Comparative Medicine
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Vol 65, No 5
October 2015
Pages 444–447

Original Research

An Effective and Reproducible Model of Ventricular Fibrillation in Crossbred Yorkshire Swine (*Sus scrofa*) for Use in Physiologic Research

James M Burgert,[1,2*] Arthur D Johnson,[1,2] Jose C Garcia-Blanco,[1] W John Craig,[2] and Joseph C O'Sullivan[2]

Transcutaneous electrical induction (TCEI) has been used to induce ventricular fibrillation (VF) in laboratory swine for physiologic and resuscitation research. Many studies do not describe the method of TCEI in detail, thus making replication by future investigators difficult. Here we describe a detailed method of electrically inducing VF that was used successfully in a prospective, experimental resuscitation study. Specifically, an electrical current was passed through the heart to induce VF in crossbred Yorkshire swine (n = 30); the current was generated by using two 22-gauge spinal needles, with one placed above and one below the heart, and three 9V batteries connected in series. VF developed in 28 of the 30 pigs (93%) within 10 s of beginning the procedure. In the remaining 2 swine, VF was induced successfully after medial redirection of the superior parasternal needle. The TCEI method is simple, reproducible, and cost-effective. TCEI may be especially valuable to researchers with limited access to funding, sophisticated equipment, or colleagues experienced in interventional cardiology techniques. The TCEI method might be most appropriate for pharmacologic studies requiring VF, VF resulting from the R-on-T phenomenon (as in prolonged QT syndrome), and VF arising from other ectopic or reentrant causes. However, the TCEI method does not accurately model the most common cause of VF, acute coronary occlusive disease. Researchers must consider the limitations of TCEI that may affect internal and external validity of collected data, when designing experiments using this model of VF.

Abbreviations: TCEI, transcutaneous electrical induction; VF, ventricular fibrillation.

The transcutaneous electrical induction (TCEI) method of inducing ventricular fibrillation (VF) in research swine is frequently used in laboratory physiologic and resuscitation studies.[11,14,15] Studies using TCEI techniques to induce VF often do not describe the procedure in detail, making replication of the technique difficult for later investigators. Furthermore, almost no studies quantify or otherwise report on the reliability of the TCEI method. Investigators designing physiologic or resuscitation experiments may be unable to find detailed models of VF in the literature. The difficulty in finding proven models of VF may lead to the unnecessary euthanasia of research animals in repeated attempts to develop appropriate models.

The model that we describe in this observational study may reduce the number of pigs euthanized and promote their wellbeing by providing a refined and humane technique of electrically inducing VF. When designing their research, investigators might find that using this model helps them to avoid unnecessary, time-consuming, and costly experimental model development. The primary objective of this observational study was to describe in detail a simple, reliable, and reproducible model of VF in laboratory swine that has been used successfully for resuscitation studies requiring VF. The secondary objective was to determine the percentage of a population of swine that converts to VF within 10 s after beginning the TCEI procedure.

Received: 05 Mar 2015. Revision requested: 07 May 2015. Accepted: 18 May 2015.
[1]The Geneva Foundation, Tacoma, Washington, and [2]Academy of Health Sciences, US Army Medical Department and School, Fort Sam Houston, Houston, Texas.
**Corresponding author. Email: James.burgert@alumni.bcm.edu*

Materials and Methods

The animals (n = 30) used during the study were male (weight, 60 to 80 kg) crossbred Yorkshire swine (*Sus scrofa*). All animals were housed and managed humanely in accordance with the *Guide for the Care and Use of Laboratory Animals*.[4] This study was conducted as an approved extension of a prospective, experimental study approved (no. BPTS-14-04) by the IACUC of Bridge PTS Laboratories (San Antonio, Texas).

The swine were premedicated with an intramuscular injection of atropine (0.05 mg/kg) and sedated with an intramuscular injection of tiletamine–zolazepam (4.4 mg/kg; Telazol, Fort Dodge Animal Health, Fort Dodge, IA). Anesthesia was induced with inhaled isoflurane, 2% to 5% in 100% oxygen, delivered by an anesthesia machine (Narkomed M, Dräger, Telford, PA). After endotracheal intubation of the pigs, the investigators reduced the isoflurane concentration to a maintenance dose (1% to 2%) for the remainder of the experiment. The left carotid artery was exposed surgically and cannulated with a 20-gauge catheter. ECG (leads II and V), arterial blood pressure, oxygen saturation, end-tidal CO_2 capnometry, and rectal temperature were monitored (MP 50, Phillips Healthcare, Andover, MA) throughout the experiment, and cardiac output was monitored continuously by using a Vigileo hemodynamic monitoring system (Edwards Lifesciences, Irvine, CA).

The materials used to perform the procedure included: 2 spinal needles (22 gauge; length, 3.5 in.; Becton-Dickinson and Company, Franklin Lakes, NJ), 3 alkaline batteries (9 V each) connected in series, two 5-mL syringes, and 2 standard alligator-lead wires (Figure 1). Each pig was placed in the supine position, with all limbs firmly secured to the operating table to prevent injury to research staff or dislodgment of monitors and supportive equipment, because the animals were expected to exhibit forceful, whole-body muscle contracture during the procedure. The spinal needles were prepared by removing the stylets from both and connecting a 5-mL syringe to each needle hub. The first (superior) spinal needle was inserted along the left sternal border, within the second intercostal space (Figure 2). Once the needle was below the skin surface, continuous aspiration of the syringe was applied to detect inadvertent vascular entry. The superior needle was advanced to a depth of approximately 1.25 in. (3.2 cm), as monitored through ultrasonography. Ultrasonography was used in the first 10 pigs to measure the insertion depth accurately and to visually confirm that the needles did not penetrate the heart or other underlying organs or vascular structures. To improve stability, the syringe was removed after needle placement. When the needle is placed correctly, it pulsates in time with the pig's heartbeat. The second (inferior) spinal needle was inserted, in the same manner as the first needle, immediately caudal to the xiphoid process to a depth of approximately 1.25 inches (3.2 cm). Alligator-lead wires were attached to the shaft of each spinal needle. The proximal clip of the inferior alligator-lead wire was connected to the exposed negative pole of the series-connected batteries (Figure 3). All research staff were cleared of the animal before proceeding with TCEI of VF. The proximal clip of the superior lead wire was tapped rapidly on the exposed positive pole of the series-connected batteries until VF was observed on ECG leads II and V and the absence of a perfusing waveform on the arterial line tracing was noted.

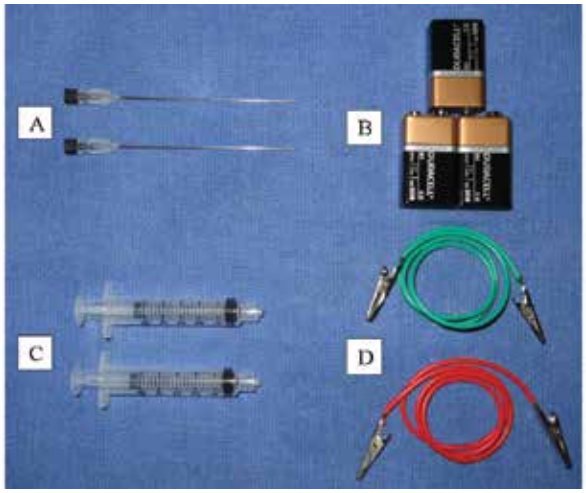

Figure 1. Materials required to perform the TCEI procedure. (A) Two spinal needles (3.5 in. long; 22 gauge). (B) Three alkaline batteries (9V each) connected in series. (C) Two 5-mL syringes. (D) Two standard alligator-lead wires.

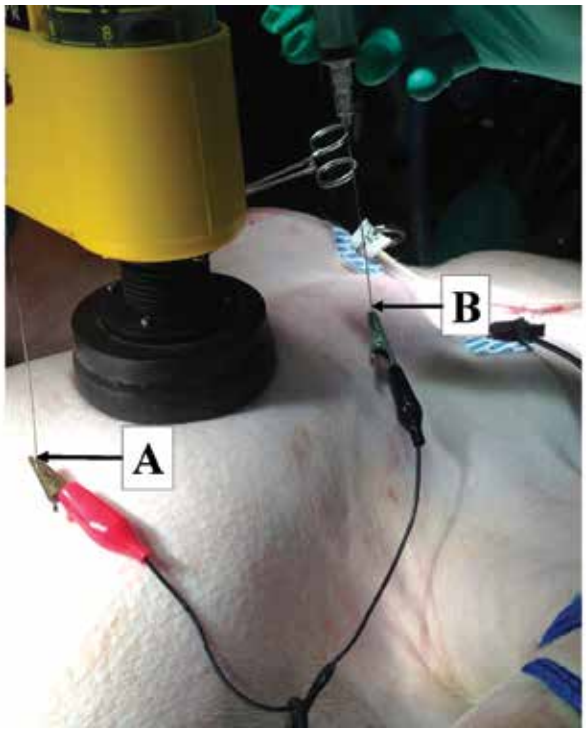

Figure 2. Spinal needles in the correct position. (A) The inferior needle is inserted immediately caudal to the xiphoid process. (B) The superior needle is inserted parasternally in the second intercostal space.

Results

Of the pigs (n = 30) that underwent TCEI, 28 (93%) converted from sinus rhythm to VF within 10 s after the beginning of the procedure. On the 2 occasions when pigs failed to convert to VF within 10 s of induction, the investigators redirected the superior spinal needle approximately 10° medially. In both instances, the affected pigs converted to VF within 10 s after redirection of the superior spinal needle. We surmise that these 2 pigs initially failed convert to VF within 10 s of induction because lateral misdirection of the needle led to inadvertent contact and discharge of electrical energy into the nearby left lung.

Pigs remained in VF for 8 to 26 min during the administration of electrical and drug interventions included in the main experimental protocol. ECG in leads II and V indicated that all pigs maintained consistent VF waveforms during the postinduction observation. The presence of a nonperfusive state during VF was confirmed by the absence of measurable arterial blood pressure, cardiac output, and end-tidal CO_2 capnometry until mechanical chest compressions and manual ventilations were initiated as conditions of the main experimental protocol.

Discussion

Several methods may be used to induce VF in laboratory animals including transcutaneous[11,14,15] and transvenous electrical manipulation,[13,16,17] coronary artery occlusion,[10,13,17] drugs,[3] hypoxia,[1] and blunt-force trauma.[7] All of these techniques successfully induce VF but differ in technical difficulty, cost, efficiency, and requirements for sophisticated training or facilities.

Transvenous electrical methods for inducing VF requires skilled operators and prolonged preparation time to place vascular sheaths and pacing wires in the right ventricle of the heart.[13,16,17] Coronary artery occlusion most closely approximates the actual cause of sudden cardiac death in humans but requires operators who have interventional cardiology skills (for directing a

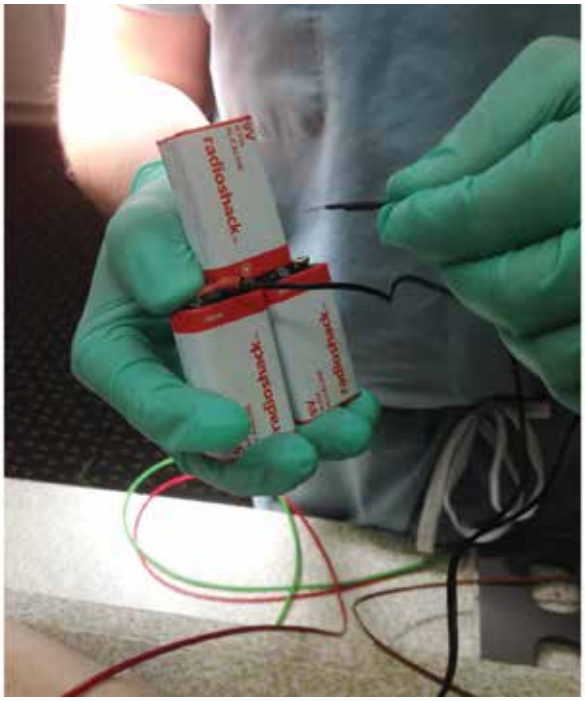

Figure 3. Inferior alligator-lead wire connected to exposed negative pole, with operator prepared to contact superior alligator-lead wire to exposed positive pole.

balloon-tipped catheter into a coronary artery), live fluoroscopy and contrast media to guide placement, and equipment as found in an electrophysiology lab.[10,13,17] Use of the occlusion technique may involve considerable preparation time, thus decreasing laboratory efficiency, and is the most costly of the methods discussed. Various drugs, such as potassium chloride, induce asystole rather than VF, thus abrogating their usefulness in studies in which VF is a necessary part of the experimental protocol.[3] Hypoxia, induced by disconnecting a subject from life-support equipment and cross-clamping the endotracheal tube, results in an unpredictable onset of VF preceded by bradyarrhythmia that occurs over a prolonged amount of time, which varies from animal to animal.[1] In addition, there a risk of causing negative-pressure pulmonary edema when inspiratory effort occurs against a clamped endotracheal tube; this side effect is suboptimal in studies where survival is an outcome measure. Furthermore, blunt-force trauma is practical as a means of inducing VF only when attempting to replicate the mechanism of injury of commotio cordis in a laboratory setting.[7]

The TCEI method that we described here offers many advantages: it is easy to perform, reproducible, and efficient and does not require advanced training or sophisticated facilities. The 93% successful rate of conversion to VF within 10 s of beginning the procedure, achieved during the current study, provides supporting evidence of the reliability of the TCEI method. Swine maintained a stable VF waveform through the 6 min before the electrical and drug therapy measures prescribed in the main experimental protocol were initiated. The duration of VF ranged from 8 to 26 min, during which pigs received standard treatment according to the American Heart Association's Advanced Cardiac Life Support VF treatment protocol.[8] Only 55% of the pigs in the main study converted from VF to a perfusing, organized rhythm after resuscitative treatment. Another team of investigators studying the stability of electrically induced VF found that VF was maintained in all pigs for 24 min before progressing to pulseless electrical activity or asystole,[6] thus indicating that electrically induced VF is long-lived in the absence of chest compressions. The results of our investigation not only support these findings but also indicate electrically induced VF is long-lived even with chest compressions and appropriate drug and electrical therapy.

The materials required for TCEI are few, reusable from animal to animal, and cost less than USD$20. Compared with other methods for inducing VF, TCEI may significantly decrease total research protocol costs, given that more repetitions of the experimental protocol can be performed each day, speeding time to experimental completion, and decreasing per-diem laboratory costs. Another cost-containment advantage of TCEI is the elimination of the necessity for time-consuming radiation safety training, radiology support personnel, and monitoring. In addition, exposure of research staff to ionizing radiation is eliminated or minimized. The use of the TCEI technique may enable additional researchers to perform physiologic experiments requiring VF, especially where access to funding, sophisticated facilities, or colleagues with skills in interventional cardiology techniques may be limited.

Although TCEI offers several advantages, investigators conducting resuscitation research need to weigh these advantages against potential limitations of the technique that may affect the internal and external validity of the study. The most obvious limitation is that TCEI does not replicate the most common cause of VF in humans, coronary artery occlusion secondary to plaque rupture.[9,13] Swine reportedly are more likely to return to spontaneous circulation and survive after electrically induced VF than after VF generated by using coronary artery occlusion.[10] A recent systematic review and meta-analysis concluded that electrically induced VF is easier to defibrillate than is ischemically induced VF.[5] This difference is especially true in untreated coronary artery occlusion, which often leads to the recurrence of VF even after successful defibrillation.[2] Furthermore, swine are 3 times more sensitive to electrical induction of VF than are humans.[12] The preponderance of this evidence suggests that data generated from resuscitation experiments using electrical induction of VF may not translate as well to human resuscitation conditions.

Although the evidence we obtained does not strongly favor the use of electrical induction of VF in resuscitation studies focusing on treatment of lethal arrythmias caused by coronary occlusive disease, the TCEI technique remains a useful and valid method of inducing VF under appropriate experimental conditions. Examples of appropriate experimental conditions may include pharmacologic studies requiring VF as an experimental condition, VF resulting from the R-on-T phenomenon as seen in prolonged QT syndrome, and VF arising from other reentrant or ectopic causes. However, researchers are not limited in using the TCEI technique in the types of studies cited in the previous examples, provided that investigators are aware of the technique's potential limitations when they generalize animal data to human populations.

TCEI of VF is a simple, effective, reproducible, and cost-effective method available to investigators using the swine model to conduct physiologic and resuscitation research. This method may be especially useful to investigators who have limited access to funding, sophisticated facilities, or colleagues with skills in interventional cardiology techniques. Investigators must consider the limitations of TCEI that may affect the internal and external validity of collected data when designing experiments using this method.

Acknowledgments

This work was funded by a postdoctoral fellowship (2014-F-13) granted by the American Association of Nurse Anesthetists Foundation. The authors declare no competing interests connected to this work. The views expressed in this work are those of the authors and do not reflect the official policy or views of the US Army, the US Department of Defense, or the US Government.

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