Proinflammatory Cytokine Inhibitor Prolongs the Survival of Rats With Heart Failure Induced by Pressure Overload

Tetsuo Shioi, MD; Akira Matsumori, MD; Tadashi Kakio, MD; Yasuki Kihara, MD; Shigetake Sasayama, MD

Although an increased expression of proinflammatory cytokines has been reported in cardiac tissue samples from patients with congestive heart failure (CHF) and in various animal models of CHF, the role of these cytokines in the disease remains to be determined. Dahl salt-sensitive (DS) rats fed a high salt diet develop hypertension, cardiac hypertrophy and eventually CHF. In the present study, DS rats were treated with FR167653 (1-[7-(4-fluorophenyl)-1,2,3,4-tetrahydro-8-(4-pyridyl)pyrazolo[5,1-c][1,2,4]triazin-2-yl]-2-phenylethanediene sulfate monohydrate), a new low molecular weight inflammatory cytokine inhibitor. Treatment with 10 mg/kg per day of FR167653 significantly prolonged the survival of the animals and also prevented the bodyweight loss associated with heart failure. In conclusion, a non-peptide proinflammatory cytokine inhibitor improved the survival of animals with heart failure. (Jpn Circ J 2001; 65: 584–585)

Key Words: Cytokine; Heart failure; p38 mitogen-activated protein kinase

P roinflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, have been shown to have negative inotropic properties, as well as promoting cardiac hypertrophy and cytotoxicity! The blood concentrations of inflammatory or anti-inflammatory cytokines are elevated in patients with heart failure1,2 and an increased expression of proinflammatory cytokines has been measured in cardiac tissue samples from patients with endstage heart failure! Furthermore, increased expression of proinflammatory cytokines in the heart tissue from animals with heart failure caused by postmyocarditis cardiomyopathy! chronic pressure overload and myocardial infarction has been reported. These observations suggest that proinflammatory cytokines play a role in the development of congestive heart failure (CHF) caused by several different mechanisms, but their functional significance remains to be clarified. This question was addressed by the present study in which rats with CHF caused by prolonged hypertension were treated with FR167653 (1-[7-(4-fluorophenyl)-1,2,3,4-tetrahydro-8-(4-pyridyl)pyrazolo[5,1-c][1,2,4]triazin-2-yl]-2-phenylethanediene sulfate monohydrate), a new cytokine inhibitor that specifically inhibits the production of IL-1β and TNF-α.

FR167653 inhibits the production of IL-1β and TNF-α from human monocytes stimulated with 10 μg/ml of lipopolysaccharide (LPS); the inhibitory concentrations that cause 50% inhibition (IC50) of each cytokine are 8.8×10^−8 mol/L and 1.1×10^−6 mol/L, respectively! At a concentration of 1×10^−6 mol/L, FR167653 inhibits neither the production of IL-6 by LPS-stimulated human monocytes nor the production of IL-2 or interferon-γ by phytohemaggutinin-M-stimulated human leukocytes. The continuous infusion of FR167653 at 0.032–0.32 mg·kg⁻¹·h⁻¹ significantly inhibits the increase in IL-1β and TNF-α in the blood of rats treated with LPS! In the present study, FR167653 was dissolved in drinking water at doses designed to inhibit the increase in both these cytokines in LPS-treated rats. The oral bioavailability of this agent is 40%.

Male, inbred Dahl salt-sensitive (DS) rats, originally obtained from Brookhaven National Laboratories, were bred and supplied by Eisai Co, Ltd, Tokyo. Following weaning, the animals were fed a diet containing 0.3% NaCl up to the age of 6 weeks, and 8% NaCl thereafter. The high-salt diet causes these rats to develop hypertension, with concentric left ventricular hypertrophy appearing at 11 weeks of age. Subsequently, between 15 and 20 weeks of age (mean, 18 weeks), the animals develop dyspnea and die from pulmonary congestion. At the stage of CHF, echocardiography reveals marked dilatation and global hypokinesis of the left ventricle.

First, the effects of FR167653 on the development of hypertension and cardiac hypertrophy were examined. At the age of 6 weeks, the rats were assigned to drinking water (group A, n=8) or drinking water containing FR167653 at either 3 mg/kg per day (group B, n=7) or 10 mg/kg per day (group C, n=8). The animals were killed at the age of 16 weeks and the weight of the left ventricle was measured. At 11 weeks of age, the mean systolic blood pressure was 195±3 mmHg in group A, 207±5 mmHg in group B and 199±6 mmHg in group C. The differences among these groups were not statistically significant. Table 1 summarizes the effects of FR167653 versus the vehicle on bodyweight (BW), left ventricular weight (LVW) and the LVW/BW ratio. At 16 weeks, the mean BW of DS rats treated with 10 mg/kg of FR167653 was significantly greater than that of the other groups (p<0.05). There was no significant difference in the LVW between the groups. The mean LVW/BW ratio of the DS rats treated with 10 mg/kg of FR167653 was significantly lower than that of the other groups (p<0.05).

In this model, the rats have a significant weight loss when...
they develop CHF, probably due to cachexia, but treatment with FR167653 attenuated this bodyweight reduction.

Next, the effects of FR167653 on survival were examined. DS rats were fed a diet containing 8% NaCl and water alone from the age of 6 weeks until the age of 11 weeks, at which time they were randomly assigned to remain on water alone (group D, n=5), or to receive water containing FR167653 at 10 mg/kg per day (group E, n=5). The rats were observed daily. At 11 weeks, the mean blood pressure and body weight were not different between the 2 groups (D and E) of animals (data not shown). Fig 1 shows that treatment with FR167653 versus the vehicle only significantly improved the survival of DS rats (p<0.05).

The concentration of IL-1β mRNA is increased in the heart tissue of DS rats fed a high-salt diet. We used quantitative polymerase chain reaction (PCR) to measure the amount of IL-1β mRNA in the heart tissue of untreated rats and those receiving FR167653 at 10 mg/kg per day and found that there was not a significant difference between the 2 groups (data not shown). The small difference in the concentration of IL-1β mRNA, which was below the sensitivity of PCR, might be the reason for the beneficial effect in this long-term experiment. More recently, FR167653 was reported as an inhibitor p38 mitogen-activated protein kinase (MAPK), which is cytotoxic in cultured cardiac myocytes. Thus, it is also possible that the beneficial effect of FR167653 observed in our experiment was the result of the modulation of other targets of p38 MAPK.

Acknowledgments
We thank Fujisawa Pharmaceutical Co, for generously providing FR167653 needed to perform these experiments. This work was supported in part by a Research Grant from the Japanese Ministry of Health and Welfare and a Grant-in-Aid for General Scientific Research from the Japanese Ministry of Education, Science, and Culture.

References

<table>
<thead>
<tr>
<th></th>
<th>Group A (vehicle, n=7)</th>
<th>Group B (FR 3 mg/kg, n=8)</th>
<th>Group C (FR 10 mg/kg, n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>350±12</td>
<td>351±13</td>
<td>381±6*</td>
</tr>
<tr>
<td>LVW (g)</td>
<td>1.12±0.03</td>
<td>1.12±0.03</td>
<td>1.07±0.02</td>
</tr>
<tr>
<td>LVW/BW ratio (×10^−3)</td>
<td>2.11±0.17</td>
<td>3.20±0.07</td>
<td>2.80±0.06*</td>
</tr>
</tbody>
</table>

Values are means±SEM. BW, bodyweight; LVW, left ventricular weight. *p<0.05 vs group A or B.