

# Hydralazine-Induced Prevention of Nitrate Tolerance: Experimental and Clinical Evidence and Potential Mechanisms

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The clinical use of hydralazine in combination with organic nitrates has resulted in a beneficial effect on survival, cardiac function, and exercise tolerance. More recently, hydralazine has been shown to prevent development of nitrate tolerance and early attenuation of nitrate-mediated hemodynamic effects in both experimental animals as well as patients with severe heart failure due to depression of left ventricular systolic function. Recent *in vitro* animal results have shown that prolonged nitroglycerin treatment results in increased production of endogenous vascular superoxide ( $\dot{c}O_2$ ) production due to a specific NADH-dependent membrane-associated oxidase and that this is at least in part

responsible for the development of nitrate tolerance. Further studies demonstrated that concomitant administration of hydralazine normalized endogenous rates of vascular  $\dot{c}O_2$  production and prevented the development of nitrate tolerance. The ability of hydralazine to inhibit vascular  $\dot{c}O_2$  anion production and to prevent the development of nitrate tolerance may provide further explanations for the benefits demonstrated in the V-HeFT studies with the hydralazine-isosorbide dinitrate combination in the treatment of patients with chronic CHF. ©1998 by Excerpta Medica, Inc.

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**R**esults of both the first and second Veterans Administration Heart Failure Trials (V-HeFT I<sup>1</sup> and II<sup>2</sup>) have established the role of hydralazine-isosorbide dinitrate (H-ISDN) in the treatment of patients with heart failure. These studies demonstrated that although angiotensin converting enzyme (ACE) inhibitors were superior to H-ISDN, use of H-ISDN in addition to diuretics and digoxin resulted in prolongation of life, improved exercise tolerance, and increased left ventricular ejection fraction. The use of H-ISDN has therefore been recommended by the new guidelines of the American Heart Association/American College of Cardiology Task Force<sup>3</sup> for patients with New York Heart Association functional class II-III heart failure when ACE inhibitors are not tolerated because of symptomatic hypotension, azotemia, hyperkalemia, cough, rash, or angioneurotic edema. The rationale for the use of H-ISDN in combination in the V-HeFT studies was based on their favorable hemodynamic effect derived from a combined arteriolar as well as venous dilatory effect, leading to a concomitant reduction in right and left ventricular filling pressures as well as an increase in cardiac output<sup>4,5</sup> (Figure 1). Recent information, however, has indicated that the development of nitrate tolerance, a major limitation of nitrate therapy, can be prevented with a concomitant administration of hydralazine.<sup>6,7</sup> These data suggest an additional potential benefit to

the concomitant use of hydralazine with organic nitrates.

The first indication for a favorable drug interaction was provided by Bauer and Fung<sup>6</sup> who studied the effect of hydralazine on the development of nitrate tolerance in an *in vivo* rat model for postmyocardial infarction congestive heart failure (CHF). Infusion of nitroglycerin to the CHF rats produced an initial reduction in left ventricular end diastolic pressure of  $46 \pm 3\%$ . However, with continuation of nitroglycerin administration, the initial hemodynamic effect was not maintained and left ventricular end diastolic pressure returned to near baseline values within 10 hours of infusion, indicating the development of nitrate tolerance. Coadministration of hydralazine given intravenously ( $2 \times 0.1$  mg bolus injections at 1.5 and 2 hours) prevented the development of nitrate tolerance (Figure 2) and maintained the nitroglycerin-mediated reduction in left ventricular end diastolic pressure throughout the 10-hour nitroglycerin infusion period. Examination of plasma concentrations of nitroglycerin and dinitrate metabolites before and after hydralazine dosing demonstrated no significant change.

## EFFECT OF HYDRALAZINE ON NITRATE TOLERANCE

Because of the potential therapeutic value of the results of the study by Bauer and Fung,<sup>6</sup> we designed a similar experiment to evaluate the effect of oral hydralazine on the development of nitrate tolerance in patients with chronic CHF.<sup>7</sup> A total of 28 patients with CHF (NYHA functional class III or IV) were studied. Patients were randomized to receive a continuous infusion of nitroglycerin for 24 hours either alone (14 patients) or concomitantly with oral hydralazine (14

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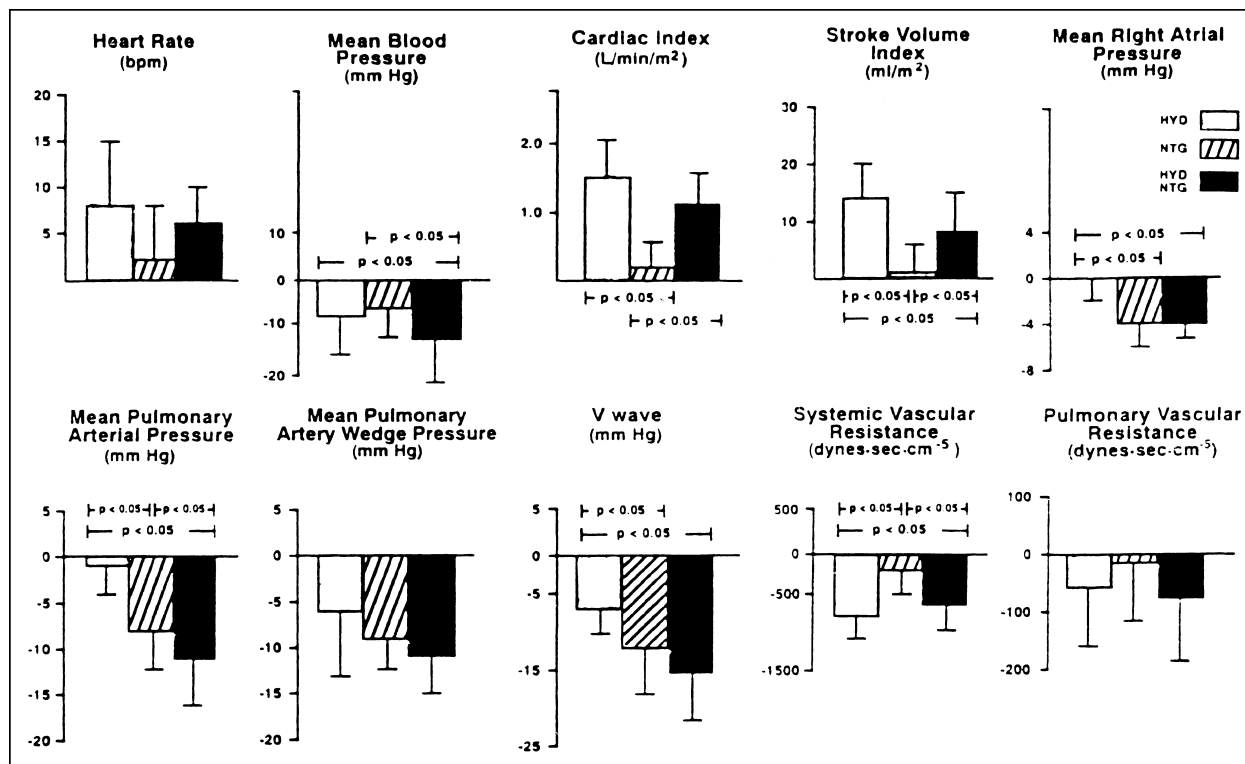


FIGURE 1. A comparison between resting hemodynamic changes from baseline on individual therapy with hydralazine (HYD), nitroglycerin (NTG), and their combination. Reprinted with permission from *Am Heart J*.<sup>5</sup>

patients) given at a dose of 75 mg 4 times daily and started at least 24 hours, before the study. Nitroglycerin was commenced in both groups at a rate of 20  $\mu\text{g}/\text{min}$  and the rate of infusion was then increased in increments of 20–60  $\mu\text{g}/\text{min}$  every 5 minutes to achieve at least a 30% reduction in mean pulmonary artery wedge pressure or until a maximum dose of 560  $\mu\text{g}/\text{min}$  was reached. The dose required to achieve the desired hemodynamic response was maintained at the same rate for 24 hours, and hemodynamic measurements were repeated periodically throughout the study in both groups.

Continuous infusion of nitroglycerin alone resulted in a significant attenuation of the initial effect 24 hours after initiation of therapy (group 1) on mean pulmonary artery pressure ( $27 \pm 4\%$  vs  $10 \pm 3\%$ ,  $p < 0.05$ ) and mean pulmonary artery wedge pressure ( $40 \pm 4\%$  vs  $16 \pm 4\%$ ,  $p < 0.05$ ; Figure 3). In contrast, in group 2 concomitant administration of oral hydralazine prevented nitroglycerin-induced hemodynamic tolerance and resulted in a persistent effect on mean pulmonary artery and wedge pressures throughout the study period ( $31 \pm 3\%$  vs  $27 \pm 4\%$ ,  $p = 0.13$  and  $37 \pm 4\%$  vs  $34 \pm 6\%$ ,  $p = 0.40$ , respectively). In addition, the initial effect on blood pressure was attenuated at 24 hours in group 1 ( $12 \pm 3\%$  vs  $5 \pm 2\%$ ,  $p < 0.05$ ) but not in group 2 ( $15 \pm 3\%$  vs  $17 \pm 2\%$ ,  $p = 0.46$ ). The results of our clinical study therefore supported the observation made by Bauer and Fung in animal experimentation,<sup>6</sup> indicating the ability of hydralazine to prevent early development of nitrate tolerance and

maintain nitrate-mediated favorable hemodynamic effects.

### HYDRALAZINE–NITRATE INTERACTION

In an attempt to evaluate the mechanism of the hydralazine–nitrate interaction demonstrated above, Unger et al<sup>8</sup> studied the effect of hydralazine on nitroglycerin-induced relaxation of aortic rings isolated from rats either previously rendered tolerant in vivo to nitroglycerin or not. These investigators demonstrated potentiation of the relaxing response to nitroglycerin in aortic rings rendered tolerant to nitroglycerin in vivo after incubation with hydralazine. At the same time, however, the effect of SIN-1 (a direct activator of guanylate cyclase), 8-bromocyclic guanylate-monophosphate (an analog of cyclic GMP), and phosphocholine (an adenylate cyclase activator) was not potentiated with hydralazine incubation. Since the relaxation to phosphocholine was unaffected, the role of the adenylate cyclase–dependent mechanism was dismissed by the investigators. Organic nitrates induce vascular smooth muscle relaxation through the activation of guanylate cyclase, an enzyme that is responsible for the formation of cyclic GMP. Unlike SIN-1, the activation of organic nitrates requires prior biotransformation involving interaction with intracellular thiol groups.<sup>9</sup> The lack of augmentation of the response of 8-bromocyclic guanylate monophosphate and SIN-1 in the presence of hydralazine suggested to the investigators that the observed interaction between

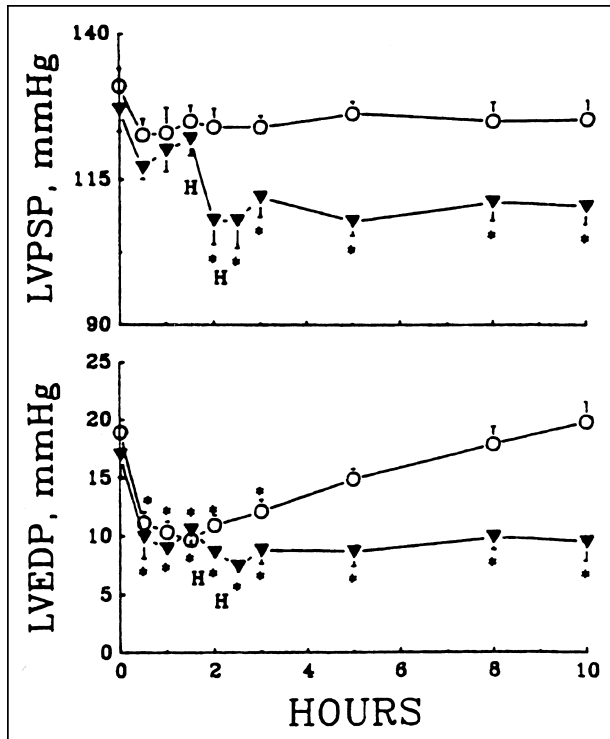


FIGURE 2. Plots of effects of nitroglycerin infusion alone (○; n = 7) or in combination with hydralazine (▽; n = 8) on left ventricular hemodynamics in rats with heart failure. Nitroglycerin was infused at 10  $\mu\text{g}/\text{min}$  in both groups. Hydralazine (H) was administered as a 0.1 mg bolus at 1.5 and 2.0 hours during nitroglycerin infusion. Nitroglycerin alone produced initial reductions in left ventricular end-diastolic pressure (LVEDP) but no effect on left ventricular peak systolic pressure (LVSP). LVEDP returned to baseline by 8 hours, indicating tolerance development. Tolerance to nitroglycerin did not occur during coadministration with hydralazine. Mean  $\pm$  SEM values are shown. \*Statistically significant differences from baseline ( $p < 0.05$ ). Reprinted with permission from *Circulation*.<sup>6</sup>

hydralazine and nitroglycerin occurs at a stage earlier than guanylate cyclase activation.

Previous studies<sup>10</sup> suggested an interaction with pyridoxal as a possible mechanism of hydralazine vasodilation. Since isoniazide also had a potentiating effect on nitroglycerin-induced relaxation in Unger's study, the investigators suggested that a common feature between isoniazide and hydralazine could be inhibition of pyridoxal-dependent reactions. Several enzymes requiring pyridoxal as a cofactor and involved in the catabolism of methionine and cysteine have been found to be inhibited by hydralazine through the formation of a hydrazone complex. It was, therefore, suggested by Unger et al<sup>8</sup> that inhibition of this pyridoxal-dependent reaction may induce sulfhydryl-containing compound accumulation and, thus, may potentiate the nitroglycerin effect and counteract tolerance.

Potential of the nitroglycerin effect with sulfhydryl groups was demonstrated by Mehra et al,<sup>11</sup> who reported augmentation of the ISDN hemodynamic effects in patients with CHF by concomitant administration of N-acetylcysteine, a sulfhydryl group donor. However, the concurrent administration of N-acetylcysteine in another study<sup>12</sup> failed to prevent the development of nitrate tolerance and the attenuation of nitroglycerin-mediated hemodynamic effect over time in a similar group of patients with CHF. The results of these studies therefore support a sulfhydryl-mediated enhancement of the nitrate effect but do not support the suggestion by Unger et al<sup>8</sup> of a sulfhydryl-mediated prevention of the nitrate tolerance.

Münzel and his coworkers<sup>13</sup> have proposed a different explanation of hydralazine-mediated prevention of nitrate tolerance. This group of investigators has demonstrated in an in vitro animal model (rabbit aortic segments) of nitrate tolerance that nitrate tolerance

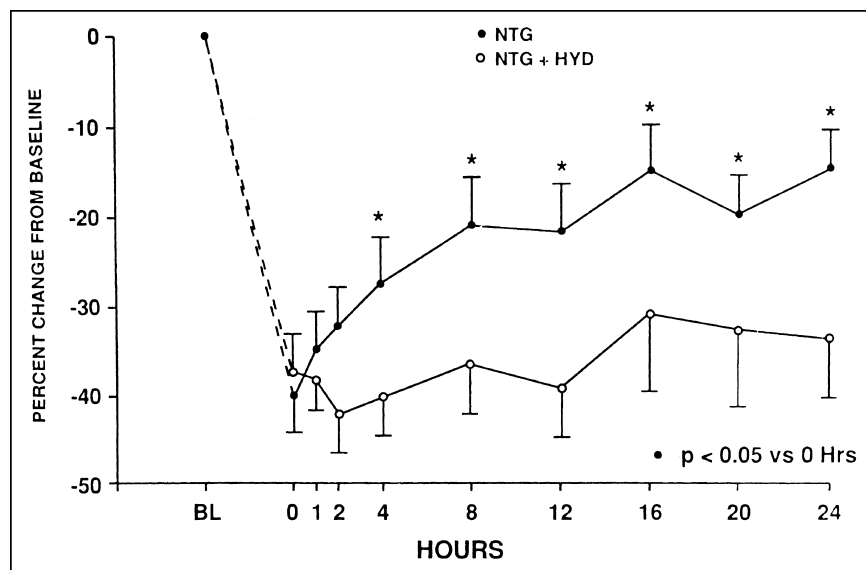
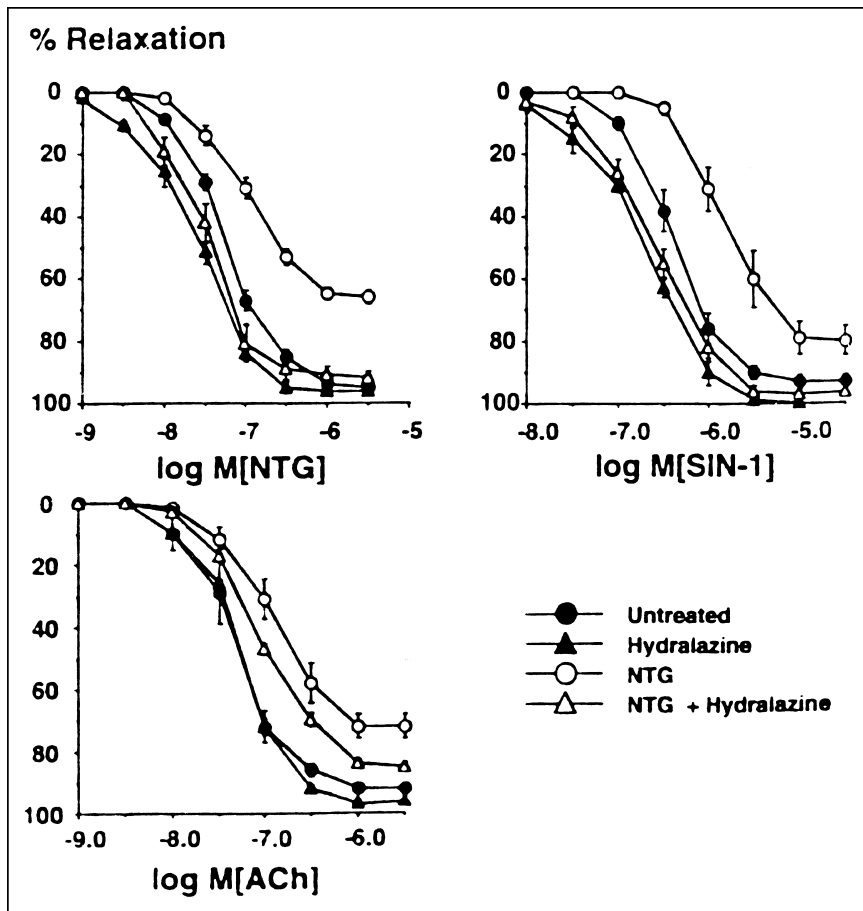


FIGURE 3. Values of mean pulmonary artery wedge pressure as measured over time during continuous administration of nitroglycerin (NTG) alone or NTG concomitantly with oral hydralazine (HYD) 75 mg q.i.d. Reprinted with permission from *J Am Coll Cardiol*.<sup>7</sup>

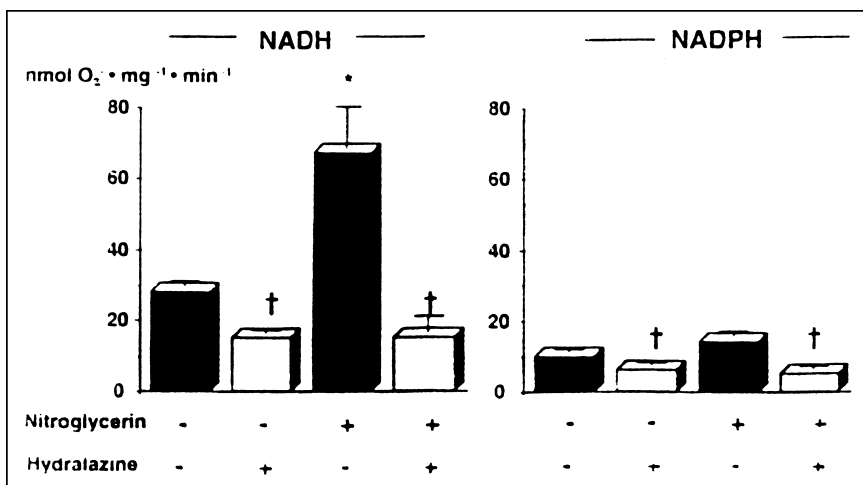
FIGURE 4. Effects of hydralazine treatment on the relaxations to nitroglycerin, the sydnonimime SIN-1, and the endothelium-dependent vasodilator acetylcholine (ACh). The segments were precontracted with phenylephrine, and relaxations to cumulative concentrations of each drug were examined. Concomitant treatment with hydralazine increased sensitivity to nitroglycerin and SIN-1 in aortae from untreated animals and corrected tolerance and cross-tolerance in nitroglycerin-treated animals. Data are expressed as mean  $\pm$  SEM of 5–9 experiments. Reprinted with permission from *J Clin Invest*.<sup>14</sup>



developed, in part, due to an NADH-oxidase mediated increase in endothelial  $\dot{c}O_2$ . The same group of investigators hypothesized that superoxide may interact with nitric oxide (NO) derived from organic nitrate to form peroxynitrite, a compound less effective than NO in activating guanylyl cyclase in vascular smooth muscle, resulting in attenuation of nitrate effects. Hydralazine caused a substantial decrease in  $\dot{c}O_2$  production in rabbits not treated with nitroglycerin and in

addition increased sensitivity of blood vessels to the relaxing effect of nitroglycerin (Figure 4).<sup>14</sup> Concomitant use of hydralazine with continuous nitroglycerin treatment completely prevented the development of nitrate tolerance and normalized endogenous rates of vascular  $\dot{c}O_2$  production. In addition, hydralazine also prevented cross-tolerance between nitroglycerin and SIN-1, another exogenous NO donor as well as NO released by endothelial stimulation with acetylcholine.

FIGURE 5. Effects of in vivo nitroglycerin treatment on NADH and NADPH oxidase activity in aortae from rabbits with or without concomitant hydralazine treatment. In vivo treatment with nitroglycerin increased superoxide  $\dot{c}O_2$  production in response to NADH almost 2.5-fold, while having no effect on NADPH oxidase activity. Concomitant treatment with hydralazine decreased the activity of both NADH- and NADPH-driven superoxide in homogenates of vessels from animals with and without nitroglycerin treatment. Each value is mean  $\pm$  SEM of 4–12 experiments. \* $p < 0.01$  untreated vs nitroglycerin-treated; † $p < 0.05$  vs without hydralazine treatment. Reprinted with permission from *J Clin Invest*.<sup>14</sup>



Further studies by the same group of investigators of vessel homogenates demonstrated an NADH-dependent membrane-associated source of  $\dot{c}O_2$  induced by nitroglycerin. Hydralazine treatment resulted in a decrease in this NADH-dependent oxidase activity in homogenates of aortae from both animals treated with nitroglycerin and those that were not (Figure 5), caused by an unknown mechanism. Since hydralazine was effective only in vivo or when incubated with intact rings but had no effect when added to the vascular homogenates, Münzel et al<sup>13</sup> postulated that hydralazine may cause prevention of the assembly of the oxidase rather than a direct inhibition of the enzyme.

1. Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Iristani FE, Dunkman WB, Jacobs W, Francis GS, Flohr KH, Goldman S, Cobb FR, Shah PM, Saunders R, Fletcher RD, Loeb HS, Hughes VC, Baker B. Effect of vasodilator therapy on mortality in chronic congestive heart failure: results of a Veterans Administration Cooperative Study. *N Engl J Med* 1986;314:1542-1547.
2. Cohn JN, Johnson G, Ziesche S, Cobb F, Francis G, Tristani F, Smith R, Dunkman WB, Loeb H, Wong M, Bhat G, Goldman S, Fletcher RD, Doherty J, Hughes CV, Carson P, Cintron G, Shabetai R, Haakenson C. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. *N Engl J Med* 1991;325:303-310.
3. American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Evaluation and Management of Heart Failure): Guidelines for the evaluation and management of heart failure. *Circulation* 1995;92:2764-2784.
4. Massie B, Chatterjee K, Werner J, Greenberg B, Hart R, Parmley WW.

Hemodynamic advantage of combined administration of hydralazine orally and nitrates nonparenterally in the vasodilator therapy of chronic heart failure. *Am J Cardiol* 1977;40:794-801.

5. Roth A, Shotan A, Elkayam U. A randomized comparison between the hemodynamic effects of hydralazine and nitroglycerin alone and in combination at rest and during isometric exercise in patients with chronic mitral regurgitation. *Am Heart J* 1993;125:155-163.
6. Bauer JA, Fung HL. Concurrent hydralazine administration prevents nitroglycerin-induced hemodynamic tolerance in experimental heart failure. *Circulation* 1991;84:35-39.
7. Gogia H, Mehra A, Parikh S, Raman M, Ajit-Uppal J, Elkayam U. Prevention of tolerance to hemodynamic effects of nitrates with concomitant use of hydralazine in patients with chronic heart failure. *J Am Coll Cardiol* 1995;26:1575-1580.
8. Unger P, Berkenboom G, Fontaine J. Interaction between hydralazine and nitrovasodilators in vascular smooth muscle. *J Cardiovasc Pharmacol* 1993;21:478-483.
9. Ahlner J, Andersson RGG, Torfard K, Axelsson KL. Organic nitrate esters: clinical use and mechanisms of action. *Pharmacol Rev* 1991;43:351-423.
10. Vidrio H. Interaction with pyridoxal as a possible mechanism of hydralazine hypotension. *J Cardiovasc Pharmacol* 1990;15:150-156.
11. Mehra A, Shotan A, Ostrzega E, Hsueh W, Vasquez-Johnson J, Elkayam U. Potentiation of isosorbide dinitrate effects with N-acetylcysteine in patients with chronic heart failure. *Circulation* 1994;89:2595-2600.
12. Dupuis J, Lalonde O, Lemieux R, Rouleau JL. Tolerance to intravenous nitroglycerin in patients with congestive heart failure: role of increased intravascular volume, neurohumoral activation and lack of prevention with N-acetylcysteine. *J Am Coll Cardiol* 1990;16:923-931.
13. Münzel T, Sayegh H, Freeman BA, Tarpey MM, Harrison DG. Evidence for enhanced vascular superoxide anion production in nitrate tolerance. A Novel mechanism underlying tolerance and cross-tolerance. *J Clin Invest* 1995;95:187-194.
14. Münzel T, Kurz S, Rajagopalan S, Thoenes M, Berrington WR, Thompson JA, Freeman BA, Harrison DG. Hydralazine prevents nitroglycerin tolerance by inhibiting activation of a membrane-bound NADH oxidase: a new action for an old drug. *J Clin Invest* 1996;98:1465-1470.