An Experimental Model of Acute and Subacute Viral Myocarditis in The Pig

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Twenty-six young pigs were infected with encephalomyocarditis virus. observed clinically, studied at intervals by noninvasive and invasive methods to assess cardiac function and eventually examined pathologically.

All infected animals appeared ill, usually manifesting diminished appetite, lethargy and fever. Spontaneous mortality occurred either 1 to 4 or 20 to 21 days after infection. Electrocardiographic abnormalities, seen in the majority of animals, comprised ST-T wave changes, conduction disturbances or ventricular ectopic rhythm. The majority of animals manifested echocardiographic evidence of left ventricular dilatation and decreased systolic function, which improved with time in some animals.

Hemodynamic studies revealed elevation of biventricular filling pressures in 3 of 10 animals; as a group, infected animals manifested significantly elevated right ventricular filling pressures. In selected animals, the feasibility of gallium scans as well as left ventriculography and coronary angiography was demonstrated.

At autopsy, heart weight/body weight ratio was significantly elevated in infected animals. The heart of all but two animals showed active myocarditis associated with fibrosis and focal calcification in the later stages.

In general, the cardiovascular manifestations were parallel with those seen in acute and subacute myocarditis in humans. It is concluded that encephalomyocarditis infection in the pig is a large animal model of viral myocarditis suitable for assessing alterations in the structure and function of the cardiovascular system and the effects of interventions.

This report presents an overview of our experience with this model, establishing its usefulness for further studies of the natural history and pathophysiology of viral myocarditis and the effects of interventions.

Methods

Experimental procedure. Outbred Yorkshire-Long-White-Duroc Hampshire pigs aged 7 to 9 weeks were used. Littermates of three to five animals were obtained. The pigs were fed a Purina chow diet ad libum.

The myocardiotropic variant of the encephalomyocarditis virus was used. Virus stock was prepared as described previously (2) and was stored at −70°C.

Eight female and 18 male young pigs weighing 11 to 14 kg were infected by deep intramuscular injection of $2.7 \times 10^7$ plaque-forming units (PFU) of encephalomyocarditis virus. The animals were shaved; permanent cutaneous markers of three to five animals were obtained. The pigs were fed a Purina chow diet ad libum. The myocardiotropic variant of the encephalomyocarditis virus was used. Virus stock was prepared as described previously (2) and was stored at −70°C.

Eight female and 18 male young pigs weighing 11 to 14 kg were infected by deep intramuscular injection of $2.7 \times 10^7$ plaque-forming units (PFU) of encephalomyocarditis virus. The animals were shaved; permanent cutaneous markers assured reproducible electrode placement. Hindlimb leads (leads I, II, III, aVR, aVL and aVF) as well as chest leads at the apex and shoulder and lead V_{10} over the seventh thoracic vertebra were recorded. Rectal temperatures and serial electrocardiograms (ECCs) were recorded throughout the study. The pigs were studied in a harness without sedation. Care was taken not to overly excite them. Some animals were killed at 2 to 3 weeks, 4 weeks and 3 months to 1 year after infection.

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Serologic tests. Neutralizing antibody titers were obtained to screen experimental animals for preexisting serum antibodies. Blood samples were drawn from an ear vein and spun down at 2,000 rpm for 10 min. The plasma was then incubated for 30 min at 50°C to inactivate complement and the sample serum was stored at 20°C for neutralizing antibody titer analysis. Equal quantities of inactivated undiluted serum and decimal dilutions of virus were incubated together at 37°C for 30 min. A 0.1-ml aliquot of the serum-virus mixture was then introduced into tube cultures of amnion fibroblast line cells. Cultures were examined microscopically for cytopathic changes after incubation for 4 days at 37°C. A serum that neutralized 1:64 of the virus was considered to contain antibody. Neutralizing antibody titers were also measured on sera obtained at the time of sudden death.

Hemodynamic studies. Pigs without marked cardiac dysfunction were studied 2 to 4 weeks after infection. They were premedicated with intramuscular ketamine (5 to 10 mg/kg body weight) and atropine (0.2 mg/kg). An intracatheter was sutured in an ear vein and thoripental administered intravenously to effect (4 to 5 mg/kg). The pig was intubated with a size 5-6 cuspidal endotracheal tube and anesthetized with a gaseous mixture of isoflurane and nitrous oxide.

A Gould ES1000 recorder with transducer amplifiers was used to record pressures at the time of catheterization. A femoral artery catheter was placed on each animal. The animal was heparinized (10,000 U of heparin sodium injection, USP) and a Millar 7F catheter passed into the left ventricle. Right heart catheterization was performed percutaneously by means of the jugular vein with use of a 5F Swan-Ganz catheter. Right and left ventricular pressures, pulmonary artery pressure and capillary wedge pressure were recorded. Cardiac output was measured by thermodilution. Cardiac catheterization was performed before infection in 10 pigs and 2 weeks or 1 month after infection in 10 pigs.

Echocardiography. Serial two-dimensional and M-mode cursor-directed echocardiograms were obtained with use of a 5-MHz transducer (Interspec Cardioscan) in awake unanesthetized animals. Precordial long-axis views were recorded. Details of the method have been reported previously in an echocardiographic study (3) of 90 healthy pigs that served as the control group for the present study.

Left ventriculography and coronary angiography. For left ventriculography, 10 ml of contrast medium was injected at 3 ml/s through a 7F Cordis angiographic four-sidehole catheter. A 7F renal catheter was used for injection into the coronary arteries. Ventriculograms and angiograms were recorded cineangiographically.

In both experimental and control animals, intracoronary injections of histamine (10 µg/kg) and intravenous injections of ergonovine (0.2 or 0.4 µg) were used in an attempt to induce coronary artery spasm.

Gallium imaging studies. Intravenous gallium-67 nitrate (6 mCi) was injected into three pigs. Seventy-two hours after injection, the pigs were sedated lightly and scanned with an Elscint Apex 410 gamma camera. Anteroposterior, posterioranterior, oblique and lateral images were obtained.

Histopathologic methods. At induced death, animals were sedated with intramuscular ketamine (50 mg/kg), lightly anesthetized with intravenous thiopental and intubated. Nitrous oxide (1%) and isoflurane (3%) were administered until a deep anesthetic plane was obtained. The chest was opened and the heart quickly excised and placed in 10% phosphate-buffered formalin solution. After sudden death, the heart was excised and placed in formalin. Sections were dehydrated in a graded ethanol series and embedded in paraffin. Sections (3 to 5 µm thick) were cut with glass knives and stained with hematoxylin-cosin. Three to five sections taken from the left and right ventricular free walls, interventricular septum, interventricular septum, atria, pulmonary artery, and aorta were examined by light microscopy. Sections from the right and left ventricles and interventricular septum were taken at the level of the mitral valve, which corresponded to the level studied echocardiographically. Samples were reviewed without knowledge of study data for the presence and severity of infiltration, necrosis, fibrosis and calcification.

Results

Signs of illness. All infected animals exhibited some signs of illness. Diminished appetite, lassitude or weakness was observed primarily in pigs that died suddenly and in those with severe cardiac dysfunction as assessed by echocardiography. All infected animals showed febrile reactions; some rectal temperatures were as high as 106.4°F. Episodes of hypothermia and circulatory collapse were noted terminally in all pigs that died of acute myocarditis. Table 1 presents the mortality data for the study. There was a bimodal distribution of death, which occurred either early (1 to 4 days) or late (20 to 21 days) after infection.

Electrocardiographic changes (Table 2). These changes were present in a majority of the pigs and occurred as early as 24 h after infection. The ECG changes included a diffuse

| Table 1. Mortality, Induced Death and Survival of 26 Infected Pigs* |
|-----------------|---|---|---|---|---|
|                | 1 | 2 | 3 | 4 | 5 |
| Deaths     |    |   |   |   |   |
| Spontaneous (no. 1) | 6 | 1 | 2 | 4 | 0 |
| Induced (no. 1)   | 0 | 3 | 4 | 6 | 4 |
| Survivors (no. 1) | 20 | 16 | 10 | 4 | 0 |
| Natural mortality (%) | 23 | 5 | 12 | 6 | 0 |

*The cumulative mortality rate in 3 weeks was 49.2%. One pig was killed on each of 4 days: days 14, 21, 28, and 34.

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| Table 2. Prevalence of Electrocardiographic Abnormalities* |
|---------------------------|---|
| T wave inversions in two or more leads | 76 |
| Premature ventricular complexes | 60 |
| Ventricular tachycardia | 24 |

*One or more abnormalities were present in all infected animals.
decrease in voltage, conduction disturbances, ventricular ectopic rhythm and ST-T wave changes. All pigs showed T wave inversions (Fig. 1), present throughout the study in some but normalizing in others.

Echocardiography. On echocardiographic examination (infected n = 26), most pigs (84%) demonstrated left ventricular enlargement with a decrease in fractional shortening (Fig. 2). There was partial return of cardiac function assessed by fractional shortening in some pigs after severe left ventricular dilation.

Gallium scans. Gallium scans were performed on three pigs within the 1st 10 days after infection. All three pigs subsequently demonstrated histopathologic findings consistent with acute myocarditis. However, only one of the three pigs had a gallium scan that was positive for myocardial inflammation (Fig. 3). Of note, this pig was scanned earliest (that is, within 5 days after infection).

Hemodynamic measurements at cardiac catheterization (Table 3). As a group, infected pigs differed from control pigs only by significant elevation of right atrial and right ventricular end-diastolic pressures. However, when individual hemodynamic profiles were analyzed, three pigs manifested elevation of both right and left ventricular filling pressures. Coronary injection of histamine and ergonovine resulted in constriction of the large coronary vessels in two infected pigs but not in the control pigs.

Pathology. Heart weight/body weight ratio at autopsy in nine pigs ranged from 3.5 to 10 x 10^-3 and averaged 5.5 x 10^-3 (SD ±2.1), almost double that reported for healthy pigs.
Figure 4. Early myocarditis 5 days after infection, demonstrating myocardial fibrosis and interstitial infiltration with mononuclear cells. Low magnification, hematoxylin-eosin stain.

Figure 5. Heart removed from a pig 30 days after infection, showing focal opalescent depressed lesions.

swine (4). The heart in animals that died ≤4 days after infection manifested diffuse pallor of the myocardium. Histologically, in early myocarditis (n = 7), there was myofiber necrosis and interstitial infiltration with mononuclear cells (Fig. 4). Foci of perivascular mononuclear cell infiltration were also present.

In the later stage of the disease (30 days after infection), focal opalescent depressed lesions could be seen grossly (Fig. 5). These foci extended into the myocardium and sometimes to the endocardium. Histologically, there was proliferation of fibrous connective tissue and calcification associated with necrosis and mononuclear infiltration in four of six pigs killed 30 days after infection. Two pigs had no evidence of histopathologic lesions: one of these had preexisting neutralizing antibodies.

None of the control pigs demonstrated significant lesions grossly or microscopically.

Neutralizing antibodies. Twenty-four of 26 pigs studied demonstrated histopathologic evidence of myocarditis. Only one demonstrated neutralizing antibodies before infection. All animals tested after infection demonstrated neutralizing antibody titers to the virus.

Late follow-up. Two pigs that had repeat catheterization at 4 months after infection demonstrated persistent systolic dysfunction. One pig studied at 1 year after infection had normal cardiac function at 30 days after severe dysfunction, but demonstrated a decrease in cardiac function on repeat echocardiography and cardiac catheterization after 1 year. In addition, a pig that did not demonstrate cardiac dysfunction during the acute and subacute stages demonstrated clear heart failure, with pleural effusion confirmed by echocardiography.

Discussion

The pig as model of virally induced myocarditis and cardiomyopathy. The traditional experimental model for studies of viral myocarditis has generally been the mouse (5), which is available in inbred strains, thus permitting selection of a strain in which a viral infection with a given strain of virus may be expressed in a desired way (that is, either as an acute disease with a high mortality rate [6] or as a milder disease with a longer survival time [7,8]). This model is therefore well suited for the study of factors enhancing as well as ameliorating the disease process. Furthermore, the use of inbred animals reduces the variability of disease expression in any one experiment. Although much of our
current knowledge about the pathogenesis and natural history of viral myocarditis has been derived from studies of murine models, it is well recognized that the disease in humans is much more variable and unpredictable (1). Furthermore, the study of cardiac pathophysiology by modern noninvasive and invasive techniques is difficult and often not feasible in small rodents.

For these reasons, it appeared desirable to develop a large animal model of myocarditis that might resemble the human disease and be suitable for pathophysiological studies. The pig was chosen as the animal because of its size, its ready availability and the similarity of its heart and circulation to those in humans. Furthermore, like the human it is an outbred mammal that might express the wide spectrum of the natural history of viral myocarditis similarly. The encephalomyocarditis virus was chosen because it is known to be myocarditic in the pig and to produce in this animal an epidemic disease, in which myocarditis is often the dominant feature.

The encephalomyocarditis virus possesses many properties of RNA viruses recently classified under the designation of picornaviruses. It is biologically similar to several well-known agents that cause central nervous system disease and myocarditis in humans and animals (polioviruses, coxsackievirus groups A and B, virus of foot and mouth disease). For reasons not fully understood, in swine, primates and mongooses encephalomyocarditis virus affects the myocardium without significant central nervous system involvement. This virus is especially suitable for experimental work because it is infectious in many animal species, but is only exceptionally pathogenic in humans (9). It is also easily handled in the laboratory.

Previous studies. Encephalomyocarditis virus was first reported as a cause of pig mortality in Panama by Murnane et al. (10). Subsequently, in Florida, the virus was repeatedly associated with pig deaths during the period between 1960 and 1966 (11). Acland and Littlejohns (12) reported an outbreak of encephalomyocarditis infection in pigs in New South Wales, Australia.

In pigs killed at 28 to 36 days after experimental infection with encephalomyocarditis virus, Craighead et al. described scattered, silver-white, shallow depressions on the epicardial surface, approximately 2 to 5 mm in diameter. Microscopic examination revealed foci of myocardial necrosis. Littlejohns and Acland (14) produced 24 experimental infections, 17 of which were fatal. Encephalomyocarditis virus in pigs was associated with gross and histological findings closely resembling those seen with coxsackievirus infections in humans. Homer and Hunter (15) produced non-suppurative myocarditis in pigs experimentally infected with encephalomyocarditis virus; all animals had gross and microscopic changes consistent with such infection. These investigators did not describe the pathophysiology or clinical course of this disease process nor the effects of therapeutic interventions.

The present study confirmed many of the clinical manifestations of the epidemic disease reported earlier. Furthermore, it revealed considerable similarity of the gross and microscopic lesions with those reported for human acute and subacute myocarditis (1, 5).

Clinical manifestations. Several striking similarities are noted between the clinical manifestations seen in the present model and those of the human disease. In the acute stage of the human disease, clinical signs have included pyrexia, tachycardia and weakness. These may be followed by rapidly progressive congestive heart failure or circulatory collapse, with or without cardiac arrhythmias. Pigs that died suddenly in the present experiments demonstrated pyrexia and tachycardia, with evident malaise and decreased activity. Ventricular arrhythmias were common. Circulatory collapse was accompanied by hypothermia and cyanosis preceding death. In contrast to this abrupt, severe and often fatal disease seen in humans, especially in the neonatal period, viral myocarditis in adolescents and adults is often subclinical in nature. Similarly, viral myocarditis produced in swine was often subclinical in nature.

Noninvasive and invasive assessment of the model. Almost all pigs demonstrated ECG changes. Gallium imaging was found to be feasible and may yield positive findings. Echocardiography proved to be the most valuable noninvasive method of study, permitting serial evaluation of the dimensions of cardiac chambers as well as ventricular function. Typically, there was significant dilation and depressed contractile function after infection (Fig. 2). Fractional shortening was decreased, but as is often the case in humans, recovered partially or fully (16–18). Cardiac catheterization documented depressed systolic and diastolic function in some pigs with normal echocardiographic findings.

Histopathology. Mononuclear infiltrates and necrosis of myofibers were the characteristic histopathologic lesions in the acute and subacute stages of the disease. The chronic stage was accompanied by myocardial fibrosis and focal calcification, but necrosis and mononuclear infiltrates often persisted. These histopathologic findings closely parallel those reported in human viral myocarditis (1, 5) and in experimental murine viral myocarditis (5, 8). However, calcific deposits are rare in humans.

Conclusions. A large animal model of acute experimental viral myocarditis was developed in the pig. This model is suitable for serial studies of the clinical and pathophysiological manifestations of the acute disease and its subsequent natural history by means of noninvasive as well as invasive methods not easily applicable to the traditional murine models of viral heart disease. This model is also suitable for the study of the effects of interventions.

The variability of viral heart disease in this model in an outbred large animal most closely resembles that seen in humans as contrasted with the uniformity of disease expression in any one strain of inbred mice.

In agreement with evidence obtained from studies of viral myocarditis in mice and humans, the present studies indicate
that acute viral myocarditis in the pig may progress to chronic dilated cardiomyopathy.

References