocker known to induce vascular production of nitric oxide, the main endothelial vasodilator. The specific mechanism of nebivolol is particularly relevant in hypertension, where nitric oxide dysfunction occurs. Indeed, nebivolol is able to reverse endothelial dysfunction. Nebivolol induces nitric oxide production via activation of β3-adrenergic receptors, which can explain the good metabolic profile observed after treatment with this drug. Moreover, nebivolol can also stimulate the β3-adrenergic receptor-mediated production of nitric oxide in the heart, and this stimulation can result in a greater protection against heart failure. In conclusion, nebivolol has a unique profile among antihypertensive drugs, adding to a very high selectivity against β1 adrenergic receptors, and an agonist action on β3 receptors and nitric oxide (NO), which has led to clinically significant improvements in hypertensive patients.

Keywords: Nebivolol, nitric oxide, vessels, heart, beta1-adrenergic receptors, beta3-adrenergic receptors

Introduction

β-blockers are among the most widely used drugs in the prevention and treatment of cardiovascular disease. Randomized controlled trials have demonstrated their efficacy in preventing cardiovascular events and improving survival in patients with hypertension, heart failure, diabetes and other cardiovascular diseases. Their main mechanism of action consists in blockade of cardiac β1-adrenergic receptors, which decrease the force and rate of contraction of the heart, with consequent reduction in arterial blood pressure and in cardiac load. Moreover, blockade of renal β1-adrenergic receptors inhibits the release of renin, with consequent reduction in angiotensin II and aldosterone plasma levels. They also have central effects, lowering total sympathetic activity.

The first β-blocker, propranolol, has been in clinical practice for over 40 years. Although it has been shown to be beneficial in hypertensive patients, it has also been associated with several side effects, mainly due to its blockade of β2-adrenergic receptors, particularly at respiratory level. Therefore, new β-blockers with greater selectivity for the β1-adrenergic receptor and a lower incidence of side effects have been developed (second generation β-blockers). However, the same blockade of β1-adrenergic receptors can lead to undesirable effects. For example, β1-adrenergic receptors are important for lipolysis in adipocytes, and β-blockers can increase lipemia. Still more relevant to hypertensive conditions, blockade of these receptors leads to vasoconstriction and consequently to an increase in vascular resistance. Thus, pharmacological research on this class of drugs has continued, in a search for β-blockers with the additional property of determining peripheral vasodilation. These are the third-generation β-blockers. Several of these compounds have been developed independently, and they differ in the molecular mechanisms by which they stimulate vasodilation.

The most used and most studied third-generation β-blocker to date is carvedilol, which is able to induce vasodilation by also blocking α1-adrenergic receptors. Randomized controlled clinical trials have shown that carvedilol can reduce mortality in patients with heart failure [Packer et al. 1996], and that it can do so more than traditional β-blockers [Poole-Wilson et al. 2003]. However, carvedilol has a limitation, in that it is not selective for β1-adrenergic receptors.
Expectedly, $\beta_2$-adrenergic related side effects, like dizziness and fatigue, have been associated with chronic treatment with carvedilol, and these side effects can lead to discontinuation of treatment [Lainscak et al. 2007].

Actually, in searching for the additional property of vasodilation, developers of third-generation $\beta$-blockers have often overlooked the importance of $\beta_1$-selectivity, so much so that only a few third-generation $\beta$-blockers have retained this characteristic. Nebivolol is the third-generation $\beta$-blocker with the greatest selectivity for $\beta_1$-adrenergic receptors, and thus for cardiac effects. Its efficacy in heart failure patients is similar to that observed with carvedilol [Lombardo et al. 2006]. In particular, it is the only $\beta$-blocker whose efficacy has been specifically demonstrated in elderly patients with heart failure, in which nebivolol reduces mortality and cardiovascular hospital admission [Edes et al. 2005; Flather et al. 2005]. At vascular level, nebivolol is able to reduce peripheral vascular resistance in hypertensive patients, after both acute [Dawes et al. 1999] and chronic [Himmelmann et al. 1996] administration. More than this, chronic treatment with nebivolol is able to reverse endothelial dysfunction, the typical trait of hypertensive patients consisting in a lower nitric oxide (NO) response to vasodilation-inducing agonists [Tzemos et al. 2001]. Nebivolol vasodilation, as opposed to that determined by carvedilol, is independent from $\alpha$-adrenergic receptors [Gao et al. 1991].

**Nebivolol and vascular nitric oxide**

So, how does nebivolol induce vasodilation? It is now clear that nebivolol exerts this action by stimulating production of NO, the main endothelial vasodilator. In fact, vasodilation to nebivolol is blocked by inhibitors of NO synthase (NOS), suggesting that stimulation of endogenous NO production is fundamental for the hemodynamic action of the drug. The first studies on nebivolol mechanisms of vasodilation were performed in isolated animal vessels, and showed that it depends on endothelial NO production [Gao et al. 1991]. Nebivolol vasodilation was blunt by the absence of endothelium and by a NOS inhibitor, but not by a COX inhibitor or a serotonin inhibitor. Moreover, it was not inhibited by an $\alpha$-adrenergic receptor antagonist, thus clarifying that nebivolol action differs from that of carvedilol. The importance of NO has been subsequently confirmed in patients. Venodilation to nebivolol, assessed by the dorsal hand vein technique, was blocked by NOS inhibitors [Bowman et al. 1994]. Moreover, also in this work on patients, the influence of $\alpha$-adrenergic receptors was ruled out. The same results were obtained in arterial circulation [Dawes et al. 1999; Cockcroft et al. 1995], thus showing that nebivolol is able to vasodilate via NO in all vascular districts. Increased NO production after treatment with nebivolol was associated with an increased activity of the endothelial isoform of NOS (eNOS), suggesting that nebivolol can induce vasodilation by stimulating this enzyme in endothelial cells [Parenti et al. 2000].

The evidence that nebivolol can induce vascular NO production is not just inferred by studies with inhibitors, which have obvious limitations. Actual NO production in vascular tissues has been observed after stimulation with nebivolol [Maffei et al. 2006] (Figure 1A). Increasing doses of nebivolol, inside the therapeutical range, increases NO production. Induction of NO production is, as expected, independent from $\beta_1$ blockade, and is not shared by other drugs of the same class.

As already mentioned, vascular NO production has an acute vasodilatory effect, leading to a greater arterial distensibility and a reduced peripheral resistance, as opposed to the increased arterial stiffness and peripheral resistance observed with other $\beta$-blocking agents. This adverse effect of traditional $\beta$-blockers is particularly severe in hypertensive patients, which already suffer of endothelial dysfunction; that is, a reduced dilation to endogenous agonists. It is
noteworthy to emphasize that the benefits of NO release by nebivolol in hypertensive patients are not limited to the acute phase, since acute NO-mediated vasodilatory action of nebivolol is also present during chronic treatment [Cosentino et al. 2001]. A consequence is that chronic treatment has been associated to a rescue of hypertension-induced endothelial dysfunction in a clinical trial [Tzemos et al. 2001]. The same observation has been repeated in patients with coronary artery disease [Lekakis et al. 2005], a particularly high-risk population. In both cases, only nebivolol, but not the traditional β-blocker atenolol, was able to improve endothelial function.

On the other hand, the long-standing action of nebivolol can be related to a particular observation, namely that nebivolol can maintain its NO-inducing effect after metabolism. In fact, nebivolol is metabolized mainly by the liver, and the main hepatic metabolites of the drug are still able to induce NO production, albeit at slightly lower levels [Maffei et al. 2006].

**Nebivolol and vascular function besides NO production**

The vascular response to NO does not depend only from its production, but also from its catabolism by reactive oxygen species (ROS), which leads to the formation of the damaging compound peroxynitrite. In fact, the decreased vascular function in hypertension seems due mostly to an excessive oxidative stress, in spite of a paradoxically increased NO production [Maffei et al. 2002]. Several lines of evidence show that nebivolol can also act as an antioxidant agent, and that this pharmacological action cooperates with the induction of NO production in contrasting vascular dysfunction. Early results obtained in animal models and in endothelial cell cultures have demonstrated that nebivolol reduces the concentration of superoxide anion, the oxidant radical most involved in NO catabolism [Mollnau et al. 2003; Cominacini et al. 2003]. The antioxidant properties of nebivolol have been subsequently confirmed in patients. In hypertensive patients, nebivolol but not atenolol, was able to decrease plasma markers of oxidative stress [Fratta Pasini et al. 2005]. This effect is particularly evident in black patients, who have increased oxidative stress and vascular dysfunction [Mason et al. 2005].

Nebivolol action on free radicals seems related to a direct ROS-scavenging action of the drug, since nebivolol concentration decreases after exposure to ROS [de Groot et al. 2004]. This hypothesis is further supported by the finding that both nebivolol enantiomers are able to reduce ROS formation [Evangelista et al. 2007]. Moreover, some hepatic metabolites of nebivolol exert the same action. It should be noted that the benefits of a decreased oxidative stress are not limited to fighting vascular dysfunction. In fact, nebivolol (but not a different β-blocker such as atenolol) has been shown to reduce the expression of human genes upregulated by oxidative stress and involved in atherosclerosis [Garbin et al. 2008]. Moreover, the decreased oxidative stress obtained with nebivolol treatment leads to a reduction in peroxynitrites [Mason et al. 2005]. Finally, the antioxidant action of nebivolol could be involved in its ability to reduce apoptosis in myocardial infarction [Mercanoglu et al. 2008].

Nebivolol affects the levels of still another molecule, which inhibits NO production and is associated with vascular dysfunction: asymmetric dimethylarginine (ADMA). Also in this case, early results have been obtained in cultured endothelial cells, where nebivolol decreases ADMA levels by increasing its catabolism by the enzyme DDAH2 [Garbin et al. 2007]. These results have been confirmed in hypertensive patients, in whom nebivolol can decrease ADMA levels and improve vascular function, whereas the traditional β-blocker atenolol cannot [Fratta Pasini et al. 2008]. Nebivolol is more beneficial on ADMA levels in hypertensive diabetic patients also when compared to another β-blocker, metoprolol [Oguz et al. 2007].

**Vascular NO production is responsible for nebivolol effects on different organs**

The above-described data clearly indicate that Nebivolol can improve vascular function by inducing NO production and reducing substances opposing NO action (Figure 2). Thus, nebivolol leads to a reduced peripheral resistance, which is involved in the antihypertensive effect of the drug. However, nebivolol action on NO production does not limit its impact to peripheral resistance.

Induction of NO production by nebivolol has been shown to decrease kidney perfusion pressure [Kakoki et al. 1999]. Consequently, nebivolol can increase renal plasma flow and glomerular filtration rate [Greven and Gabriëls, 2000]. These effects are dose-dependent and need activation of
NOS. The increase in glomerular filtration rate augments urine flow, and thus urinary excretion of sodium and chloride. Therefore, the NO-inducing ability of nebivolol seems to give it also a small diuretic action, which can contribute to the hypotensive effects of nebivolol in addition to its β-blocking action. Nebivolol has also demonstrated its efficacy against kidney injury. In fact, nebivolol was able to decrease proteinuria and tubular necrosis in an animal model of nephropathy, probably thanks to induction of NO [Toprak et al. 2008]. Moreover, long-term administration of nebivolol but not atenolol was able to reduce renal fibrosis in another study [Pires et al. 2007], indicating that nebivolol protection against renal injury is independent of its β-blocking action. Given the importance of renal mechanisms on the regulation of blood pressure, an additional renal effect of nebivolol would amplify its efficacy in hypertensive patients. Human trials in patients with renal failure should be planned to investigate this issue.

Moreover, NO is involved in vascular cell proliferation. Nebivolol reduces proliferation of smooth muscle and endothelial cells [Brehm et al. 2001] by a NO-dependent mechanism [Ignarro et al. 2002]. These findings can be related to the antiatherogenic effects observed with nebivolol, but not carvedilol, in an animal model of hypercholesterolemia [de Nigris et al. 2008]. In another animal model, nebivolol but not metoprolol was able to inhibit neointima formation after balloon injury [Wolf et al. 2007], indicating once more that the specific NO-inducing ability of nebivolol make it a unique β-blocker particularly suited for treatment of complicated patients.

Platelet aggregation is another physiological response mediated by NO. NOS is present in platelets, and the resulting NO inhibits their aggregation. Nebivolol has been identified as the most powerful inhibitor of aggregation of human platelets among β-blockers, and the mechanism of action is partially dependent on NO [Falciani et al. 2001].

Finally, recent blockbuster drugs have highlighted the importance of NO against erectile dysfunction, a common adverse event of antihypertensive therapy. In this regard, several trials have shown that treatment with Nebivolol can avoid the decrease in sexual activity observable with different blood-pressure-lowering drugs [Brixius et al. 2007; Boydak et al. 2005].

**Nebivolol-induced production of nitric oxide is realized via activation of β3 adrenergic receptors**

Recent studies have elucidated the mechanism by which nebivolol is able to induce vascular NO production. Actually, it depends on the activation of β3 adrenergic receptors, since NO production is blocked by selective β3 receptor antagonists. This finding was first observed in rats [de Groot et al. 2003]. The same mechanism is also present in humans, as demonstrated in coronary arteries [Dessy et al. 2005] and subsequently confirmed in other vascular districts [Rozec et al. 2006]. Another study seems to suggest a different mechanism, showing that the endothelium-dependent vasodilation by nebivolol in rat arteries is inhibited by an antagonist of an estrogen receptor [Garbán et al. 2004]. Nebivolol may well act through both these mechanisms, since complete inhibition of NO production by nebivolol in endothelial cells can be achieved by the simultaneous blockade of estrogen receptor and β3 adrenergic receptor [Ladage et al. 2006]. Further studies are needed to verify whether estrogen receptors participate in nebivolol action on NO in patients treated with this drug.

As regarding β3 adrenergic receptors, the issue is much clearer. Actually, the notion that Nebivolol is able to activate β3 receptors in endothelial cells was already present in an earlier study [Gosgnach et al. 2001], although this latter did not link β3 activation with NO production. This link could have been hypothesized. On this issue, β3 adrenergic receptor is known to be present in the endothelium, and to induce vasodilation [Shen et al. 1996]. In isolated vessels, inhibition of this vasodilation has been observed not only after selective
blockade of $\beta_3$ adrenergic receptors, but also after addition of NO synthase inhibitors. These findings, demonstrating the involvement of endogenous NO production in $\beta_3$-induced vasodilation, have been found in both conductance [Trochu et al. 1999] and resistance [Dessy et al. 2004] arteries, and even in veins [Mallem et al. 2003], as observed with nebivolol. Of note, $\beta_3$ adrenergic receptors have been observed to induce NO production in coronary arteries [Dessy et al. 2004], with consequent vasodilation and greater blood flow to the myocardium, which can bring to improved heart function.

While $\beta_3$ adrenergic receptors are present and active in the cardiovascular system, their main site of expression in the human body is the adipose tissue. In adipocytes, activation of $\beta_3$ adrenergic receptors increases lipolysis, thermogenesis and energy expenditure, thus leading to a reduction in body weight and in adiposity. On this issue, biomedical research is evaluating the possibility to use $\beta_3$ adrenergic receptor agonists as drugs against diseases linked to adipose dysfunction, such as obesity and diabetes mellitus [Yen, 1994]. Since nebivolol has a partial agonism on $\beta_3$ adrenergic receptors, it might exert beneficial actions on adipose metabolism. Indeed, nebivolol has been shown to differ from other $\beta$-blockers for its effects on metabolic parameters. A study in hypertensive hyperlipidemic patients has attested that, while atenolol increases plasma levels of triglycerides and lipoprotein(a), the same reduction in blood pressure accomplished by nebivolol was associated with a decrease in triglycerides and no variation in lipoprotein(a) [Rizos et al. 2003]. Moreover, nebivolol improved insulin sensitivity as assessed by HOMA index, a parameter unaffected by atenolol. A similar difference in insulin sensitivity between patients treated with nebivolol or atenolol was found also in another study, strengthening the scientific evidence of this observation [Poirier et al. 2001]. Thus, treatment with nebivolol may be particularly indicated in hypertensive patients with metabolic disturbances. In summary, nebivolol ability to stimulate $\beta_3$ adrenergic receptors gives this drug particular advantages over other $\beta$-blockers in the treatment of hypertensive patients (Figure 3).

**Nebivolol and nitric oxide in the heart**

The effect of nebivolol on blood flow in coronary arteries, besides $\beta_1$ adrenergic blockade, may
explain its efficacy in elderly patients with heart failure. Indeed, nebivolol treatment has been shown to increase coronary blood reserve, while at the same time it reduces collateral flow, in both control patients and patients with coronary artery disease [Togni et al. 2007]. This is in line with the observation that nebivolol can rescue endothelial function in patients with coronary artery disease, as mentioned above [Lekakis et al. 2005].

But is that all? As a matter of fact, NO synthase is also present in the heart, produced by both endothelium and cardiomyocytes [Massion et al. 2005]. β3 adrenergic receptors have been demonstrated to stimulate NO production also in cardiomyocytes [Gauthier et al. 1998]. Based on these findings, we have looked for a possible action on cardiac NO production by nebivolol. We found that nebivolol, but not other β1-blockers, is able to stimulate NO production in the myocardium [Maffei et al. 2007] (Figure 1B). As observed in vessels, cardiac NO production depends on activation of β3 adrenergic receptors, since it is blocked by SR59230A, a selective β3 antagonist. We also found that nebivolol induces this β3-mediated NO production by increasing levels of the inducible isoform of NO synthase (iNOS), and that pharmacological blockade of this isoform inhibited the action of nebivolol. This finding may appear odd, since the endothelial isoform eNOS has been shown to be activated by nebivolol in vessels. However, it is confirmed by another study, which found that nebivolol is unable to activate eNOS in human myocardium [Brixius et al. 2006].

NO production may participate in the overall effect of nebivolol on the heart. However, the exact role of NO in the heart is presently confused; it varies according to the amount of NO produced, to the isoform of NO synthase that produces it and to its cellular and subcellular compartmentalization [Massion et al. 2005]. It has been suggested that constitutive NO production is a positive regulator of inotropy, while hyperstimulation of NO has the opposite effect on cardiac force. In particular, NO has been shown to decrease the positive inotropy induced by β1 adrenergic receptors, thus acting as an ‘endogenous β-blocker’ in the heart [Hare et al. 1995a]. Counteraction of β1-mediated cardiac effects by NO has been also observed in patients with left ventricular dysfunction [Hare et al. 1995b], and the isoform responsible has been recognized as iNOS [Ziolo et al. 2004]. On this issue, iNOS is overexpressed in human failing hearts, as consistently seen in several studies [Fukuchi et al. 1998; Haywood et al. 1996]. Such overexpression has been initially considered to participate to the pathophysiology of heart failure. However, recent findings suggest that an increase in iNOS-derived NO production can be a compensatory mechanism, which is used by cardiac tissue to respond to the pathological conditions leading to heart failure. In fact, absence of iNOS in animal models has been shown to enhance myocardial damage [Zingarelli et al. 2002]. A therapeutically more relevant observation is that iNOS overexpression protects against the cardiac injury determined by myocardial infarction, and this protection is mediated by COX-2 [Li et al. 2003]. In a related study, the protection induced by iNOS overexpression has been demonstrated in the medium term (2 months), without apparent side effects such as inflammation or reduced contractility [Li et al. 2006]. The approach used by these researchers is gene therapy, which is known to be transient. Thus, a pharmacological treatment able to constantly increase iNOS levels in the heart, such as nebivolol, might be particularly beneficial in heart failure, without the complications associated with NO donors. A definite conclusion on this subject is at the moment premature, and more studies are needed to provide a better understanding about such a possibility. Other observations suggest that an increase in iNOS activity in the heart can be protective against cardiovascular disease. iNOS has been demonstrated to mediate the beneficial effects of ischemic preconditioning [Bolli et al. 1997].

Other suggestive findings indicate that in the heart NO limits oxygen consumption [Suto et al. 1998], improving the mechanic efficacy of cardiac contraction even when blood flow is reduced [Saavedra et al. 2002]. On this issue, nebivolol is able to limit myocardial oxygen consumption in patients [Togni et al. 2007], and an involvement of NO in this effect may be supposed. Moreover, NO can decrease cardiomyocyte apoptosis, and a reduction in apoptosis has been observed after treatment with nebivolol in an animal model of myocardial infarction [Mercanoglu et al. 2008]. Finally, NO has been shown to protect the heart against insults as varied as ischemia/reperfusion [Elrod et al. 2006] and septic shock [Ichinose et al. 2007]. A peculiar observation is that NO can protect the heart from arrhythmia [Wainwright and Martorana, 1993]. This could be due to the observed modulation of β1 and β2 adrenergic...
receptor activity by NO. Such an effect of NO could underlie the observed efficacy of nebivolol in both animal models of ischemia [Lu et al. 1994] and in patients, where it is able to decrease QT dispersion [Galetta et al. 2005].

Conclusions
In summary, in the last few years nebivolol has been shown to differ from traditional \( \beta \)-blockers in that it not only reduces blood pressure by selectively blocking \( \beta_1 \) receptors in heart, kidney and brain, but it can also induce production of NO from both vessels and heart. This activity provides particular cardiovascular benefits to patients treated with this drug (Figure 4). In both cases, such induction of NO production is realized by stimulating \( \beta_3 \) adrenergic receptors, whose activity has been advocated as a protective factor against metabolic disturbances.

Reduced NO production in response to endogenous agonists, particularly in vessels (endothelial dysfunction), is a hallmark of hypertension and can contribute to its pathological consequences. Nebivolol treatment could restore NO availability in hypertensive patients, leading to functional improvements. Moreover, nebivolol has been demonstrated to reverse endothelial dysfunction, allowing a normal NO response to physiological agonists in presence of elevated blood pressure levels.

This ability could offer advantages to hypertensive patients treated with nebivolol, increasing its efficacy. This could be particularly true in some subclasses of patients, such as those with heart failure or metabolic disease. Moreover, stimulation of \( \beta_3 \) adrenergic receptors and NO can limit the adverse events usually associated with treatment with drugs of the same class. These benefits have been revealed by a recent meta-analysis [Van Bortel et al. 2008], showing that nebivolol has a greater efficacy than all other antihypertensive drugs combined, while the incidence of adverse events is comparable to placebo. In conclusion, Nebivolol has a unique profile amongst antihypertensive drugs, adding to a very high selectivity against \( \beta_1 \) adrenergic receptors, an agonist action on \( \beta_3 \) receptors and NO, which lead to clinically significant improvements in hypertensive patients.

Conflict of interests statement
The Authors have received by Menarini research grants, now expired, for the research described in [Maffei et al. 2006; Maffei et al. 2007].

References


other antihypertensive drugs: A meta-analysis. *Am J Cardiovasc Drugs* 8: 35–44.


