Effects of Vesnarinone on Peripheral Circulating Levels of Cytokines and Cytokine Receptors in Patients With Heart Failure*

A Report From the Vesnarinone Trial

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Study objectives: Proinflammatory cytokines may contribute to disease progression in heart failure by virtue of the direct toxic effects that these molecules exert on the heart and the circulation. Accordingly, there is interest in developing therapeutic agents with anticytokine properties that might be used as adjunctive therapy to modulate proinflammatory cytokine levels in patients with heart failure. Previous experimental studies suggested that vesnarinone has potent anticytokine properties in vitro. Therefore, we examined the effects of vesnarinone on circulating levels of cytokines and cytokine receptors in a large-scale, multicenter, clinical trial of patients with moderate-to-advanced heart failure: the Vesnarinone Trial (VEST).

Methods: Circulating levels of tumor necrosis factor (TNF)-α, soluble TNF-receptor type 1, soluble TNF-receptor type 2, as well as interleukin (IL)-6 and soluble IL-6 receptor (sIL-6R) were measured on plasma samples by enzyme-linked immunosorbent assay at baseline and at 24 weeks in patients who were receiving placebo (n = 352), 30 mg of vesnarinone (n = 367), and 60 mg of vesnarinone (n = 327).

Results: Treatment with 30 mg and 60 mg of vesnarinone had no effect on circulating levels of cytokines or cytokine receptors in patients with advanced heart failure over a 24-week period.

Conclusions: In contrast to the potent anticytokine effects observed with vesnarinone in experimental studies in vitro, the results of this clinical study suggest that vesnarinone does not have any measurable anticytokine effects in vivo in patients with moderate-to-advanced heart failure.

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Key words: cytokine; heart failure; interleukin-6; receptor; soluble interleukin-6 receptor; soluble tumor necrosis factor-receptor type 1; soluble tumor necrosis factor-receptor type 2; tumor necrosis factor-α; vesnarinone

Abbreviations: ANOVA = analysis of variance; ELISA = enzyme-linked immunosorbent assay; IL = interleukin; NYHA = New York Heart Association; sIL-6R = soluble interleukin-6 receptor; sTNFR1 = soluble tumor necrosis factor-receptor type 1; sTNFR2 = soluble tumor necrosis factor-receptor type 2; TNF = tumor necrosis factor; VEST = Vesnarinone Trial

Previous studies suggest that proinflammatory cytokines may contribute to disease progression in heart failure by virtue of the direct toxic effects that these molecules exert on the heart and the circulation. Accordingly, there has been increasing interest in developing therapeutic agents with anticytokine properties that might be used as adjunctive therapy for patients with moderate-to-advanced heart failure. Vesnarinone is a positive inotropic agent that enhances cardiac contractility through multiple mechanisms, including an increase in the inward calcium current attributable to the phosphodiesterase inhibitory properties of this compound. Previous small-scale clinical studies with vesnarinone have shown salutary effects on the quality of life, as well as morbidity and mortality in patients with advanced heart failure. However, the mechanisms for these beneficial effects are unknown.

Relevant to the above discussion is the observation that vesnarinone inhibits the production of proinflammatory cytokines and cytokine receptors in patients with heart failure. This inhibitory effect may be mediated by the phosphodiesterase inhibitory properties of vesnarinone, which could lead to a reduction in the release of proinflammatory cytokines and cytokine receptors from activated immune cells. However, further studies are needed to confirm this hypothesis and to evaluate the clinical effectiveness of vesnarinone in reducing inflammation in patients with heart failure.
Materials and Methods

Patient Population

The protocol specified that the first 1,200 patients enrolled in the VEST were to be included in the analysis of cytokines and cytokine receptors. The design, patient demographics, and results of the VEST have been reported previously.15

Circulating Levels of Cytokines and Cytokine Receptors

Plasma tumor necrosis factor (TNF)-α, interleukin (IL)-6, soluble TNF-receptor type 1 (sTNFR1), soluble TNF-receptor type 2 (sTNFR2), and soluble IL-6 receptor (sIL-6R) levels were obtained at baseline and at 24 weeks of follow-up for each patient. If the patient had a recent infection, the cytokine and cytokine receptors were drawn 2 weeks after the resolution of the most recent infection. All patients were receiving stable doses of angiotensin-converting enzyme inhibitors, diuretics, digoxin, and/or vasodilators for 30 days prior to obtaining baseline measurements. Circulating levels of cytokines and cytokine receptors were measured using an enzyme-linked immunoassay (ELISA; R&D Systems; Minneapolis, MN) that measures "total" TNF and IL-6 (ie, free [unbound] cytokine and cytokine bound to receptors).16–18 There are several aspects of the methodology used in the analysis of this large clinical database that bear further emphasis. First, plasma samples at all 189 centers in the VEST were collected using a standard protocol that was designed to minimize ex vivo production and/or catabolism of cytokines. That is, plasma samples were immediately stored on ice, the cells were rapidly separated from the plasma (<30 min), and endotoxin-free ethylenediaminetetra-acetic acid tubes were used to collect samples.19 To safeguard against the presence of heterophile antibodies,21 which can lead to spuriously high levels of cytokines in up to 15 to 20% of heart failure patients (Douglas Mann, MD; unpublished observations; June 1996), all cytokine assays were performed using at least one serial dilution to ensure that the samples diluted appropriately; subsequent dilutions were performed as necessary. All ELISAs were performed by two experienced senior technicians who were familiar with the assays, and who were blinded with respect to the treatment protocol of the patient. All plasma samples were stored at −70°C at the Core Cytokine Laboratory at the Houston Veterans Affairs Medical Center in Houston, TX. In preliminary control experiments, we determined that the process of storing and shipping the plasma samples to the Core Laboratory resulted in negligible change in the detectable levels of each cytokine tested. After the completion of the cytokine analysis, the relevant demographic and clinical data corresponding to these samples were obtained from the VEST Data Coordinating Center at the University of Wisconsin in Madison, WI.

Results

Patient Population

Of the 1,200 patients included in the analysis of cytokines and cytokine receptors, 154 patients were excluded based on either inadequate blood samples for the baseline and/or 24-week time points (n = 136) or incorrect New York Heart Association (NYHA) class (n = 18). There were 67 deaths within the first 24 weeks (18 deaths in the placebo group, 19 deaths in the 30-mg vesnarinone group, and 30 deaths in the 60-mg vesnarinone groups). The patients who died within the first 24 weeks were not included in the analysis. The final data set consisted of a total of 1,046 patients with NYHA class III/IV heart failure. Of the 1,046 patients included, 352 received placebo, 367 received 30 mg of vesnarinone, and 327 patients received 60 mg of vesnarinone.

Table 1 shows the demographic characteristics of the study population. The mean age of the patient cohort was 62 years, of which most (77%) were men. Approximately 90% were in NYHA class III functional status at the time of enrollment, and 60% of the patients were categorized as having an ischemic cardiomyopathy. As shown, there were no significant differences in age, sex, etiology of heart failure, ejection fraction, serum sodium level, and weight among patients in the placebo, 30-mg vesnarinone, and 60-mg vesnarinone groups. There was, however, a higher proportion of NYHA class IV patients in the vesnarinone groups (p = 0.02). These characteristics are similar to those previously reported for the entire cohort of VEST patients.15

Statistical Analysis

Neither the cytokine nor the cytokine-receptor data were normally distributed; therefore, the data were subjected to logarithmic transformation prior to all statistical analyses. However, in order to permit comparison with results from other studies, both the cytokine and cytokine-receptor data are presented as mean ± SEM on the untransformed scale. Analysis of variance (ANOVA) was used to compare continuous variables in the placebo, 30-mg vesnarinone, and 60-mg vesnarinone groups at baseline. The χ2 test was used for comparison of categoric variables. Cytokine levels at baseline and at 24 weeks were compared between the treatment groups using a repeated-measures factorial ANOVA. The fold change in cytokine and cytokine-receptor levels (ie, ratio of levels at 24 weeks to levels at baseline) as a function of survival was compared between the treatment groups using a factorial ANOVA. Post hoc analysis with the Tukey's test was performed where appropriate. Data were analyzed using software (SAS system for Windows, version 6.12; SAS Institute, Cary, NC). A significant difference was said to exist at p < 0.05.
Baseline values. As shown in Figure 2, changes in IL-6 and sIL-6R at 24 weeks compared to placebo, neither treatment with 30 mg of vesnarinone nor 60 mg of vesnarinone led to a significant decrease in circulating levels of cytokines or cytokine receptors among the three treatment groups. Figure 1 shows the changes in TNF-α and TNF receptors at 24 weeks compared to baseline values. Figure 1, top, A shows that there was a small increase in the circulating levels of TNF-α in each of the three treatment groups at 24 weeks, which was statistically significant in only the 30-mg vesnarinone group (p = 0.04). However, there was no significant difference in the levels of TNF-α as a function of time among patients who received placebo, 30 mg of vesnarinone, or 60 mg of vesnarinone (p = 0.77). Figure 1, middle, B, and bottom, C shows that although there was a significant increase in the circulating levels of sTNFR1 and sTNFR2 in each of the three treatment groups at 24 weeks (p < 0.001 for each group), there was no significant difference in the levels of sTNFR1 or sTNFR2 as a function of time between patients who received placebo, 30 mg of vesnarinone, or 60 mg of vesnarinone (p = 0.99 and p = 0.77, respectively). Figure 2 shows the changes in IL-6 and sIL-6R at 24 weeks compared to baseline values. As shown in Figure 2, left, A, there was a decrease in IL-6 levels in the placebo and 30-mg vesnarinone groups at 24 weeks (statistically insignificant) and a trend toward a significant increase in IL-6 at 24 weeks in the 60-mg vesnarinone group (p = 0.07). However, there was no significant difference in the levels of IL-6 as a function of time between patients who received placebo, 30 mg of vesnarinone, or 60 mg of vesnarinone (p = 0.15). Although there was an increase in sIL-6R levels in the placebo (p = 0.03) group and no significant change in the 30-mg vesnarinone (p = 0.88) or 60-mg vesnarinone (p = 0.14) groups at 24 weeks, there was no significant difference (p = 0.21) in the levels of sIL-6R as a function of time among patients who received placebo, 30 mg of vesnarinone, or 60 mg of vesnarinone (Fig 2, right, B). Thus, when compared to placebo, neither treatment with 30 mg of vesnarinone nor 60 mg of vesnarinone led to a significant decrease in circulating levels of cytokines or cytokine receptors at 24 weeks.

Since pentoxifylline, another phosphodiesterase inhibitor, was effective in reducing levels of TNF-α in patients with idiopathic dilated cardiomyopathy in a previous study,22 we also examined vesnarinone-induced changes in cytokine levels in relation to the etiology of heart failure. Figure 3 shows the fold change in TNF-α and IL-6 levels from baseline to 24 weeks (ie, 24 weeks/baseline levels), in relation to the etiology of heart failure in the three treatment groups. As shown in the Figure 3, the fold change in TNF-α and IL-6 did not differ significantly between

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### Table 1—Patient Demographics*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo (n = 352)</th>
<th>Vesnarinone, 30 mg (n = 367)</th>
<th>Vesnarinone, 60 mg (n = 327)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>63.0 ± 0.6</td>
<td>61.7 ± 0.6</td>
<td>61.7 ± 0.6</td>
<td>0.26</td>
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<td>Male sex, %</td>
<td>76.4</td>
<td>76.8</td>
<td>79.2</td>
<td>0.65</td>
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<td>Ischemic cause of heart failure, %</td>
<td>60.5</td>
<td>57.8</td>
<td>59</td>
<td>0.76</td>
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<tr>
<td>NYHA class III, %</td>
<td>92.9</td>
<td>89.9</td>
<td>86.5</td>
<td>0.02</td>
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<tr>
<td>NYHA class IV, %</td>
<td>7.1</td>
<td>10.1</td>
<td>13.5</td>
<td></td>
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<tr>
<td>Ejection fraction, %</td>
<td>21.1 ± 0.3</td>
<td>20.8 ± 0.3</td>
<td>20.7 ± 0.3</td>
<td>0.58</td>
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<tr>
<td>Serum sodium, mEq/L</td>
<td>138.5 ± 0.2</td>
<td>138.7 ± 0.2</td>
<td>138.6 ± 0.2</td>
<td>0.71</td>
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<tr>
<td>Weight, kg</td>
<td>81.7 ± 1.0</td>
<td>81.3 ± 1.1</td>
<td>84.0 ± 1.1</td>
<td>0.16</td>
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*Data are presented as mean ± SEM.

### Table 2—Baseline Cytokine and Cytokine Receptor Levels*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo (n = 352)</th>
<th>Vesnarinone 30 mg (n = 367)</th>
<th>Vesnarinone, 60 mg (n = 327)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, pg/mL</td>
<td>6.4 ± 0.2</td>
<td>6.2 ± 0.2</td>
<td>6.2 ± 0.2</td>
<td>0.29</td>
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<tr>
<td>IL-6, pg/mL</td>
<td>6.3 ± 0.5</td>
<td>6.4 ± 0.5</td>
<td>5.7 ± 0.3</td>
<td>0.80</td>
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<tr>
<td>sTNFR1, pg/mL</td>
<td>1,922.1 ± 50.5</td>
<td>1,793.5 ± 47.1</td>
<td>1,824.7 ± 51.4</td>
<td>0.10</td>
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<tr>
<td>sTNFR2, pg/mL</td>
<td>4,609.5 ± 116.2</td>
<td>4,323.8 ± 108.4</td>
<td>4,364.0 ± 106.4</td>
<td>0.08</td>
</tr>
<tr>
<td>sIL-6R, pg/mL</td>
<td>38.6 ± 0.5</td>
<td>38.9 ± 0.5</td>
<td>39.0 ± 0.6</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM.
patients with ischemic and nonischemic etiology of heart failure in the three treatment groups.

Change in Cytokine Levels and Patient Mortality

Because vesnarinone had been shown to have a dose-dependent adverse effect on mortality, we next examined vesnarinone-induced changes in cytokines in relation to patient mortality. During follow-up from the 24th week to a maximum of 78 weeks, there were 43 deaths (12.2%) in the placebo group, and 58 deaths (15.8%) and 48 deaths (14.7%) in the 30-mg vesnarinone group and 60-mg vesnarinone group, respectively. In order to determine if the changes in the cytokines levels were different in the patients who died during the follow-up period, we compared the fold change in cytokine and cytokine-receptor levels from baseline to 24 weeks in the three treatment groups. Figure 4 shows the fold change in TNF-α and IL-6 levels from baseline to 24 weeks in relation to mortality in the three treatment groups. As shown in Figure 4, left, A, the fold change in TNF-α levels was similar in the survivors and nonsurvivors in the three different treatment groups. Figure 4, right, B shows that the fold change in IL-6 levels was not significantly different in the survivors in the three different treatment groups. In contrast, there was a significant (p < 0.01) dose-dependent increase in IL-6 levels in the nonsurvivors who received vesnarinone. Post hoc testing revealed a statistically significant increase in IL-6 levels in nonsurvivors in the 60-mg vesnarinone group when compared with the nonsurvivors in the placebo group. There were no significant differences in the fold change of soluble TNF-α or IL-6 receptor levels in relation to mortality among the three treatment groups (data not shown).

Discussion

The results of this clinical study, in which we examined the anticytokine properties of vesnarinone in patients with moderate-to-advanced heart failure, suggest that neither the 30-mg/d dosage nor 60 mg/d dosage of vesnarinone had any measurable effect on the peripheral circulating levels of cytokines and cytokine receptors. The finding that vesnarinone had no discernible effect on circulating levels of TNF-α and IL-6 in humans in vivo differs from experimental data which showed that vesnarinone inhibited the production of proinflammatory cytokines. Indeed, several in vitro studies10–12 have shown that vesnarinone suppresses the production of TNF-α and IL-6 in various human cell lines, including peripheral lymphocytes, monocytes, T-cell lines, and microglial cells. Moreover, an in vivo study23 of endotoxemia in rabbits showed that IV vesnarinone reduced the circulating levels of TNF-α. Although the reasons for the discrepancy between the previous in vitro/in vivo findings are not clear, there are several possible explanations. First, the doses of vesnarinone used in the VEST may have been less than those used in

![Figure 1. Circulating levels of TNF-α and TNF-α receptors in patients with moderate-to-advanced heart failure. Circulating levels of TNF-α (top, A), sTNFR1 (middle, B), and sTNFR2 (bottom, C) were measured on plasma samples by ELISA at baseline and at 24 weeks in patients who were receiving placebo, 30 mg of vesnarinone, and 60 mg of vesnarinone. Patients receiving placebo are depicted by the closed circles, patients receiving 30 mg of vesnarinone are depicted by the open circles, and patients receiving 60 mg of vesnarinone are depicted by the open squares.](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21965/)

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experimental studies in vitro or in vivo. Second, circulating levels of cytokines may not mirror changes in cytokine levels in tissues. Thus, we cannot exclude the possibility that vesnarinone decreased cytokine levels in the myocardium and/or other tissue beds. Third, phosphodiesterase inhibitors such as vesnarinone may not be able to suppress cytokine synthesis once cytokine production has already been initiated in patients with heart failure. Nevertheless, the recent experience with the phosphodiesterase inhibitor pentoxifylline, which was shown to significantly decrease TNF-\(\alpha\) levels in patients with idiopathic dilated cardiomyopathy, would argue against this possibility. However, we did not find any decrease in levels of TNF-\(\alpha\) in patients with an ischemic or nonischemic etiology of heart failure in this study. Fourth, the short-term effects of vesnarinone observed in experimental studies may not be sustained following longer-term use of the drug; that is, there may be an "escape phenomenon" following long-term clinical use of vesnarinone. Finally, the metabolism of vesnarinone in patients with heart failure may favor the generation of one or more metabolites that lack anticytokine properties. It

Figure 2. Circulating levels of IL-6 and sIL-6R in patients with moderate-to-advanced heart failure. Circulating levels of IL-6 (left, A) and sIL-6R (right, B) were measured on plasma samples by ELISA at baseline and at 24 weeks in patients who were receiving placebo, 30 mg of vesnarinone, and 60 mg of vesnarinone. Patients receiving placebo are depicted by the closed circles, patients receiving 30 mg of vesnarinone are depicted by the open circles, and patients receiving 60 mg of vesnarinone are depicted by the open squares.

Figure 3. Changes in cytokine levels in relation to etiology of heart failure. The fold change in circulating levels of TNF-\(\alpha\) (left, A) and IL-6 (right, B) from baseline to 24 weeks are depicted for patients who received placebo, 30 mg of vesnarinone, and 60 mg of vesnarinone. Patients were classified based on ischemic (solid bars) and nonischemic (open bars) etiology of heart failure. There were no significant differences (p > 0.05) in the fold change in cytokine levels among the three treatment groups by etiology of heart failure.
bears emphasis that the results of the present study are entirely analogous to those that were reported recently with amiodarone, which was shown to suppress TNF-α production in endotoxin-stimulated mononuclear cells, but which had no measurable effect on peripheral circulating levels of TNF-α in a subset of patients with nonischemic cardiomyopathy enrolled in the Survival Trial of Antiarrhythmic Therapy in Congestive Heart Failure.

A second interesting and potentially important finding in this study is that there was a significant dose-dependent increase in IL-6 levels in the vesnarinone-treated patients who died, when compared to the nonsurvivors who received placebo (Fig 4, right, B). Although the mechanism for the increase in IL-6 levels in the nonsurvivors who were treated with vesnarinone is not known, it is interesting to note that previous reports have shown that the treatment of patients with agents that elevate cyclic adenosine monophosphate levels, such as dobutamine and pentoxifylline, has resulted in increased circulating levels of IL-6. Accordingly, it is possible that an increase in the levels of cyclic adenosine monophosphate mediated by the phosphodiesterase-inhibiting effect of vesnarinone was responsible for the higher IL-6 levels seen in patients treated with vesnarinone. The question of whether elevated IL-6 levels may be used as a serologic marker for patients who are likely to have an untoward outcome following the sustained use of phosphodiesterase inhibitors will require further study.

**Conclusion**

As noted at the outset, there has been increased interest in exploring the role of anticytokine strategies for patients with heart failure. Historically, the use of vesnarinone in the VEST was one of the first attempts to modulate proinflammatory cytokine levels in patients with heart failure. Although the present study suggests that the doses of vesnarinone used in VEST did not have anticytokine effects in patients with heart failure, the concept that anticytokine therapy may be useful in patients with heart failure is suggested by two studies. As one example, treatment of patients with dilated cardiomyopathy with pentoxifylline for 6 months was associated with an improvement in left ventricular ejection performance and clinical outcomes, as well as a decrease in circulating levels of TNF-α when compared to a comparable group of age-matched placebo control subjects. Moreover, we have previously shown that use of a soluble TNF-α antagonist can lead to an improvement in clinical outcomes and a decrease in the circulating levels of biologically active TNF-α in patients with moderate heart failure. While the results of these two clinical studies must be regarded as provisional because of the relatively small numbers of patients and the relatively short duration of follow-up, these studies do suggest that it is both safe and potentially feasible to modulate proinflammatory cytokine levels in patients with heart failure.
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REFERENCES