Propranolol ameliorates and epinephrine exacerbates progression of acute and chronic viral myocarditis


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The interaction of neurohumoral and inflammatory mediators in the development of heart failure has not been extensively studied (28). Recent investigations have shown that inflammatory cytokines play a critical role in the pathophysiology of heart failure (23). Catecholamines are neurotransmitters that may result in cardiotoxicity (7, 10, 31). Administration of catecholamines induces myocardial hypertrophy and may produce cardiomyopathy (2, 7, 11). Activation of the β-adrenergic system and neurohumoral mediators is considered a hallmark of congestive heart failure (17) and a major determinant of prognosis (3). Here, we use an experimental model of viral myocarditis to study the interaction of neurohumoral and inflammatory mediators in the development of acute and chronic viral myocarditis.

Encephalomyocarditis virus (EMCV), a picornavirus biologically similar to Coxsackie virus, can cause severe myocarditis in experimental animals. The disease in mice is characterized by myocardial necrosis and inflammation in the acute stage followed by myocardial fibrosis and hypertrophy in the chronic stage (1, 14). The disease process includes damage to cardiomyocytes that eventually heals or initial myocyte damage followed by activation of autoimmune mechanisms driven by T cells, B cells, cytokines, and immune complexes. In the final stage of dilated cardiomyopathy, viral and immunologic processes are no longer active, and heart failure predominates (25).

Several drugs have been investigated in murine models to treat viral myocarditis (14). Steroids, nonsteroidal anti-inflammatory drugs, immunosuppressive therapy, and other therapeutic modalities have been disappointing (1, 14, 26). Any benefits of these therapies were not distinguishable from spontaneous improvement, and some of the drugs were associated with an exacerbation of the disease.

Detection of activating autoantibodies against β1-adrenoceptors (18, 37) in human myocarditis has stimulated new interest in the potential benefits and mechanisms of β-blocking agents in inflammatory heart disease. Blockade of adrenergic receptors, especially β-receptors, inhibits immunosuppression caused by catecholamines and has an immunomodulating effect (9, 21, 22). A few studies have demonstrated beneficial effects of β-blockers in viral myocarditis. Tominaga et al. (36) reported that the β-blocker carteolol had beneficial effects on dilated cardiomyopathy induced by EMCV but had no effect on the early stage of myocarditis. Nishio et al. (30) reported that carvedilol improved the survival of mice infected with EMCV. Others, however, found that metoprolol actually increased the mortality of mice with myocarditis (30, 33). The interpretation of these findings in viral-induced myocarditis is complicated by the participation of neurohumoral and inflammatory responses.

Accordingly, in the present study, we investigate the influence of the β-adrenergic system on the time course of cytokine gene expression, myocardial morphology, arrhythmogenesis, and survival in mice with myocarditis induced by EMCV.
Virus Preparation and Inoculation of Mice

Methods

Virus Preparation and Inoculation of Mice

The M variant of EMCV (American Type Culture Collection, Manassas, VA) was used in this study. Viral stock was prepared as previously described (38). Briefly, human amnion cell monolayers were infected with EMCV and harvested when cytopathic effects were complete. The viral titers were determined by plaque formation on human amnion cell monolayers. The viral stock was stored at −70°C until used. BALB/c mice (Charles River Laboratories, Wilmington, MA) were inoculated with one intraperitoneal injection of 140 plaque-forming units (0.1 ml) of EMCV diluted in Eagle’s minimum essential medium. Control BALB/c mice were injected with 0.1 ml of Eagle’s minimum essential medium (sham inoculated).

All animal experiments were performed in accordance with National Institutes of Health guidelines. Protocols were approved by the Animal Care and Use Committees of Beth Israel Deaconess Medical Center and Harvard Medical School.

Epinephrine Administration

EMCV inoculation with epinephrine for 7 days and followed for 30 days. Four-week-old male EMCV-inoculated or sham-inoculated mice were divided into five treatment groups to examine the effects of epinephrine as follows: 50 EMCV-inoculated mice received 0.3 mg/kg epinephrine (group 1), 50 EMCV-inoculated mice received 0.6 mg/kg epinephrine (group 2), 20 sham-inoculated mice received 0.3 mg/kg epinephrine (group 3), 20 sham-inoculated mice received 0.6 mg/kg epinephrine (group 4), and 30 EMCV-inoculated mice received saline.

Administration of epinephrine or saline was commenced immediately after EMCV inoculation and sham inoculation and continued for 7 days. Mortality was monitored daily for 30 days. Five EMCV-inoculated mice and 5 EMCV-inoculated mice treated with 0.3 mg/kg epinephrine were killed on days 7, 14, and 30 to determine myocardial histopathological changes.

A single dose of epinephrine 120 days after EMCV inoculation. We previously showed that EMCV-inoculated BALB/c mice develop dilated cardiomyopathy at the later stage of chronic myocarditis (>90 days after EMCV inoculation) (38). Therefore, we chose 120 days after EMCV inoculation to test the response of EMCV-inoculated mice to epinephrine. Accordingly, 4-wk-old male EMCV-inoculated or sham-inoculated mice were divided into three treatment groups 120 days after EMCV or sham inoculation to determine the acute effect of a single intraperitoneal dose of epinephrine as follows: 10 EMCV-inoculated mice received a single dose of 0.3 mg/kg epinephrine (group 1), 10 EMCV-inoculated mice received a single dose of 0.6 mg/kg epinephrine (group 2), and 10 sham-inoculated mice received a single dose of 0.6 mg/kg epinephrine (group 3) and served as controls.

Propranolol Administration

EMCV-inoculated mice treated with propranolol beginning 7 days before, concomitant with, or 4 days after inoculation, and continuing for 30 days. Four-week-old male EMCV-inoculated mice were divided into four groups to examine the effects of the timing of commencement of daily treatment with propranolol (Sigma Chemical, St. Louis, MO) at 3 mg/kg ip as follows: 40 EMCV-inoculated mice received propranolol 7 days before EMCV inoculation (group 1), 40 EMCV-inoculated mice received propranolol beginning immediately after EMCV inoculation (group 2), 40 EMCV-inoculated mice received propranolol beginning 4 days after EMCV inoculation (~4 days coincides with peak viremia in this model (5, 38); (group 3)), and 40 mice received no propranolol but received saline beginning immediately after EMCV inoculation (group 4).

Propranolol or saline administration was continued daily for 30 days after EMCV inoculation. Five animals from each group were killed on days 7, 14, and 30 to determine myocardial histopathological changes. Mortality was monitored daily for 30 days.

A single dose of propranolol and epinephrine administered 120 days after EMCV inoculation. Four-week-old male EMCV-inoculated or sham-inoculated mice were divided into three treatment groups 120 days after EMCV inoculation to examine the acute benefit of a single dose of propranolol before a single dose of epinephrine as follows: 10 EMCV-inoculated mice received a single dose of 3 mg/kg propranolol 30 min before a single dose of 0.3 mg/kg epinephrine (group 1), 10 EMCV-inoculated mice received a single dose of 3 mg/kg propranolol 30 min before a single dose of 0.6 mg/kg epinephrine (group 2), and 10 sham-inoculated mice received a single dose of 0.6 mg/kg epinephrine (group 3).

Gene Expression Analysis

We used gene-array analysis to determine whether EMCV, epinephrine, and propranolol affected gene expression of cytokines. We obtained tissue from mouse hearts treated as follows: 1) EMCV-inoculated mice, 2) EMCV-inoculated and epinephrine-treated (0.3 mg/kg) mice, 3) EMCV-inoculated and propranolol-treated (3 mg/kg) mice, 4) EMCV-inoculated and epinephrine (0.3 mg/kg) + propranolol-treated (3 mg/kg) mice, and 5) saline-treated control mice. Daily intraperitoneal injections of epinephrine, propranolol, and epinephrine + propranolol were immediately commenced after EMCV inoculation and continued for 14 days. Tissue was obtained in duplicate to ensure the repeatability of the gene expression observations. Total RNA was extracted from heart tissue by TriReagent (Sigma Chemical). Two micrograms of RNA were used to perform cytokine analyses via GEArray Q Series Mouse Common Cytokine Gene Array (SuperArray Bioscience), which contains 96 common mouse cytokine genes. Analyses were performed according to the protocol of the manufacturer (SuperArray Bioscience), and the data were analyzed with a GEArray analyzer. Gene comparisons were expressed as a ratio adjusted for background and housekeeper gene expression. We selected TNF-α, IL-6, and IL-10, which are the cytokines commonly linked to the inflammation process (29, 34).

ECG Acquisition and Analysis

A subgroup (n = 19) of 4-wk-old male BALB/c mice (Charles River Laboratories, Wilmington, MA) were anesthetized with ketamine (45 mg/kg ip) and xylazine (5 mg/kg ip), and a telemetry transmitter (model ETA-F20, Data Sciences International, St. Paul, MN) was implanted in the abdomen of each mouse to obtain long-term ambulatory ECGs. Bipolar electrodes were sutured on the epicardium of the left and right ventricles in a typical lead II configuration. The animals were allowed to recover from surgery for ~7 days before EMCV (n = 13) or sham (n = 6) inoculation. ECGs were continuously recorded using a telemetry receiver and Dataquest ART version 1.10 acquisition software (Data Sciences International) for 2 wk or until the time of death.

Histopathology

Apical transverse sections of the left ventricle were fixed in 10% formalin and embedded in paraffin, cut into 3-µm-thick sections, and stained with hematoxylin and eosin. Several sections of each heart were scored blindly. Histological evidence of myocarditis and inflammation was classified in terms of the degree of cellular infiltration and myocardial cell necrosis and graded from 0 to + for each myocardial sample (26).

Statistical Analysis

Values are means ± SE. Survival of mice was analyzed by the Kaplan-Meier method. Comparison within and between groups was performed using one-way ANOVA. Differences were considered significant with P < 0.05.
RESULTS

Effects of Epinephrine on Viral Myocarditis

Epinephrine administered for 7 days after EMCV inoculation increased mortality, myocardial necrosis, and cell infiltration. Epinephrine treatment significantly increased EMCV-induced mortality during all three phases of myocarditis. The survival rate in EMCV-inoculated mice followed for 30 days was 70% for those treated with saline, 50% for those treated with 0.3 mg/kg epinephrine, and 40% for those treated with 0.6 mg/kg epinephrine (Fig. 1). Myocardial necrosis and inflammatory cell infiltration were found in EMCV-inoculated mice killed on day 7 (Fig. 2). Cardiomyocyte necrosis and inflammation were significantly more prominent, confluent in some areas, and multifocal in other areas in EMCV-inoculated mice killed on days 14 and 30 (Table 1). The myocardial histopathological scores were significantly increased in epinephrine-treated EMCV-inoculated mice killed on days 7, 14, and 30 (Table 1). Survival was 100% for sham-inoculated mice treated with epinephrine (0.3 and 0.6 mg/kg, no virus) and followed for 30 days (Fig. 1). There were no significant myocardial histopathological findings in sham-inoculated mice treated with epinephrine.

A single dose of epinephrine 120 days after EMCV inoculation caused sudden death. Seventy percent of EMCV-inoculated mice died within 10 min after a single injection of 0.6 mg/kg epinephrine, and 40% died after a single injection of 0.3 mg/kg epinephrine 120 days after EMCV inoculation. Epinephrine did not cause any mortality in sham-inoculated control mice. Histopathological examinations showed dilatation of the left ventricle and myocardial fibrosis in all mice that died (Fig. 3).

Effects of Propranolol on Viral Myocarditis

Propranolol administered 7 days before, concomitant with, and 4 days after EMCV inoculation decreased mortality, myocardial necrosis, and cell infiltration. All three regimens of propranolol administration, 7 days before, concomitant with, and 4 days after EMCV inoculation, significantly reduced mortality compared with untreated EMCV-inoculated mice (Fig. 4). The myocardial histopathological scores were significantly decreased in propranolol-treated EMCV-inoculated mice killed on days 7, 14, and 30 compared with untreated EMCV-inoculated mice (Table 2).

Propranolol administered before a single dose of epinephrine 120 days after EMCV inoculation reduced sudden death. Propranolol administered 30 min before a single dose of epinephrine 120 days after EMCV inoculation significantly decreased mortality caused by epinephrine administration in mice. Propranolol reduced the incidence of death from 70% to 33% (P < 0.05) in EMCV-inoculated mice given 0.6 mg/kg epinephrine and from 41% to 25% (P < 0.05) in EMCV-inoculated mice given 0.3 mg/kg epinephrine. Myocardial histopathology showed a dilated left ventricle in all mice that died. Epinephrine did not cause any mortality in sham-inoculated control mice.

Gene Expression of Cytokines

We used gene-array analysis to determine gene expression of cytokines in hearts from EMCV-inoculated mice, EMCV-inoculated mice treated with epinephrine (0.3 mg/kg), EMCV-inoculated mice treated with propranolol (3 mg/kg), EMCV-inoculated mice treated with epinephrine (0.3 mg/kg) + propranolol (3 mg/kg), and saline-treated control mice. EMCV increased the expression of TNF-α (5.5-fold), IL-6 (4.7-fold),
and IL-10 (13.7-fold) compared with saline-treated mice. Epinephrine further increased the expression of TNF-α (9.6-fold), IL-6 (7.7-fold), and IL-10 (16.5-fold). Propranolol, however, blunted the expression of TNF-α (2.0-fold), IL-6 (3.1-fold), and IL-10 (3.0-fold) in EMCV-inoculated mice. Furthermore, propranolol attenuated the expression of TNF-α (2.7-fold), IL-6 (3.2-fold), and IL-10 (6.3-fold) in EMCV-inoculated and epinephrine treated-mice compared with saline-treated mice.

**Complex Arrhythmias in EMCV-Inoculated Mice**

Eight of the 13 EMCV-infected mice died during the 2-wk period of ECG monitoring, but none of the sham-inoculated mice died. Figure 5 shows characteristic ECG recordings from an EMCV-inoculated mouse on the day of death. Bradycardia, S-T segment elevation, conduction block, premature contractions, and idioventricular rhythm were observed in the mice that died. Arrhythmias increased with time after EMCV inoculation and were complex on the day of death. Uninfected control animals did not manifest arrhythmias.

**DISCUSSION**

Myocarditis can be viewed as a three-phase process, beginning with an acute phase within a few days after virus inoculation (14). The acute phase includes early pathological evidence of myocardial damage. Cell-mediated immunity may follow and activate a subacute phase of myocardial and endothelial damage after viral invasion between around day 7 and around day 30 after inoculation. The chronic phase is characterized by gradual myocyte loss, myocardial fibrosis, and progression to dilated cardiomyopathy (23). In agreement with previous studies (5, 16, 34), we found that the subacute phase of myocarditis was characterized by extensive myocardial necrosis and inflammation and increased TNF-α, IL-6, and IL-10 gene expression. Mortality reached 30% in the EMCV-inoculated group. The occurrence of arrhythmias and death in EMCV-inoculated mice correlated temporally with maximal infiltration and necrosis scores. Two-thirds of the EMCV-inoculated mice monitored for ECG disturbances exhibited an idioventricular rhythm and died within 2 wk. Arrhythmias have

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**Table 1. Histological grading in epinephrine-treated EMCV-inoculated mice on days 7, 14, and 30**

<table>
<thead>
<tr>
<th></th>
<th>Infiltration</th>
<th>Necrosis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>EMCV</td>
<td>0.7±0.1</td>
<td>1.8±0.1</td>
</tr>
<tr>
<td>EMCV+epinephrine</td>
<td>1.1±0.2*</td>
<td>2.3±0.2*</td>
</tr>
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</table>

Values are means ± SE; n = 5. Epinephrine was administered at 0.3 mg/kg. Histological scoring of hearts ranged from 0 to 4+ in each of the categories of infiltration and necrosis: 0, no or questionable presence of myocardial lesions in each category; ≥1, limited focal distribution of myocardial lesions; ≥2, to ≥3, presence of multiple myocardial lesions; ≥4, presence of coalescent and extensive lesions over the entire examined heart tissue. EMCV, encephalomyocarditis virus. *P < 0.05 vs. EMCV alone.

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**Fig. 3.** Apical transverse sections of left ventricle from a sham-inoculated control mouse (A) and an EMCV-inoculated mouse (B) after 120 days. Dilated cardiomyopathy was present in the EMCV-inoculated mouse after 120 days. Hematoxylin-eosin staining. Magnification, ×20.

**Fig. 4.** Survival in EMCV-inoculated mice treated with propranolol (0.3 mg/kg) 7 days before, concomitant with, and 4 days after EMCV inoculation. Propranolol was administered daily for 30 days after EMCV inoculation. Percent survival in all propranolol-treated mice was significantly higher than in untreated EMCV-inoculated mice (*P < 0.05).
been previously demonstrated in murine models of viral myocarditis, including sinus arrest, second- or third-degree atrioventricular block, atrial premature complexes, and ventricular tachycardia (33).

A main finding of our study is that short-term (7 days) epinephrine administration exacerbated histopathology, cytokine gene expression, and mortality during myocarditis in EMCV-inoculated mice. Percent survival was significantly lower in EMCV-inoculated mice treated with the low and high dose of epinephrine than in untreated EMCV-inoculated mice during the acute, subacute, and chronic phases of viral myocarditis. No detrimental sequelae were observed in sham-inoculated mice treated with epinephrine. We further demonstrated that propranolol, a nonspecific \( \beta \)-adrenergic antagonist, ameliorated the detrimental effects of EMCV infection on histopathology, cytokine gene expression, and survival during the subacute phase of myocarditis. Propranolol, moreover, also attenuated the detrimental effects of epinephrine in EMCV-inoculated mice. Irrespective of the timing of treatment with propranolol, whether before, concomitant with, or 4 days after EMCV inoculation, propranolol had significant beneficial effects on overall survival and extent of histopathological changes 30 days after viral infection.

It is well known that catecholamines modulate T cell function by \( \beta \)-adrenergic receptor stimulation and \( \beta \)-adrenergic receptor-independent mechanisms (4, 20). Catecholamines act through \( \beta \)-adrenergic receptors to regulate the expression of the cytokine receptors (8). Epinephrine and norepinephrine have immunosuppressive effects (13). Chronic \( \beta \)-adrenergic activation influences the expression of cardiac TNF-\( \alpha \) and IL-1\( \beta \) in experimental heart failure models (28, 30). Various cytokines may induce left ventricular dysfunction, left ventricular remodeling, fetal gene expression, and cardiomyopathy (23, 25). The detection of activating autoantibodies against \( \beta_1 \)-adrenergic receptors in patients with myocarditis and cardiomyopathy (12, 18, 37) has heightened interest in the pleiotropic interactions of the \( \beta \)-adrenergic system.

Chronic myocarditis with dilated cardiomyopathy occurred 90 days after EMCV inoculation (38), with increased myocardial expression of TNF-\( \alpha \) and IL-1\( \beta \) (34). It is known, moreover, that cardiomyopathy increases the cardiovascular response to epinephrine (10, 31). We demonstrated that epinephrine challenge of EMCV-inoculated mice at 120 days resulted in sudden death; increased sensitivity to catecholamines could cause arrhythmias and explain the high mortality in epinephrine-challenged EMCV-inoculated mice (15). The histopathological examination showed a dilated left ventricle in all mice that died. Propranolol treatment 30 min before epinephrine administration to EMCV-inoculated mice significantly reduced sudden death.

Left ventricular dysfunction and dilated cardiomyopathy have been described in transgenic mice with sustained TNF-\( \alpha \) expression (6) comparable to the levels we measured in EMCV-inoculated mice. Myocardial dysfunction leads to chronic adrenergic stimulation, which precipitates myocyte hypertrophy, myocyte damage, fibrosis, and apoptosis (19). The mechanisms mediating the detrimental effects of excessive \( \beta \)-receptor stimulation may include protein kinase A-induced hyperphosphorylation of the sarcoplasmic reticulum (SR) Ca\(^{2+} \) release channel, resulting in permanent leakage of Ca\(^{2+} \) (23). Uncoordinated SR Ca\(^{2+} \) release and diastolic Ca\(^{2+} \) oscillations may increase diastolic tone, reduce systolic force generation, and trigger arrhythmias (27). \( \beta \)-Adrenergic receptor blockade has been shown to correct the defective interaction of the SR Ca\(^{2+} \) release channel (ryanodine receptor) with its ligand FKBP 12.6 (32). The detrimental effects of acute and chronic epinephrine administration may be due to excessive protein kinase A-induced phosphorylation of the SR Ca\(^{2+} \) release
channel. Accordingly, there are multiple pathways by which propranolol may exert therapeutic effects.

In conclusion, this study demonstrated that administration of epinephrine exacerbated myocarditis and increased mortality in EMCV-inoculated mice. Treatment with the β-blocker propranolol reduced the severity of myocarditis and mortality. These beneficial effects of propranolol were conferred irrespective of whether propranolol was administered as a prophylactic or a treatment.

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