Glutaraldehyde Inactivation of Exotic Animal Viruses in Swine Heart Tissue

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Glutaraldehyde, 0.2%, in a 1:100 (wt/vol) ratio, inactivated four animal viruses (foot-and-mouth disease, swine vesicular disease, African swine fever, hog cholera) in swine heart tissues during 11-day exposures at 22 to 26°C.

Surgical replacement of defective human heart valves with glutaraldehyde (GA)-fixed swine aortic valve bioprostheses has been a successful xenograph procedure (2, 10, 12, 13). Intensified use of the technique dramatically increased demands for swine hearts of optimal sizes. However, requirements for valves from swine weighing 100 to 200 kg cannot be economically met with domestic swine. Consequently, swine hearts must be imported from foreign sources where exotic animal diseases, such as foot-and-mouth disease, may exist.

Because GA is used as a compatible fixative for porcine aortic valves (2) before they are mounted in prosthetic units, experiments were performed to determine the GA treatments required to inactivate exotic animal viruses. The test viruses were selected on the basis of their ability to replicate or remain viable in swine tissues, the availability of sensitive assay systems, prodromal periods long enough to permit distant transport of infectious tissues from clinically normal animals, and the range of biochemical diversity. The selected viruses were: foot-and-mouth disease virus (FMDV), a rhinovirus; swine vesicular disease virus (SVDV), an enterovirus; hog cholera virus (HCV), a pestivirus; and African swine fever virus (ASFV), a putative iridovirus (7). The FMDV type A, subtype 3, strain Mecklenburg was a mixture of bovine vesicular fluid and macerated vesicular epithelium diluted 1:10 in minimum essential medium used as inoculum to infect swine. The SVDV was a 10% suspension of swine vesicular material. The HCV was a 10% suspension of defibrinated acute-phase blood from swine experimentally infected with HCV. The ASFV was a 10% suspension of defibrinated acute-phase blood from swine experimentally infected with ASFV.

Line-bred Tamworth swine weighing 175 to 250 kg were used for infectivity assays and as donors of infective aortic valve units. Feeding and housing practices used to maintain swine under secure experimental conditions have been described elsewhere (3).

Primary bovine kidney (1), a swine kidney cell line (MVPK) (4, 6), and swine buffy coat cultures were used for infectivity assays of FMDV, SVDV, and ASFV. The BK and MVPK cells were prepared as monolayer cultures in 120-ml prescription bottles for plaque assays of FMDV (7) and SVDV (4). The ASFV was assayed for infectivity as described by Hess and Pan (9). Virus-neutralizing antibodies were assayed as described elsewhere (5, 11).

Donor swine were infected with FMDV or SVDV by inoculation into the foot pads with 10% suspensions of macerated infectious vesicular material previously described. Swine were infected with HCV and ASFV by intramuscular inoculations of 10% defibrinated viremic blood previously described. Within 2 to 7 days after the viruses were inoculated, swine showed acute clinical signs of the expected disease and were killed. Hearts from four donor swine for each disease were grossly excised, placed in cold, sterile phosphate-buffered saline, and transported to biological hoods for dissection. Aortic valve units were excised, carefully trimmed of extraneous tissue, and weighed, and a 1-g section of each valve unit was taken for infectivity titer. Trimmed valves were rinsed thoroughly in cold, sterile phosphate-buffered saline and transferred to flasks containing phosphate-buffered 0.2% GA. (Fresh buffered GA was obtained from Hancock Laboratories, Anaheim, Calif.) The volume of GA was adjusted to yield a 1:100 (wt/vol) ratio, and the flasks were stored in the dark at room temperature for 24 h. The first GA solutions were then discarded and replaced with an equal volume of fresh 0.2% buffered GA. Valves were exposed to the second GA treatment for 10 more days at room temperature.

After 11 days of exposure to 0.2% GA, the valves were individually rinsed three times in cold, sterile phosphate-buffered saline and pre-

pared as 1:10 suspensions in minimum essential medium in a high-speed tissue homogenizer. The minimum essential medium contained 0.34% sodium bisulfite to neutralize free GA. Each valve suspension was centrifuged, and the clarified supernatant was pooled with the other three suspensions in its set and used for inoculum in four test swine.

For detection of surviving HCV or ASFV, each test swine was intramuscularly inoculated with 5 ml of clarified valve suspension. For detection of infectious FMDV and SVDV, each test swine was inoculated with 0.5 ml of clarified valve suspension in the hind foot pads and 5 ml intramuscularly. After the swine were inoculated with the suspensions, they were observed for 14 days for clinical signs of virus-specific disease. Swine not reacting to these injections were killed on day 14, and serum samples were assayed for antibody to the suspected virus.

Preliminary trials indicated that the 72-h treatments with 0.2, 0.4, and 1% GA did not inactivate FMDV, SVDV, HCV, and ASFV. The weight-to-volume ratio used in these trials was 1:10 without change of GA solutions. These trials also showed that valves fixed at GA concentrations greater than 0.2% lost optimal flexibility and thus were not suitable for use in biosynthetic units.

In the definitive experiments, valve specimens were exposed to fresh 0.2% GA (1:100) for 10 more days. All FMDV, SVDV, and ASFV donor swine had substantial infectivity titers in their blood, valve tissue, or both at the time of harvest (Table 1). Because results of preliminary trials show that valve units from HCV-infected swine contained infectious HCV, we assumed that this was also true in the definitive experiment. After 11 days of exposure to buffered 0.2% GA, none of the valves contained detectable levels of FMDV, SVDV, HCV, and ASFV as determined by animal inoculations. Thus, we concluded that this treatment inactivated the four test viruses in swine aortic valve specimens. Although detailed kinetic studies were not done due to cost factors, the 11-day fixation period is compatible with manufacturing processes.

Results of infectivity assays (Table 1) indicate that high concentrations of FMDV, SVDV, and ASFV may be found in heart tissues of infected swine. Several infectivity titers (5 logs/g) were especially notable because the trimmed valves contained minimal amounts of residual myocardium. Also notable is the fact that valve tissue from SVDV-infected swine contained more infectious virus than found in the blood. These data support data reported by S. S. Lai (Ph.D. thesis, Cornell University, Ithaca, N.Y., 1977)

TABLE 1. *Inactivation of test viruses in swine aortic valves after exposure to 0.2% glutaraldehyde for 11 days*

Donor virus	Donor response[a]		Swine response[b]	
	Viremia	Aortic valve titers	Clinical	14-day serology (VN)
FMDV	4.8, 4.5 4.5, 4.2	<1.0, 2.8 5.2, 2.2	NEG	NEG
SVDV	3.3, <1.0 <1.0, <1.0	5.3, 3.2 4.7, 5.0	NEG	NEG
HCV	NT	NT	NEG	NT
ASFV	>4.0	>3.0	NEG	NEG

[a] Four samples of donor blood and aortic valve tissue were taken immediately after euthanasia. FMDV and SVDV titers are expressed as $\log_{10}$ plaque-forming units per milliliter or gram; ASFV titers are expressed as $\log_{10}$ hemadsorption units per milliliter or gram.

[b] Four test swine were observed for virus-specific disease for 14 days after inoculation with donor aortic valve suspension. NT, Not tested; NEG, negative for clinical disease after inoculation with 10% aortic valve tissue suspension from valves exposed for 11 days to 0.2% glutaraldehyde, or no virus neutralization (VN) antibody detected.

and suggest that SVDV may replicate in swine heart tissues rather than be "in transit" due to viremia. Also, it has been postulated (8) that SVDV may be a mutant of coxsackie B-5 virus, which causes myocardial lesions in humans.

In conclusion, low concentrations of GA will inactivate infective viruses in heart tissues if the weight-to-volume ratio is high and when the holding time is long enough. Also, the four test viruses in swine heart tissue were inactivated with a fixation procedure compatible with requirements for xenograph transplants into humans (2). Although clinically diseased swine would not likely be selected as donors of aortic valves for biosynthetic heart valve units, fixation in buffered 0.2% GA as described here should obviate any biohazards associated with their importation from areas where animal diseases foreign to the United States may exist.

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