Vesnarinone, a New Inotropic Agent, Inhibits Cytokine Production by Stimulated Human Blood From Patients With Heart Failure

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Background Vesnarinone, a quininoline derivative, is a recently synthesized positive inotropic agent that has been shown to dramatically improve the survival of patients with heart failure. However, the mechanism of action of vesnarinone remains unknown. Reversible neutropenia complicated with vesnarinone therapy suggests that vesnarinone may modulate the production of cytokines. Because tumor necrosis factor (TNF)-α and other cytokines have been shown to depress myocardial contractility, we investigated the effects of vesnarinone on the production of various cytokines.

Methods and Results We studied the effects of vesnarinone on cytokine production by lipopolysaccharide (LPS)-stimulated whole blood from seven patients with heart failure and from five healthy volunteers. Heparinized blood was diluted in RPMI and stimulated with LPS. Vesnarinone was added in a range of 1 to 30 μg/mL, the blood was incubated for 24 hours, and interleukin (IL)-1α, IL-1β, IL-6, TNF-α, interferon (IFN)-γ, and granulocyte colony-stimulating factor (G-CSF) were measured by an enzyme-linked immunosorbent assay. LPS stimulation induced a more prominent increase in TNF-α in patients with heart failure than in healthy volunteers. Vesnarinone inhibited the production of TNF-α and IFN-γ both in healthy volunteers and in patients with heart failure. IL-1α and IL-1β were also suppressed in healthy volunteers, but this response was variable, and a significant reduction was not seen in patients with heart failure. Marked inhibition of G-CSF and other cytokines by vesnarinone was observed in one patient who had developed neutropenia as a result of vesnarinone therapy.

Conclusions Although the number of study patients was small and the results are preliminary, these findings provide evidence that vesnarinone plays an important role in the regulation of cytokines and suggest that the reduction of cytokine release may contribute to the beneficial effects of the drug in the treatment of heart failure. Furthermore, the measurement of cytokines may be useful in predicting the occurrence of neutropenia, which has been occasionally reported in patients treated with vesnarinone. (Circulation. 1994;89:955-958.)

Key Words • vesnarinone • heart failure • cytokines

According to the recent concept of heart failure, the primary goals in the management of the disease are to improve the quality of life and to prolong life. Recently developed inotropic agents that increase intracellular cAMP by either stimulating β-adrenergic receptors or inhibiting phosphodiesterase have produced dramatic short-term hemodynamic benefits in patients with advanced heart failure. However, long-term treatment with these agents results in an unfavorable outcome, with an acceleration of the disease process and an adverse effect on survival. In contrast to these agents, vesnarinone, a quininoline derivative, is a recently synthesized positive inotropic agent that has been shown to improve both the quality of life and the prognosis of heart failure. In particular, a recent report by Feldman et al demonstrated a dramatic improvement in survival during a 6-month study in patients with heart failure. They pointed out that reversible neutropenia was a complication in 2.5% of the patients treated with vesnarinone. This complication has also been associated with vesnarinone treatment in Japan. These findings strongly suggest that vesnarinone has an additional effect of modulating the production of the cytokines that play a substantial role in the pathogenesis of heart failure.

Although it has been suggested that the mechanism of action of vesnarinone is related to a slight inhibition of phosphodiesterase III, increased inward calcium current, and reduced potassium current, the true mechanism by which the drug reduces mortality has not been clarified. One possible alternative reason for the beneficial effects of vesnarinone is the recent report that this agent might inhibit the production of some cytokines. In this study, we investigated the effects of vesnarinone on cytokine production in blood from patients with heart failure and from healthy volunteers.

Methods

Seven patients with congestive heart failure (four men and three women; age range, 42 to 74 years; mean±SD age, 58±12 years) were studied. Cause of heart failure was cardiomyopathy in six patients and valvular heart disease in one patient. Three patients were in New York Heart Association (NYHA) class II and four patients were in NYHA class III. Heart rate was 70±11 beats per minute, (range, 60 to 90 beats per minute), ejection fraction was 22±7% (range, 12% to 29%), cardiac index was 2.0±0.6 L·min⁻¹·m⁻² (range, 1.5 to 3.1 L·min⁻¹·m⁻²), mean pulmonary capillary wedge pressure was 16±10 mm Hg (range, 7 to 32 mm Hg), and left ventricular end-diastolic pressure was 16±7 mm Hg (range, 4 to 22
mm Hg) at diagnostic catheterization. Patient height was 157 ± 5 cm (range, 150 to 163 cm), body weight was 48 ± 7 kg (range, 38 to 59 kg), and none of the patients were cachectic. Five patients were receiving diuretics, four were receiving angiotensin-converting enzyme inhibitors, four were receiving β-adrenergic blockers, and one was receiving digitalis. Three patients with consecutive ventricular ectopic beats on Holter monitor ECGs were receiving antiarrhythmic agents such as propranolol, mexiletine, or amiodarone.

Human whole blood was used in this study as an ex vivo model of local cytokine production. Blood from five healthy male volunteers and seven patients with heart failure was drawn into heparinized syringes (20 U/mL of heparin) and diluted in RPMI (GIBCO) at a ratio of 1:10. Increasing concentrations of vesnarinone, dissolved in 1 N HCl, diluted with FCS, and neutralized by 1 N NaOH, were added to blood stimulated with 100 ng/mL of lipopolysaccharide (LPS, Difco). The blood was incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO₂, and plasma was harvested for the analysis of cytokines. Interleukin (IL)-1α, IL-1β, IL-6, tumor necrosis factor (TNF)-α, interferon (IFN)–γ, and granulocyte colony-stimulating factor (G-CSF) were measured by an enzyme-linked immunosorbent assay using commercially available kits and kits developed by Otsuka Pharmaceutical Co., Ltd. A dose-response study was performed, in which vesnarinone in a final concentration range of 1 to 30 μg/mL was added to LPS-stimulated whole blood from five healthy male volunteers. Kruskal-Wallis test was used to compare the data for various concentrations of vesnarinone in healthy volunteers, and Mann-Whitney test was performed to analyze the values in patients with heart failure and to compare the data for LPS stimulation in healthy volunteers with those for patients with heart failure.

Results

Fig 1 demonstrates that the markedly suppressed levels of IL-1α, IL-1β, TNF-α, and IFN-γ release observed at vesnarinone concentrations of 1 to 30 μg/mL were significantly different than the control levels (70 pg/mL for IL-1α, 593 pg/mL for IL-1β, 234 pg/mL for TNF-α, and 348 pg/mL for IFN-γ, median; P < .05). Vesnarinone caused a dose-dependent reduction in TNF-α release. Reduction of IFN-γ release was prominent, and 1 μg/mL of vesnarinone suppressed IFN-γ to below the detectable level of 20 pg/mL. Elevated levels of IL-6 (33 pg/mL) and G-CSF (21 pg/mL) by LPS stimulation were observed in three individuals, and 1 μg/mL of vesnarinone suppressed IL-6 and G-CSF to below the detectable levels (20 pg/mL).

Because a profound reduction in the release of various cytokines was evident in the specimens from healthy volunteers containing 10 μg/mL of vesnarinone, speci-
moms from patients with heart failure were studied using this dose of vesnarinone. Median values for LPS-stimulated cytokines in patients with heart failure were 99 pg/mL of IL-1α, 667 pg/mL of IL-1β, 523 pg/mL of TNF-α, and 64 pg/mL of IFN-γ (Fig 2). A significant reduction in TNF-α and IFN-γ levels was observed on incubation with vesnarinone in patients with heart failure (P<.05). LPS induced an increase of G-CSF in three patients (114 pg/mL), and vesnarinone reduced G-CSF to below the detectable levels in two of these patients. LPS-stimulated production of IL-1α, IL-1β, TNF-α, and G-CSF tended to be higher in patients with heart failure than in healthy volunteers (IL-1α, 97 versus 70 pg/mL; IL-1β, 667 versus 593 pg/mL; TNF-α, 523 versus 234 pg/mL; and G-CSF, 114 versus 21 pg/mL), and the increase in TNF-α production was significantly more prominent in patients with heart failure (P<.05).

Marked inhibition of G-CSF (from 205 to <20 pg/mL), IL-1α (from 84 to <10 pg/mL), IL-1β (from 1589 to 58 pg/mL), TNF-α (from 593 to 20 pg/mL) and IFN-γ (from 291 to <20 pg/mL) was observed in one patient with valvular heart disease who had developed neutropenia as a result of vesnarinone therapy and had recovered by treatment with recombinant G-CSF (Fig 2, white dots).

**Discussion**

In this study, we demonstrated that vesnarinone inhibited the production of TNF-α and IFN-γ by LPS-stimulated whole blood from patients with heart failure and healthy volunteers. Although IL-1α and IL-1β were also suppressed in the healthy volunteers, a significant reduction was not found in patients with heart failure. LPS stimulation induced a more prominent increase in TNF-α in patients with heart failure. These results suggest that a different mechanism of cytokine regulation may exist in heart failure. Cytokine levels were unaffected by vesnarinone treatment or tended to be high in a patient with dilated cardiomyopathy with congestive heart failure. Although the importance of these changes is unclear, vesnarinone may have different regulatory effects on the production of cytokines in some patients with heart failure.

Cytokines are being increasingly recognized as essential mediators of normal and pathological immune responses. It is widely accepted that cytokines are involved in the cascade of events that lead to a wide range of biological responses to exogenous and endogenous pathogens. An elevated concentration of TNF-α has been reported in patients with chronic heart failure, and patients with high concentrations of TNF-α have had cardiac cachexia. TNF-α has been reported to depress myocardial contractility, alter muscle membrane potential, lower blood pressure, and precipitate pulmonary edema. Repeated TNF-α infusion may lead to a permanent decrease in myocardial contractility and ultimately result in dilated cardiomyopathy. Recently, the direct effects of proinflammatory cytokines on the contractility of mammalian heart were studied. TNF-α, IL-6, and IL-2 inhibited the contractility of isolated hamster papillary muscles in a concentration-dependent, reversible manner. The nitric oxide synthase inhibitor Nω-monomethyl-L-arginine blocked these negative inotropic effects. L-Arginine reversed the inhibitory effect of Nω-monomethyl-L-arginine. These findings demonstrate that the direct negative inotropic effect of cytokines is largely mediated by myocardial nitric oxide synthase. Thus, the regulation of proinflammatory cytokines may provide new therapeutic strategies in the treatment of cardiac diseases. Although the number of study patients was small and the results are preliminary, these findings provide evidence that vesnarinone plays an important role in the regulation of cytokines and suggest that the reduction of cytokine release contrib-
utes to the beneficial effects of the drug in the treatment of heart failure.

The marked inhibition of G-CSF observed in one patient with heart failure who had developed neutropenia as a result of vesnarinone therapy suggests that vesnarinone caused neutropenia by the suppression of G-CSF. This result suggests that the measurement of cytokines may be useful in predicting the occurrence of neutropenia in patients with heart failure who are treated with vesnarinone.

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