Prevention of Nitrate Tolerance With Angiotension Converting Enzyme Inhibitors

Richard J. Katz, MD; Warren S. Levy, MD; Leslie Buff, RN; and Alan G. Wasserman, MD

Background. Activation of neurohumoral hormones or sulfhydryl group depletion may contribute to the development of nitroglycerin tolerance. In an attempt to prevent nitrate tolerance, this study evaluated the interaction of nitroglycerin with angiotensin converting enzyme (ACE) inhibitors with and without a sulfhydryl group.

Methods and Results. Thirty-four subjects were randomized to a 7-day regimen of enalapril 10 mg b.i.d., captopril 25 mg t.i.d., or placebo. Venodilator response to nitroglycerin was assessed with forearm plethysmography by measuring the change in venous volume after administration of 0.4 mg sublingual nitroglycerin. Plethysmographic measurements were obtained serially 1) at baseline, 2) after 4 days of ACE inhibitor or placebo, 3) 2 hours after application of a 10 mg/24 hr nitroglycerin patch, and 4) 74 hours after continuous nitropatch application. ACE inhibition alone caused no significant change in the response to sublingual nitroglycerin. Nitrate response remained unchanged after 2 hours (“acute”) of nitropatch exposure in all three groups. After 74 hours (“chronic”) of continuous nitropatch application, the venodilator response to sublingual nitroglycerin was reduced by 40% in the placebo group, 10% in the enalapril group, and 2% in the captopril group. This attenuation was significant only in the placebo group (p<0.01). Pairwise comparison of nitrate response between groups was significantly different between the captopril and placebo groups (p<0.01) and between the placebo and enalapril groups (p<0.05). Plasma renin levels increased equally in the enalapril and captopril groups. Body weight increased only in the placebo group, suggesting prevention of nitrate-induced volume expansion in the ACE inhibitor groups.

Conclusions. This study demonstrates that ACE inhibitors may prevent nitrate tolerance to long-term nitrate therapy. (Circulation 1991;83:1271–1277)

Continuous administration of organic nitrates has been shown to result in rapid development of drug tolerance.1–6 Intermittent nitrate therapy has been recommended as an approach to preserve pharmacological action; however, a nitrate-free interval provides efficacy for only 8–12 hours.7,8 Alternative approaches to circumventing nitrate tolerance possibly can be achieved by modification of either of the two mechanisms proposed to cause this phenomenon: depletion of critical intracellular sulfhydryl groups9,10 and activation of counter-regulatory hormones.11,12 Preliminary investigation has suggested that administration of sulfhydryl donors, N-acetylcysteine and methionine, may potentiate the vasodilator effects of nitroglycerin after acute drug exposure and during chronic nitrate delivery.13–17 Experience with neurohumoral blockade for prevention of nitrate tolerance, thus far, has been limited. Neurohumorally mediated rebound vasoconstriction seen after acute nitroprusside withdrawal can be blocked by pretreatment of patients with captopril.18 Van Gilst et al.19 reported the direct coronary vasodilator effect of captopril alone and a synergistic effect with isosorbide dinitrate. Their study design was limited to the isolated rat heart after acute isosorbide dinitrate exposure without assessment of nitrate-tolerant hearts or the effects of angiotensin converting enzyme (ACE) inhibition in an intact circulatory system. Of note, captopril, a sulfhydryl-containing ACE inhibitor, potentiated isosorbide dinitrate action; whereas ramipril, a non-sulfhydryl-containing ACE inhibitor, had no independent or synergistic vasodilating effects. These findings suggest that the nitrate-potentiating action of captopril may be due solely to its sulfhydryl moiety rather than any specific ACE-inhibiting action.

It was the purpose of this study to assess the effects of ACE inhibitors on tolerance produced during con-
Persistent administration of transdermal nitroglycerin in humans. We further compared the relative effects of a sulfhydryl-containing ACE inhibitor, captopril, with a nonsulfhydryl-containing ACE inhibitor, enalapril.

Methods

Patient Population

The study population was composed of 34 normal volunteers (22 men and 12 women), ranging in age from 23 to 48 years. No subject had known cardiovascular disease, and none had been exposed to any nitrate compounds or other cardiac medications. Three subjects were taking estrogen preparations. Written, informed consent for participation in this study was obtained from all subjects, and the protocol was approved by the George Washington University Medical Center Committee on Human Investigation. Exclusion criteria included a resting heart rate greater than 120 beats/min, systolic blood pressure less than 100 mm Hg, or pregnancy.

Assessment of Venodilator Response to Nitroglycerin

An established technique to evaluate the venodilator response to nitroglycerin is forearm plethysmography.20,21 We adapted this in vivo methodology for testing nitrate tolerance from the previous work of Zelis and Mason.21 The effect of nitroglycerin on venous tone was evaluated by measuring the change of venous volume after a sublingual nitroglycerin dose. Venous volumes were determined by the equilibrium technique of forearm plethysmography.22,23 Forearm circumference was measured with a calibrated single-strand mercury-in-Silastic strain gauge.24 The signal was balanced with a plethysmograph (model 271, Parks Medical Electronics, Aloha, Ore.) and recorded on a strip chart recorder. The hand was isolated from the circulation by inflating a wrist cuff to suprasystolic pressure. A forearm occlusion cuff was inflated to 30 mm Hg above cuff zero. Cuff zero is that pressure below which forearm volume remains unchanged and above which forearm volume increased. After inflation of the forearm cuff, the venous volume increased for 3–4 minutes until reaching a plateau. This value was considered to be the baseline (prenitroglycerin) venous tone (VV [30], cc/100 cc arm). Immediately on attaining equilibration, 0.4 mg sublingual nitroglycerin was administered with continued forearm volume recording until a new plateau was achieved or for a maximum of 5 minutes. The change in forearm volume from the first plateau to the second plateau was considered to be the venodilatory effect of nitroglycerin. Arterial blood pressure and heart rate were measured every 3 minutes from the contralateral arm using an automated blood pressure device (Colin, Japan). All subjects were studied recumbent at the same time of day in a warm, quiet environment.

Study Protocol

The study design (Figure 1) consisted of a series of measurements of plethysmographic venodilator re-

![Figure 1. Study design indicating serial venous volume (VV) measurements (*) obtained day 1 before any concomitant therapy at baseline (BI), day 4 before nitropatch (Pre NTP), day 4 2 hours after nitropatch application (NTP 2h), and day 7 74 hours after nitropatch application (NTP 74h). *VV measurement before and after sublingual nitroglycerin.](image-url)
minutes, samples for plasma renin were drawn, cold centrifuged, and sent for analysis to Nichols Institute Reference Laboratories (San Juan Capistrano, Calif.).

Statistical Analysis

Nonparametric analysis was performed because of the nonnormality of data distribution. Differences within each group were evaluated by a Wilcoxon signed-rank test. A Wilcoxon ranked-sum test was applied to assess differences between groups. The Dunn method for multiple comparisons was used to locate differences in paired treatment groups. Results are expressed as mean±SD, and for all analyses, a p value of 0.05 or less was considered significant.

Results

Thirty-four subjects entered the study, and two subjects terminated prematurely because of side effects. Both volunteers had been randomized to the enalapril arm and had dizziness; one on ACE inhibitor alone and one during the combination of ACE inhibitor and nitropatch. These two subjects were replaced by additional volunteers to maintain intergroup balance (10 placebo, 11 enalapril, and 11 captopril completers). No other subjects had any adverse symptoms.

Baseline Venous Measurements

Venous tone and venodilator response to sublingual nitroglycerin were similar in all three groups before ACE inhibitor or placebo administration. Baseline venous volume (cc/100 cc arm) increased after nitroglycerin administration from 2.57±0.57 to 3.37±0.62 in the placebo group, from 2.88±0.88 to 3.77±1.03 in the enalapril group, and from 2.65±0.60 to 3.44±0.76 in the captopril group (Table 1). These increases of venous volume of 34%, 32%, and 30%, respectively, were not statistically different.

Venous Measurements on ACE Inhibitor Alone

Neither enalapril nor captopril alone altered the resting venous volume or the venodilator response to sublingual nitroglycerin. After 4 days of ACE inhibitor therapy, resting venous volume (cc/100 cc arm) was 3.02±1.03 in the enalapril group and 2.71±0.70 in the captopril group, an insignificant change from the baseline day for either group (Table 1). After sublingual nitroglycerin, venous volume increased in the enalapril group to 3.88±1.25 (+30%) and to 3.47±0.88 (+29%) in the captopril group, p=NS compared with day 1 (Figure 2). Similarly, the parallel placebo group had reproducible resting venous volume and response to sublingual nitroglycerin when tested on the fourth day of the study (Figure 2).

Acute Venodilator Response to Transdermal Nitroglycerin

The resting venous volume was unaltered after 2 hours of exposure to a 10 mg/24 hr transdermal nitroglycerin patch in all groups (Table 2). In addition, the

<table>
<thead>
<tr>
<th>Day 1: Baseline</th>
<th>Placebo (n=10)</th>
<th>Enalapril (n=11)</th>
<th>Captopril (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre sublingual NTG</td>
<td>2.57±0.57</td>
<td>2.88±0.88</td>
<td>2.65±0.60</td>
</tr>
<tr>
<td>Post sublingual NTG</td>
<td>3.37±0.62</td>
<td>3.77±1.03</td>
<td>3.44±0.76</td>
</tr>
<tr>
<td>Change (pre–post sublingual NTG)</td>
<td>0.80±0.20</td>
<td>0.89±0.23</td>
<td>0.79±0.22</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are mean±SD cc/100 cc arm.
Sublingual NTG, 0.4 mg sublingual nitroglycerin; ACEI, angiotensin converting enzyme inhibitor.
*p=NS vs. day 1 before sublingual NTG values; †p=NS vs. change day 1.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Bar graph of change in venous volume (VV) (cc/100 cc arm) after 0.4 mg sublingual nitroglycerin on day 1 (baseline) versus day 4 with concomitant placebo or angiotensin converting enzyme inhibitor only.
2-hour postpatch venodilator response to sublingual nitroglycerin was comparable across groups and was the same as the prepatch measurements.

**Venodilator Response to Chronic Transdermal Nitroglycerin**

Nitrate tolerance was observed in the placebo group after 74 hours of continuous application of transdermal patches. At the end of this chronic phase, the placebo group venodilator response to sublingual nitroglycerin was attenuated 40% (Figure 3), only increasing 0.45±0.16 compared with the acute 2-hour postpatch increase of 0.69±0.34, \( p<0.01 \) (Table 2). Only a 10% reduced venodilator response to sublingual nitroglycerin was observed in the enalapril group (Figure 3), increasing 0.70±0.36 on the chronic patch compared with 0.73±0.29 on the acute patch, \( p=\text{NS} \). Pairwise comparison of nitrate response between the placebo and enalapril groups demonstrated a significant intergroup difference (\( p<0.05 \)). No attenuation to sublingual nitroglycerin was noted in the captopril group after 74 hours of continuous nitropatch treatment (Figure 3). Sublingual nitroglycerin increased the venous volume 0.65±0.23 on the acute patch similar to an increase of 0.68±0.16 on the chronic patch, \( p=\text{NS} \). A pairwise comparison of the acute with chronic patch response to sublingual nitroglycerin was statistically different between the captopril group and placebo group, \( p<0.01 \). No difference was noted in the pairwise comparison of the acute with chronic nitrate response between the enalapril and captopril groups.

**Plasma Renin Measurements**

Pretreatment plasma renin values were similar for all three groups. After 4 days of treatment with ACE inhibitor alone, the renin measurements increased significantly in the enalapril and captopril groups (Table 3). Renin levels remained elevated after 74 hours of nitropatch treatment in both ACE inhibitor groups but were unaltered from the ACE inhibitor alone phase. The renin response to ACE inhibitor with enalapril and captopril was comparable both on day 4 after ACE inhibitor alone and on day 7 after combined ACE inhibitor and 74 hours of nitropatch treatment. No change in renin values was noted in the placebo group throughout the study even after the development of nitrate tolerance.

**Weights**

Body weight increased 0.3±0.3 kg in the placebo group during continuous nitropatch application (Table 4). When a nitropatch was worn with concomitant ACE inhibitor, body weight was unchanged in the enalapril group and decreased 0.2±0.6 kg in the captopril group. Pairwise comparison of weight change (from day 4 to day 7) revealed a significant difference between the placebo and both ACE inhibitor groups (\( p<0.05 \)) but no significant change comparing enalapril with captopril.

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**Table 2. Postpatch Venous Volume Measurements**

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=10)</th>
<th>Enalapril (n=11)</th>
<th>Captopril (n=11)</th>
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<tbody>
<tr>
<td>Day 4: 2 hr after patch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre sublingual NTG</td>
<td>2.57±0.55</td>
<td>3.12±0.102</td>
<td>2.60±0.62</td>
</tr>
<tr>
<td>Post sublingual NTG</td>
<td>3.38±0.77</td>
<td>3.85±1.20</td>
<td>3.28±0.71</td>
</tr>
<tr>
<td>Change (pre-post sublingual NTG)</td>
<td>0.69±0.34</td>
<td>0.63±0.29</td>
<td>0.65±0.23</td>
</tr>
<tr>
<td>( p )</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Day 7: 74 hr after patch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre sublingual NTG</td>
<td>2.58±0.41</td>
<td>3.21±1.04</td>
<td>2.71±0.52</td>
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<tr>
<td>Post sublingual NTG</td>
<td>3.03±0.48</td>
<td>3.91±1.33</td>
<td>3.40±0.55</td>
</tr>
<tr>
<td>Change (pre-post sublingual NTG)</td>
<td>0.45±0.15*</td>
<td>0.70±0.36†</td>
<td>0.68±0.10†</td>
</tr>
<tr>
<td>( p )</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are mean±SD cc/100 cc arm.

Sublingual NTG, 0.4 mg sublingual nitroglycerin.

\( *p<0.01 \) change vs. 2 hr after patch; \( †p=\text{NS} \) change vs. 2 hours after patch.
In Table 3, serial renin measurements are presented for Placebo and Enalapril groups. The measurements are shown for Day 1: Baseline, Day 4: Placebo or ACEI only, and Day 7: After patch treatment. The data are given as mean ± SD ng/ml/hr. ACEI, angiotensin converting enzyme inhibitor.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=10)</th>
<th>Enalapril (n=11)</th>
<th>Captopril (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1: Baseline</td>
<td>4.6±2.0</td>
<td>3.7±2.9</td>
<td>2.8±1.5</td>
</tr>
<tr>
<td>Day 4: Placebo or ACEI only</td>
<td>5.1±5.8</td>
<td>26.1±13.5*</td>
<td>17.8±12.4*</td>
</tr>
<tr>
<td>Day 7: After patch</td>
<td>4.1±2.4</td>
<td>36.4±27.4*</td>
<td>15.9±11.0*</td>
</tr>
</tbody>
</table>

Data are mean±SD ng/ml/hr. ACEI, angiotensin converting enzyme inhibitor.

*p<0.01 change from day 1.

Discussion

Tolerance to organic nitrates has been demonstrated in vitro and in vivo in animal and human models. In a study by Zelis and Mason, complete tolerance to chronic administration of isosorbide dinitrate was demonstrated with forearm plethysmography. With this technique, the venodilator response to sublingual nitroglycerin was totally abolished in subjects after a 1-month treatment with isosorbide dinitrate 20 mg orally q.i.d. In a recent study testing interaction of transdermal nitroglycerin with methionine, a sulfhydryl donor, we observed attenuation to the venodilator effects of sublingual nitroglycerin by plethysmography after only 3 days of continuous patch application. Similarly, in the present study, rapid nitrate tolerance was produced in the placebo group. Venous response to sublingual nitroglycerin, which was fully reproducible when retested after 4 days, was attenuated by 40% after 74 hours of patch application. This partial tolerance compared with the Zelis and Mason study may have been due to our use of a small-dose nitroglycerin patch. The 10 mg/24 hr patch had been selected because it is the most commonly prescribed size.

Selection of ACE inhibitors for evaluation to maintain nitroglycerin effect was derived from the proposed mechanisms of nitrate tolerance. Drug-induced vasodilatation produces reflex stimulation of renin and catecholamines that can attenuate vasodilator action. In clinical studies using nitrates at doses to produce mild hypotension, there was rapid development of tolerance. Drug-induced elevation of angiotensin II reverses the preload and afterload effects of nitroglycerin directly by increasing sodium reabsorption in the proximal tubule and indirectly by increased aldosterone and ADH production. In addition, angiotensin II produces arteriolar vasoconstriction and increased sympathetic outflow.

Clinical studies using long-acting nitrates for angina and congestive heart failure have shown an association of drug tolerance with stimulation of the renin–angiotensin system. Agabiti-Rosei et al measured renin, aldosterone, and catecholamine levels after treatment with a single 24-hour nitroglycerin patch in patients with angina. Neurohormonal levels were elevated in the group of patients who failed to exhibit a persistent anti-ischemic response to nitrate therapy. Packer et al observed increased plasma renin levels in patients with congestive heart failure treated continuously for 48 hours with intravenous nitroglycerin. By introducing a nitrate-free interval, renin stimulation was prevented, and hemodynamic tolerance was avoided.

Not all clinical studies of nitrate tolerance have observed increased plasma renin levels. In our study, plasma renin levels did not increase in the placebo group during nitropatch therapy. This may have been due to the low-dose nitroglycerin patches used in this study. In addition, tissue renin measurements may be required to accurately reflect renin activation. Several studies have provided evidence of volume expansion during chronic nitrate therapy. In three studies with continuous intravenous or transdermal nitroglycerin, Lis et al, Bennett et al, and Levy et al noted a progressive fall in the hematocrit level, possibly due to volume expansion and hemodilution. Significant weight gain during nitrate therapy was further noted in studies by Levy et al and Packer et al. Increased weight also was noted in the present study but only in the placebo group. Pretreatment with enalapril or captopril prevented weight change during nitroglycerin administration.

Certain ACE inhibitors may potentiate the vasodilator action of organic nitrates directly by serving as sulfhydryl donors. Van Gilst and coworkers used the isolated, perfused heart model to assess the effects of ACE inhibitors, thereby eliminating

Table 4. Serial Weights

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=10)</th>
<th>Enalapril (n=11)</th>
<th>Captopril (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1: Baseline</td>
<td>69.1±12.1</td>
<td>69.3±8.8</td>
<td>69.9±12.5</td>
</tr>
<tr>
<td>Day 4: Placebo or ACEI only</td>
<td>69.5±11.9</td>
<td>69.2±9.0</td>
<td>69.9±12.5</td>
</tr>
<tr>
<td>Day 7: After patch, 74 hr</td>
<td>69.9±12.2</td>
<td>69.2±9.1*</td>
<td>69.7±12.2*</td>
</tr>
</tbody>
</table>

Data are mean±SD kg. ACEI, angiotensin converting enzyme inhibitor.

*p<0.05 weight change in nitropatch (day 4 to day 7) versus placebo (pairwise comparison).
any action mediated by systemic angiotensin activity. Captopril, a sulfhydryl-containing ACE inhibitor, potentiated the increase of coronary blood flow produced by isosorbide dinitrate alone. Similar synergistically increased flow was observed with the sulfhydryl donor cysteine; but ramipril, a nonsulfhydryl-containing ACE inhibitor, did not augment coronary flow. This pharmacological interaction parallels that reported with N-acetylcysteine and methionine.\textsuperscript{13–17,33}

Captopril or enalapril independently did not increase the venodilator response to sublingual nitroglycerin before nitropatch administration. In our previous study using methionine, a sulfhydryl donor, small but significant potentiation to sublingual nitroglycerin was observed.\textsuperscript{17} Although captopril has a sulfhydryl moiety, absence of nitroglycerin venodilator potentiation may be due to the relatively small amount of sulfhydryl groups available in captopril 25 mg t.i.d. Alternatively, it may be that venodilation after administration of sublingual nitroglycerin is near maximal in the nontolerant state. Because of a limit to venodilatation, no further effect may be possible.

Nitrates tolerance after 3 days of continuous therapy was completely prevented by concomitant enalapril or captopril therapy. Attenuation of venodilator response to sublingual nitroglycerin was only 10% in the enalapril group, \( p < 0.05 \) compared with placebo. No attenuation to chronic nitroglycerin was seen in the captopril group, \( p < 0.01 \) compared with placebo.

The similar results in the enalapril and captopril groups were paralleled by similar magnitudes of ACE inhibition. Plasma renin levels increased comparably in both groups with a slightly greater elevation in the enalapril group. In addition, prevention of plasma volume expansion by long-acting nitroglycerin, as estimated by weight change, was similar with both drugs.

The importance of the sulfhydryl group in captopril in preventing nitrate tolerance remains in question. Addition of captopril during acute nitrate exposure did not potentiate the venodilatory effect of sublingual nitroglycerin compared with our previous observations with methionine.\textsuperscript{17} Furthermore, in this study, prevention of nitrate tolerance with the nonsulfhydryl-containing ACE inhibitor enalapril was similar to that with captopril. These observations may be due to the relatively small amount of sulfhydryl groups provided by captopril 25 mg t.i.d. compared with the large single doses of N-acetylcysteine (200 mg/kg) and methionine (5 g) required to show pharmacological effect. The results of this study suggest that ACE inhibitors may be helpful as concomitant therapy to maintain the venodilatory effects of long-acting nitrates. Clinical studies in patients with angina pectoris and congestive heart failure are needed to substantiate these preliminary observations.

Acknowledgments

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KEY WORDS • nitroglycerin • captopril • nitrate tolerance • angiotensin converting enzyme inhibitors • enalapril
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