Limitations of Animal Electrical Cardiac Safety Models

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***Introduction* — Human electrical safety standards are based almost exclusively on animal studies and there is an unjustified assumption that ventricular fibrillation (VF) thresholds in animals are the same as those in humans.**

***Methods and Results* — We analyzed differences between animals and humans in cardiac stimulation. A broad literature survey revealed that swine are a fragile electrophysiologic research species and have a dense intramural Purkinje fiber network, which is not found in some other species, including humans. Anesthesia agents have to be chosen carefully as swine are prone to malignant hyperthermia. Cardiac stimulation thresholds depend on weight and capture rates. Thus, the animal weight has to be representative of the weight of human subjects. Studies have shown significant ECG differences between humans and other species, including swine and canine. At least one study suggested that rabbit hearts tend to develop VF in a manner more similar to that seen in humans.**

***Conclusion* — Animal studies can play a role in conservatively evaluating cardiac safety. However, while still abiding by the precautionary principle, animal study design has to take into account the significant anatomical and electrophysiological differences between humans and other mammals. Data from multiple animal models may offer broader perspectives. If attempts are made to extrapolate animal results to humans then appropriate numerical correction factors should be applied, such as some of those discussed in this article.**

I. Introduction

Some conflicting and inconsistent results from animal studies have raised questions about the suitability of extrapolating findings from animal models to humans for cardiac safety [1]. The primary issue in cardiac safety is the induction of VF (ventricular fibrillation) since VF is the lethal consequence that can occur with the lowest currents [2]. Decades ago, the canine model was preferred for electrical safety research. However, legal and political restrictions have led to the dominance of the swine model as it is considered an agricultural vs. a companion animal [3].

II. Important Electroanatomical Aspects of Animal Models

A. Distribution of Purkinje fibers.

The cardiac conduction system consists of a network of specialized myocardial cells that generates the cardiac rhythm and assures its organized propagation through the heart, resulting in an efficient contraction of the heart. In mammalian hearts, the cardiac conduction system includes the sinus node, the atrioventricular node, the atrioventricular (His) bundle, its right and left branches, and the network of Purkinje fibers [4]. The sinus node represents the 'pacemaker' of the heart. It fires the original activation impulses that form the activation sequence of the heart chambers. The atrioventricular node delays the ventricular activation with respect to that of the atria. The His bundles are the conductive pathways between the atria and ventricles They are formed of fast conducting tissue that carries the activation to the right and left bundle branches which, in turn, rapidly spread the activation through the ventricles. The Purkinje fibers are the terminal part of the cardiac conduction system. They originate from the bundle branches and are structured as a3-D subendocardial and intramural network [4]. Their function is to distribute the activation to the myocardial muscle responsible for the ventricular contraction. The Purkinje set of fibers include: the subendocardial network, (which has connection to the bundle branches and assure the apex-to-base activation of the ventricle) and a variably present intramural component [4]. Subendocardial fibers have been found, although with different morphology, in all mammalian hearts. Intramural fibers are morphologically distinguishable only in some species, such as ovine, bovine, or porcine which are members of the order Artiodactyla (2 or 4-toed ungulates) [4]. Fig. 1 illustrates diagrammatically the difference in Purkinje fiber spread between swine (1a) and human (1b) hearts. Given that their role is to accelerate transmural conduction, it is very important to note that intramural Purkinje fiber networks have not been found in some other species, including mouse and humans [4]. Fig. 2 illustrates the Sedmera *et al*. findings of intramural Purkinje fibers in swine.

Taking the above morphological differences into account, Dosdall *et al*. studied aspects of post-shock conduction in swine hearts using plunge needles with 12 electrodes and basket catheters with 32 bipolar recording sites [5]. They determined that Purkinje activations were recorded prior to local myocardial activation in 15% of plunge needles during the first post-shock activation cycle and concluded that the Purkinje system is active during the early post-shock activation cycles following defibrillation shocks [5].

The above reports corroborate previous studies that have suggested the Purkinje system may be responsible for the onset of arrhythmias and may be important in the maintenance of VF [5]. There have been several reports of Purkinje system triggered ventricular tachycardia (VT) and VF [6]. With a significantly denser innervation of Purkinje intramural fibers, swine hearts are likely to be more prone to induction and maintenance of fast cardiac rhythms.

B. Sensitivity to anesthetics.

Anesthetics have been known for their pro-arrhythmic effects, for example propofol and isoflurane [7]. Malignant hyperthermia is the dramatic rise in body temperature triggered

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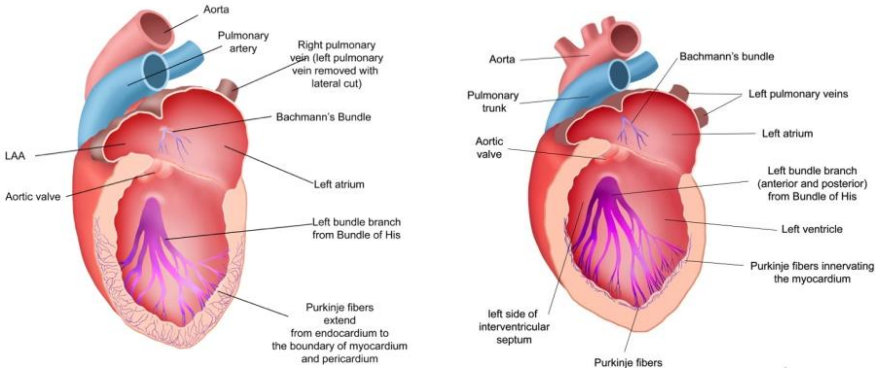
by inhaled anesthetic, most commonly by halothane. In swine, known as porcine stress syndrome, the condition may be an inherited, autosomal recessive disorder due to a defective ryanodine receptor leading to huge calcium influx, muscle contractions, and increase in metabolic load.



(a) (b)

Fig. 1. (a) Illustration of Purkinje fiber intramural myocardial spread in swine; (b) Human hearts do not have intramural Purkinje innervations.

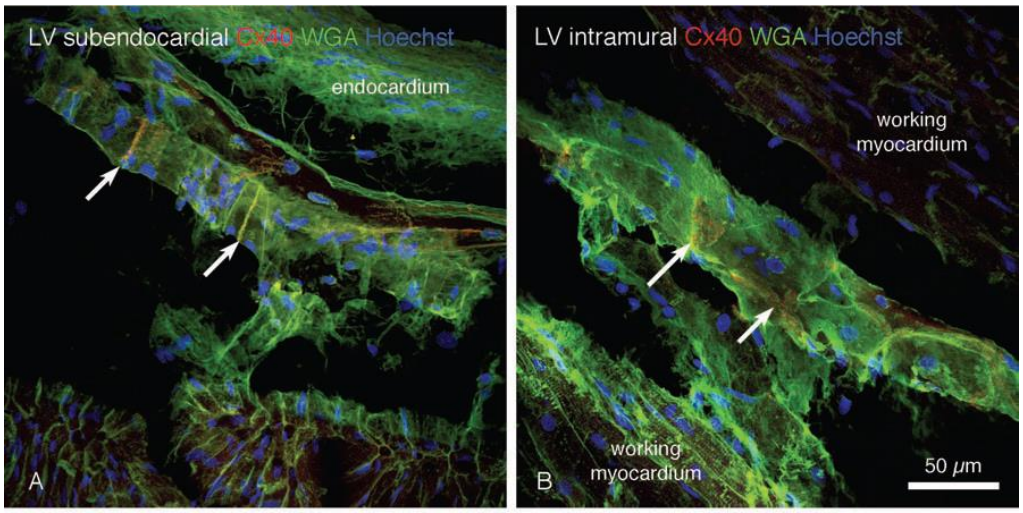


Fig. 2. Intramural presence of Purkinje fibers in swine heart. Connexin40 staining labels specifically both subendocardial (A) and intramural (B)[4].

This can be triggered by stress under anesthesia with halothane and makes the animal significantly more sensitive to developing tachycardias. Additionally, Rao *et al*. found that older swine had an increased likelihood of arrhythmia developments during halothane anesthesia [8]. Propofol has been known to prolong effective refractory periods [9]. As such, when combined with other potentially hazardous cardiac conditions, propofol displayed pro-arrhythmic properties [9, 10]. Purrinos *et al*. have shown that swine anesthetized with isoflurane had significantly lower arterial pressures and were more difficult to be maintained in homeostasis [10]. Also, the incidence of VF was 50% higher in the group of swine anesthetized using isoflurane [10].

As anesthesia is inevitable when using animal models, it is important to develop an appropriate plan that minimizes the anesthetic potentially biasing effects on the study results.

C. Electrophysiology aspects.

The nature and progression of VF may not be the same in all species, so results in canine or swine should be extrapolated to humans with caution. It is known that the QRS of swine is different from that of carnivores or primates. Hamlin *et al*. have shown that during the terminal 30 ms of ventricular activation, the basilar third of the interventricular septum is activated in a general apico-basilar direction [11]. Unlike in primates, swine displayed an epicardial-to-endocardial activation pattern in regions of both the right and left ventricular free-walls [11]. These different ventricular activation patterns may be the result of Purkinje fiber intramural penetration, which, as discussed above, is not found in primates [11]. As a result, swine display long QT intervals and a short repolarization reserve [12]. Cheng *et al*. have shown that the activation patterns during 10-minute long VF episodes are different

between canine and swine [13]. They determined that in swine the epicardium was still being activated during the last 7 minutes of VF instead of becoming silent as in canines. Consistent with these differences, Panfilov showed that, although canine and swine had been historically considered the best experimental models for human VF because of the heart size similarity, the number of reentrant VF sources are quite dissimilar [14]. He found that the number of reentrant VF sources in rabbit hearts were 0.7 to 0.8 of the number of sources in the human heart. In contrast, both canines and swine had a much higher number of reentrant VF sources, 2 – 2.9 times and 2.5 – 4 times that in the human heart, respectively. He suggested that wave patterns during VF in the human heart are similar to those in the rabbit heart, whereas VF in swine and canine hearts have a more complex organization [14].

D. VF thresholds.

In 1936, Ferris *et al*. showed that swine can be more easily fibrillated than other mammals of similar weight [15]. After correcting for weight, swine were fibrillated at lower currents than bovine or ovine. Using data from 104 animals of several species (rabbits, primates, canines, goats, ponies), Geddes *et al*. found the threshold current for fibrillation, at 5 s exposures, varies almost as the square root of body weight (W in kg) [16].

$$I_f = b \cdot W^a \ \ mA_{RMS} \ \ (60 \text{ Hz AC})$$

where *b* and *a* are parameters based on electrode position only (e.g. *b*=29.7, *a*=0.51 for right forelimb-to-left hind limb path). For 3 s exposures, Dalziel determined that the 0.05 and 50 percentile fibrillation currents for swine were lower than the respective currents for ovine and bovine [17]. Fig. 3 summarizes the dependence of fibrillating currents on the weight of the subject.

As shown in Table 1, the international standard IEC 60479-3 also provides similar ranges for fibrillating currents for livestock, including swine [30]. Table 1 also shows that swine have lower fibrillating thresholds than livestock of other species.

Many recent electrical safety animal studies used smaller swine. Lakkkireddy *et al*. reported data from two studies conducted on 13 swine weighing 34.4 ± 6.95 kg [18, 19]. Nanthakumar *et al*. reported data from a study conducted on 6 swine weighing between 45–55 kg [20]. Similarly, Dennis *et al*. used 6 swine weighing between 22 – 46 kg [21]. Walter used swine weighing 25–71 kg [22].Valentino used swine weighing 25–36 kg [23]. Dawes used swine weighing 36.7–38.6 kg [24, 26]. At average swine weights significantly less than 50 kg, as used by studies cited above, the expected average 60 Hz AC fibrillating current is less than 175 mA_{RMS}. The two animals in which Dennis *et al*. induced VF weighed 29 and 31 kg, at the low end of the ranges. In contrast, Stratton *et al*. reports that the average weight of subjects exposed to electrical stimulation was 92 ± 18 kg [25]. Given the large weight reported by Stratton *et al*. and the fibrillating current ranges described above, the expected 60 Hz AC VF induction threshold relevant for the average human subjects is over 300 mA_{RMS} [27]. By comparison, on average, this threshold is between 71% to over 300% higher than that for the swine used in the animal research discussed above just based on the weight difference.

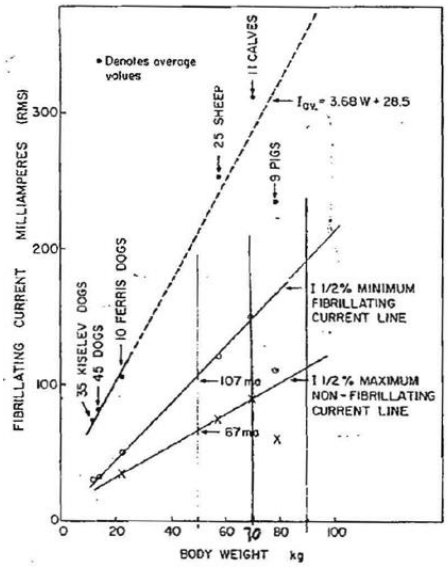


Fig. 3. Fibrillation currents as function of weight [17].

Swine are also extremely sensitive to higher frequency currents. Cardiac ablation with radio frequencies is routinely performed clinically without problems yet these same frequencies will result in VF in swine. Pak *et al*. have shown that radiofrequency ablation targeting the posterior papillary muscle resulted in incessant VF in swine, but not in canines [28].

TABLE I. Livestock VF thresholds at 50/60 Hz currents applied for 3 s [30].

Species	Average weight		Minimum fibrillating current	
	Body kg	Heart g	Average A	Range A
Pig	79	300	0,24	0,17 to 0,27
Sheep	56	270	0,25	0,16 to 0,39
Calf	70	420	0,31	0,21 to 0,47
Pony	115	–	0,3	0,16 to 0,41
NOTE – Too little data is available for horses to be included in this table.				

A possible cause (beyond the transmural Purkinje fibers) for this difference lies with significant ion channel differences. Although the repetition rate for some stimulators is about 19 pulses/s, the leading edge of the applied current is much more rapid and results in a spectrum with higher frequency content [29]. According to research such as Pak's *et al*., swine are significantly more susceptible than other species and may respond with fast ventricular rhythms to the increased stimulator signal frequency content.

The studies analyzed above imply that in order to extrapolate animal results to humans some appropriate numerical correction factors have to be considered. Computer modeling can be useful in further understanding stimulation effects on humans [31].

III. CONCLUSION

Animal studies can play a role, but their design has to take into account the significant differences with respect to the human anatomy and electrophysiology. While still abiding by

the precautionary principle, researchers should be aware that animal studies of electrical safety using small animals may significantly exaggerate the risk to adult humans. This is especially true for the swine model. Perhaps no one single animal model may be best suited to accurately estimate effects of electrical stimulation on humans. Data from multiple models may offer a broader perspective. However, the clinical relevance has to be studied carefully before extrapolation to humans is attempted.

IV. Disclosure

Dr. Panescu is a paid consultant to TASER International, Inc. (TASER). Dr. Kroll is a consultant to TASER, and a member of the TASER Scientific and Medical Advisory Board (SMAB) and Corporate Board. Mr. Brave is a TASER employee and legal advisor to the SMAB and Training Advisory Board.

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