Animal Models for Therapeutic Trials of Viral Myocarditis

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Introduction

Clinically, viral myocarditis may appear in a wide variety of forms, ranging from a total lack of clinical manifestations to sudden, unexpected death. Myocarditis may be subacute or even chronic, leading to progressive myocardial failure and death [1, 2]. We have developed animal models for viral myocarditis using encephalomyocarditis (EMC) virus in which congestive heart failure developed in a range of the acute to subacute stages [5], and dilatation and hypertrophy as seen in dilated cardiomyopathy developed in the chronic stage [6]. Histologically, myocardial necrosis became apparent on day 7, cellular infiltration was most marked on day 14, and thereafter, inflammatory reaction decreased, and fibrosis appeared; calcification persisted to day 28. Marked myocardial fibrosis and hypertrophy of myocardial cells became evident on day 90. We have investigated the natural history and pathogenesis of viral myocarditis and assessed diagnostic methods and therapeutic and preventive interventions in these models [7–20, 23, 26–28, 30, 31, 34]. This review discusses our recent therapeutic trials of viral myocarditis.

Virus Vaccine and Passive Immunization

Passive immunization with hyperimmune rabbit sera given early after virus inoculation had a protective effect in EMC virus myocarditis [10]. Virus-specific vaccine prevented development of myocarditis due to EMC virus. After subsequent challenge with the virus, all vaccinated mice survived without developing myocarditis [10]. Vaccination of mothers before pregnancy completely inhibited myocardial virus replication and had a protective effect on EMC virus infection in offspring [11]. Such vaccines may not be required for widespread use in any entire population but could be important for specifically targeted high-risk groups.
Antiviral Agents

In an animal model of EMC virus myocarditis, we demonstrated that human leukocyte interferon-α A/D given 1 day before or simultaneously with inoculation with EMC virus inhibited multiplication of virus in the heart and protected mice from developing myocarditis. Prevention was dependent on dosage and on the time of initiation of treatment. When treatment was started previous to or simultaneously with virus inoculation, interferon-α A/D at a dose of $10^7$ U/kg per day effectively reduced both inflammatory response and myocardial damage [12].

Ribavirin (virazole, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic nucleoside analogue, structurally related to inosine and guanosine, and has broad antiviral activity against RNA and DNA viruses. Clinically, its efficacy has been demonstrated in measles, influenza, and respiratory syncytial virus. Ribavirin at a dose of 200 mg/kg inhibited viral replication in the heart and improved survival and myocardial damage when treatment was started simultaneously with infection [8]. Ribavirin was also effective at a dose of 400 mg/kg beginning 1 day or 3 days after inoculation. Ribavirin also had beneficial effects on coxsackievirus B3 myocarditis. The minimal effective dose required for effect against coxsackievirus myocarditis was smaller than that for EMC virus myocarditis [13].

As described above, both interferon-α A/D and ribavirin were effective, but large quantities of these agents were necessary to obtain beneficial effects. The side effects of these drugs are usually dose related; synergistic combination of drugs may allow effective treatment at lower drug concentrations. This strategy has been highly successful in the treatment of a number of bacterial diseases and cancers. Since the mode of action of interferon may differ from that of ribavirin, we investigated the possibility that recombinant interferon-α A/D is synergistic with ribavirin. Ribavirin at 100 mg/kg or interferon at $10^6$ U/kg alone did not inhibit EMC virus replication in the heart. When used together, 100 mg/kg ribavirin and $10^6$ U/kg interferon per day achieved a striking effect. The use of the synergistic combination of interferon and ribavirin to treat mice infected with EMC virus produced an enhanced survival. The myocardial virus concentration, the inflammatory response, and myocardial damage were also effectively reduced in mice treated with the above combination of ribavirin and interferon.

We further investigated the effects of interferon-α and ribavirin on coxsackievirus B3 myocarditis in mice. In this experiment, 4-week-old C3H mice were inoculated with the Nancy strain of coxsackievirus B3. The combination treatment of ribavirin 100 mg/kg plus interferon $5 \times 10^6$ U/kg effectively reduced viral replication on day 4 even when the treatment was started 1 day after infection. This combination achieves greater effects at lower concentrations than either drug used alone, and there is potential for reduction in frequency of undesirable side effects of both drugs. The use of combinations of antiviral agents deserves further careful study for other serious viral infections.