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Effect of Metoprolol in Acute Coxsackievirus B3 Murine Myocarditis

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Recent studies suggest that beta-adrenergic blocking agents show promise in the management of cardiomyopathies; however, their role in acute myocarditis is unknown. One hundred 3 week old mice were infected with coxsackievirus B3 and were given either metoprolol (n = 50) or normal saline solution (n = 50) intraperitoneally for 10 days. Twenty mice from each group were observed for mortality for 30 days. Of the remaining 60 mice, 10 from each group were killed on day 3, 6 or 10 and examined for heart viral titers and pathologic changes.

Mortality rate in the metoprolol group was 60% compared with 0% in the saline group ($p < 0.005$). Viral titers on day 10 of infection were $10^{2.6 \pm 0.2}$ median tissue culture infective dose for the metoprolol group versus $10^{2.1 \pm 0.1}$ for the saline group ($p < 0.05$). Whereas pathologic changes at days 3, 6 and 10 of infection were similar in both groups, on day 30 of infection, inflammation, necrosis and mineralization scores (mean ± SEM) were 1.1 ± 0.3, 2.1 ± 0.4, 2.2 ± 0.5 for the metoprolol group versus 0.3 ± 0.1, 0.4 ± 0.3, 0.4 ± 0.3 for the saline group, respectively ($p < 0.01$). Six noninfected mice received metoprolol intraperitoneally for 10 days; there was no mortality during 30 days of observation. In conclusion, metoprolol administration exerts deleterious effects in acute coxsackievirus B3 murine myocarditis.

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Viral cardiomyopathy is a disease caused by multiplication of virus in the myocardium, pericardium or endocardium that temporarily or permanently affects the normal anatomy and physiologic functions of the heart (1). The overall prevalence of myocarditis in patients with suspected viral illness ranges from 2.3 to 5% (2). In the acute phase of viral myocarditis, patients may suffer from congestive heart failure and valvular insufficiency, whereas in the chronic phase they may develop chronic heart failure, constrictive pericarditis or valvular insufficiency (3).

Several drugs have been investigated in experimentally induced coxsackievirus murine myocarditis to explore their therapeutic potential in modulating the course of the disease. Trials with corticosteroids (4), ibuprofen (5), sodium salicylate and indomethacin (6) either did not alter the disease or even had deleterious effects. Traditionally, beta-adrenergic blocking agents have been considered to be contraindicated in patients with congestive heart failure; however, recent studies have suggested that these agents improve survival rates in patients with cardiomyopathy (7) and that their withdrawal results in clinical deterioration (8). Moreover, exercise was found to exert a deleterious effect on the course of viral myocarditis in a murine model (9). We hypothesized that beta-receptor blockade might exert a beneficial effect on viral myocarditis by placing the heart at rest. Furthermore, a beta-blocker is occasionally used in the course of viral myocarditis to control arrhythmias, palpitation or incidental hypertension, however, its role in viral myocarditis has not been previously investigated. Therefore, we sought to determine the effect of metoprolol on acute coxsackievirus B3 myocarditis in mice.

Methods

Virus. Coxsackievirus B3 (nancy strain) was used to produce the experimental infection after its passage in tissue cultures, as has been described previously (10). Each mouse was injected intraperitoneally with 0.1 ml of minimal essential medium of stock virus containing $10^{5.2}$ median tissue culture infective dose.

Drug administration. Metoprolol (CIBA-Geigy Corp.) solution was prepared by dissolving the powder in sterile water for injection. The solution was sterilized with a 4.5 μm filter, and each mouse was injected with 0.013 mg/g intraperitoneally at 7:00 AM and 5:00 PM, and 0.0065 mg/g at 12 noon, daily for 10 days starting on day 0 of the study. The dose was

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Table 1. Study Groups

Study of acute phase of myocarditis
Group I: infected, receiving metoprolol (n = 30)
Group II: infected, receiving saline solution (n = 30)
Mortality study
Group III: infected, receiving metoprolol (n = 20)
Group IV: infected, receiving saline solution (n = 20)
Toxicity study
Noninfected, receiving metoprolol (n = 6)
Control group
Noninfected, nontreated (n = 2)

decided on through a series of pilot studies in 3 week old noninfected mice to achieve a reduction in heart rate of 40 to 50% throughout most of the day.

Experimental design (Table 1). A total of 108 3 week old cesarean-derived-1 (CD1) Swiss Webster mice (Charles River Laboratory) was utilized in the study. Two noninfected, nontreated mice were kept in a separate cage and served as a control for the study groups.

Group I consisted of 30 mice infected with coxsackievirus B3 on day 0 of the study; they received metoprolol for 10 days starting at day 0. Group II consisted of 30 mice infected with coxsackievirus B3 on day 0 of the study; they received saline solution for 10 days starting on day 0. Ten mice from each group were killed on day 3, 6 or 10. Groups I and II served to study the effect of metoprolol on the acute phase of myocarditis. Group III consisted of 20 mice infected with coxsackievirus that received metoprolol for 10 days starting on day 0 of the study, and Group IV consisted of 20 mice infected with coxsackievirus that received saline solution for 10 days starting on day 0 of the study. Groups III and IV were observed for mortality for 30 days. Six noninfected mice were entered into a toxicity study.

Autopsy protocol. On the day they were killed, the mice were anesthetized by ether and exsanguinated by way of the axillary artery. Death assurance was performed by cervical dislocation and the thoracic cavity was explored; the heart was excised and examined for gross pathologic changes. The apex of the heart was cut and minced with a tissue grinder in 0.5 ml minimal essential media, and viral assay was performed as described previously (11).

The heart was then fixed in 10% formalin solution and processed for histologic examination. Transverse sections at the mid-left ventricular cavity level were obtained, and histologic sections were stained with hematoxylin-eosin. Each section was examined for evidence of mononuclear and polymorphonuclear cellular infiltration, necrosis and mineralization, and was given a histologic score between 0 (no involvement noted) to 4+ (100% involvement), with 1+, 2+ and 3+ representing 25, 50 and 75% involvement of the histologic section, respectively. A similar semiquantitative scoring system was utilized previously to assess the extent of myocarditis (6). Histologic examination was done without the knowledge of the treatment given.

Toxicity study. A group of six 3 week old noninfected mice was injected with metoprolol in the same dose utilized in the study and was observed for 30 days for weight gain and mortality.

Statistical analysis. Statistical analysis was done by the Mann-Whitney test for the viral titers, Mantel-Haenszel test for the comparison of survival of curves and Student's *t* test for degree of histopathologic involvement. Data are expressed as mean ± SEM.

Table 2. Viral Titers

	Group I: Metoprolol	Group II: Saline	p Value*
Day 3	$10^{4.7\pm0.6}$	$10^{4.4\pm0.4}$	NS
Day 6	$10^{4.1\pm0.4}$	$10^{3.7\pm0.3}$	NS
Day 10	$10^{2.6\pm0.2}$	$10^{2.1\pm0.1}$	<0.05

NS = not significant.

Results

Viral titers during the acute phase of myocarditis (Table 2). On days 3 and 6 of infection, viral titers were similar in the infected control group and infected metoprolol group; however, on day 10 of infection, the viral titer was $10^{2.6 \pm 0.2}$ in the metoprolol group versus $10^{2.1 \pm 0.1}$ in the control group ($p < 0.05$).

Histopathologic examination (Table 3). Heart sections revealed focal myocardial involvement with mononuclear and polymorphonuclear cellular infiltrates, necrosis and areas of dystrophic mineralization. Histopathologic scores for days

Table 3. Histopathologic Scores

	Group I: Metoprolol			Group II: Normal Saline		
Day	I	N	M	I	N	M
3	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0	0	0
6	2.7 ± 0.2	2.5 ± 0.4	1.9 ± 0.2	2.8 ± 0.2	2.2 ± 0.4	1.9 ± 0.3
10	2.9 ± 0.4	2.3 ± 0.4	2.4 ± 0.3	2.7 ± 0.2	1.9 ± 0.4	1.9 ± 0.4
30	1.1 ± 0.3	2.1 ± 0.4	2.2 ± 0.5	0.3 ± 0.1*	0.4 ± 0.3*	0.4 ± 0.3*

*$p < 0.01$. I = inflammation; M = mineralization; N = necrosis.

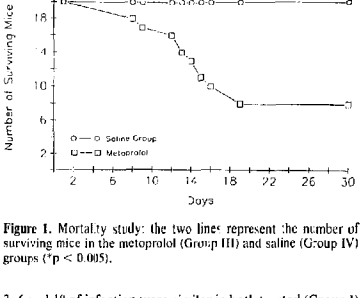

Figure 1. Mortality study: the two lines represent the number of surviving mice in the metoprolol (Group III) and saline (Group IV) groups (*$p < 0.005$).

3, 6 and 10 of infection were similar in both treated (Group I) and untreated (Group II) groups; however, on day 30 of infection, inflammation, necrosis and mineralization scores were 1.1 ± 0.3, 2.1 ± 0.4 and 2.2 ± 0.5 for the metoprolol group (Group III) versus 0.3 ± 0.1 ($p < 0.01$), 0.4 ± 0.3 ($p < 0.01$) and 0.4 ± 0.3 ($p < 0.01$) for the control group (Group IV), respectively.

Mortality study (Fig. 1). In the metoprolol-treated group, 8 (40%) of the 20 mice in Group III survived, whereas in the saline group (Group IV), all 20 mice (100%) survived ($p < 0.005$). Half of the dead mice exhibited autolysis at the time of death and autopsy was not performed. In the remaining dead mice, autopsy revealed the heart to be grossly involved with exudates typical of myocarditis.

Toxicity study. Six mice that were not infected but received metoprolol continued to thrive normally; the average weight increased from 18 to 20 g at the beginning of the study to 26 to 30 g 1 week later (average weight gain about 50%). There were no deaths in this group or in the two control noninfected, nontreated mice during 30 days of observation.

Discussion

Deleterious effect of beta-blockade therapy. Chronically increased sympathetic tone contributes to increased arterial vascular resistance and heart rate. Such an increase in afterload and heart rate may contribute to hemodynamic deterioration in the failing heart and, in fact, some preliminary data (7) appear to favor the use of beta-blocking agents in the management of patients with congestive heart failure. The effect of beta-blockers, however, in acute myocarditis is not known. This study shows that metoprolol, in a dose sufficient to produce a beta-receptor blockade effect on normal mice without any evidence of toxicity, is deleterious to mice infected with coxsackievirus B3. Infected, treated mice had significantly greater mortality in the first 30 days after infection, and the increased number of deaths was associated with more viral replication on day 10 of infection and more myocardial necrosis on day 30 of infection as detected by histopathologic studies.

Mechanism. The mechanism of the metoprolol effect in our study is not known; however, it is possible that it either interfered with body defense against viral replication or unfavorably changed the hemodynamics of the infected heart. It is possible that metoprolol did interfere with the natural defense lines against infection, thus making the mice more susceptible to infection. It was demonstrated that propranolol (another beta-blocker) can inhibit the phagocytic activity of polymorphonuclear leukocytes and monocytes (12), and may suppress mitogenic activation of lymphocytes (13); however, the effect of the drug on lymphocytes and interferon production in our model was not investigated.

Conclusions. Metoprolol administration in acute murine myocarditis leads to significantly increased mortality, increased viral replication on day 10 of infection and more cardiac damage on day 30 of infection.

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