Differential Effects of Nebivolol and Metoprolol on Insulin Sensitivity and Plasminogen Activator Inhibitor in the Metabolic Syndrome

Katie Ayers, Loretta M. Byrne, Anthony DeMatteo, Nancy J. Brown

Abstract—Early-generation β-blockers lower blood pressure and reduce cardiovascular mortality in coronary artery disease and congestive heart failure but worsen glucose homeostasis and fibrinolytic balance. Nebivolol is a third-generation β-blocker that increases the bioavailability of nitric oxide. We compared the effect of nebivolol (5 mg/d) and the β1-selective antagonist metoprolol (100 mg/d) on glucose homeostasis and markers of fibrinolysis in 46 subjects with metabolic syndrome. Subjects underwent a frequently sampled IV glucose tolerance test after 3-week washout and placebo treatment and after randomized treatment with study drug. After 12-week treatment, nebivolol and metoprolol equivalently decreased systolic blood pressure, diastolic blood pressure, and heart rate. Neither drug affected β-cell function, disposition index, or acute insulin response to glucose. Metoprolol significantly decreased the insulin sensitivity index. In contrast, nebivolol did not affect insulin sensitivity, and the decrease in sensitivity was significantly greater after metoprolol than after nebivolol (−1.5±2.5×10−4×min−1 per milliunit per liter versus 0.04±2.19×10−4×min−1 per milliunit per liter after nebivolol; P=0.03). Circulating plasminogen activator inhibitor also increased after treatment with metoprolol (from 9.8±6.8 to 12.3±7.8 ng/mL) but not nebivolol (from 10.8±7.8 to 10.5±6.2 ng/mL; P=0.05 versus metoprolol). Metoprolol, but not nebivolol, increased F2-isoprostane concentrations. In summary, treatment with metoprolol decreased insulin sensitivity and increased oxidative stress and the antifibrinolytic plasminogen activator inhibitor 1 in patients with metabolic syndrome, whereas nebivolol lacked detrimental metabolic effects. Large clinical trials are needed to compare effects of nebivolol and the β1 receptor antagonist metoprolol on clinical outcomes in patients with hypertension and the metabolic syndrome. (Hypertension. 2012;59:893-898.)

Key Words: clinical science ■ insulin resistance ■ hypertension ■ cardiovascular pathophysiology ■ antihypertensive therapy

The prevalence of obesity and the metabolic syndrome has reached epidemic proportions in developed countries and conveys an increased risk of cardiovascular mortality. Elevated circulating concentrations of plasminogen activator inhibitor 1 (PAI-1), the major physiological inhibitor of fibrinolysis in vivo, are a hallmark of insulin resistance and the metabolic syndrome and, in turn, are associated with an increased risk of thrombotic cardiovascular events. Insulin resistance and impaired fibrinolysis contribute to increased cardiovascular morbidity and mortality in the metabolic syndrome. Importantly, commonly used antihypertensive agents differ in their impact on insulin sensitivity and biomarkers of impaired fibrinolysis. For example, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers improve glucose homeostasis in observational studies and some prospective trials. Angiotensin-converting enzyme inhibitors also decrease PAI-1 antigen and activity under conditions in which the renin-angiotensin-aldosterone system is activated. Angiotensin receptor blockers may have a transient beneficial effect on fibrinolytic balance, but this effect is not sustained. In contrast, diuretics impair glucose homeostasis and increase PAI-1 antigen and activity. Early-generation β-blockers can worsen glucose homeostasis and have little effect or a detrimental effect on fibrinolytic balance. Nebivolol is a third-generation β-blocker that increases the bioavailability of endogenous nitric oxide (NO). NO decreases the expression of PAI-1 and improves insulin sensitivity and muscle glucose uptake. Based on the mechanism of action of nebivolol, we hypothesized that nebivolol would have a relatively favorable effect on insulin sensitivity and fibrinolytic balance compared with an earlier-generation β-blocker.

Methods

Subjects

Subjects between the ages of 18 to 70 years with the metabolic syndrome were studied. All of the subjects gave written informed
consent, and the study was approved by the institutional review board and implemented according to the Declaration of Helsinki. Metabolic syndrome was defined using the National Cholesterol Education Program criteria of ≥3 of the following: fasting plasma glucose of ≥100 mg/dL (5.5 mmol/L), serum triglycerides of ≥150 mg/dL (1.7 mmol/L), serum high-density lipoprotein cholesterol <40 mg/dL (1.04 mmol/L) in men or 50 mg/dL in women, untreated blood pressure of ≥130/85 mmHg, or waist girth of >102 cm in men or >88 cm in women. Subjects with significant cardiovascular (other than hypertension), renal, pulmonary, endocrine (other than insulin resistance or hyperlipidemia), or hematologic disease were excluded, as were pregnant women. Patients with diabetes mellitus, defined by a fasting glucose of 126 mg/dL (7 mmol/L) or medication use, were also excluded.

Study Protocol
After screening history and physical examination, all of the antihypertensive medications were discontinued for 3 weeks (Figure 1, top). Spironolactone was discontinued 4 weeks before study initiation. After washout, subjects were treated with placebo in a single-blind fashion for 21 days. For the last 3 days they were provided a nitrate-controlled diet. On the 20th day, subjects provided a 24-hour urine collection for measurement of electrolytes and NO metabolites. The subjects reported to the Clinical Research Center the following day for measurement of fibrinolytic, endocrine, and inflammatory biomarkers. Two baseline samples were collected 10 minutes apart for the measurement of fibrinolytic markers and for a frequently sampled intravenous glucose tolerance test performed on each study day.

After the first study day, patients were randomly assigned to double-blind treatment with 5 mg of nebivolol once daily or 100 mg of metoprolol succinate ER once daily for 12 weeks. These doses were chosen based on published comparative studies indicating similar effects on blood pressure.\(^\text{17,22}\) Randomization was stratified by the presence or absence of hypertension and by race. Subjects returned for blood pressure checks and pill counts 1, 2, 4, 6, 8, and 10 weeks after randomization to active study medication. After 12 weeks of study drug, subjects then repeated the nitrate-controlled diet, urine collection, and study day with IV glucose tolerance test. Using the appearance and complete disappearance of the Korotkoff sounds (K1 and K5) as systolic and diastolic blood pressures. The mean of 3 supine measurements was used. During the IV glucose tolerance test, blood pressures were collected with an automated oscillometric recording device (Dinamap, Critikon, Carlsbad, CA).

Insulin-Modified Frequently Sampled Intravenous Glucose Tolerance Test
Subjects reported to the Clinical Research Center between 7:00 AM and 8:00 AM after a 12-hour fast and were studied in the supine position. Thirty minutes to 1 hour after taking their last dose of study medication, subjects rested in the supine position for 15 minutes before their blood pressure was measured as described above. Intravenous catheters were then placed and, 30 minutes later, blood was obtained through a catheter for the measurement of fibrinolytic, endocrine, and inflammatory biomarkers. Two baseline samples were collected 10 minutes apart for measurement of glucose and insulin (Figure 1, bottom). At t=0 minutes, a bolus of 300 mg of glucose per kilogram of body weight was administered in a 25% glucose-saline solution over 1 minute. At t=20 minutes, a bolus of 0.02 U/kg of body weight of regular insulin (Actrapid, Novo Nordisk, Princeton, NJ) was given intravenously. Blood samples were collected for measurement of glucose and insulin at time t=2, 3, 4, 5, 6, 8, 10, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 90, 110, 130, 150, 170, and 180 minutes. Plasma glucose was measured by glucose oxidase method with a Beckman glucose analyzer at every time point. Plasma insulin concentrations were determined by radioimmunoassay.

The acute insulin response to glucose, the area under the insulin curve between 0 and 10 minutes, and sensitivity index, the capacity for insulin to promote the disposal of glucose and to inhibit the endogenous production of glucose, were calculated using a modified version of the program MINMOD based on the Bergman minimal model.\(^\text{23}\) Disposition index, representing the overall ability of islet cells to secrete insulin normalized to the degree of insulin resistance, was also calculated using this model. \(\beta\)-Cell function was assessed by the computer model from the residual insulin secretion after the initial IV dextrose infusion.

Laboratory Analysis
Blood samples were collected on ice and centrifuged immediately at 0°C for 20 minutes. All of the plasma or serum were separated and stored at −80°C for 20 minutes. Serum and plasma were separated and stored at −80°C for 20 minutes. Serum and plasma were separated and stored at −80°C for 20 minutes. Serum and plasma were separated and stored at −80°C for 20 minutes. Serum and plasma were separated and stored at −80°C for 20 minutes.

Hemodynamic Measurements
Blood pressure was measured with an aneroid sphygmomanometer (Tycos 767, Welch Allyn, Skaneateles Falls, NY) during office visits, using the appearance and complete disappearance of the Korotkoff sounds (K1 and K5) as systolic and diastolic blood pressures. The mean of 3 supine measurements was used. During the IV glucose tolerance test, blood pressures were collected with an automated oscillometric recording device (Dinamap, Critikon, Carlsbad, CA).
Twelve-week treatment with either metoprolol or nebivolol significantly decreased systolic blood pressure, diastolic blood pressure, and heart rate (P<0.05), and the hemodynamic effects of the 2 drugs were similar (Figure 2). Metoprolol (from 8.4±0.76 to 0.50±0.52 ng of angiotensin I per milliliter per minute; P=0.007) and nebivolol (from 0.67±0.72 to 0.23±0.31 ng of angiotensin I per milliliter per minute; P=0.009) similarly reduced plasma renin activity (P=0.61 for metoprolol versus nebivolol.) Metoprolol significantly reduced serum aldosterone (from 9.6±2.9 to 8.3±2.8 ng/dL; P=0.006). The effect of nebivolol on aldosterone was not statistically significant (from 9.5±3.0 to 8.5±2.3 ng/dL; P=0.057) but similar to that of metoprolol (P=0.82 for metoprolol versus nebivolol). Twenty-four-hour urine sodium excretion was also similar in the 2 groups at baseline (123.3±59.1 mmol in the metoprolol group and 117.2±47.3 mmol in the nebivolol group) and after 12 weeks of treatment (108.0±49.7 mmol in the metoprolol group and 123.9±40.8 mmol in the nebivolol group).

Metabolic Effects of Metoprolol and Nebivolol

Table 2 shows the effect of treatment with metoprolol or nebivolol on measures of insulin sensitivity and β-cell function calculated from the IV glucose tolerance test. Twelve-week treatment with metoprolol significantly decreased the insulin sensitivity index. Nebivolol did not affect insulin sensitivity. Thus, the change in insulin sensitivity index differed significantly in the metoprolol and nebivolol treatment groups (−1.5±2.5×10−4×min−1 per milliunit per liter after 12 weeks of metoprolol versus 0.04±2.19×10−4×min−1 per milliunit per liter after nebivolol; P=0.03).

Effects of Metoprolol and Nebivolol on Fibrinolytic Balance

Pretreatment PAI-1 antigen concentrations were similar in the metoprolol (9.8±6.8 ng/mL) and nebivolol (10.8±7.8 ng/mL) groups, but PAI-1 antigen concentrations were significantly higher in the metoprolol-treated subjects after 12 weeks of therapy (12.3±7.8 versus 10.5±6.2 ng/mL in nebivolol-treated subjects; P=0.05 after controlling for race and pretreatment PAI-1). There was a significant relationship between pretreatment PAI-1 antigen and posttreatment PAI-1 antigen (P=0.001). There was a significant effect of race on PAI-1 antigen in the nebivolol treatment group (P=0.017). The change

Table 1. Subjects Characteristics Before Randomization

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nebivolol Group (N=23)</th>
<th>Metoprolol Group (N=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>41.3±11.5</td>
<td>47.4±8.5*</td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>8:15</td>
<td>8:15</td>
</tr>
<tr>
<td>Race, black:white:other</td>
<td>6:16:1</td>
<td>5:17:1</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>133.8±11.7</td>
<td>138.9±14.4</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>83.5±9.2</td>
<td>85.8±9.2</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>75.8±9.8</td>
<td>75.6±12.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>37.0±7.4</td>
<td>36.6±7.0</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>119.5±16.6</td>
<td>113.7±11.4</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>124.0±49.8</td>
<td>124.4±44.9</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>39.0±7.4</td>
<td>42.0±8.0</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>95.7±9.3</td>
<td>98.2±10.9</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; BMI, body mass index; HDL, high-density lipoprotein.

*P=0.045 vs nebivolol group.
in PAI-1 concentrations after 12 weeks of treatment also differed significantly in the 2 treatment groups (Figure 3).

During β-blockade, PAI-1 antigen correlated with fasting insulin concentration \((r=0.45; P=0.002)\), the change in fasting insulin from pretreatment \((r=0.44; P=0.003)\), and the change in fasting glucose from pretreatment \((r=0.38; P=0.01)\). t-PA antigen paralleled PAI-1 antigen concentrations. Hence, metoprolol treatment increased t-PA antigen concentrations from 11.2±2.3 to 12.8±3.8 ng/mL \((P=0.04)\), although posttreatment t-PA antigen concentrations did not differ significantly between the 2 groups.

### Effects of Metoprolol and Nebivolol on NO Metabolites and Oxidative Stress

Neither metoprolol nor nebivolol treatment altered plasma concentrations of NO metabolites (Table 3). Metoprolol increased urine NO metabolites, whereas nebivolol significantly increased plasma cGMP; however, the change in urine NO metabolites and plasma cGMP did not differ between treatment groups. Plasma cGMP correlated inversely with NO metabolites (Table 3) and metoprolol treatment increased urine F₂-isoprostanes from 1.78±0.83 ng/mg Cr \((P=0.02)\). Nebivolol did not significantly affect F₂-isoprostanes (1.78±0.83 ng/mg Cr pretreatment and 1.95±1.63 ng/mg Cr posttreatment).

Neither metoprolol nor nebivolol altered plasma concentrations of asymmetric dimethylarginine (Table 3) or inflammatory cytokines (data not shown).

### Discussion

This study tested the hypothesis that the β-blockers nebivolol and metoprolol differ in their effects on insulin sensitivity and fibrinolytic balance. At doses that were equipotent with respect to reductions in blood pressure, heart rate, and renin activity, metoprolol treatment decreased insulin sensitivity, increased PAI-1 antigen concentrations, and increased oxidative stress, whereas nebivolol treatment did not.

Metoprolol is a β₁-receptor–selective antagonist widely used to prevent cardiovascular disease. Like nonselective β-blockers, metoprolol has been reported to increase fasting glucose concentrations and/or insulin concentrations and to decrease insulin sensitivity as measured by homeostasis model assessment-insulin resistance. The mechanism through which metoprolol decreases insulin sensitivity is not known but may involve decreased blood flow because of unopposed α-receptor–mediated vasoconstriction. It follows that nebivolol, a vasodilator, did not reduce insulin sensitivity; previous studies have reported that nebivolol reduces homeostasis model.
assessment-insulin resistance.¹⁷ Poirier et al²⁸ compared the effects of atenolol and nebivolol and found that, like metoprolol, atenolol significantly reduced insulin sensitivity (insulin-induced glucose disposal rate/mean insulin concentration ratio), but nebivolol did not.

This study is the first to compare the effects of nebivolol and an early-generation β-blocker on fibrinolytic balance. Earlier studies reported that β₁-receptor–selective antagonists have no impact or a negative impact on PAI-1. For example, Boman et al¹² reported that 36-week treatment with atenolol increases PAI-1 activity in patients with hypertension and left ventricular hypertrophy, as we observed with metoprolol in individuals with the metabolic syndrome. Only 2 previous studies have examined the effect of nebivolol on fibrinolytic balance in humans. In an uncontrolled study in hypertensive patients, nebivolol decreased the PAI-1/α2-PI ratio but did not affect PAI-1 antigen or activity concentrations.²⁹ Vyssoulis et al³⁰ reported that nebivolol and celiprolol reduced PAI-1, whereas carvedilol did not, in patients with uncomplicated hypertension; however, 20% of patients were also taking hydrochlorothiazide, which increases PAI-1.

Circulating PAI-1 concentrations are increased during insulin resistance and both glucose and insulin stimulate response elements in the PAI-1 promoter.³¹,³² In the current study, PAI-1 antigen concentrations correlated with the effect of β-blockade on both insulin and glucose concentrations, suggesting that increased insulin resistance contributed to the increase in PAI-1 concentrations during metoprolol. We hypothesize that the preservation of fibrinolytic balance during nebivolol treatment reflected preserved insulin sensitivity. Stimulation of NO synthase during nebivolol treatment would also be expected to moderate PAI-1 concentrations; NO decreases PAI-1 expression through a cGMP-dependent mechanism.²⁰ Circulating cGMP and NO metabolite concentrations are imperfect measures of NO production in humans, however, and we did not find evidence of an effect of nebivolol on vascular NO production.

Increased oxidative stress contributes to cardiovascular risk in the metabolic syndrome.³³ Nebivolol has been reported to reduce oxidative stress in vivo in rodent models. In vivo, liquid chromatography-mass spectrometry measurement of F₂-isoprostanes has become the gold standard for assessing oxidative stress.³⁴ For the most part, studies using less accurate ELISA assays for F₂-isoprostanes report no effect of atenolol, carvedilol, or metoprolol on F₂-isoprostanes in hypertensive or diabetic patients.³⁵,³⁶ Fahlbusch et al³⁷ also reported no effect of 6-day treatment with either carvedilol or metoprolol on urinary F₂-isoprostane excretion, measured by liquid chromatography-mass spectrometry, in healthy volunteers. Fratta et al³⁸ reported that nebivolol reduced plasma F₂-isoprostanes, measured using a commercially available ELISA, in patients with essential hypertension. Troost et al³⁹ reported that 7-day treatment with nebivolol decreased urinary F₂-isoprostanes, measured by liquid chromatography-mass spectrometry, in healthy volunteers. In the present study in subjects with the metabolic syndrome, the finding that metoprolol increased F₂-isoprostanes whereas nebivolol had no effect may reflect the high baseline levels of F₂-isoprostanes in this obese study population.

Perspectives
The prevalence of obesity and the metabolic syndrome has reached epidemic proportions in developed countries.¹² Although metoprolol and other early-generation β-blockers have been shown to reduce cardiovascular mortality in patients with coronary artery disease⁴⁰ and congestive heart failure,²⁰ this is not true in hypertension without these conditions,⁴¹ and negative effects of these drugs on insulin resistance, plasminogen activator-inhibitor, and oxidative stress may diminish their beneficial effects in the obese.¹¹,¹⁷ The present randomized study in individuals with the metabolic syndrome suggests that nebivolol has a favorable effect on fibrinolytic balance compared with metoprolol and lacks negative effects on insulin sensitivity and oxidative stress. Large clinical trials are needed to compare the effects of these 2 drugs on cardiovascular outcomes in obese patients with the metabolic syndrome.

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References


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