IBUTILIDE AS A FIRSTLINE ANTIARRHYTHMIC DRUG IN A PORCINE MODEL OF OUT-OF-HOSPITAL CARDIAC ARREST

By

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Abstract

Objectives: Class III antiarrhythmics have been demonstrated to lower defibrillation threshold (DFT). The impedance threshold valve (ITV) has been demonstrated to improve hemodynamics during cardiopulmonary resuscitation (CPR). We hypothesize that administration of the class III antiarrhythmic drug ibutilide, and use of the ITV during CPR after induction of ventricular fibrillation (VF) will lead to greater short-term survival vs. control.

Methods: VF was induced in 30-35 kg pigs and left untreated for 4 minutes. CPR was performed for 8 minutes using automatic ventilation. 0.005 mg/kg IV ibutilide was administered during CPR. Electrophysiologic and hemodynamic variables were measured at baseline, during CPR, and post-resuscitation.

Results and Conclusions: Ibutilide increased DFT (161.1±33.3 J vs. 200±54.8 J, $p=0.044$). Ibutilide did not significantly change any other variable measured. The ITV did not significantly change any variable measured. Ibutilide appears inactive in the pig, while the ITV may not function properly when used with automatic ventilators.

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Introduction

Cardiac Arrest, Ventricular Tachycardia and Ventricular Fibrillation.

Cardiac arrhythmias are a major cause of morbidity and mortality. Every year in the United States sudden cardiac death, often due to ventricular fibrillation, claims the lives of more than 450,000 people[1]. The two arrhythmias believed to be most often associated with sudden death and cardiac arrest are ventricular tachycardia (VT) and ventricular fibrillation (VF). VT is defined as a rapid heart rate, conventionally 100 beats per minute or more, originating in the ventricle. VF is defined as exceedingly rapid, uncoordinated contractions of the ventricles. VT is an organized ventricular rhythm, and there is an associated cardiac contraction. VT becomes a serious, potentially fatal arrhythmia when the ventricular rate is so fast that the ventricle is unable to fill with blood between beats, and thus, cardiac output becomes so low that there is no palpable pulse or effective circulation of the blood. VF, by contrast, is a completely disorganized electrical rhythm with no organized cardiac contraction and no cardiac output. Left untreated both pulseless VT and VF will lead to death. Given that at present, cardiac arrest survival rates typically range between 2 and 5 percent, it is desirable to find ways to both prevent cardiac arrest, and improve the treatment of cardiac arrest[77,78].

Antiarrhythmic drugs are commonly employed to manage patients believed to be at risk for potentially lethal arrhythmias such as VT and VF. Since action potential generation is dependant on the activity of ion channels that allow for depolarizing and repolarizing currents, drugs that alter these ionic currents, either depolarizing or

repolarizing, can function effectively as antiarrhythmic agents to either suppress the generation, or the maintenance of arrhythmias[2,3].

The clinical use and development of antiarrhythmic drugs was greatly impacted by the Cardiac Arrest Suppression Trial (CAST). The major hypothesis of this study was that the suppression of ventricular ectopic activity by drugs with Vaughan-Williams class I antiarrhythmic (Na^+ channel blocking) action would reduce the risk of sudden cardiac death after myocardial infarction[4-6]. Quite the opposite, it was found that the use of encainide and flecainide in CAST I[5] and moricizine in CAST II[6] were not associated with a survival benefit, and were associated with increased mortality in patients after myocardial infarction. This finding shifted attention away from class I action and towards the use and development of drugs with class III action (agents which prolong action potential duration (APD) and refractoriness) for ventricular arrhythmias[7]. Agents with 'pure' class III action would be expected to prolong refractoriness without affecting conduction velocity. This action was predicted to suppress re-entrant arrhythmias[8].

Refractoriness and Re-entry

The concepts of refractoriness and re-entry are central to all the experiments performed for this thesis. The term refractory period describes a period after the depolarization of excitable cells during which a second stimulus of the same magnitude cannot elicit a second depolarization. Excitable cells must repolarize, at least to a degree, before they can be depolarized again. The refractory period can further be broken down into several divisions. The first to consider is the absolute refractory period (ARP). This refers to the earliest portion of the action potential after depolarization during which a

second stimulus cannot cause a second depolarization, regardless of the magnitude of the second stimulus. During the ARP it is impossible to cause an additional depolarization. The ARP is followed by the relative refractory period (RRP). During the RRP an additional stimulus can cause a second depolarization, but the second stimulus must be of greater magnitude than the original stimulus in order to elicit a second depolarization. This second depolarization will not propagate as quickly as the first because relatively refractory cells have fewer sodium channels that have completely recovered from the previous depolarization as compared with fully repolarized tissue, and thus conduction is slowed. This slowed conduction is an important factor in the development of re-entry. Impedance is also higher in relatively refractory tissue as compared to repolarized tissue. Another interval of interest is the effective refractory period (ERP). This refers to the longest interval between two stimuli unable to cause a second depolarization. The ERP is elicited by pacing using twice the diastolic pacing threshold and adding an early stimulus to measure the earliest point at which the extra stimulus is able to cause a depolarization; the longest interval unable to cause a depolarization is the effective refractory period (fig I.). The ERP is used as a measure of refractoriness in the intact heart, and ERPs obtained at a given paced rate both before and after an intervention, such as the administration of an antiarrhythmic drug, can be used to evaluate the impact if the intervention on refractory period, and, indirectly, action potential duration. Also important is the functional refractory period (FRP). The FRP is defined as the minimum interval possible between successive responses to stimulation of a tissue, that is to say, the shortest interval possible between two stimuli that will allow the second to cause a depolarization. The greatest distinction between ERP and FRP is that ERP is a static measure – it is

determined by introducing a single early beat and determining the longest possible interval between the early beat and the preceding beat that will not allow the early beat to capture the heart. FRP, on the other hand, is a dynamic measure. The FRP is determined using steady-state pacing and is equal to the shortest pacing cycle length that produces 1:1 capture.

Re-entry is a common mechanism of arrhythmia related to refractoriness. In order for re-entry to be possible several conditions must be met. First, there must be either a branch point, either structural or functional, in a conductive pathway, or a circular conductive pathway. Each of the two arms of the bifurcated pathway must also possess certain characteristics, namely one pathway must exhibit unidirectional block – that is an impulse or wave of depolarization must not be able to traverse this pathway in one direction, but must be able to traverse the pathway when travelling in the opposite direction. This unidirectional block may be only temporary, for example, in the case of recently stimulated tissue, which, at that instant, is absolutely refractory to further stimulation. Additionally the second arm must display slowed conduction such that when an impulse travels down this second pathway, and propagates back up the first (blocked) arm of the bifurcated pathway and reaches the point of bifurcation, the tissue has had enough time to repolarize such that it can be depolarized by the impulse. In this way a re-entrant circuit has been formed and, once begun, a single impulse has the potential to travel around this circuit indefinitely, provided the refractoriness of the tissue does not change (fig II.). Refractoriness, and the above discussed refractory periods become very important in this process. The ARP and RRP are important in initiating re-entrant arrhythmias as they usually provide conditions necessary for the unidirectional block

(ARP) and slowed conduction (RRP) necessary to initiate the arrhythmia. The FRP is important in the maintenance of the arrhythmia, insofar as the time necessary for an impulse to make its way around the circuit must be longer than the FRP. Because re-entrant circuits are not usually defined by a particular anatomic structure, such as the His-Purkinje system of the heart, functional re-entry is most common. Impulses will conduct through any pathway that is no longer refractory. In this way re-entrant circuits can evolve and develop during the arrhythmia. The relative refractory period, and the functional refractory period are very important in determining if and where the wave of depolarization will be conducted. The impulse will be conducted from one cell into any adjoining cell that is no longer refractory.

Class III antiarrhythmic activity is defined as prolonging action potential duration and refractoriness. Drugs with class III antiarrhythmic action are often used to prevent or interrupt the re-entrant processes described above. They do this by prolonging the refractory period of the tissue in the re-entrant circuit enough that the propagating wave of depolarization encounters tissue that is refractory to depolarization by the wave, and thus dies out (fig III.). These principles and processes form the cornerstone for all the experiments preformed for this thesis.

This thesis details the investigation of drugs with class III antiarrhythmic activity for use as first line antiarrhythmic drugs during out-of-hospital cardiac arrest. Along with this investigation a mechanical device designed to be used as an adjunct to closed-chest CPR was tested. The Inspiratory Impedance Threshold Valve was tested in conjunction with the ibutilide experiments to examine its ability to improve blood flow during CPR,

and whether or not combining this mechanical adjunct to CPR with antiarrhythmic drugs can improve cardiac arrest survival to a greater extent than either therapy alone.

Antiarrhythmic Drugs in Cardiac Arrest

As previously mentioned, sudden death, often due to ventricular fibrillation, is a major cause of cardiac morbidity and mortality, claiming the lives of at least 450,000 persons annually in the United States[1]. The American Heart Association recognizes 4 major steps in their 'Chain of Survival'. These are: 1) early access to emergency medical services, 2) prompt cardiopulmonary resuscitation (CPR), 3) early access to cardiac defibrillation, and 4) early access to advanced medical care[63]. Despite recent attempts to improve all components in this chain of survival, survival rates remain poor, generally 2-5% in urban centers[77,78].

The average interval between onset of symptoms and the activation of the emergency medical system, or 911, to the arrival of advanced life support personnel generally averages 7-10 minutes, even in the best equipped systems[77]. In general, fewer than 50%, and at times as few as 15-25% of patients found to have ventricular fibrillation as the initial cardiac rhythm survive to hospital admission. Of this group, a further 75-80% die in hospital, the majority within the first 24 hours[77].

Class Ia antiarrhythmic drugs such as lidocaine and bretylium have long been heralded and used as potentially beneficial agents in the resuscitation of shock-resistant out-of-hospital ventricular fibrillation[63]. In spite of their use, there is no evidence, either experimental or clinical, to demonstrate consistently that the administration of either

lidocaine or bretylium is associated with improved short- or long-term outcomes in either laboratory or clinical settings; quite the opposite, there is evidence that sodium channel blocking agents may cause harm after VF, in the form of causing increased defibrillation thresholds[80,81,156]. In sharp contrast to this, intravenous amiodarone has been shown in animal studies to be far superior in achieving successful resuscitation from shock resistant VF[82].

Amiodarone

Among the treatments that can be provided by advanced medical care are anitarrhythmic drugs. These medications, including lidocaine, bretylium, magnesium and procainamide were classified as "acceptable, probably helpful" treatment for ventricular tachyarrhythmias that are unresponsive to greater than three shocks under American Heart Association Advanced Cardiac Life Support guidelines prior to 2000[64]. The 2000 American Heart Association (AHA)/International Liaison Committee On Resuscitation (ILCOR) guidelines added amiodarone to the list of drugs "to be considered" in this setting. In the ARREST trial, a randomized, double-blind, placebo-controlled clinical trial of bolus IV amiodarone during Advanced Cardiac Life Support (ACLS) in patients with out-of-hospital cardiac arrest due to shock-refractory ventricular fibrillation or pulseless ventricular tachycardia[65], amiodarone was found to improve survival to hospital admission, compared to placebo. Based on the ARREST trial, amiodarone is recommended for consideration as an alternative to lidocaine in shock-refractory ventricular fibrillation/pulseless ventricular tachycardia cardiac arrest[66]. The ALIVE trial (Amiodarone vs. Lidocaine In pre-hospital Ventricular fibrillation Evaluation) was

recently published in the New England Journal of Medicine[76]. This study compared IV amiodarone to IV lidocaine using a randomized, double-blind, double-dummy technique, in patients with persistent or recurrent out-of-hospital VF resistant to three defibrillation shocks. Survival to admission to hospital was improved from 11% in the lidocaine group to 22.7% in the amiodarone group ($p=0.0043$)[76]. Amiodarone can thus be reasonably considered as antiarrhythmic therapy of first choice in shock refractory VF. Antiarrhythmic therapy such as amiodarone may be useful in increasing the probability of successful defibrillation and/or reducing the risk of fibrillation recurrence after restoration of a perfusing rhythm.

The mechanism of these two effects may be different. With respect to the first, it is reasonable to hypothesize that a reduction in defibrillation threshold (DFT) will be correlated with an increased probability of defibrillation with energies available from standard transthoracic defibrillators. Defibrillation energy requirements increase with increased duration of VF[67]. This may be due to progressive myocardial hypoxia, acidosis, and extracellular hyperkalemia as VF continues. These metabolic changes lead to slowed intracardiac conduction, and slowed conduction via sodium channel block is known to lead to increases in DFT[80,80,156]. In addition, myocardial ATP depletion during VF leads to activation of ATP coupled potassium channels (I_K-ATP opening), leading to increases in repolarizing current which shortens APD, another variable associated with increases in DFT[68]. Interventions which increase myocardial blood flow and oxygen supply during CPR will reduce/delay the electrophysiological consequences of myocardial ischemia that contribute to the time dependent increase in DFT during VF. Drugs that prolong APD and refractoriness can decrease DFT acutely[67,68]. Although the

mechanism is not entirely known, drugs that block the I_{K1}, I_{to}, I_{Kr}, and I_{Ks} repolarizing potassium channels (alone or in combination) are all associated with decreases in DFT in a variety of experimental models[69,70]. Intravenous amiodarone decreases the E_{50} (energy associated with a 50% probability in defibrillation) in dogs subject to 15-second episodes of VF[71].

Many studies examining the ability of various drugs to alter parameters such as defibrillation threshold, shortening of refractoriness as a result of VF, and short-term survival employ study designs that involve the administration of the study drug before the induction of VF. This method does not reflect real-world resuscitation attempts. Patients suffering from VF rarely have antiarrhythmic agents in their circulation prior to their cardiac arrest. For this reason this study will administer antiarrhythmic drugs after the onset of ventricular fibrillation in the midst of CPR and the continuing resuscitation efforts. One study has shown that the administration of class III antiarrhythmic drugs during ventricular fibrillation can significantly lower DFT in anaesthetized dogs[79].

Amiodarone appears to be a particularly promising agent for use as a first line antiarrhythmic. Prior to the advent of amiodarone, lidocaine was the gold standard for the treatment of shock refractory ventricular fibrillation; despite the fact that no evidence exists demonstrating it is effective. Quite the opposite, evidence does exist showing that sodium channel blockers such as lidocaine increase mortality from ventricular fibrillation, largely as a result of harmful effects such as slowing impulse conduction, and decreasing impulse formation, which leads to higher incidences of asystole post-defibrillation, as well as increasing defibrillation threshold[80,81]. By contrast, in a small

study of dogs subjected to coronary ligation-induced shock resistant ventricular fibrillation, intravenous amiodarone was shown to be greatly superior to lidocaine in achieving return of spontaneous circulation – 87% vs. 14%[82]. Amiodarone has also been shown to demonstrate less rate dependence than is seen in many 'pure' class III drugs, and thus is more effective at higher heart rates[83]. These observations, combined with those from the ARREST[65] and ALIVE[76] trials demonstrate that amiodarone is far superior to any drug used previously for the treatment of ventricular fibrillation.

It must be noted that although amiodarone is superior to other available antiarrhythmic drugs for the treatment of cardiac arrest, it is not ideal. The greatest drawback to the use of amiodarone is the fact that it has very low water solubility. Commercially available amiodarone is dissolved in Tween 80 (polyoxyethylene sorbitan mono-oleate). Tween 80 has been found to cause hypotension[65,139] in humans. In dogs Tween 80 has been found to cause a 60% decrease in blood pressure, tachycardia, and a 60% decrease in cardiac contractility[140-142]. With this in mind the initial plan was to evaluate an aqueous formulation of amiodarone being developed. Development of the aqueous formulation of amiodarone was terminated by the drug company due to local venous toxicity (phlebitis) at the injection site and thus was no longer available for this study. At this point it was decided to proceed with the study using the commercially available formulation of amiodarone (fig. VI).

In clinical studies involving cardiac arrest patients, amiodarone has been demonstrated to be superior to both placebo and intravenous lidocaine with respect to survival to hospital admission[65,76]. Although amiodarone is superior to the other available

options, unfortunately it is not a perfect drug for use in cardiac arrest; there are severe limitations to it use. Amiodarone is not water-soluble, and needs to be drawn up into a syringe and slowly diluted before injection – a process that requires approximately 10 minutes in the field[76]. Additionally, rapid injection of IV amiodarone results in vasodilation, at least in part due to the effects of the diluent polysorbate-80, as well as negative inotropy and chronotropy, increasing the risk of post-resuscitation bradycardia and hypotension[63,65,76].

As previously mentioned, drugs that prolong action potential duration and prolong refractoriness (class III action) can decrease defibrillation threshold acutely. The mechanism of this decrease in DFT is not entirely known, yet drugs which block I_{K1}, I_{to}, I_{Kr} and I_{Ks} repolarizing potassium channels, alone or in combination, are all associated with decreases in DFT in a variety of experimental models[69,70,98,99,100].

Ibutilide

Ibutilide fumarate is a substituted methanesulfonamide derivative with structural similarities to the antiarrhythmic agent sotalol. Ibutilide is an agent with 'pure' Vaughan-Williams class III antiarrhythmic activity, meaning that its primary mechanism of action, with respect to its antiarrhythmic properties, is the prolongation of the action potential duration, and that it has little or no effects from the other Vaughan-Williams classes. Clinically, ibutilide has been approved for use in recent onset atrial fibrillation and atrial flutter, can be rapidly injected, and has no direct hemodynamic effects[101-106]. The mechanism by which ibutilide exerts its class III effects is unique. Most drugs with class III effects block the I_{Kr} current – the rapidly activating delayed rectifier potassium

current. The current research indicates that ibutilide probably blocks I_{Kr}[103,159-161], but its primary mechanism of action is the activation of a slow inward current, carried mostly by sodium, during the plateau phase of the action potential[162,163]. Ibutilide does not appear to affect cardiac output, pulmonary artery pressure, pulmonary capillary wedge pressure, blood pressure, or heart rate, even in patients with depressed ventricular function[164]. In humans, ibutilide has class III effects on the ventricle that appears to demonstrate less reverse rate-dependence than other 'pure' class III agents (I_{Kr} blockers) such as dofetilide and d-sotalol[102].

In reproductive studies in rats, orally administered ibtilide was both teratogenic and embryocidal[166]. The excretion of ibutilide into breast milk has not been studied. Ibutilide has not been shown to be genotoxic in multiple tests, including the Ames assay, although no animal studies have been conducted to determine its carcinogenic potential[167].

Ibutilide is rapidly and extensively distributed, with an estimated volume of distribution of 9-13 l/kg. It is approximately 40% protein bound and is primarily eliminated through hepatic biotransformation to eight renally excreted metabolites, none of which has significant antiarrhythmic properties[165]. Ibutilide has a high systemic clearance with a variable elimination half-life ranging between 2 and 12 hours with a mean of 6 hours. The metabolic pathways for ibutilide have not been completely determined, but they do not appear to involve the cytochrome P450 isozymes CYP3A4 or CYP 2D6. Co-administration of digoxin, calcium channel blockers, or β-adrenergic receptor blockers with ibutilide has no apparent effect on the pharmacokinetics, safety, or

efficacy of the drug in clinical trials[167]. Clearance appears to be unaltered by either a reduction in creatinine clearance or left ventricular dysfunction. Currently there is no recommended dosage change in the presence of either renal or hepatic dysfunction, however, abnormal liver function would likely lead to reduced clearance and a prolongation of pharmacological effect and could necessitate longer periods of monitoring after ibutilide administration[167]. In normal volunteers and patients with atrial fibrillation and atrial flutter, ibutilide pharmacokinetics are not influenced by patient age, sex, or type of arrhythmia[103,167,168].

Animal studies have shown that ibutilide may be useful in the prevention and treatment of VF. Pre-treatment with ibutilide in Langendorff-perfused rabbit hearts was demonstrated to significantly reduce the incidence of VF as a result of hypoxia and reperfusion[106]. Ibutilide was also demonstrated to be capable of chemical defibrillation when added to the perfusion medium after the electrical induction of VF[106]. Later studies conducted using intact dogs demonstrated that ibutilide can significantly decrease defibrillation threshold after short-duration VF[101,104,106]. These studies also observed that many animals treated with ibutilide exhibited one or more episodes of spontaneous defibrillation[101]. All of these studies have also demonstrated that ibutilide prolongs effective refractory period and monophasic action potential duration at 90% repolarization both before the induction of VF, and after defibrillation[101,104,106]. For these reasons it is expected that ibutilide will be able to lower the defibrillation threshold when administered during CPR, after the onset of VF in a fashion similar to that observed with the investigational class III agent MS-551[107]. We also believe that the prolonged action potential duration and refractory period resultant from ibutilide administration will act in

a protective fashion, reducing the likelihood of refibrillation after initial successful defibrillation. If the refractory period is prolonged post-defibrillation, it should be less likely that ectopic beats that occur should develop into re-entrant arrhythmias such as VT or VF. Together these effects should result in the earlier reestablishment of a perfusing rhythm, since the initial 150 J biphasic shock (as recommended in the ACLS guidelines[63]) is more likely to be effective. It is also conceivable that the post-shock action potential duration prolonging effects of ibutilide (fig. VII) will enhance cardiac contractility by extending the time in which Ca^{2+} can enter ventricular cells during each cardiac cycle.

It is known that defibrillation energy requirements increase with increasing duration of VF[108,109]. This may be due to progressive myocardial hypoxia, acidosis and extracellular hyperkalemia as VF continues. These changes lead to slowed intracardiac conduction; slowed conduction due to sodium channel block is known to lead to increases in defibrillation threshold[81]. The depletion of ATP during VF leads to the activation of ATP-coupled potassium channels (I_K-ATP opening), which, in turn, leads to increases in repolarizing current, which causes APD to shorten. This APD shortening is also associated with increases in defibrillation threshold[68,110]. Interventions which result in increased myocardial blood flow and oxygen supply during CPR should reduce or delay the electrophysiological consequences of myocardial ischemia that contribute to the time dependent increase in defibrillation threshold during VF.

The final steps to irreversible cell injury in cardiac arrest results from cellular hypoxia and acidosis, ultimately resulting in Ca^{2+} overload and action potential duration

shortening in the heart[111,112]. Both in the heart and in the brain, these consequences will be indirectly or directly inhibited by improving cardiac output, blood flow, and oxygen delivery (impedance threshold valve), and directly prolonging action potential duration (ibutilide). Re-fibrillation occurs in at least 40 % of patients within the first minute following defibrillation from out-of-hospital cardiac arrest[113], and up to 30 % of patients with VF require multiple defibrillation shocks, resulting in prolonged VF and possibly shock-induced myocardial dysfunction[111]. Reducing defibrillation threshold and preventing re-fibrillation may be extremely important components of the integrated therapies tested in these experiments.

The ibutilide experiments will be carried out in combination with the ITV experiments. These two interventions have either been demonstrated (ITV) or are hypothesized (ibutilide) to improve cardiac output during CPR (ITV), improve defibrillation (ibutilide), decrease re-fibrillation, and/or improve contractile function following defibrillation (both ibutilide and ITV). It is also believed that neither of these therapies alone will produce optimal resuscitation in experimental or clinical cardiac arrest.

Impedance Threshold Valve

Standard CPR (manual active chest compressions and passive decompression) delivers only approximately 35 % of normal coronary perfusion and only approximately 25 % of normal blood flow to the brain[72]. Despite this poor ability to generate blood flow, a brief period of CPR prior to defibrillation attempts is associated with better outcomes as compared to patients who receive defibrillation immediately upon the arrival of

paramedics[84]. In addition to this, it has been observed that interrupting chest compressions during CPR is detrimental in experimental cardiac arrest[85,86]. Observations such as these suggest that CPR is a more important link in the chain of survival than previously thought. In this light, effective CPR can be seen as "priming the pump" by providing a brief period of coronary flow, resulting in an improvement in the metabolic state, and the potential for better contractile recovery of the myocardium after defibrillation[87].

Newer methods of CPR have recently been developed, which are expected to increase coronary and cerebral perfusion. Based on the observation that intermittent, yet complete occlusion of the airway during the decompression phase of mechanical chest compressions resulted in greater decreases in intrathoracic pressure and greater venous return than with an open airway[88], the impedance threshold valve was designed as a one-way valve to allow air to exit the lungs during the compression phase, but preventing the passive entry of air back into the lungs during the decompression phase. The ITV is a small, one-way valve that is placed between the endotracheal tube or facemask and the ventilation bag, thus becoming part of the respiratory circuit. The end result is the maintenance of a negative intrathoracic pressure up to a 'cracking pressure' of –22 cmH_2O during the decompression phase of chest compressions[88]. This has been demonstrated to develop greater negative intrathoracic pressure resulting in increased venous return, and in turn, greater cardiac output[87,88]. The ITV has been demonstrated to improve both survival and neurological function after cardiac arrest and resuscitation in both laboratory animal models as well as humans.

Animal studies have consistently demonstrated that the use of the ITV improved left ventricular blood flow by between 39% and 71% vs. control, cerebral blood flow between 20% and 47% vs. control, and coronary perfusion pressure by approximately 40%[73,74,95]. A subsequent animal study demonstrated that the above noted improvements in vital organ perfusion translate into greater 24-hour survival, and significantly better neurological performance 24 hours post-resuscitation[96]. This same study demonstrated significant increases in peak end-tidal carbon dioxide during CPR, as well as significantly higher 'systolic' blood pressures during chest compressions.

Only two clinical studies have been completed to date. One of these studies, carried out by Plaisance et al. in Paris, involved 21 patients experiencing cardiac arrest and attended to by mobile intensive care units. With such a small group it was not possible for this study to demonstrate a significant difference in terms of survival benefit, but they were able to demonstrate a significant increase in end-tidal carbon dioxide, coronary perfusion pressure, and diastolic blood pressure[72]. This study also demonstrated a significantly shorter interval from intubation to return of spontaneous circulation in the ITV group as compared to control. The second study was carried out by Wolcke et al. in Mainz, Germany. In this study 210 patients were included in the final analysis. This study did demonstrate a significant increase in return of spontaneous circulation, 1-hour survival, and 24-hour survival in the ITV group vs. the control group. This study also observed a non-significant trend towards better overall neurological function in the ITV group vs. the control group[97]. Other clinical studies of the ITV are currently underway, but not other clinical data is currently available.

In this study, active compression-decompression (ACD) CPR will be performed. Active compression-decompression CPR was developed after a case report of a man who used a common household plunger on multiple different occasions to resuscitate his father[89]. By using a suction device to actively pull up on the chest wall, ACD CPR serves to increase negative intrathoracic pressure during the decompression phase of chest compressions. This, in turn, enhances venous return and minute ventilation. Animal and human studies have demonstrated that vital organ blood flow is significantly increased using ACD CPR as compared with standard CPR[90-94]. Active compression-decompression (ACD) CPR plus an inspiratory impedance threshold valve (ITV) has recently been described as a method to improve vital organ, including myocardial, blood flow and the currently poor resuscitation rates in patients suffering from cardiac arrest[73].

The primary objective of this study is to assess the effects of intravenous class III antiarrhythmic drugs and the ITV on short-term survival defined as a successful return of spontaneous circulation (ROSC) after short term VF in a cardiac arrest model with ACD CPR[73,75] in pigs. Secondary objectives include examination of the impact of class III antiarrhythmic drugs and the ITV on defibrillation energy requirements, incidence of re-fibrillation after initially successful defibrillation, and the ability of class III antiarrhythmic drugs to blunt the post-resuscitation electophysiological consequences of VF such as action potential duration shortening and effective refractory period shortening.

Objectives

Although amiodarone appears to be the most promising agent currently available for the treatment of VF, it is not ideal. Amiodarone is not water-soluble. The diluent, tween 80, that it is prepared in is known to cause vasodilatation, and negative inotropy and chronotropy in humans[63,65,76]. In other species, such as dogs, tween 80 is known to cause severe hypotension, tachycardia, and decreased cardiac contractility. In order to negate the effects of the diluent required for intravenous administration of amiodarone, the studies were conducted with ibutilide, a water-soluble antiarrhythmic drug with 'pure' class III action. Ibutilide has also been shown to lower defibrillation threshold acutely[101,104,106]. We hypothesize that ibutilide will have many of the same beneficial effects expected from amiodarone – that the administration of ibutilide during CPR will decrease defibrillation energy requirements, decrease the likelihood of re-fibrillation, blunt post-resuscitation APD shortening, and result in increased short-term survival. The ibutilide study was carried out in conjunction with the ITV study. Previous data has shown that the ITV improves hemodynamics during CPR and improves cardiac arrest survival[73-75,87-93]. We hypothesize that the ITV will improve coronary perfusion and antiarrhythmic drug circulation during CPR, and that when combined with ibutilide, the ITV will act additively, or perhaps synergistically to improve short-term survival after experimental cardiac arrest to a greater extent than either therapy can alone. The primary objective of this combined ibutilide/ITV study is to examine the effects of ibutilide and the ITV, alone and in combination on short term survival after experimental cardiac arrest. Secondary objectives include examining the effects of ibutilide and the ITV, alone and in combination on defibrillation energy requirements, decrease the likelihood of re-

fibrillation, improve peripheral blood pressure during CPR, improve coronary perfusion during CPR, and blunt post-resuscitation APD shortening.

Figures

The Action Potential and Refractory Periods

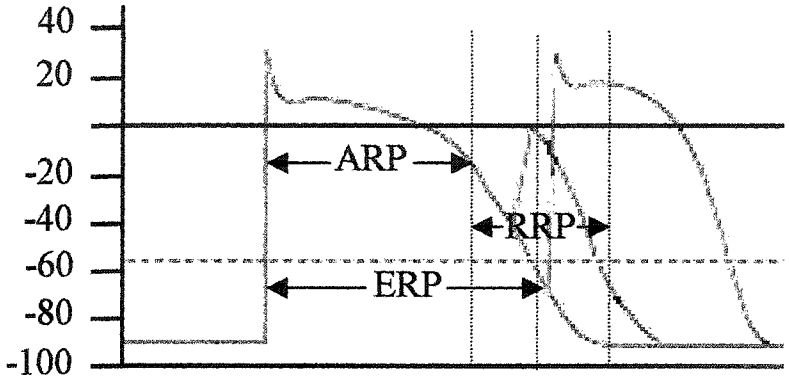

Figure I. An illustration of the action potential showing the absolute refractory period (ARP) during which a second stimulus of any magnitude cannot elicit a second depolarization, the relative refractory period (RRP), during which a stronger stimulus can elicit a slower, weaker depolarization, and the effective refractory period (ERP), the longest interval between successive stimuli in which the second stimulus fails to capture.

The Development of Re-entrant Arrhythmias

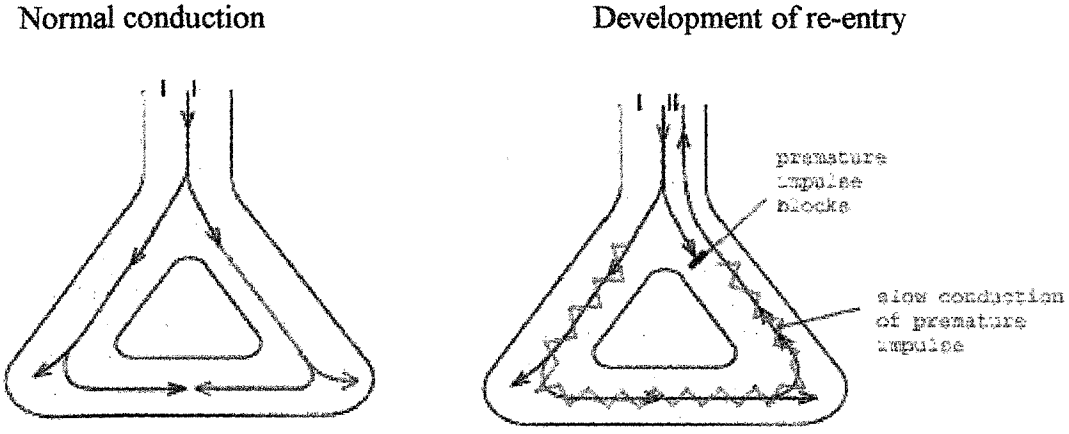

Figure II. The development of a re-entrant circuit. A conductive pathway bifurcates, either anatomically or functionally. In one arm the impulse is blocked. In the other arm conduction is slowed. This allows enough time to pass for the tissue at the bifurcation point to repolarize so it can be depolarized again, initiating a re-entrant circuit.

Class III Effect on the Action Potential and Re-entrant Circuits

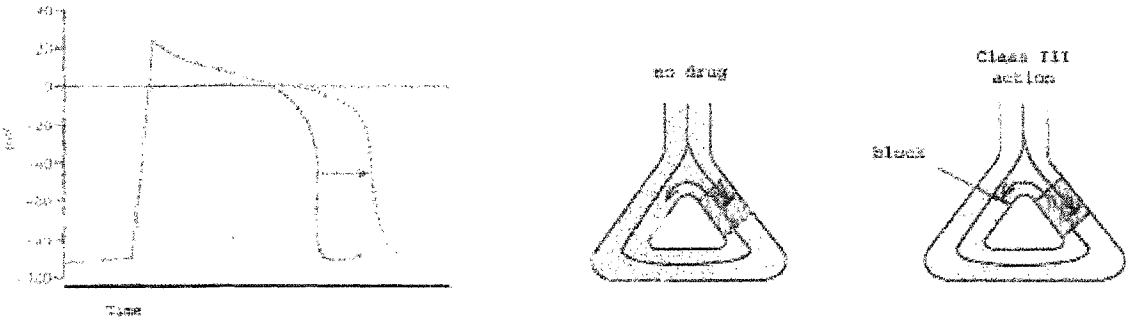

Figure III. Class III action prolongs the action potential duration and refractory period. In the figure on the left, the action potential prior to the addition of the drug is shown with the solid line. The dotted line shows how a drug with class III action would prolong the refractory period. The figure on the right shows how a drug with class III action can interrupt the re-entrant circuit by causing prolonging the refractory period enough that the wave of depolarization will encounter an area that is still refractory and not be conducted, terminating the arrhythmia.

The Chemical Structure of Ibutilide

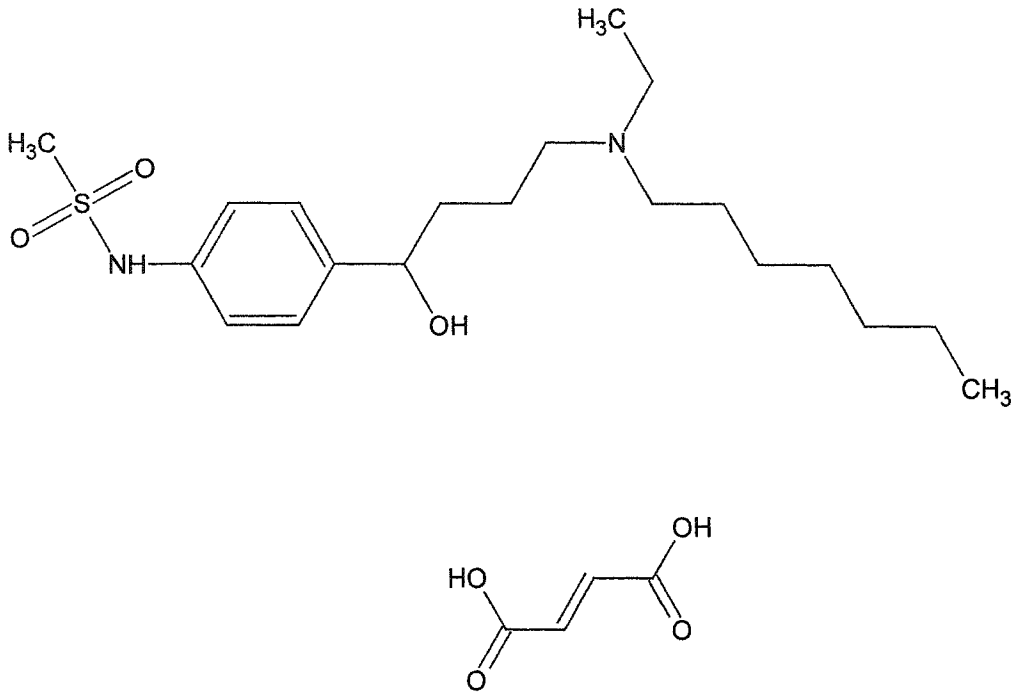

Figure IV. The chemical structure of ibutilide fumarate.

EXPERIMENTAL DESIGN, PROCEDURE, AND METHODS OF APPROACH

All experiments were approved by the Animal Care Committee at St. Michael's Hospital and were demonstrated to conform to the guiding principles of the Canadian Council on Animal Care prior to beginning any experiments.

EXPERIMENTAL MODEL

A pig model was selected for this study for several reasons. Given that in dogs Tween 80 has been found to cause a 60% decrease in blood pressure, tachycardia, and a 60% decrease in cardiac contractility[140-142], as well as the prohibitive cost of dogs for research, a dog model was ruled out. Pigs are currently the standard model used for CPR research[73,74,87,89,93,96]. Pigs are also the most likely candidate in the search for donor cardiac tissue for xenotransplantation to humans[114-116]. Additionally, ibutilide has been administered to pigs and has been observed to prolong right and left atrial ERP at doses of 0.0015 mg/kg[169]. Based on the above evidence it is reasonable to assume that the anatomy and physiology of the pig heart bears enough resemblance to that of the human heart that it would be an appropriate model for this line of investigation.

In order to better mimic the situation encountered by paramedics it was decided that the ibutilide would be administered during CPR as opposed to before the onset of ventricular fibrillation. One study by Marakawa et al. addressed the specific question of can antiarrhythmic drugs be effective if administered during CPR as opposed to before the onset of VF. This study demonstrated clearly that the class III antiarrhythmic drug MS-551 was able to decrease the defibrillation threshold acutely when administered during ventricular fibrillation[107]. The ibutilide drug studies were conducted along with the

ITV study. It is believed that the combination of ibutilide with the ITV will produce greater rates of return of spontaneous circulation than either therapy will produce alone.

Based on the above we believed that a pig model of CPR in which amiodarone or ibutilide is administered during CPR in order to simulate the EMS response to out-of-hospital cardiac arrest is a reasonable model for this study.

The primary objective of this study is to assess the effects of intravenous ibutilide and the ITV on short-term survival defined as a successful return of spontaneous circulation (ROSC) after short term VF in a cardiac arrest model with ACD CPR[12,14] in pigs. Secondary objectives include examination of the impact of ibutilide and the ITV on defibrillation energy requirements, and the ability of ibutilide to blunt the post-resuscitation electophysiological consequences of VF such as action potential duration shortening and effective refractory period shortening.

General Methods:

Pigs (25-35 kg) were fasted overnight and sedated with ketamine (20 mg/kg IM). Anaesthesia was induced with thiopental 8mg/kg IV via the ear vein and maintained with isoflurane 1-3% (The reported minimum alveolar concentration [MAC] value for isoflurane in pigs is 1.2-2.04%[170]) and nitrous oxide 50% for the duration of the surgical procedure In order to minimize any possible drug effect on defibrillation, isoflurane administration was stopped 5 minutes before cardiac arrest is induced. End-tidal isoflurane levels were not measured. Instead signs of recovery from general anaesthesia (increasing muscular tone in the jaw muscles, increasing blood pressure and heart rate) were used to indicate that circulating isoflurane levels were decreasing prior to the

initiation of cardiac arrest. Circulating isoflurane levels should be further decreased prior to defibrillation by the 8 minutes of cardiopulmonary resuscitation preformed without the delivery of isoflurane. The anaesthetic regimen differs from that previously described[73,75] in order to better simulate an out-of-hospital cardiac arrest. Pentobarbital has been demonstrated to block sodium channels in human ventricular myocytes[124]. Based on this evidence, pentobarbital anaesthesia is not appropriate. The chosen anaesthetic regimen listed above uses drugs that are known to cause minimal cardiac effects. Isoflurane causes a slight depression of the maximum rate of rise of action potential upstroke, but has minimal effects on intraventricular conduction[126]. In addition to this, isoflurane is reported to have "a terminal half-life of 10.0 ± 5.57 minutes"[127] in human patients undergoing elective surgery, and thus should be present at low levels at the time of defibrillation, 18 minutes after the administration of isoflurane was terminated. Pigs were placed in the dorsal recumbent position and intubated by standard endotracheal intubation technique. They were ventilated during the preparatory phase of the experiment and at the end of the experiment by a Ventimeter® Ventilator (Airshields Inc., Hatboro, PA), with a tidal volume and rate set to maintain the pH, pCO_2 and pO_2 in the physiological range (pH 7.35-7.45, pCO_2 35-45 mmHg, pO_2>100 mmHg) as measured in arterial blood samples. Normothermia was maintained using a humidifier (Bourns Medical Systems Inc., Riverside, CA) to heat inspired gas to 37°C. Normal saline was infused at a rate of 2-4 mL/kg/hr to prevent volume depletion.

Defibrillation patch electrodes (EDGE Quik-Combo®, Physio-Control, Redmond, WA) for defibrillation and cardiac monitoring were adhered to the left and right chest. A monophasic action potential (MAP) catheter (EP Technologies Inc., Sunnyvale, CA) was

positioned at the apex of the right ventricle via the right femoral vein to allow for the recording of monophasic action potentials, pacing and measurement of ventricular effective refractory period (VERP). A micromanometer-tipped catheter (Mikro-Tip® Transducer, Millar Instruments, Inc., Houston, TX) was placed in the left ventricle via the right femoral artery to allow for the recording of left ventricular pressure. A catheter introducer was inserted in the left femoral artery for continuous arterial pressure monitoring (by a Statham transducer P23Id, Bionetics Inc., Toronto, ON). Once all the introducing sheaths were in place, 2500 International Units of heparin was administered in order to fully anticoagulate the animal prior to catheter placement. Three limb leads of the surface electrocardiogram, unipolar electrograms from the endocardial MAP catheter, endocardial MAP electrograms, and blood pressure were amplified using a custom-made amplifier (Cartesian Labs, Toronto, ON) and recorded using a custom-made computer software program, (Electrophysiological Recording System – Acqui2, Cartesian Labs, Toronto, ON). The MAP signals were DC coupled and filtered with a 500 Hz low pass filter, the unipolar electrograms were filtered at 0.05 – 500 Hz. Additionally a 7 French Swan-Ganz catheter (Edwards Lifesciences, Irvine, CA) was floated into the pulmonary artery via a catheter introducing sheath in the right jugular vein to measure cardiac output by the standard thermodilution technique using the Vigilance® monitor (Edwards Lifesciences, Irvine, CA).

Five minutes prior to the induction of ventricular fibrillation the administration of isoflurane was terminated. Ventricular fibrillation was induced using two seconds of 7.5 volts (V) of fully rectified 60 Hz current via right endocardial electrodes. The point at which ventricular fibrillation is induced is termed "time 0". The endotracheal tube was

immediately disconnected from the mechanical ventilator and tube cuff pressure was assessed to ensure that it was adequate to seal the trachea. After 4 minutes of ventricular fibrillation, during which time no chest compression or ventilation takes place, CPR is begun. At this point, the pigs were assigned randomly to initially receive either ACD CPR alone, or ACD CPR plus the ITV. Ventilatory support was provided at the same rate and tidal volume used during the surgical phase of preparation using the Ventimeter® Ventilator (Airshields Inc., Hatboro, PA), and ACD chest compressions were performed for 6 minutes. After 6 minutes of CPR, the study drug (ibutilide (0.005 mg/kg IV bolus)) or saline placebo was administered, and CPR was continued for another 2 minutes. As shown in the experiment flowcharts below (figures **V-XII**), it consisted of pre-CPR phase (4 min), CPR_1 phase (6 min), drug administration, CPR_2 phase (2 min), and defibrillation.

Specific Methods:

Inspiratory Impedance Threshold Valve:

The Inspiratory Impedance Threshold Valve (ITV) in this study consists of a -22 cm H_2O threshold valve (ITV value, CPRx Inc.) connected in series between the endotracheal tube and the ventilator such that during the decompression phase, but in the absence of active ventilation, the valve opens only with greater the -22 cm H_2O of inspiratory pressure[74]. In this fashion, more than –22 cm H_2O of intrathoracic pressure is required for inspiration of respiratory gases during performance of CPR with the ITV.

Cardiopulmonary Resuscitation:

Active Compression-Decompression Cardiopulmonary Resuscitation (ACD CPR) was performed using a silicone suction cup attached to an automated piston device

(internal diameter 8 cm, ACD Controller, Ambu International)[75]. The rate of compression was 80/min with a 50% duty cycle, a depth of 25% of the anterior-posterior diameter of the chest wall, and a velocity of 7.5 inches/sec. The applied force was kept constant using a force transducer with a visible gauge on the device. This process is the same, regardless of ITV status.

During CPR, ventilation was provided by a Ventimeter® Ventilator (Airshields Inc., Hatboro, PA), and was delivered at the same settings of rate and tidal volume used during the surgical phase of preparation to maintain pH and pCO_2 in the physiologic range. Ventilatory support was continued throughout all experiments with 100% oxygen supplementation. End-tidal carbon dioxide ($ETCO_2$) levels in the expiratory gas were measured throughout the experiment using the $ETCO_2$ module of the Lifepak 12™ (Medtronic-PhysioControl, Redmond, WA) defibrillator.

Defibrillation:

Defibrillation was attempted using an external defibrillator (Lifepak 12™ 3D Biphasic, Medtronic-PhysioControl, Redmond, WA) delivering biphasic shocks. Animals received one shock at 150 J. If the 150 J shock was not successful, two additional shocks (250 J and 300 J respectively) were delivered within 15 seconds of the previous shock. If spontaneous circulation was not restored, vasopressin (0.8 U/kg) was administered, CPR performed or another 90 seconds and a maximum of three successive shocks (360 J, 360 J, 360 J) were then delivered. After the third 360 J shock (a total of 6 biphasic shocks), no further efforts were made to resuscitate the animal.

Study Drug Administration:

The pigs were assigned randomly to receive ibutilide (fig. IV), or saline placebo in both the ACD CPR and ACD CPR plus the ITV groups. 10 min. after the start of VF (4 min VF with no intervention followed by 6 min CPR, 2 min prior to 1st defibrillation shock), either 0.005 mg/kg ibutilide, or vehicle was administered by IV bolus through the venous catheter, followed by a 10 mL normal saline flush. The dose of ibutilide (0.005 mg/kg) is ½ the recommended adult human dose. Given the moderate steady-state apparent volume of distribution (6.6-13.4 L) and short elimination half-life (3-6 hours) we believe that an appropriate dose for pigs is lower than that for humans, given their higher body fat percentage[103,105].

Electrophysiologic Measurements:

Heart rate was determined as the mean sinus R-R interval of 5 successive beats as recorded by the surface ECG, and conduction velocity was determined as the mean sinus QRS duration of 5 successive beats, as QRS duration has previously been determined to be a reliable surrogate for direct measurements of conduction velocity[134].

"Frequency" or "cycle length" of VF (VFCL) was determined as the mean activation-activation interval for the last 10 intervals prior to the first defibrillation shock from the surface ECG electrode, which allows activation recording independent of direction of impulse spread in the endocardial plane. VFCL is a reliable index of local refractoriness during VF[134].

Ventricular effective refractory period was measured to the nearest 2 msec by the incremental extrastimulus technique, delivering an S_2 stimulus at twice diastolic threshold following an 8-beat pacing train, after a 30 seconds of constant pacing at 350

msec cycle length. Monophasic Action Potential duration (MAPD) was measured using the Ag-AgCl electrode (EP Technologies, Inc.) in the right ventricular endocardium and displayed on screen and stored to hard drive. $MAPD_{90}$ was measured as action potential duration to 90% repolarization after 30 sec. of constant ventricular pacing at 300 msec cycle length. All measures were done just prior to VF induction, as well as 3 min and 10 min after the return of spontaneous circulation (ROSC).

Statistical Methods

All non-binary data were tested for normality and were found to follow a normal distribution. Differences between groups in blood pressure, heart rate, end-tidal CO_2, cardiac output, VFCL, VF amplitude, VERP, $MAPD_{90}$, and DFT were assessed before the onset of VF, during CPR, and 10 minutes after defibrillation, where appropriate, by comparing the means of the groups, for example, when comparing ibutilide treatment vs. saline, or active vs. sham ITV, using a paired t-test analysis. Where more than two groups were being compared, for example, when comparing the 4 independent treatment groups (control, ibutilide only, ITV only, ibutilide + ITV), single factor analysis of variance (ANOVA) was performed to compare the means of the groups.

Results are presented as mean ± SD in the data tables. Differences were considered to be statistically significant if p-values were found to be less than 0.05.

Sample size calculations were performed based on anticipated changes in proportional survival in animals treated with ibutilide vs. control animals. These calculations were made using a two-sided binomial distribution. That is to say, these

sample size calculations were based on proportions, not means, using the following equation:

$\alpha = 0.05$
$\beta = 0.80$
$Z_{\alpha/2} = 1.96$
$Z_{\beta} = 0.84$
p1 (control) = 40%
p2 (Ibutilide-treated) = 80%

$n=(Z_{\alpha} + Z_{\beta})^2(p1q1 + p2q2)/(p2-p1)^2$
$n=(1.96 + 0.84)^2((0.4)(0.6)+(0.8)(0.2))/(0.8-0.4)^2$
$n=(7.84)(0.4)/(0.16)$
$n=19.6$

Based on the above calculation, groups of 20 animals were required in order to demonstrate the anticipated improvement from 40% survival in the control group to 80% survival in the ibutilide-treated group with a significance level of 0.05 and 80% power.

RESULTS

IBUTILIDE STUDY

Prior to commencing the ibutilide experiments in earnest a series of 10 pilot experiments were performed to evaluate the behaviour of ibutilide in the pig model of CPR. Of these 10 pigs, 4 received ibutilide and 6 were control experiments. Of the control experiments, none achieved a return of spontaneous circulation. Four of the six ended the experiment in pulseless electrical activity (PEA). In the other two VF remained after several episodes of successful defibrillation followed by early refibrillation. These six animals had a mean blood pressure prior to the initiation of VF of 76.2 ± 10.8 mmHg, a mean defibrillation threshold of 200 ± 77.5 J, and a mean blood pressure after defibrillation of 14.8 ± 2.8 mmHg. See table **1**.

Of the 4 animals receiving ibutilide, the first received 0.005 mg/kg – ½ the recommended dose for adult humans in atrial fibrillation/atrial flutter. In this animal ROSC was achieved with a single 150 J shock, and over the next 2 minutes the mean arterial pressure rose to 100 mmHg. After this the mean arterial pressure continued to rise over the next 15 minutes, eventually peaking at 282 mmHg. The pig was monitored for an additional 10 minutes as the mean arterial pressure gradually fell to 260 mmHg. At this time the pig was sacrificed. See table **1**.

Given that hypertension is a reported side effect of ibutilide[162], the extreme hypertension observed in the first test-pig was believed to be a result of ibutilide overdose. Based on this evaluation the second ibutilide pilot pig received 0.0015 mg/kg. This pig was defibrillated after a second shock to PEA.

The two remaining pigs received ibutilide at 0.005 mg/kg and were readily defibrillated to ROSC, one receiving a single shock of 150 J, the other requiring a second 250 J shock. Both these pigs achieved systolic blood pressures greater than 90 mmHg within 2 minutes of achieving ROSC and did not suffer any episodes of refibrillation. Based on the above findings that 100% of the 6 control animals failed to achieve ROSC, while 75% of the 4 animals receiving ibutilide achieved ROSC with systolic blood pressures over 90 mmHg it was decided that the study should proceed using an ibutilide dose of 0.005 mg/kg. Together the 4 ibutilide treated animals had a mean blood pressure prior to defibrillation of 83.5 ± 9 mmHg, a mean defibrillation threshold of 175 ± 50 J, and a mean blood pressure post defibrillation of 73.3 ± 55.7 mmHg. The only of these to be significantly different from the control group is the post defibrillation blood pressure (14.8 ± 2.8 mmHg vs. 73.3 ± 55.7 mmHg, p=0.048). Unfortunately these experiments were performed during a period when the recording system was being repaired so blood pressure and defibrillation threshold data is all that is available, see table **1**.

The experiments proper were performed in a randomized, double blind fashion. A total of 20 animals were randomized into 4 treatment groups; control (no ibutilide, no ITV), ibutilide only, ITV only, ibutilide and ITV, such that a total of 10 animals received Ibutilide and 10 did not. Likewise, 10 animals received an active ITV, and 10 received a sham ITV. Before beginning the study it was hypothesized that ibutilide administration would result in lower defibrillation energy requirements, less re-fibrillation, greater survival compared to control, and less electrophysiological disruption compared to control (less post-resuscitation shortening of VERP and $MAPD_{90}$).

At baseline it was found that all measured variables were normal for all animals used in the study. Furthermore it was found that none of the measured variables were significantly different at baseline between the control group and the ibutilide treated group. See table **2**.

We found that the mean defibrillation threshold for animals treated with ibutilide was higher than that for animals not receiving the drug (211 ± 60 J vs. 161 ± 33.3 J, p=0.044). Ten minutes post defibrillation no significant difference was found between the two groups on any other variables. Contrary to our hypothesis, ibutilide did not favour survival. In the group receiving ibutilide, ROSC was achieved initially in 3 of 10 animals, (one of these 3 was hypotensive with a blood pressure of 20/12 and was asystolic within 5 minutes), while in the control group, ROSC was achieved in 7 of 10 animals, p= ns at 10 minutes post-defibrillation, see table **3**. Parameters recorded during CPR show that both the ibutilide treated group and the control group received comparable CPR, see table **4**. Ibutilide did not mitigate the electrophysiological disruption induced by ventricular fibrillation. In all animals, the ventricular effective refractory period (VERP) and the monophasic action potential duration at 90% repolarization ($MAPD_{90}$) were measured at a paced cycle length of 300 ms prior to the induction of ventricular fibrillation. In all animals that achieved ROSC, these measures were repeated 10 minutes post-ROSC. The $MAPD_{90}$ was shortened by 11.9 ± 17.4 % compared with baseline in the group receiving ibutilide, and shortened by 11.6 ± 22.9% (p=ns) compared with baseline in the control group. It was also found that the VERP in the group receiving ibutilide was prolonged by 7.1 ± 5.4% compared with baseline while

the VERP of the control group was shortened by 7.1 ± 8.2% compared with baseline (p=ns), see table 5.

Table 7 summarizes the data collected prior to VF, during CPR, and post defibrillation in the ibutilide study. There were no significant differences with regards to any of the measured variables prior to the initiation of VF. During CPR the ibutilide treated group had a significantly higher cardiac output, but no other variables were significantly different. This difference ought to favour greater survival in the ibutilide treated group, but this was not the case. Post defibrillation the only variable showing a statistically significant difference were defibrillation threshold [the higher in the ibutilide treated group (161.1 ± 33.3 vs. 200 ± 54.8 J, p=0.044)].

Based on these findings this experimental series was terminated and investigations into the reasons for this apparent lack of response to ibutilide were begun. 3 animals were treated with open-label ibutilide in sinus rhythm at doses ranging from 0.005 mg/kg to 0.03mg/kg. VERP and $MAPD_{90}$ were measures at paced cycle length 300 ms both prior to and 12 minutes after the administration of ibutilide. This coincides to the time point 10 minutes after defibrillation used in the cardiac arrest experiments where the drug is administered 2 minutes prior to the first defibrillation shock. At dose 0.005 mg/kg (n=1), the same dose used in the cardiac arrest experiments it was noted that VERP was prolonged from 178 ms before drug administration to 188 ms 12 minutes after drug administration, a difference of 5.6 %. The $MAPD_{90}$ was found to be 190 ms prior to drug administration and 184 ms 12 minutes after drug administration, a shortening of 3.5 %. At an ibutilide dose of 0.01 mg/kg (n=1) VERP was noted at 200 ms prior to drug

administration and 214 ms 12 minutes after drug administration, a prolongation of 7 %, while $MAPD_{90}$ was found to be 210 ms prior to drug administration and 208 ms 12 minutes after drug administration, a shortening of 1 %. At an ibutilide dose of 0.03 mg/kg (n=1) VERP was found to be 196 ms prior to drug administration and 186 ms 12 minutes after drug administration, a shortening of 5.1 % while $MAPD_{90}$ was found to be 204 ms prior to drug administration and 200 ms 12 minutes after drug administration, a shortening of 2 %. Based on these observations it was concluded that ibutilide has virtually no effect on VERP and $MAPD_{90}$ in pigs within the dose range tested, see table **6**. This is very different from the effects of ibutilide in the human ventricle where strong class III effects have been noted[102,105,162].

Tables and Figures

Hemodynamic Data from Ibutilide Pilot Studies.

	ibutilide dose mg/kg	Baseline mean BP mmHg	CPR mean BP mmHg	DFT J	Post VF mean BP mmHg	Outcome
Pig #						
2	0	82	35	150	16.0	PEA Dead
4	0	89	38	300	n/a	VF Dead
5	0	61	23	150	13.0	PEA Dead
7	0	66	39	300	14.0	PEA Dead
9	0	83	26	150	12.0	PEA Dead
10	0	76	35	150	19.0	VF Dead
mean		76.2	32.7	200.0	14.8	
SD		10.8	6.6	77.5	2.8	
3	0.0015	84	32	250	15.0	PEA Dead
1	0.005	78	38	150	149.0	ROSC
6	0.005	76	36	150	67.0	ROSC
8	0.005	96	11	150	62.0	ROSC
mean		83.3	28.3	150.0	92.7	
SD		11.0	15.0	0.0	48.9	
p values		0.381	0.551	0.316	0.009	

Table 1 – Data from the ten pilot experiments performed prior to beginning the ibutilide study. The post defibrillation blood pressure was significantly higher in the ibutilide treated group. These preliminary data suggest that ibutilide may offer some benefit and pursuing the study is worthwhile. Data collected was not as extensive as was the data collected for the ibutilide study proper because the computer acquisition and recording system was being retrofitted at the time these experiments were performed. BP=blood pressure, DFT=defibrillation threshold.

Baseline Data from All Pigs in the Ibutilide Study.

	sys. BP mmHg	dias. BP mmHg	HR BPM	ETCO2 mmHg	CO L/min	MAPD90 msec	VERP @ 300 msec	Ibutilide
PIG #								
1.0	105	68	206	42.0	3.4	190.0	190.0	No
3.0	105	70	127	N/A	5.3	256.8	202.0	No
5.0	117	73	116	37.6	4.4	188.6	192.0	No
6.0	107	74	139	N/A	3.2	229.2	218.0	No
10.0	105	64	130	41.0	4.4	252.2	186.0	No
12.0	115	66	135	41.0	4.5	229.2	192.0	No
15.0	104	67	113	40.0	3.4	201.6	192.0	No
16.0	128	86	141	47.2	3.9	233.2	180.0	No
18.0	119	86	119	42.4	4.4	199.4	214.0	No
19.0	121	87	158	46.0	4.8	200.8	186.0	No
mean	112.5	74.0	138.5	42.1	4.2	218.1	195.2	
SD	8.4	9.0	27.4	3.1	0.7	25.2	12.4	
2.0	131	83	190	N/A	5.5	173.8	184.0	Yes
4.0	100	59	130	36.7	4.9	220.8	202.0	Yes
7.0	108	65	124	35.5	5.1	212.8	234.0	Yes
8.0	104	68	126	39.0	4.0	225.0	184.0	Yes
9.0	113	82	143	34.8	4.1	239.4	200.0	Yes
11.0	118	78	138	41.4	4.8	240.2	194.0	Yes
13.0	113	65	171	39.8	4.7	235.2	194.0	Yes
14.0	117	66	201	38.0	4.1	160.2	194.0	Yes
17.0	124	88	136	43.2	4.1	239.0	166.0	Yes
20.0	97	52	195	47.0	5.7	139.4	148.0	Yes
mean	112.3	70.5	155.5	39.5	4.7	208.6	190.0	
SD	10.8	11.6	30.5	3.9	0.6	37.1	22.7	
p values	0.976	0.451	0.206	0.144	0.096	0.510	0.533	

Table 2 – At baseline during normal sinus rhythm, measured variables including Blood pressure, heart rate, end-tidal CO2 cardiac output and electrophysiolocial parameters for all animals were normal. There were no statistically significant differences between the control group and the ibutilide treated group. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, LVP sys=systolic left ventricular pressure, LVP dias=diastolic left ventricular pressure, CVP sys=systolic central venous pressure, CVP dias=diastolic central venous pressure, HR=heart rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, MAPD90=monophasic action potential at 90% repolarization, VERP=ventricular effective refractory period, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation.

Data collected 10 Minutes Post-Defibrillation – Ibutilide Study.

	sys. BP mmHg	dias. BP mmHg	HR BPM	ETCO2 mmHg	CO L/min	MAPD90 msec	VERP @ 300 msec	DFT J	refib	# of shocks	Outcome	Drug ?	AVP ?
PIG #													
1.000	128	73	192	38	4.4	N/A	172.0	150	2	3.000	ROSC	No	No
3.000	118	74	192	N/A	3.5	155.0	200.0	250	0	2.000	ROSC	No	No
5.000								150	0	1.000	PEA dead	No	No
6.000										6.000	VF dead	No	Yes
10.000	77	44	150	42	2.9	230.6	180.0	150	0	1.000	ROSC	No	No
12.000	114	72	187	39	3.6	173.6	180.0	150	1	2.000	ROSC	No	No
15.000	106	71	184	44	3.8	260.0	190.0	150	1	2.000	ROSC	No	No
16.000								150	0	1.000	PEA dead	No	No
18.000	130	94	207	38	2.6	176.8	162.0	150	0	1.000	ROSC	No	No
19.000	107	57	188	56	5.2	171.0	178.0	150	2	3.000	ROSC	No	No
mean	111.4	69.3	185.8	42.8	3.7	194.5	180.3	161.1	0.7	2.2			
SD	17.8	15.5	17.4	6.9	0.9	41.1	12.2	33.3	0.9	1.5			
2.000	95	57	214	N/A	2.9	174.6	190.0	250	0	2.000	ROSC	Yes	No
4.000	20	12	28	N/A*	N/A*	N/A*	N/A*	150	2	3.000	ROSC temp	Yes	No
7.000								250	0	2.000	asystole dea	Yes	No
8.000								150	0	1.000	PEA dead	Yes	No
9.000								250	1	3.000	PEA dead	Yes	No
11.000								250	0	2.000	PEA dead	Yes	No
13.000								300	1	6.000	VF dead	Yes	Yes
14.000								150	3	6.000	VF dead	Yes	Yes
17.000	98	55	189	32	3.3	181.2	184.0	150	0	1.000	ROSC	Yes	No
20.000										6.000	VF dead	Yes	Yes
mean	71.0	41.3	143.4	32.0	3.1	177.9	187.0	211.1	0.8	3.2			
SD	44.2	25.4	100.7	#DIV/0!	0.3	4.7	4.2	60.1	1.1	2.0			
p values	0.061	0.060	0.273		0.378	0.607	0.486	0.044		0.233			

Table 3 – Data 10 minutes post defibrillation. The top portion of the table is data from control animals, the bottom portion of the table is data from animals treated with ibutilide. p values at the bottom indicate results from a two-tailed t-test between ibutilide-treated and control groups. The only parameters to reach significance (<.05) were defibrillation threshold – 161.1 ± 33.3 vs. 211.1 ± 60.1 p=0.044 (lower in the control group), and survival at 10 minutes – 7 of 10 vs. 2 of 10 p=0.024 (higher in the control group). Spaces are left blank for animals that were not resuscitated. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, HR=heart rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, MAPD90=monophasic action potential at 90% repolarization, VERP=ventricular effective refractory period, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation, ROSC= return of spontaneous circulation, PEA= pulseless electrical activity, N/A indicates this datum could not be collected because it was outside the range of detection of the recording device ($ETCO_2$) or because of poor signal quality (MAPD90), N/A* indicates that this datum could not be collected because the animal died during the collection of data at this timepoint.

Data collected during CPR – Ibutilide Study.

	sys. BP mmHg	dias. BP mmHg	comp rate	ETCO2 mmHg	CO L/min	VFCL msec	VF amp mV	DFT J	refib?	# of shocks	Outcome	Ibutilide ?
PIG #												
1.000	55	19	80	37	0.9	62.5	0.306	150	Yes x2	3	ROSC	No
3.000	67	24	80	25	1.1	76.9	0.232	250	No	2	ROSC	No
5.000	44	14	80	25	1.0	62.5	0.200	150	No	1	PEA dead	No
6.000	65	21	80	N/A	0.9	47.6	0.122			6	VF dead	No
10.000	83	28	80	41	1.2	58.8	0.310	150	No	1	ROSC	No
12.000	78	30	80	35	1.3	52.6	0.162	150	Yes x1	2	ROSC	No
15.000	62	28	80	35	1.2	62.5	0.172		Yes x1	2	ROSC	No
16.000	49	23	80	29	1.1	58.8	0.148	150	No	1	PEA dead	No
18.000	58	31	80	35	0.9	66.7	0.284	150	No	1	ROSC	No
19.000	85	30	80	36	1.0	83.3	0.178	150	Yes x2	3	ROSC	No
mean	64.6	24.8		33.1	1.1	63.2	0.211	162.5		2		
SD	13.9	5.6		5.5	0.1	10.5	0.068	35.4		2		
2.000	105	28	80	N/A	1.4	90.9	0.234	250	No	2	ROSC	Yes
4.000	75	27	80	32	1.0	76.9	0.308		Yes x2	3	ROSC temp	Yes
7.000	44	14	80	25	0.8	71.4	0.160	250	No	2	asystole dead	Yes
8.000	115	29	80	31	2.1	50.0	0.304	150	No	1	PEA dead	Yes
9.000	95	32	80	37	1.2	62.5	0.130		Yes x1	3	PEA dead	Yes
11.000	51	26	80	42	1.3	90.9	0.290		No	2	PEA dead	Yes
13.000	70	17	80	35	1.6	62.5	0.210		Yes x1	6	VF dead	Yes
14.000	58	20	80	27	1.0	111.1	0.240	150	Yes x3	6	VF dead	Yes
17.000	109	34	80	47	1.5	76.9	0.356	150	No	1	ROSC	Yes
20.000	51	21	80	47	1.2	47.6	0.114			6	VF dead	Yes
mean	77.3	24.8		35.9	1.3	74.1	0.235	190.0		3		
SD	26.7	6.5		8.1	0.4	19.7	0.081	54.8		2		
p-values	0.199	1.000		0.408	0.046	0.143	0.498	0.290		0.233		

Table 4 – Data collected during CPR. This data was taken prior to the administration of ibutilide. p values at the bottom indicate results from a two-tailed t-test between ibutilide-treated and control groups. This data suggests that both groups received comparable CPR, and that differences in CPR cannot sufficiently explain differences in survival between the ibutilide treated group and control group. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, HR=heart rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, VFCL=ventricular fibrillation cycle length, VF amp=ventricular fibrillation amplitude, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation, ROSC=return of spontaneous circulation, PEA=pulseless electrical activity, N/A indicated that this datum could not be collected because the value was outside the range of detection of the device.

Data Collected 10 Minutes Post Defibrillation - Ibutilide Study.

	MAPD90 pre msec	MAPD90 post msec	% Change pre to post	VERP @ 300 pre msec	VERP @ 300 post msec	% Change pre to post	Ibutilide
PIG #							
1.0	190.0	N/A		190.0	172.0	-9.5	No
3.0	256.8	155.0	-39.6	202.0	200.0	-1.0	No
5.0	188.6			192.0			No
6.0	229.2			218.0			No
10.0	252.2	230.6	-8.6	186.0	180.0	-3.2	No
12.0	229.2	173.6	-24.3	192.0	180.0	-6.3	No
15.0	201.6	260.0	29.0	192.0	190.0	-1.0	No
16.0	233.2			180.0			No
18.0	199.4	176.8	-11.3	214.0	162.0	-24.3	No
19.0	200.8	171.0	-14.8	186.0	178.0	-4.3	No
mean	218.1	194.5		195.2	180.3	-7.1	
SD	25.2	41.1		12.4	12.2	8.2	
2.0	173.8	174.6	0.5	184.0	190.0	3.3	Yes
4.0	220.8	N/A*		202.0	N/A*		Yes
7.0	212.8			234.0			Yes
8.0	225.0			184.0			Yes
9.0	239.4			200.0			Yes
11.0	240.2			194.0			Yes
13.0	235.2			194.0			Yes
14.0	160.2			194.0			Yes
17.0	239.0	181.2	-24.2	166.0	184.0	10.8	Yes
20.0	139.4			148.0			Yes
mean	208.6	177.9	-11.9	190.0	187.0	7.1	
SD	37.1	4.7	17.4	22.7	4.2	5.4	
p values	0.510	0.607	0.989	0.225	0.486	0.060	

Table 5 – Pigs treated with ibutilide were expected to show less shortening of $MAPD_{90}$ and VERP as compared to control. This table shows $MAPD_{90}$ and VERP before induction of VF and 10 minutes after defibrillation. There is no significant difference between animals treated with ibutilide and control. p values are from two tailed t tests comparing the percent change of control animals to that of ibutilide treated animals. These failed to reach a level of signifance (<.05) for both $MAPD_{90}$ and VERP. Spaces were left blank for animals that were not resuscitated. Calculations were based on n=7 surviving for control group and n=3 surviving for ibutilide treated group. MAPD90=monophasic action potential at 90% repolarization, VERP=ventricular effective refractory period, pre=prior to cardiac arrest, post=after defibrillation, blank spaces indicate that the animal in question was dead at this timepoint, N/A indicates that this datum could not be collected because poor signal quality, N/A* indicates that this datum could not be collected because the animal died as a result of trying to collect this datum.

Effect of Increasing Doses of Ibutilide on MAPD90 and VERP in Normal Sinus Rhythm.

	MAPD90 pre	MAPD90 post	% change	VERP pre	VERP post	% change
	msec	msec	pre to post	msec	msec	pre to post
DOSE mg/kg						
0.005	190	184	-3.2	178	188	5.6
0.01	210	208	-1.0	200	214	7.0
0.03	204	200	-2.0	196	186	-5.1

Table 6 – $MAPD_{90}$ and VERP prolongation observed with increasing doses of ibutilide. Doses up to 6 times that administered to pigs during the study, and 3 times the maximum recommended dose for human patients show no consistent or appreciable prolongation of $MAPD_{90}$ and VERP, showing no class III activity in pigs. n=1 for each dose. MAPD90=monophasic action potential at 90% repolarization, VERP=ventricular effective refractory period, pre= prior to cardiac arrest, post=after defibrillation.

Summary Data From the Ibutilide Study

		sys. BP	dias. BP	HR	comp rate	ETCO2	CO	MAPD90	VFCL	VF amp	VERP@300	DFT	# of shocks
		mm-Hg	mm-Hg	BPM		mm-Hg	L/min	msec	msec	mV	msec	J	
	Condition												
t=0 baseline	Control Pre VF n=10	112.5 +/- 8.4	74 +/- 9	138.5 +/- 27.4	n/a	42.1 +/- 3.1	4.2 +/- 0.7	218.1 +/- 25.2	n/a	n/a	195.3 +/- 12.4	161.1 +/- 3	2.2 +/- 1.5
	Ibutilide Pre VF n=10	112.3 +/- 10.8	70.5 +/- 11.6	184.2 +/- 92.8	n/a	39.5 +/- 3.9	4.7 +/- 0.6	208.6 +/- 37.1	n/a	n/a	190 +/- 22.7	200 +/- 54.	3.2 +/- 2
	p values	0.982	0.448	0.133		0.198	0.507	0.510			0.533	0.044	0.483
t=7 min during CPR	Control CPR n=10	64.6 +/- 14	24.8 +/- 5.6	n/a	80	33.1 +/- 5.5	1.1 +/- 0.1	n/a	63.2 +/- 10.5	0.2 +/- 0.07	n/a	161.1 +/- 3	2.2 +/- 1.5
	Ibutilide CPR n=10	77.3 +/- 26.7	24.8 +/- 5.6	n/a	80	35.9 +/- 8.1	1.3 +/- 0.4	n/a	74.1 +/- 19.9	0.24 +/- 0.08	n/a	200 +/- 54.	3.2 +/- 2
	p values	0.199	1.000			0.408	0.061		0.144	0.708		0.044	0.483
t=22 min post-defibrillation	Control Post VF n=7	111.4 +/- 17.8	69.3 +/- 15.5	234 +/- 71	n/a	42.9 +/- 6.9	3.7 +/- 0.9	194.5 +/- 41.1	n/a	n/a	180.3 +/- 12.2	161.1 +/- 3	2.2 +/- 1.5
	Ibutilide Post VF n=2	71 +/- 44.2	41.3 +/- 25.4	143.4 +/- 100.7	n/a	32 (n=1)	3.1 +/- 0.3	177.9 +/- 4.7	n/a	n/a	187 +/- 4.2	200 +/- 54.	3.2 +/- 2
	p values	0.061	0.060	0.136			0.378	0.607			0.486	0.044	0.483

Table 7 – At baseline there were no significant differences between the control and ibutilide treated groups. The only measured variable to reach significance was the defibrillation threshold, and ibutilide caused the defibrillation threshold to be higher, opposite of what was expected. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. Survival was significantly higher in the control group, 7 of 10 vs. 2 of 10 p=0.024. sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, HR=heart rate, comp rate=compression rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, MAPD90=monophasic action potential at 90% repolarization, VFCL=ventricular fibrillation cycle length, VF amp=ventricular fibrillation amplitude, VERP=ventricular effective refractory period, DFT=defibrillation threshold.

ITV STUDY

As previously noted, the impedance threshold valve (ITV) experiments were performed in combination with the ibutilide experiments, so these experiments were also performed in a randomized, double blind fashion. A total of 20 animals were randomized into 4 treatment groups; control (no ibutilide, no ITV), ibutilide only, ITV only, ibutilide and ITV, such that a total of 10 animals were treated with the active ITV and 10 were treated with a sham ITV. Baseline values for animals treated with the ITV and control (no ITV) groups were not significantly different, see table **8**. Prior to commencing these experiments it was hypothesized that animals treated with the active ITV would show better hemodynamic values during CPR and would experience better vital organ blood flow during CPR resulting in greater survival.

It was found that the active ITV resulted in very little difference in hemodynamic values during CPR. Table **9** shows measurements taken during CPR. There is no significant difference between the control group and the ITV treated group on any of the measured parameters. Data collected 10 minutes post defibrillation, shown in table **10**, show that there was no difference found in any of the hemodynamic or electrophysiologic values measured at 10 minutes post defibrillation. It was also found that the active ITV offered no survival benefit. 6 of 10 animals treated with the active valve achieved ROSC while 4 of 10 animals treated with the sham valve achieved ROSC (p=ns).

Table **11** summarizes the data collected prior to VF, during CPR, and post defibrillation in the ITV study. In this study there were no significant differences found

between the control group and the ITV treated group on any of the measured variables at any of the time points, prior to the initiation of VF, during CPR, or post defibrillation.

Table **12** summarizes the data broken down into the four treatment groups – control, ibutilide only, ITV only, and both ibutilide and ITV. One-way ANOVA indicates that there were no statistically significant differences between any of the four groups at any time point. It should be noted that all the animals in the ibutilide only group were dead 10 minutes post defibrillation, so they were left out of the statistical analysis for this time point.

Tables and Figures

Baseline Data From ITV Study.

	sys. BP mmHg	dias. BP mmHg	HR BPM	ETCO2 mmHg	CO L/min	MAPD90 msec	VERP @ 300 msec	ITV ?
PIG #								
3.0	105	70	127	N/A	5.3	256.8	202.0	No
4.0	100	59	130	37	4.9	220.8	202.0	No
6.0	107	74	139	N/A	3.2	229.2	218.0	No
8.0	104	68	126	39	4.0	225.0	184.0	No
10.0	105	64	130	41	4.4	252.2	186.0	No
11.0	118	78	138	41	4.8	240.2	194.0	No
14.0	117	66	201	38	4.1	160.2	194.0	No
16.0	128	86	141	47	3.9	233.2	180.0	No
19.0	121	87	158	46	4.8	200.8	186.0	No
20.0	97	52	195	47	5.7	139.4	148.0	No
mean	109.9	70.3	148.5	42.0	4.5	215.8	189.4	
SD	10.1	11.2	27.7	4.2	0.7	38.5	18.4	
1.0	105	68	206	42	3.4	190.0	190.0	Yes
2.0	131	83	190	N/A	5.5	173.8	184.0	Yes
5.0	117	73	116	38	4.4	188.6	192.0	Yes
7.0	108	65	124	36	5.1	212.8	234.0	Yes
9.0	113	82	143	35	4.1	239.4	200.0	Yes
12.0	115	66	135	41	4.5	229.2	192.0	Yes
13.0	113	65	171	40	4.7	235.2	194.0	Yes
15.0	104	67	113	40	3.4	201.6	192.0	Yes
17.0	124	88	136	43	4.1	239.0	166.0	Yes
18.0	119	86	119	42	4.4	199.4	214.0	Yes
mean	114.9	74.2	145.4	39.6	4.4	210.9	195.8	
SD	8.5	9.4	32.7	3.0	0.6	23.7	18.0	
p values	0.246	0.400	0.821	0.182	0.659	0.737	0.441	

Table 8 – Baseline Blood pressure, heart rate, end-tidal CO2 cardiac output and electrophysiolocial parameters for all animals were normal. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. There were no significant differences between the control group (top portion of table) and the ITV treated group (bottom portion of table). sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, HR=heart rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, MAPD90=monophasic action potential at 90% repolarization, VERP=ventricular effective refractory period, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation, ITV=impedance threshold valve, N/A indicates that the datum was outside the range of detection of the device.

Data Collected During CPR – ITV Study.

	sys. BP mmHg	dias. BP mmHg	comp rate	ETCO2 mmHg	CO L/min	VFCL msec	VF amp mV	ITV ?
PIG #								
3.000	67	24	80	25	1.1	76.9	0.232	No
4.000	75	27	80	32	1.0	76.9	0.308	No
6.000	65	21	80	N/A	0.9	47.6	0.122	No
8.000	115	29	80	31	2.1	50.0	0.304	No
10.000	83	28	80	41	1.2	58.8	0.310	No
11.000	51	26	80	42	1.3	90.9	0.290	No
14.000	58	20	80	27	1.0	111.1	0.240	No
16.000	49	23	80	29	1.1	58.8	0.148	No
19.000	85	30	80	36	1.0	83.3	0.178	No
20.000	51	21	80	47	1.2	47.6	0.114	No
mean	69.9	24.9		34.4	1.2	70.2	0.2	
SD	20.5	3.6		7.5	0.3	21.2	0.1	
1.000	55	19	80	37	0.9	62.5	0.306	Yes
2.000	105	28	80	N/A	1.4	90.9	0.234	Yes
5.000	44	14	80	25	1.0	62.5	0.200	Yes
7.000	44	14	80	25	0.8	71.4	0.160	Yes
9.000	95	32	80	37	1.2	62.5	0.130	Yes
12.000	78	30	80	35	1.3	52.6	0.162	Yes
13.000	70	17	80	35	1.6	62.5	0.210	Yes
15.000	62	28	80	35	1.2	62.5	0.172	Yes
17.000	109	34	80	47	1.5	76.9	0.356	Yes
18.000	58	31	80	35	0.9	66.7	0.284	Yes
mean	72.0	24.7		34.6	1.2	67.1	0.2	
SD	24.0	7.8		6.6	0.3	10.5	0.1	
p-values	0.836	0.942		0.974	0.969	0.684	0.926	

Table 9 – Data collected during CPR. No significant difference was noted on any of the measured parameters. The ITV valve does not appear to have any effect on either hemodynamic variables or electrophysiologic variables during CPR. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, comp rate=compression rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, VFCL=ventricular fibrillation cycle length, VF amp=ventricular fibrillation amplitude, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation, ITV=impedance threshold valve, N/A indicates that the datum was out of the range of detection of the device.

Data Collected 10 Minutes Post Defibrillation – ITV Study.

	sys. BP mmHg	dias. BP mmHg	HR BPM	ETCO2 mmHg	CO L/min	MAPD90 msec	VERP @ 300 msec	DFT J	# of shocks	Outcome	ITV ?
PIG #											
3.000	118	74	192	N/A	3.5	155.0	200.0	250	2	ROSC	No
4.000	20	12	28	N/A	N/A	N/A*	N/A*	150	3	ROSC temp	No
6.000									6	VF dead	No
8.000								150	1	PEA dead	No
10.000	77	44	150	42	2.9	230.6	180.0	150	1	ROSC	No
11.000								250	2	PEA dead	No
14.000								150	6	VF dead	No
16.000								150	1	PEA dead	No
19.000	107	57	188	56	5.2	171.0	178.0	150	3	ROSC	No
20.000									6	VF dead	No
mean	80.5	46.8	139.5	49.0	3.9	185.5	186.0	175.0	3.1		
SD	43.9	26.2	76.7	9.9	1.2	39.8	12.2	46.3	2.1		
1.000	128	73	192	38	4.4	N/A	172.0	150	3	ROSC	Yes
2.000	95	57	214	N/A	2.9	174.6	190.0	250	2	ROSC	Yes
5.000								150	1	PEA dead	Yes
7.000								250	2	asystole dea	Yes
9.000								250	3	PEA dead	Yes
12.000	114	72	187	39	3.6	173.6	180.0	150	2	ROSC	Yes
13.000								300	6	VF dead	Yes
15.000	106	71	184	44	3.8	260.0	190.0	150	2	ROSC	Yes
17.000	98	55	189	32	3.3	181.2	184.0	150	1	ROSC	Yes
18.000	130	94	207	38	2.6	176.8	162.0	150	1	ROSC	Yes
mean	111.8	70.3	195.4	38.2	3.4	193.2	179.7	195.0	2.3		
SD	14.9	14.0	12.0	4.3	0.6	37.4	11.0	59.9	1.5		
p values	0.137	0.098	0.108	0.078	0.496	0.792	0.455	0.449	0.344		

Table 10 – Data collected 10 minutes post defibrillation. There is no significant difference between the control group and the ITV treated group on any parameter. The ITV does not appear to improve post-resuscitation hemodymanics, electrophysiologic parameters, defibrillation threshold, nor does it appear to improve short-term cardiac arrest survival. Spaces were left blank for animals that were not resuscitated. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. Calculations based on n=3 for control group and n=6 for ITV treated group (p=ns). sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, HR=heart rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, MAPD90=monophasic action potential at 90% repolarization, VERP=ventricular effective refractory period, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation ITV=impedance threshold valve, AVP=arginine vasopressin, ROSC=return of spontaneous circulation, PEA=pulseless electrical activity, blank spaces indicate that the animal was dead at the indicated timepoint, N/A indicates that the datum was out of range of the recording device, N/A* indicates that the attempt to record the datum resulted in the death of the animal.

Summary Data From ITV Study.

		sys. BP	dias. BP	HR	comp rate	ETCO2	CO	MAPD90	VFCL	VF amp	VERP@300	DFT	#of shocks
		mmHg	mmHg	BPM		mmHg	L/min	msec	msec	mV	msec	J	
	Condition												
t=0 baseline	Control Pre VF n=10	109.9 +/- 10.1	70.2 +/- 11.2	178.2 +/- 94.4	n/a	42 +/- 4.2	4.5 +/- 0.7	215.8 +/- 38.5	n/a	n/a	189.4 +/- 18.4	175 +/- 46.	3.2 +/- 2.1
	ITV only Pre VF n=10	114.9 +/- 8.57	74.2 +/- 9.4	145.4 +/- 32.7	n/a	39.6 +/- 3	4.3 +/- 0.7	210.9 +/- 38.7	n/a	n/a	195.8 +/- 18	195 +/- 59.	2.3 +/- 1.5
	p values	0.265	0.456	0.820		0.203	0.659	0.351			0.441	0.449	0.344
t=7 min during CPR	Control CPR n=10	69.9 +/- 20.5	24.9 +/- 3.6	n/a	80	34.4 +/- 7.5	1.2 +/- 0.3	n/a	70.2 +/- 21.2	0.2 +/- 0.08	n/a	175 +/- 46.	3.2 +/- 2.1
	ITV only CPR n=10	72 +/- 24	24.7 +/- 7.8	n/a	80	34.6 +/- 6.6	1.2 +/- 0.3	n/a	67.1 +/- 10.5	0.2 +/- 0.07	n/a	195 +/- 59.	2.3 +/- 1.5
	p values	0.703	0.942			0.974	0.984		0.605	0.924		0.449	0.344
t=22 min post-defibrillation	Control Post VF n=3	80.5 +/- 43.9	46.7 +/- 26.2	169.9 +/- 117.5	n/a	49 +/- 9.9	3.9 +/- 1.2	185.5 +/- 39.8	n/a	n/a	186 +/- 12.2	175 +/- 46.	3.2 +/- 2.1
	ITV only Post VF n=6	111.8 +/- 14.9	70.3 +/- 14	213.4 +/- 58.8	n/a	38.3 +/- 4.2	3.4 +/- 0.6	193.2 +/- 37.4	n/a	n/a	179.7 +/- 11	195 +/- 59.	2.3 +/- 1.5
	p values	0.188	0.098	0.601		0.078	0.496	0.796			0.455	0.449	0.344

Table 11 – None of the variables measures at any time point were significantly different between the control and ITV treated groups. Survival was not statistically different between the control group and the ITV treated group, 3 of 10 vs. 6 of 10. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, HR=heart rate, comp rate=compression rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, MAPD90=monophasic action potential at 90% repolarization, VFCL=ventricular fibrillation cycle length, VF amp=ventricular fibrillation amplitude, VERP=ventricular effective refractory period, DFT=defibrillation threshold.

Summary Data From the 4 Treatment Groups, Control, Ibutilide Only, ITV Only, and Ibutilide + ITV.

		sys. BP	dias. BP	HR	comp rate	ETCO2	CO	MAPD90	VFCL	VF amp	VERP @300	DFT	# of shocks
		mmHg	mmHg	BPM		mmHg	L/min	msec	msec	mV	msec	J	
	Condition												
t=0 baseline	Control Pre VF n=5	113 +/- 10.5	76.1 +/- 10.1	139 +/- 12.3	n/a	44.7 +/- 3.3	4.3 +/- 0.8	234.4 +/- 22.2	n/a	n/a	194.4 +/- 15.5	175 +/- 50	2.6 +/- 2.1
	Ibutilide only Pre VF n=5	106.8 +/- 9.7	64.6 +/- 9.7	217.5 +/- 117.6	n/a	40.4 +/- 4.1	4.7 +/- 0.7	197.1 +/- 44.4	n/a	n/a	184.4 +/- 21.3	175 +/- 50	3.6 +/- 2.3
	ITV only Pre VF n=5	111.9 +/- 7	72 +/- 8.2	137.9 +/- 39.2	n/a	40.6 +/- 1.2	4 +/- 0.6	201.8 +/- 16.3	n/a	n/a	196 +/- 10.1	150 +/- 0	1.8 +/- 0.8
	Ibutilide +ITV Pre VF n=5	117.9 +/- 9.6	76.5 +/- 10.8	152.9 +/- 27	n/a	38.3 +/- 3.9	4.7 +/- 0.6	220 +/- 28.1	n/a	n/a	195.6 +/- 25	225 +/- 50	2.8 +/- 1.9
	p values	0.339	0.217	0.242		0.150	0.385	0.211			0.737	0.053	0.522
t=7 min during CPR	Control CPR n=5	69.8 +/- 14.7	25.2 +/- 3.7	n/a	80	32.7 +/- 71.	1 +/- 0.1	n/a	65.1 +/- 14.6	0.2 +/- 0.07	n/a	175 +/- 50	2.6 +/- 2.1
	Ibutilide only CPR n=5	70 +/- 27	24.6 +/- 3.9	n/a	80	35.8 +/- 8.3	1.3 +/- 0.4	n/a	75.3 +/- 27.1	0.3 +/-0.08	n/a	175 +/- 50	3.6 +/- 2.3
	ITV only CPR n=5	59.4 +/- 12.4	24.4 +/- 7.5	n/a	80	33.4 +/- 4.8	1.1 +/- 0.1	n/a	61.4 +/- 5.2	0.2 +/- 0.07	n/a	150 +/- 0	1.8 +/- 0.8
	Ibutilide +ITV CPR n=5	84.6 +/- 27.3	25 +/- 9	n/a	80	36 +/- 9	1.3 +/- 0.3	n/a	72.8 +/- 11.8	0.2 +/- 0.09	n/a	225 +/- 50	2.8 +/- 1.9
	p values	0.354	0.997			0.883	0.288		0.531	0.755		0.053	0.522
t=22 min post-defibrillation	Control Post VF n=3	100.7 +/- 21.2	58.3 +/- 15	217.2 +/- 85.3	n/a	49 +/- 4.4	3.9 +/- 1.2	185.5 +/- 39.8	n/a	n/a	186 +/- 12.1	175 +/- 50	2.6 +/- 2.1
	Ibutilide only Post VF n=0	all dead	all dead	all dead	all dead	all dead	all dead	all dead	all dead	all dead	all dead	all dead	all dead
	ITV only Post VF n=4	119.5 +/- 11.5	77.5 +/- 11	246.6 +/- 68.8	n/a	39.7 +/- 2.9	3.6 +/- 0.7	203.5 +/- 49	n/a	n/a	176 +/- 11.9	150 +/- 0	1.8 +/- 0.8
	Ibutilide +ITV Post VF n=2	96.5 +/- 2.1	56 +/- 1.4	201 +/- 17.6	n/a	32 (n=1)	3.1 +/- 0.3	177.9 +/- 4.7	n/a	n/a	187 +/- 4.2	225 +/- 50	2.8 +/- 1.9
	p values	0.192	0.107	0.733		0.132	0.650	0.767			0.428	0.053	0.522

Table 12 – One-way ANOVA tests were used to examine the possibility that there were statistically significant differences between each of the four treatment groups: Control (no Ibutilide, no ITV), Ibutilide only, ITV only, Ibutilide and ITV together. It was found that prior to VF, and during CPR there were no significant differences between any of the groups on any of the measured variables. 10 minutes post defibrillation all the animals in the ibutilide only group were dead, but there were no significant differences between any of the other three groups, and even though all the animals in the ibutilide only group were dead, one way ANOVA revealed no significant differences in survival between the groups. Left ventricular pressures were incorrectly measured and thus omitted. This was later traced to a problem in our recording and acquisition system. This problem has since been corrected. Central venous pressures also were incorrectly measured. The source of these elevated readings is unknown. Central venous pressures are now recorded using a long introducer placed in the caudal vena cava via the right femoral vein rather than via the proximal port of the swan-ganz catheter. sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, HR=heart rate, comp rate=compression rate, ETCO2=end-tidal carbon dioxide level, CO=cardiac output, MAPD90=monophasic action potential at 90% repolarization, VFCL=ventricular fibrillation cycle length, VF amp=ventricular fibrillation amplitude, VERP=ventricular effective refractory period, DFT=defibrillation threshold.

Experimental Flow—All Animals Not Receiving Ibutilide

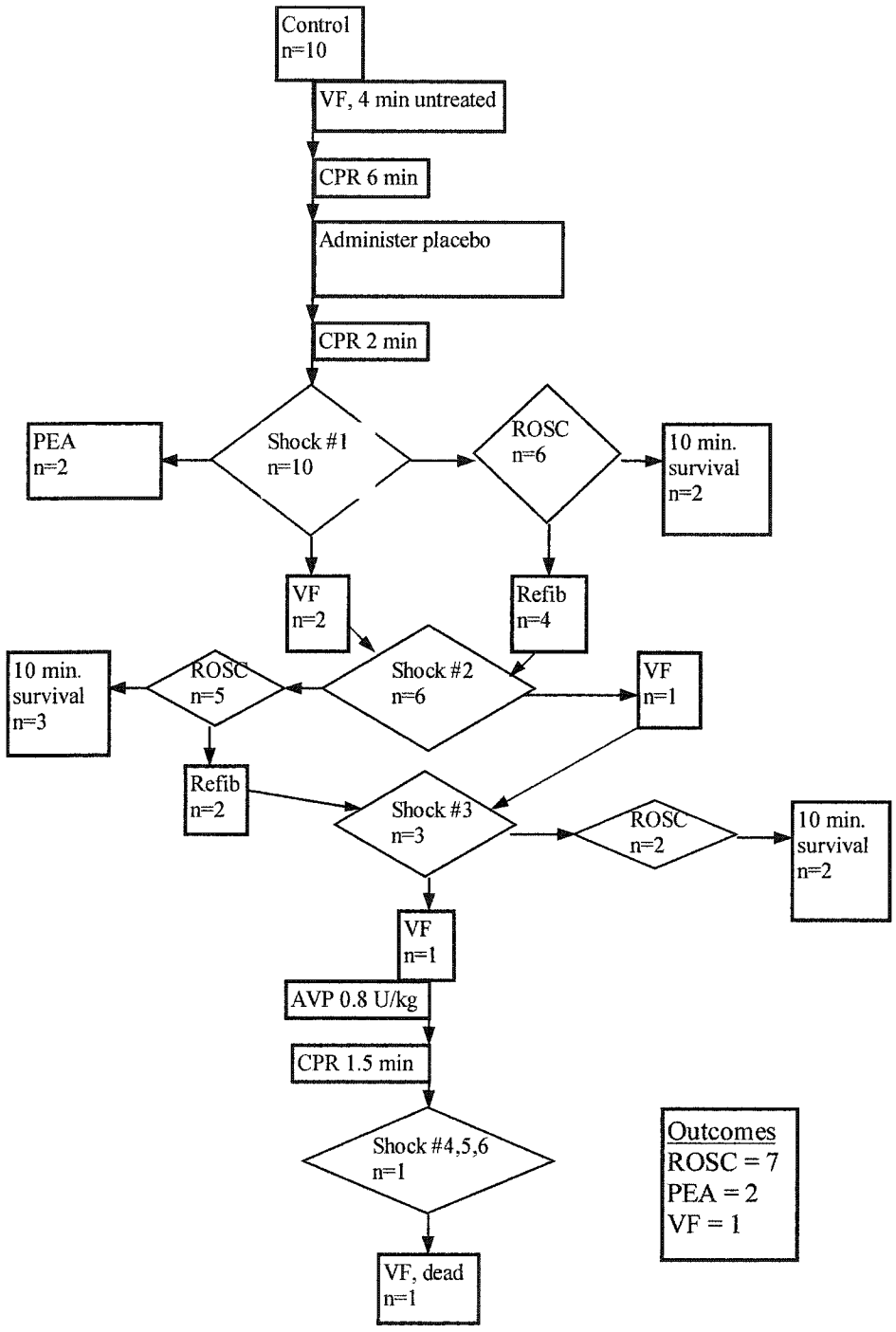

Figure V – Flowchart detailing experimental timeline and outcomes for control animals in ibutilide study. VF=ventricular fibrillation, PEA=pulseless electrical activity, NSR=normal sinus rhythm, ROSC=return of spontaneous circulation, refib=refibrillation after initial successful defibrillation, AVP=arginine vasopressin.

Experimental Flow—All Animals receiving Ibutilide

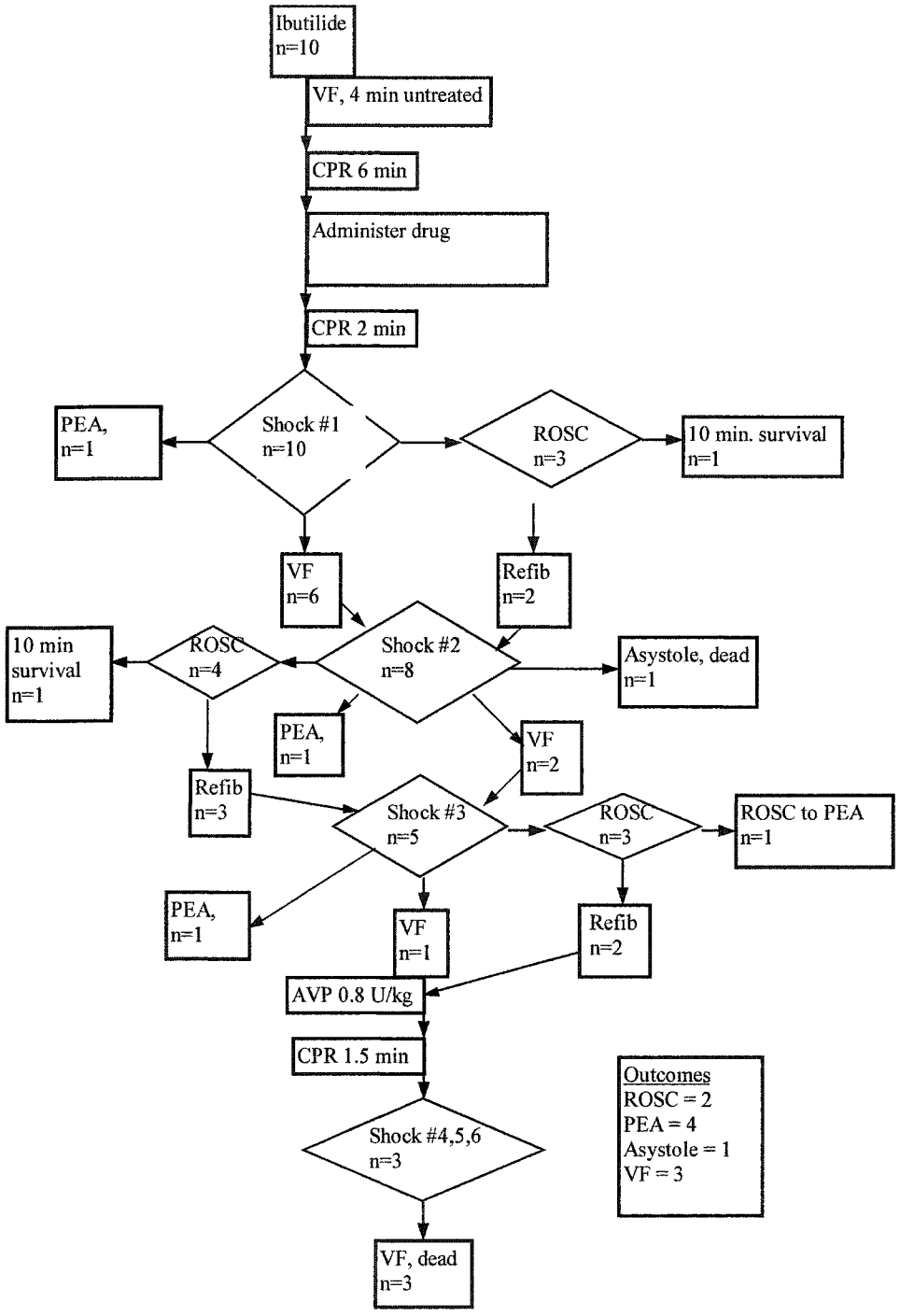

Figure VI - Flowchart detailing experimental timeline and outcomes for treated animals in ibutilide study. VF=ventricular fibrillation, PEA=pulseless electrical activity, NSR=normal sinus rhythm, ROSC=return of spontaneous circulation, refib=refibrillation after initial successful defibrillation, AVP=arginine vasopressin.

Experimental Flow—All Animals Not Treated With the ITV

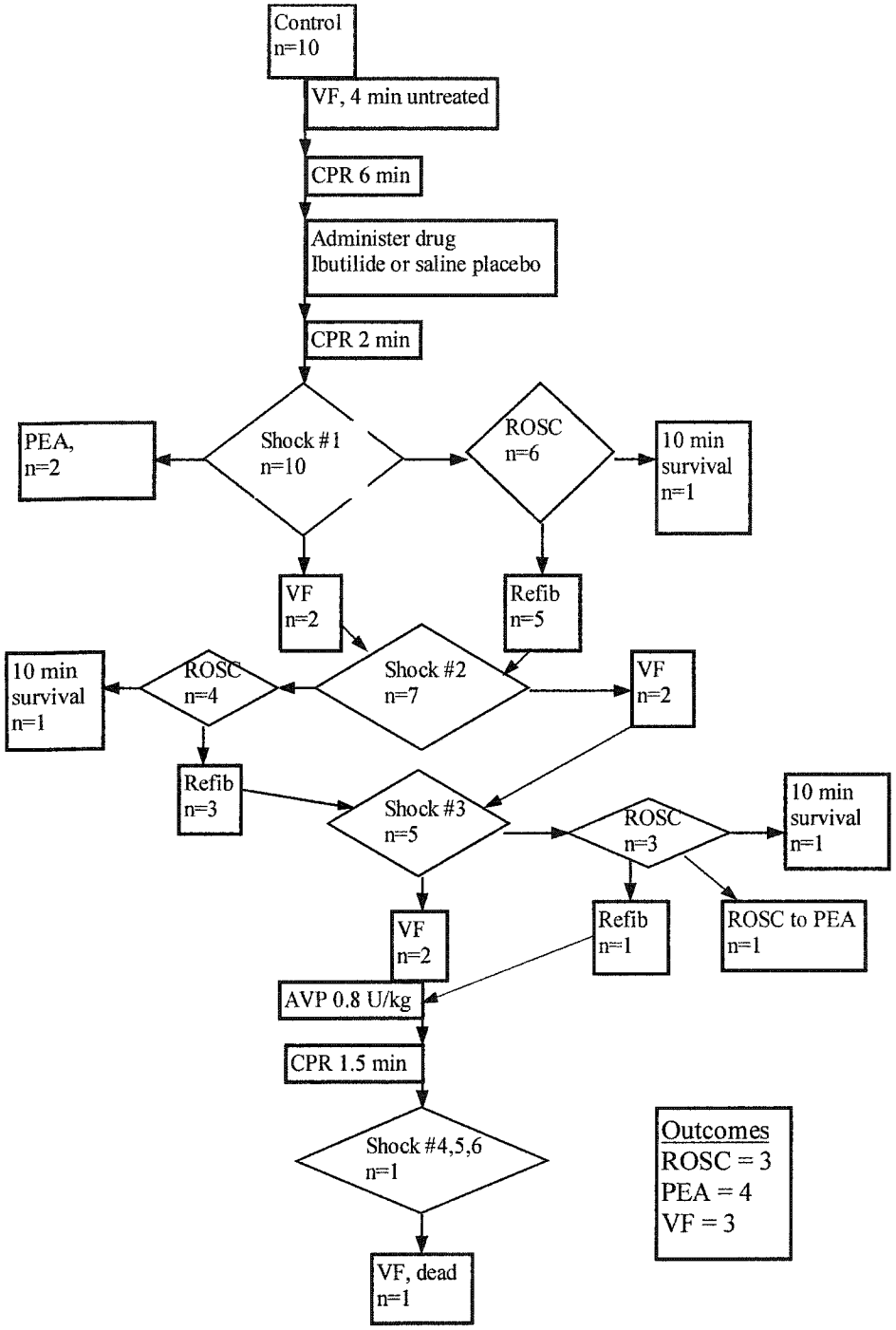

Figure VII - Flowchart detailing experimental timeline and outcomes for control animals in ITV study. VF=ventricular fibrillation, PEA=pulseless electrical activity, NSR=normal sinus rhythm, ROSC=return of spontaneous circulation, refib=refibrillation after initial successful defibrillation, AVP=arginine vasopressin.

Experimental Flow—All Animals Treated With the ITV

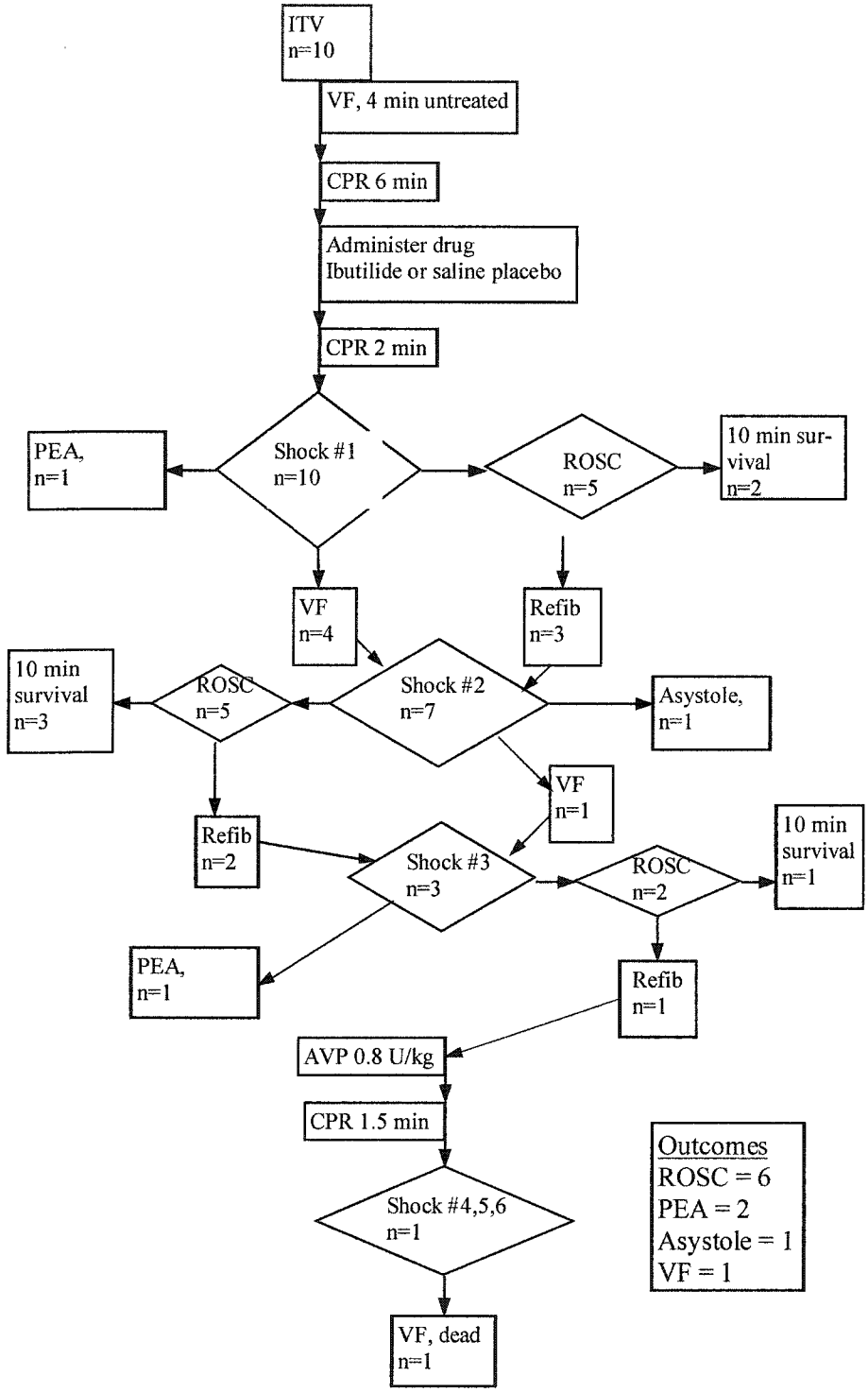

Figure VIII - Flowchart detailing experimental timeline and outcomes for treated animals in ITV study. VF=ventricular fibrillation, PEA=pulseless electrical activity, NSR=normal sinus rhythm, ROSC=return of spontaneous circulation, refib=refibrillation after initial successful defibrillation, AVP=arginine vasopressin.

Experimental Flow—No Ibutilide, No ITV

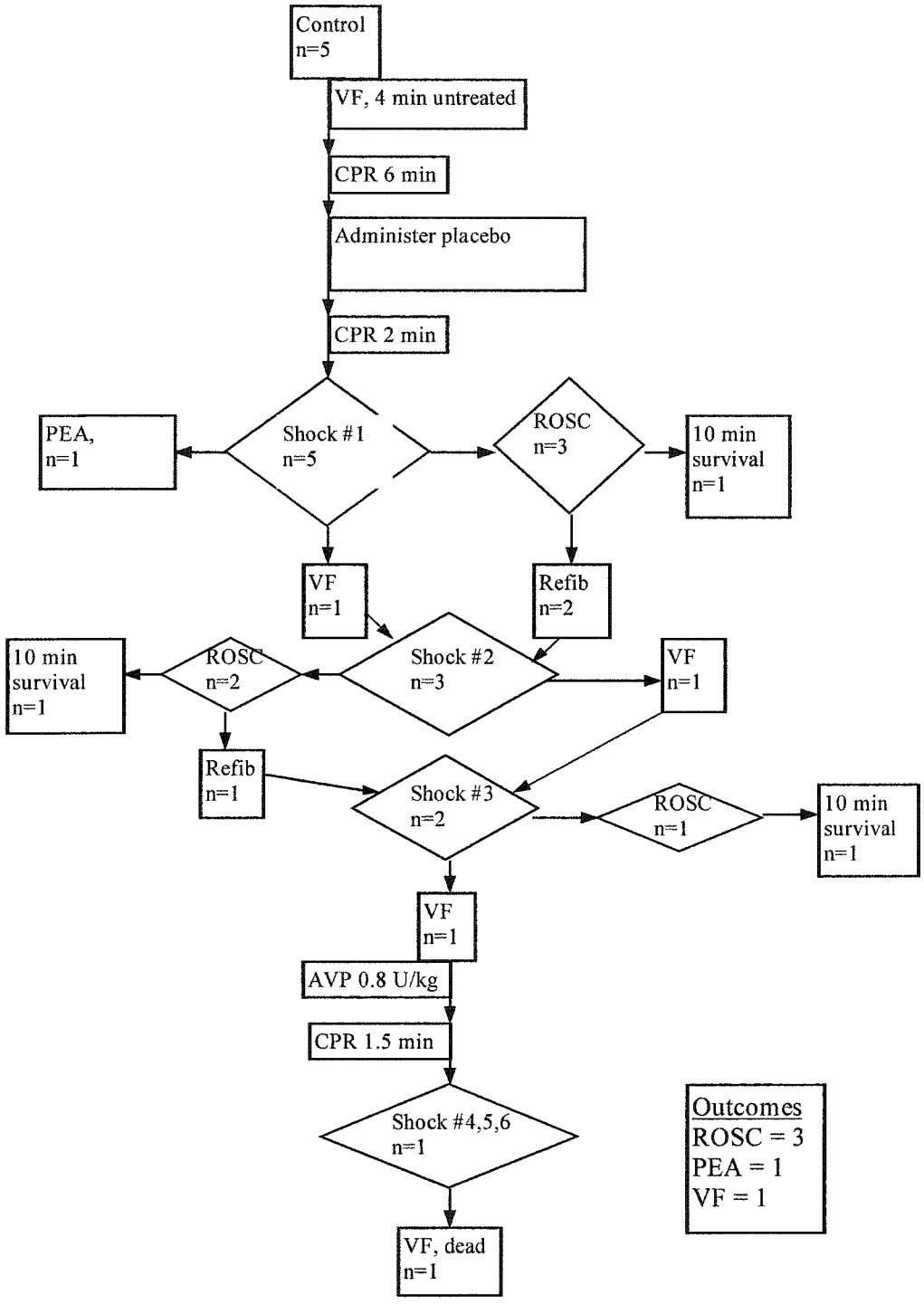

Figure IX - Flowchart detailing experimental timeline and outcomes for control animals in combined Ibutilide and ITV study. VF=ventricular fibrillation, PEA=pulseless electrical activity, NSR=normal sinus rhythm, ROSC=return of spontaneous circulation, refib=refibrillation after initial successful defibrillation, AVP=arginine vasopressin.

Experimental Flow—Ibutilide Only No ITV

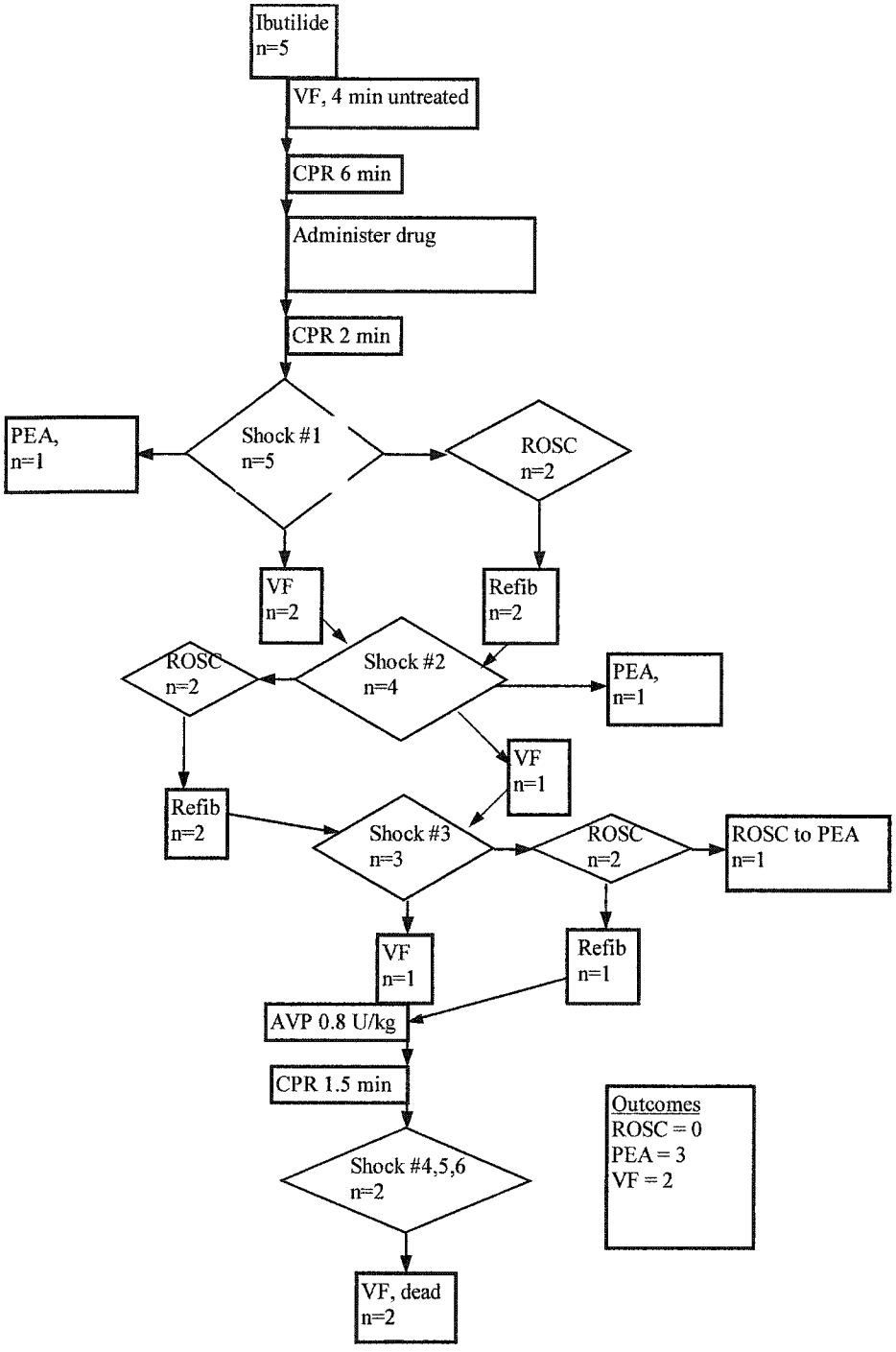

Figure X - Flowchart detailing experimental timeline and outcomes for Ibutilide only treated animals in combined Ibutilide and ITV study. VF=ventricular fibrillation, PEA=pulseless electrical activity, NSR=normal sinus rhythm, ROSC=return of spontaneous circulation, refib=refibrillation after initial successful defibrillation, AVP=arginine vasopressin.

Experimental Flow—ITV Only No Ibutilide

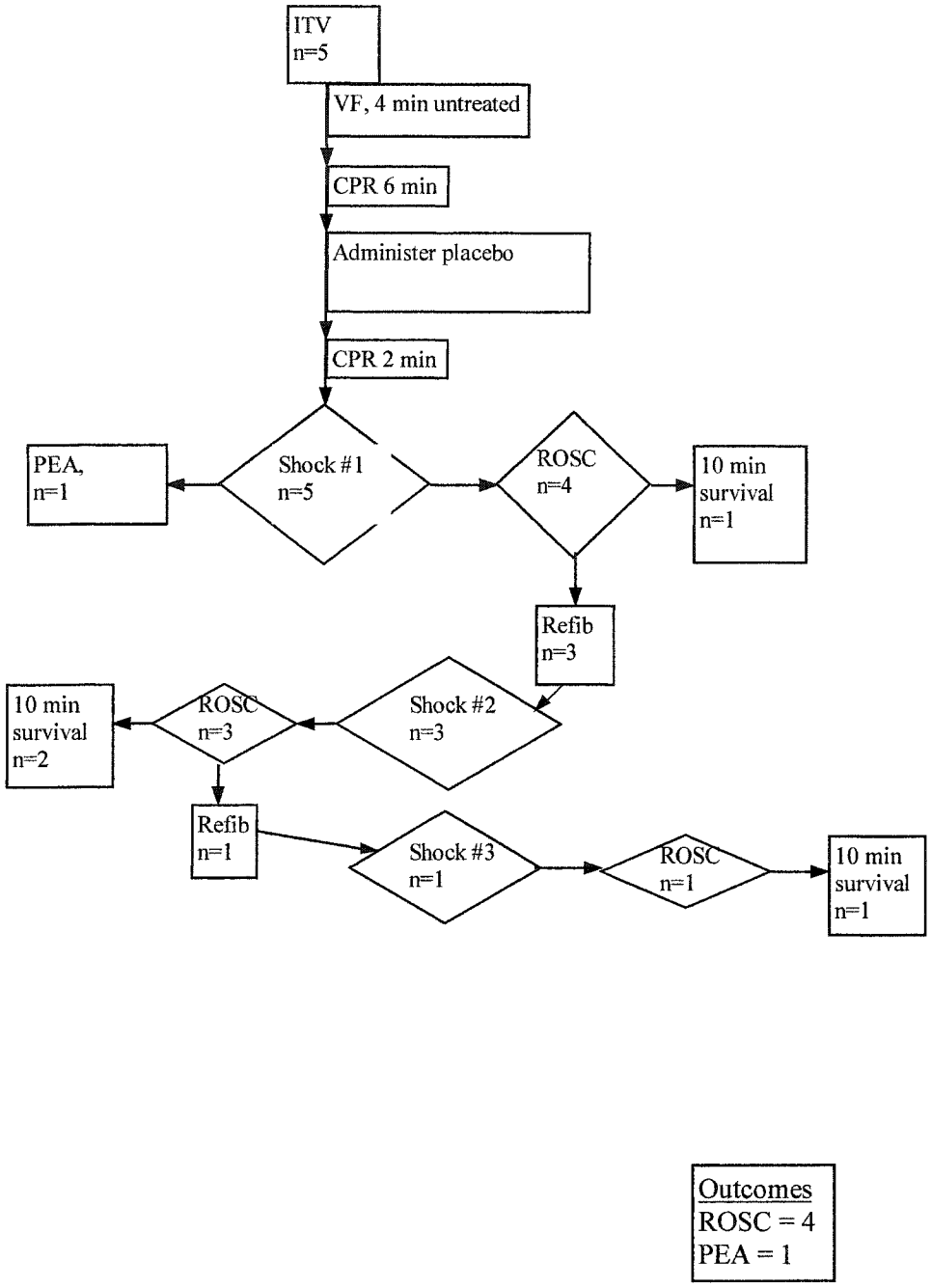

Figure XI - Flowchart detailing experimental timeline and outcomes for ITV only treated animals in combined Ibutilide and ITV study. VF=ventricular fibrillation, PEA=pulseless electrical activity, NSR=normal sinus rhythm, ROSC=return of spontaneous circulation, refib=refibrillation after initial successful defibrillation, AVP=arginine vasopressin.

Experimental Flow—ITV and Ibutilide

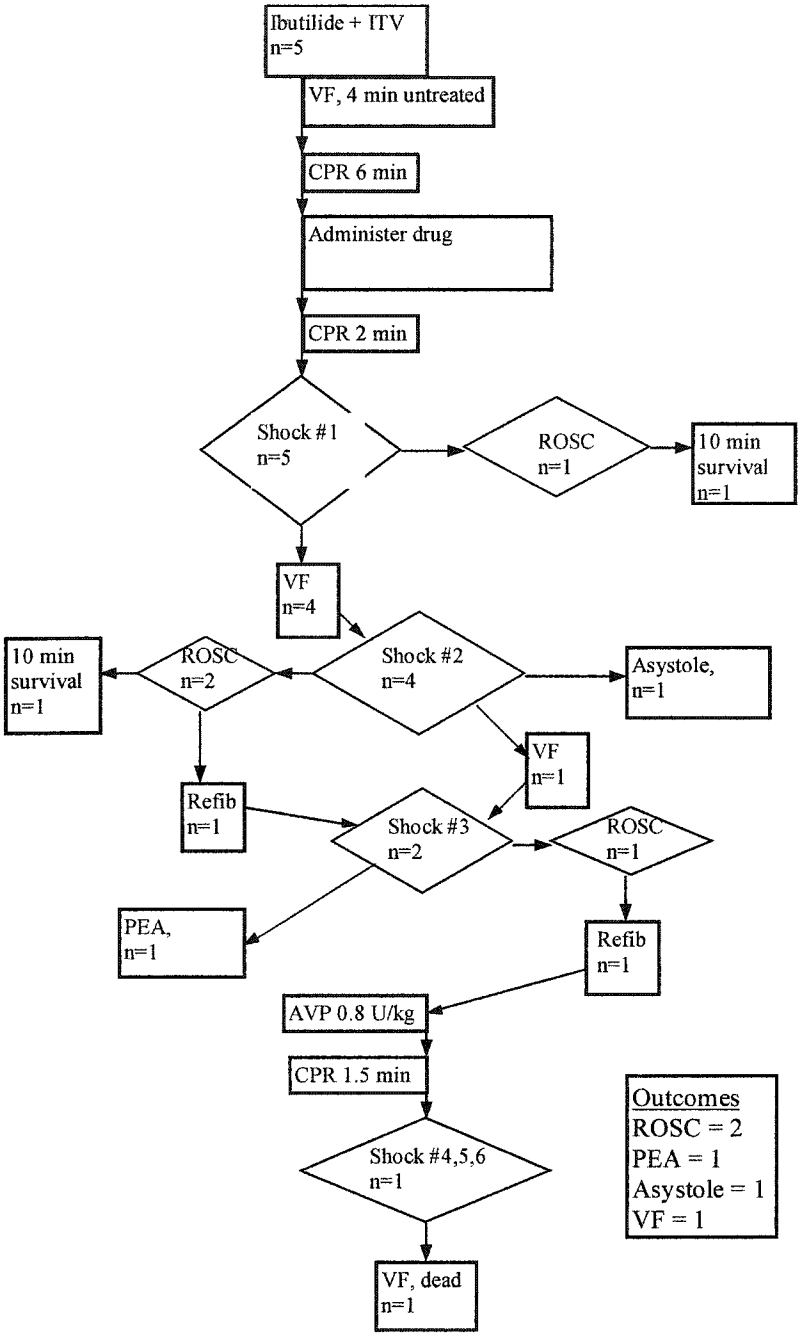

Figure XII - Flowchart detailing experimental timeline and outcomes for Ibutilide ITV only treated animals in combined Ibutilide and ITV study. VF=ventricular fibrillation, PEA=pulseless electrical activity, NSR=normal sinus rhythm, ROSC=return of spontaneous circulation, refib=refibrillation after initial successful defibrillation, AVP=arginine vasopressin.

DISCUSSION

IBUTILIDE STUDY

Principal Findings

The principal finding of this study was that ibutilide showed no class III antiarrhythmic action in pigs, despite the fact that it shows strong class III action in humans[102]. Ibutilide displayed none of the effects we expected; it did not prolong the action potential duration during normal sinus rhythm, did not lower the defibrillation threshold (in fact it increased the defibrillation threshold), did not increase the ventricular fibrillation cycle length (VFCL), did not increase cardiac arrest survival. These observations appear to be species-dependant. Ibutilide has been tested in other animal species, including dogs, rabbits, guinea pigs, as well as humans. Ibutilide has been shown to prolong refractory periods in all these species[101-106], but does not appear to consistently prolong action potential duration or effective refractory periods in pigs, even at high doses. It must be conceded that there are many potential causes for these observations: that the ibutilide dose at which this study was conducted was insufficient to produce a prolongation of APD, that ibutilide did produce a small, but significant prolongation of APD that this study was not adequately powered to detect, or that ibutilide truly has no APD prolonging effects in pigs, due to either pharmacokinetic or pharmacodynamic factors. It should also be noted that the pharmacokinetics and pharmacodynamics of ibutilide on ventricular electrophysiologic function in pigs has not been studied to date. It is unknown how ibutilide distributes in pigs, or how it is cleared. It is possible that pigs are somehow able to clear ibutilide, be that through metabolism or excretion, before it is able to have an effect. It is also possible that the ion channels with which ibutilide must

interact in order to exert its electrophysiologic effects (I_{Kr} and others) are different enough in their structure or morphology that ibutilide is unable to act in the pig. Because of the unknown pharmacokinetics and pharmacodynamics of ibutilide in the pig, I believe that if this study were to be continued, or if follow-up work were to be performed, one of the first steps should be thorough dose-response profiling. It would be prudent to examine serum levels of ibutilide in the pig after various doses, and at various time points, in order to better understand how ibutilide distributes in the pig, and how quickly it is cleared. I believe it would also be appropriate to examine how these blood levels relate to measures of refractoriness, be that VERP or $MAPD_{90}$. If it were found that ibutilide is able to prolong refractoriness at some dose, then it might be appropriate to repeat the cardiac arrest study using a dose that is known to be effective in pigs.

I believe that it is unlikely that simply increasing the dose of ibutilide is likely to produce a prolongation of APD in pigs. Given that ibutilide given to humans at a dose of 0.01 mg/kg to a maximum dose of 1.0 mg prolonged VERP by approximately 16%[102], in ferret ventricular tissue ibutilide at a concentration of 1.0μM prolonged ERP by 20%[171], in adult male rabbits an ibutilide dose of 50 nmol/kg resulted in 47% prolongation of APD[172], and the epicardial administration of ibutilide to dogs at a does equivalent to 3.5 μg/kg produced ERP prolongation of approximately 20%[104]. Taken together, these results suggest that our administration of 0.005 mg/kg should have been sufficient to produce a prolongation of APD and ERP. Additionally, subsequent experiments were performed for this thesis using doses of 0.01 mg/kg, and 0.03 mg/kg. Neither of these doses, which are all equivalent to, or higher than those noted above to prolong ERP

and/or APD were not found to prolong either APD or ERP in pigs (data presented above 13 on page 42).

Strengths and Weaknesses of the Study

The greatest weakness of this study appears to be the choice of experimental model. As mentioned above in the section detailing the experimental model, we did believe that pigs were an appropriate model for this study. In the previous studies (see appendix 1 & 2) the drug itself, be it HMR 1556 or amiodarone, was not the real source of the problem, but rather the diluent needed to dissolve these two water-insoluble drugs. Additionally, dofetilide is a highly selective blocker of the I_{Kr} channel[23]. In the case of this experiment, ibutilide is water soluble, and it has a novel mechanism of action. Ibutilide is believed to both block the I_{Kr} channel, and activate a slow, inward sodium current during the plateau phase of the action potential[101-106]. Even if pigs were assumed to respond uniquely to I_{Kr} block as was seen with dofetilide (see appendix 2), it was reasonable to assume that because of its dual action, ibutilide would still be expected to display some APD- and ERP-prolonging effects

Some of the more recent studies examining the mechanism by which ibutilide exerts its class III antiarrhythmic effects suggest that the activation of the slow, inward sodium current by ibutilide may not be as important as its I_{Kr}-blocking effects[161,169]. Data collected by us and presented in appendix 2 suggests that I_{Kr} block by dofetilide in the pig ventricle does not produce a dose-dependent prolongation of action potential duration. If this observed lack of prolongation of refractoriness in the pig ventricle is not limited to dofetilide, but rather is generalized to all I_{Kr}-blocking drugs, and ibutilide is primarily an I_{Kr} blocker, this could explain the lack of effect with ibutilide observed here.

Unfortunately this question is very difficult to resolve, both because the true mechanism of action of ibutilide is not fully understood, and the presence and activity of the ion channels in porcine ventricular myocytes has not been characterized.

It should also be noted that the group sizes used in this study were relatively small. Consequently, it is possible that small, but real differences were not detected in these experiments. At its inception, this study was designed and powered to detect a difference in survival between control animals and ibutilide-treated animals. It was predicted that approximately 40% of control animals would survive, and approximately 80% of ibutilide-treated animals would survive. Based on calculations using a 2-sample binomial distribution, it was found that in order to achieve 95% confidence with an 80% power to detect a difference between control and ibutilide-treated animals, groups of 20 animals were required (see Statistical Methods above). During the course of the study, after 10 animals had been randomized into each group, an interim analysis was performed. Upon analysis of the data presented above, that survival in the control group was approximately 70%, while survival in the ibutilide-treated group was only 20%. By simply looking at the raw data, it is apparent that ibutilide did not improve short-term cardiac arrest survival. Additionally, given that survival in the control group was 70%, even if the all the ibutilide-treated animals initially required for the study were to survive, and all the remaining control animals were to die, we would end up with 35% survival in the control group and 60% survival in the treated group. This would be insufficient to demonstrate a significant effect of ibutilide on survival. In fact, given that the interim data showed that the survival rate in the ibutilide-treated group is only 29% of that observed in the control group, that there are ethical considerations that must be addressed

before continuing to sacrifice animals in a study showing poorer survival with a therapy designed to improve survival.

At this point it was decided that it was appropriate to terminate the study. It must be conceded that as a consequence of terminating the study prematurely, small but real differences may have been rendered undetectable, but that continuing a study that was extremely unlikely to show a significant improvement with ibutilide as compared to control was not worthwhile, especially given that we were unable to demonstrate that ibutilide had any ability to prolong refractoriness in normal sinus rhythm.

Strengths & Weaknesses in Relation to Other Studies

The chosen anaesthetic regimen may have played a role in observed lack of response to ibutilide. Isoflurane has been shown to depress the ventricular L-type calcium channel in isolated guinea pig ventricular myocytes[150]. Lee and Lee proposed that the slow inward sodium current enhanced by ibutilide is carried through the L-type calcium channel[151], so presumably it might be possible for isoflurane to interfere with the APD and ERP prolonging effects of ibutilide. I believe that this is unlikely, since Camara et al. showed that ibutilide has a Kd of 0.71 ± 0.02 mM for the depression of the L-type calcium current, and that this dose showed a hyperpolarizing shift in steady state inactivation[150]. This would suggest that very high levels of isoflurane would need to be present in the ventricle to generate this effect, and that the effect would tend to favour the inward passage of positive ions, thus increasing rather than decreasing the effect of ibutilide.

Unanswered Questions & Future Research

The results from this study demonstrate clearly that if pigs are to be used for antiarrhythmic drug research that their cellular electrophysiology must be examined in detail. Clearly there are differences in the way various species respond to the same drug. Varying methods of anaesthesia do not appear to be sufficient to explain the lack of response to ibutilide observed in this study. Since anaesthetized pigs are the standard model for CPR research[73,74,87,89,93,96] it would be prudent to examine the cardiac physiology of the pig in greater detail before accepting results obtained from pig models of CPR. Granted, the pig heart is anatomically very similar to the human heart[114-116], but this study clearly demonstrates that other cardiac studies in pigs whose basis is anything other than purely anatomical may be seriously flawed, simply through the choice of a pig model.

ITV STUDY

Principal Findings

The principal finding of this study was that the impedance threshold valve did not have any significant impact on any variables measured during CPR, nor did it have any significant impact on survival, or any variables measured post-resuscitation. This is in stark contrast to several previous studies investigating the impedance threshold valve for use during CPR. Previous studies have demonstrated that the ITV can significantly improve hemodynamics during CPR, cardiac arrest survival, and post-resuscitation hemodynamic and neurological outcomes[73,74,87,89,93,96].

Strengths and Weaknesses of the study

The ITV functions by generating greater negative pressures within the thorax during chest compressions[74]. In order for this to be possible several things must be accomplished. Firstly, the airway must be completely controlled. If any of the connections between the ventilation apparatus and the cuff of the endotracheal tube fail to seal completely the ITV will not have any effect. Secondly, it must be noted that the decreased intrathoracic pressure developed by the ITV is not developed instantaneously, but rather is developed slowly with each successive compression, and reaches a plateau at approximately -15 cmH_2O[73-75]. Therefore it is important that sufficient time be allowed between ventilations for the valve to generate its effects. Unfortunately we did not fully understand or appreciate the way in which the valve functioned in relation to the ventilatory cycle. In this study, because of the small number of people present in the lab during the experiments, ventilation was provided using the ventilator on the anaesthesia cart, using the same settings that were used during the preparatory phase of the

experiment rather than a bag-valve device, as would be used by paramedics. Thus the pigs were ventilated at a rate between 12 and 16 breaths per minute with a tidal volume equal to 10 ml/kg body weight, and the inspiration:expiration ratio (I:E ratio) was set to 1:2. The end result was that the pigs were being over-ventilated – not hyperventilated, but rather too great a portion of any given minute was spent ventilating the pigs for the ITV to be able to exert its beneficial effects.

It should also be noted that the group sizes used in this study were relatively small. Consequently, it is possible that small, but real differences were not detected in these experiments. Given that 30% of the control animals survived vs. 60% survival in the ITV-treated animals, it is conceivable that a significant difference in terms of survival could have been demonstrated had the study been taken to its conclusion. The primary goal of this study was to demonstrate that ibutilide was able to improve survival vs. control, with demonstration of any added benefit of the ITV being a secondary objective. The possibility of demonstrating that the ITV can improve cardiac arrest survival was not compelling enough reason to continue the study in light of the unfavourable results found in the ibutilide study, especially given that previous studies by other investigators have already demonstrated that the ITV offers a survival benefit when used during CPR.

Strengths & Weaknesses in Relation to Other Studies

Unlike the other studies presented in this thesis, uncontrollable variable introduced as a result of porcine physiology is not the major cause of the lack of positive results. Pigs were and are used as the laboratory model of choice for studying the ITV. The apparent reason for these results is our ventilation technique. Having visited the Lurie laboratory, and having had the opportunity to see how similar experiments are

performed in their lab, I know firsthand that a positive pressure demand valve is used for ventilation during CPR in their studies. Since we had no such device here, and lacked enough personnel to have someone operate a bag-valve device during CPR we opted to use the surgical ventilator during CPR.

This indicates that ventilatory strategy has a profound impact on the function of the ITV. No previous study has examined how the ITV functions in conjunction with mechanical ventilation. In one previous study Lurie et al. report considering mechanical ventilation, but opted for manual ventilation primarily because it was easier to interpose ventilations with the decompression phase of chest compressions[74]. To date no study has examined how the ITV functions when ventilations are not interposed with chest compressions. This study demonstrates that in order for the ITV to function properly that ventilations must be interposed with chest compressions; that ventilations must be relatively short in duration, no longer than the decompression interval between 2 adjacent compressions; and that ventilations must be relatively infrequent when compared to the frequency of chest compressions. Unpublished results from this lab obtained very recently demonstrates that the ITV can generate negative intrathoracic pressures and improve blood pressures during CPR when ventilation is performed using a bag-valve device at a compression:ventilation ratio of 5:1. We have also found that the intrathoracic pressure can be made more negative, and blood pressures during CPR increased further by altering the compression:ventilation ratio to 15:1.

Implications for Clinicians & Policymakers

At first glance these results may not appear to have much clinical relevance, but I believe that if the ITV were to become routinely used during resuscitative attempts that these results could become important. These results, take in conjunction with previous results from Lurie et al. [73,74,87,89,93,96] demonstrate clearly that exactly how a patient is ventilated has profound impact on the ability of the valve to function. The ITV requires relatively long periods of uninterrupted chest compressions between short ventilations, such as are developed by the use of a bag-valve device in order to function properly. Without several compressions between ventilations, or in the presence of extended ventilations, such as are provided by automatic ventilators, insufficient time exists for the ITV to be able to develop sufficient negative intrathoracic pressure to have any impact on hemodynamics during CPR. This may mean that any potential benefit offered by the ITV could be completely negated by automatic ventilators.

Unanswered Questions & Future Research

Based on the preponderance of evidence in the literature I believe that the ITV is a device that may prove to be very beneficial in the treatment of cardiac arrest. Given the results of this study I believe it is important that the limitations of the ITV be examined closely. Current studies underway in our lab have shown that using a bag-valve device for ventilation during chest compressions does cause greater negative intrathoracic pressures (unpublished preliminary data). This would suggest that there exists a threshold in ventilation above which the valve does not function. It would be useful to determine this threshold, as well as to determine if continuing to decrease ventilation will further improve the function of the ITV. If decreasing ventilation improves the function of the ITV it would also become important to determine at what point the patient begins to

suffer due to inadequate ventilation. This way ventilatory strategies can be developed which optimize the function of the ITV without compromising the ventilatory needs of the patient.

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Appendices

Appendix 1: Additional Data

Amiodarone for the Treatment of Cardiac Arrest

As mentioned in the body of the thesis, amiodarone was also investigated as a first line antiarrhythmic for the treatment of cardiac arrest. Tween 80, the diluent in commercially available amiodarone, was found to cause profound hypotension in pigs similar to prior observations in dogs[144]. This section contains the results obtained from this study prior to its being terminated, and a discussion of those results.

EXPERIMENTAL DESIGN, PROCEDURE, AND METHODS OF APPROACH

The same methods were employed in this study as are detailed in the body of the thesis with the exception of the administration of the study drug. Below are the details pertaining to the administration of amiodarone as the study drug.

Study Drug Administration, Amiodarone:

The pigs were assigned randomly to receive amiodarone (fig. III), or saline placebo in both the ACD CPR and ACD CPR plus the ITV groups. 10 min. after the start of VF (4 min VF with no intervention followed by 6 min CPR, 2 min prior to 1st defibrillation shock), either 5 mg/kg amiodarone, or vehicle was administered by IV bolus through the venous catheter, followed by a 10 mL normal saline flush. The dose of amiodarone (5 mg/kg) is based on previous studies performed in dogs[121,123], in which it was reported that this singe dose had the most pronounced effects on QT intervals and ventricular effective refractory periods[121,123].

RESULTS

Prior to commencing the experiments to evaluate the usefulness of amiodarone as a first line antiarrhythmic for the treatment of cardiac arrest preliminary studies were undertaken to evaluate the stability of the pig model of cardiopulmonary resuscitation (CPR). Given that there was no literature describing the behaviour of amiodarone in pigs experiments were also undertaken to ensure that the effects of amiodarone on pigs would be comparable to the effects of amiodarone on humans.

The stability of the model was verified on 4 pigs. All 4 were defibrillated to pulsatile rhythms, and 3 of the 4 had femoral blood pressures exceeding 90 mmHg systolic within 2 minutes of return of spontaneous circulation (ROSC), see table **13**.

Once it had been determined that the pig model of CPR was stable and could be successfully defibrillated the effects of amiodarone in this model were tested for feasibility. Amiodarone was administered to 2 pigs during CPR 2 minutes prior to the first defibrillation shock. The first animal required a total of 7 defibrillation shocks ranging from 200 J biphasic to 360 J biphasic, only the last of which was successful. The 7^{th} shock terminated ventricular fibrillation and converted the pig directly to a pulseless electrical rhythm indicative of electrical-mechanical dissociation (PEA). The second pig required a total of 8 shocks ranging from 150 J to 360 J biphasic, all of which were successful. This pig temporarily achieved ROSC with a mean arterial pressure of 22 mmHg but refibrillated within 2 minutes. Each successive shock resulted in shorter and shorter intervals of ROSC were achieved, followed by episodes of refibrillation. The 8^{th} shock resulted in PEA.

At this point we theorized that the pigs were hypersensitive to the diluent, Tween 80, that commercially available intravenous amiodarone is dissolved in – an agent which is known to cause vasodilatation and decreased blood pressure. It was observed in a study by Grund et al. that the reduction in coronary flow, blood pressure, left ventricular pressure and stroke volume in response to tween 80 was confined mainly to a first injection of tween 80. If subsequent injections were made, the effects on cardiac parameters was much less pronounced[143]. Based on this observation 2 further pigs were treated with a modified protocol. In an effort to 'desensitize' the pigs to the hypotensive effects of tween 80: the 150 mg dose of amiodarone was split into 2 doses of 75 mg, 1 of which was administered approximately 2 hours prior to the induction of ventricular fibrillation, and the other was administered during CPR 2 minutes prior to the first defibrillation shock. The first of these two animals received a single shock of 150 J biphasic and achieved ROSC with a mean femoral blood pressure of 23 mmHg, which degenerated into PEA within 2 minutes. The second pig also received a single shock of 150 J biphasic and was immediately converted to PEA.

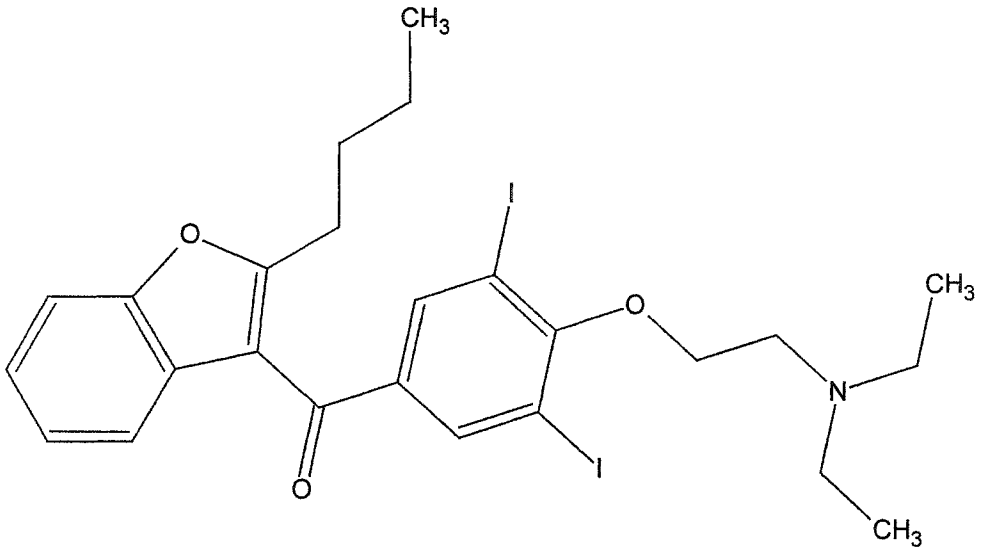
One further experiment was performed to verify that the hypotensive effects observed in pigs receiving amiodarone were the result of tween 80 one pig was administered 300 mg of tween 80 during CPR. This dose is equal to the amount of tween 80 in a 150 mg dose of amiodarone. This pig received a single shock of 150 J biphasic and achieved ROSC with a mean femoral arterial pressure of 15 mmHg, which degenerated to PEA within 1 minute.

There was no statistically significant difference between the control animals and those treated with amiodarone at baseline, See table **14**. Values recorded during CPR also show no statistically significant difference between the groups, leading us to believe that the two groups received comparable CPR, see table **15**. Post defibrillation it is readily apparent that the blood pressure in all animals treated with amiodarone is too low to sustain life, see table **16**.

Based on these observations it was determined that commercially available amiodarone formulations which include tween 80, when administered to pigs, results in blood pressures too low to sustain life. Based on this it was decided that amiodarone could not be properly evaluated in pigs and a water-soluble antiarrhythmic should be selected for investigation.

Tables and Figures

The Chemical Structure of Amiodarone



HCl

Figure XIII. The chemical structure of amiodarone HCl

Tables and Figures

Feasibility of a Pig Model of CPR.

	Amio dose	sys. BP	dias. BP	HR	MAPD90	DFT	refib?	# of shocks	Outcome
	mg/kg	mmHg	mmHg	BPM	msec	J			
PIG #									
1.0	control	98	56	117	217.4	150	0	1	ROSC temp
2.0	control	94	62	101	286.8	150	0	1	ROSC
3.0	control	81	57	130	256.6	360	0	8	ROSC
4.0	control	137	89	103	274.8	300	0	3	ROSC
mean		102.5	66.0	112.8	258.9	240.0	0.0	3.3	
SD		24.1	15.6	13.5	30.3	106.8	0.0	3.3	
1.0	control	26	21	67	176.4	150	0	1	ROSC temp
2.0	control	178	157	115	261.0	150	0	1	ROSC
3.0	control	92	76	228	noisy	360	0	8	ROSC
4.0	control	117	72	120	256.4	300	0	3	ROSC
mean		103.3	81.5	132.6	231.3	240.0	0.0	3.3	
SD		62.9	56.2	68.3	47.6	106.8	0.0	3.3	
p values		0.983	0.614	0.590	0.387	1.000		1.000	

Table 13 – Results from 4 pigs used to verify that a pig model of CPR was feasible in our laboratory. The top portion of the table contains results prior to VF, and the bottom portion contains results from the same animals post defibrillation. These results demonstrate that the pig model of CPR is feasible and stable in our laboratory. Amio=Amiodarone, sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, HR=heart rate, MAPD90=monophasic action potential at 90% repolarization measured during pacing at 350 ms cycle length, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation.

Baseline Data from Amiodarone Experiments.

	Amio dose	sys. BP	dias. BP	HR	MAPD90	DFT	refib?	# of shocks	Outcome
	mg/kg	mmHg	mmHg	BPM	msec	J			
PIG #									
1.0	control	98	56	117	217.4	150	0	1	ROSC temp
2.0	control	94	62	101	286.8	150	0	1	ROSC
3.0	control	81	57	130	256.6	360	0	8	ROSC
4.0	control	137	89	103	274.8	300	0	3	ROSC
mean		102.5	66.0	112.8	258.9	240.0	0.0	3.3	
SD		24.1	15.6	13.5	30.3	106.8	0.0	3.3	
5.0	5 mg/kg	98	76	76	358.6	250	4	8	PEA
6.0	5 mg/kg	106	78	110	327.2	150	6	8	PEA
7.0	5 mg/kg split	105	78	106	261.0	150	0	1	ROSC low BP
8.0	5 mg/kg split	102	73	108	314.8	250	0	2	ROSC low BP
9.0	Tween 80 300 mg	92	72	104	n/a	150	0	1	ROSC low BP
mean		100.6	75.4	100.8	315.4	190.0	2.0	4.0	
SD		5.7	2.8	14.0	40.7	54.8	2.8	3.7	
p values		0.803	0.220	0.238	0.065	0.389		0.760	

Table 14 – Baseline data from preliminary amiodarone studies. Prior to the induction of VF there was no significant difference between those pigs used as control animals (top portion of table) and those treated with amiodarone (bottom portion of table). Amio=amiodarone, sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, LVP sys=systolic left ventricular pressure, LVP dias=diastolic left ventricular pressure, HR=heart rate, MAPD90=monophasic action potential at 90% repolarization, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation.

Data Collected During CPR – Amiodarone Experiments.

	Amio dose	sys. BP	dias. BP	comp rate	VFCL	VF amp	DFT	refib	# of shocks	Outcome
	mg/kg	mmHg	mmHg	comp/min	msec	mV	J			
PIG #										
1.0	control	65	42	80	62.8	0.742	150	0	1	ROSC temp
2.0	control	79	38	80	76.6	0.512	150	0	1	ROSC
3.0	control	66	31	80	93.5	0.763	360	0	8	ROSC
4.0	control	58	23	80	81.2	0.807	300	0	3	ROSC
mean		67.0	33.5	80.0	78.5	0.706	240.0	0.0	3.3	
SD		8.8	8.3	0.0	12.7	0.132	106.8	0.0	3.3	
5.0	5 mg/kg	62	43	80	71.6	0.603	250	4	8	asystole
6.0	5 mg/kg	62	28	80	70.2	0.637	150	6	8	asystole
7.0	5 mg/kg split	68	32	80	81.9	0.520	150	0	1	ROSC low BP
8.0	5 mg/kg split	46	24	80	78.2	0.537	250	0	2	ROSC low BP
9.0	Tween 80 300 mg	53	22	80	n/a	n/a	150	0	1	ROSC low BP
mean		58.2	29.8	80.0	75.5	0.574	190.0	2.0	4.0	
SD		8.7	8.3	0.0	5.5	0.055	54.8	2.8	3.7	
p values		0.176	0.529		0.681	0.115	0.389		0.760	

Table 15 – There was no statistically significant difference in the variables recorded during CPR between the control group and the group treated with amiodarone. This eliminates inadequate CPR as an explanation for the differences observed in outcome and variables recorded post-defibrillation. Amio=amiodarone sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, LVP sys=systolic left ventricular pressure, LVP dias=diastolic left ventricular pressure, comp rate=compression rate, VFCL= ventricular fibrillation cycle length, VF amp=ventricular fibrillation amplitude, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation.

Data Collected Post Defibrillation – Amiodarone Study.

	Amio dose	sys. BP	dias. BP	HR	MAPD90	DFT	refib	# of shocks	Outcome
	mg/kg	mmHg	mmHg	BPM	msec	J			
PIG #									
1.0	0.0	26	21	67	176.4	150	0	1	ROSC temp
2.0	0.0	178	157	115	261.0	150	0	1	ROSC
3.0	0.0	92	76	228	N/A	360	0	8	ROSC
4.0	0.0	117	72	120	256.4	300	0	3	ROSC
mean		103.3	81.5	132.6	231.3	240.0	0.0	3.3	
SD		62.9	56.2	68.3	47.6	106.8	0.0	3.3	
5.0	5 mg/kg	17	17	n/a	N/A	250	4	8	asystole
6.0	5 mg/kg	21	21	n/a	N/A	150	6	8	asystole
7.0	5 mg/kg split	25	23	36	272.0	150	0	1	ROSC low BP
8.0	5 mg/kg split	24	22	37	209.0	250	0	2	ROSC low BP
9.0	Tween 80 300 mg	16	15	37	N/A	150	0	1	ROSC low BP
mean		20.6	19.6	36.7	240.5	190.0	2.0	4.0	
SD		4.0	3.4	0.3	44.5	54.8	2.8	3.7	
p values		0.020	0.041	0.065	0.838	0.389		0.760	

Table 16 – Post defibrillation the blood pressure was significantly lower in the group treated with amiodarone. It can also be seen that the blood pressures recorded from all animals in the amiodarone treated group were too low to sustain life, even though the heart was still beating in some of these animals. Amio=amiodarone, sys. BP=systolic blood pressure, dias. BP=diastolic blood pressure, LVP sys=systolic left ventricular pressure, LVP dias=diastolic left ventricular pressure, HR=heart rate, MAPD90=monophasic action potential at 90% repolarization, DFT=defibrillation threshold, refib=number of episodes of refibrillation after initial defibrillation.

DISCUSSION

Principal Findings

The principal finding of this study was that Tween 80, the diluent in commercially available intravenous amiodarone, causes vasodilatation and extreme hypotension in pigs. This did not appear to affect our ability to defibrillate the animals, pigs treated with amiodarone were readily defibrillated, but the systolic blood pressure post-defibrillation was consistently below 25 mmHg.

Strengths and Weaknesses of the Study

This study was designed to investigate the utility of amiodarone as a first line antiarrhythmic drug for use during CPR. In order to answer the questions asked in this study a model must be chosen which is appropriate for both experimental CPR and examination of the study drug. Pigs are the standard model used for experimental CPR [ex. 71-74], but are a novel model for antiarrhythmic drug research. As noted in the previous section on the combination potassium channel block study, the cellular electrophysiology of pigs has not been explored. Nonetheless pigs were chosen as an appropriate model for this study.

Methods for performing CPR on pigs are well established, and hemodynamic results during CPR can readily be compared with those obtained by other groups doing CPR research. Additionally we perceived that the major problem with the combination potassium channel block study, and the prime reason for having abandoned it, was the profound ERP shortening observed with the administration of the DMSO/PEG diluent necessary for the administration of the I_{Ks} blocker HRM 1556. We believed, perhaps

naively, that pigs would respond to antiarrhythmic drugs as humans do. Furthermore, the antiarrhythmic effects of amiodarone are not the source of the problems in this study, but rather the tween 80 diluent necessary for the IV administration of amiodarone.

Strengths & Weaknesses in Relation to Other Studies

A previous study suggested that the administration of tween 80 to pigs was relatively benign. Pigs were given 6 ml of a 0.02% tween 80 solution. This resulted in a brief (0,5 to 3 min) reduction in coronary flow, and minor decreases in left ventricular pressure, left ventricular dP/dt, aortic pressure, and stroke volume, as well as a minor increase in heart rate[143]. However, commercially available amiodarone contains 10% tween 80, so the administration of 150 mg (3 ml) of amiodarone contains 250 times more tween 80 than was administered in the Grund study referred to above.

The profound drop in blood pressure observed in this study is not unique. The administration of tween 80 to dogs at a dose of 10 mg/kg over 5 minutes caused a 60% decrease in left ventricle dP/dt and a 60 % drop in blood pressure lasting for 30 minutes or longer. Similar effects were observed with the administration of commercially available intravenous amiodarone. This observation prompted the conclusion that tween 80 is not an inert substance, but rather is a potent cardiac depressant and the major cause of the severe hypotension observed with the administration of intravenous amiodarone[144]. These results support the observations from this study, and the hypothesis that the effects of tween 80 are species-specific since human studies have shown that tween 80 has only mild negative inotropic effects on humans, and is mostly associated with vasodilation[145-147].

The vasodilatation observed with the administration of amiodarone can be explained by the histamine-releasing properties of tween 80. Histamine is a paracrine hormone secreted by basophils and mast cells that acts as a vasodilator and has been shown to be released after the intravenous administration of many drugs in animals and humans[148,149]. Miaini et al. demonstrated that the administration of tween 80 to dogs increases histamine release, and that both systolic and diastolic blood pressures drop in response to this histamine release, and return to baseline along with the histamine plasma clearance[149]. The same study demonstrated that tween 80 administered to rat mast cells resulted in a histamine release. Together these results indicate that tween 80 acts as a histamine releaser, and that this histamine release is likely to be responsible for the hemodynamic changes observed with the administration of tween 80.

Unanswered Questions & Future Research

This study did not evaluate the histamine release associated with tween 80 or amiodarone administration in pigs. The severe hypotension and hemodynamic collapse observed in this study as a result of amiodarone administration was not anticipated. More work needs to be done in order to examine whether tween 80 administration in pigs triggers a histamine release, and if this histamine release is sufficient to explain the severe hypotension observed after the administration of amiodarone and tween 80. It might also be worthwhile to explore the possibility that the administration of a histamine receptor antagonist might be able to attenuate the hypotensive effects of amiodarone. If this hypotension could be lessened, or prevented perhaps it could be used as a protective therapy for patients in need of rapid infusion of intravenous amiodarone. This might have

the potential to further enhance the utility of amiodarone for the treatment of cardiac arrest in humans.

Given that the antiarrhythmic effects of amiodarone are not the source of the problems in this study, but rather the tween 80 diluent necessary for the IV administration of amiodarone is the source of problems we chose to continue our investigation of the usefulness of drugs with class III antiarrhythmic action during cardiac arrest using a water-soluble drug. Ibutilide was chosen for the continuation of this study because it is water soluble, and operated by a unique mechanism of action. Ibutilide appears to both block the rapidly activating component of the delayed rectifier potassium current (I_{Kr}), and enhance an inward sodium current during the action potential plateau phase[101-106]. This should mean that even if the results obtained using dofetilide in the earlier study detailed in this thesis are representative of the way pig react to selective I_{Kr} blockers in general, ibutilide should still be effective because of its unique mechanism of action.

Appendix 2: Other Experiments

Combination Potassium Channel Block and Reverse Rate-Dependence

A study was initiated to investigate the hypothesis that combining drugs in order to block both the I_{Kr} and I_{Ks} repolarizing potassium currents would result in greater prolongation of action potential duration at fast heart rates, and display less reverse rate-dependence than is observed with I_{Kr} block alone. This study was terminated due to physiological problems with the pig model in which the study was conducted. This section details the background information and results that were obtained from this study before it was abandoned.

INTRODUCTION

Drugs with class III action appear to be more effective than class I agents in treating re-entrant ventricular tachycardia[9]. Drugs with class III action are also much more effective in preventing the recurrence of atrial fibrillation[9,10] and in terminating atrial flutter[8]. The majority of available drugs with class III action are K^+ channel blockers. Cardiac myocytes possess many different K^+ channels that are possible targets for antiarrhythmic drugs. These include I_{K1}, the inward rectifier K^+ current; I_K, the delayer rectifier K^+ current; I_{to}, the transient outward K^+ current; and in atrial tissue I_{Kur}, the ultra-rapid delayed rectifier[3,8,11-13].The channels responsible for I_K are of particular interest with respect to arrhythmias and drugs with class III antiarrhythmic action.

In 1990 Sanguinetti and Jurkiewicz separated guinea pig ventricular I_K into two component currents based on different sensitivity to E-4031, a methanesulphonanilide with class III action, and on distinct kinetics for E-4031 sensitive and resistant

components[14]. The E-4031-sensitive component activated rapidly and displayed inward rectification positive to 0mV, and was termed I_{Kr}[14]. The E-4031-resistant component activated more slowly than I_{Kr} and did not attain a true steady-state level, and thus was termed I_{Ks}[14]. A range of other evidence points towards the existence of two delayed rectifier currents in not only guinea pig, but a range of other species including rabbits, dogs and humans[15-20].

The 'ideal' drug with class III antiarrhythmic action would be most effective at prolonging the ventricular APD at fast cycle lengths[2,3,21,22]. Unfortunately a number of agents with 'pure' class III effects, including d-sotalol, E-4031 and dofetilide, highly selective I_{Kr} blockers[14,23-27], show the opposite profile with respect to rate: APD prolongation is greatest at slower rates, rather than faster rates. This phenomenon is termed 'reverse rate-dependence' (RRD)[2,21,22,28]. RRD is of concern because the excessive APD prolongation generated at slow rates can lead to early after-depolarizations (EAD's). These cellular electrophysiological events[29-31] are believed to underlie *torsades des pointes* (TdP) polymorphic ventricular tachycardias. The proarrhythmic risk of 'pure' I_{Kr} blocking drugs was brought to light by the SWORD trial, which showed increased mortality due to presumed arrhythmic death in post-infarction patients taking d-sotalol[32]. Therefore it is clear that selective I_{Kr} blockade has potential to be both antiarrhythmic and proarrhythmic.

The exact basis of the observed phenomenon of reverse rate dependence is a topic still debated. Several different possible explanations have been suggested. The simplest of these explanations involves the idea that drugs bind to the I_{Kr} channel in the resting

state between action potentials and dissociate during membrane depolarizations[21,33]. This would result in lessening block as rate increases based on the shorter intervals between action potentials in which the drugs would have the opportunity to bind. Unfortunately several lines of evidence contradict this theory. Both dofetilide and almokalant are reported to block the I_{Kr} channel in the open state and possess slow kinetics of recovery from block[23,34].

Another theory is the so-called 'I_{Ks} accumulation' hypothesis. An important study with regards to the nature of reverse rate dependence demonstrated that dofetilide produced reverse-rate dependent effects on guinea pig ventricular action potentials, but produced rate-independent block of the I_{Kr} channel[24]. The same study observed that repetitive stimulation did not increase the magnitude of I_{Kr}, but did increase the magnitude of I_{Ks}. Jurkiewicz and Sanguinetti then proposed that reverse-rate dependence might be a product of the interaction between these two currents at different rates. At slower rates I_{Kr} might be the dominant repolarizing current, while at faster rates I_{Ks} might become dominant owing to its incomplete deactivation between successive action potentials, leading to the accumulation of I_{Ks}. Under this hypothesis selective I_{Kr} block would have a greater effect on action potential duration at slower rates versus faster rates. This theory also appears consistent the previous observation that isoprenaline antagonized the class III effect of E-4031 on the action potentials elicited from guinea pig myocytes, increasing the magnitude of I_{Ks}, but not affecting I_{Kr}[35]; as well as the later observation that the class III effect of I_{Kr} blockers is diminished under conditions of adrenergic drive[36-38]. This could also explain the observations that purkinje fibres and mid-myocardial cells appear more prone to rate dependant action potential prolongation,

since they have less I_{Ks}[39-42]. This mechanism would also suggest that drugs with class III action selective for I_{Ks}, or which block both I_K components would be expected to exhibit less reverse rate-dependence than pure I_{Kr} blockers. This is supported by the observation that the selective I_{Ks} blocker chromanol 293B is reported to prolong action potential duration independent of rate in human and guinea pig ventricular myocytes[43].

Although there are clear problems with this hypothesis, such as the observation that the channel kinetics of I_{Kr} and I_{Ks} are strikingly different in dogs as compared to guinea pigs such that in canine myocytes I_{Kr} is deactivated slowly while I_{Ks} deactivated rapidly, yet E-4031 still prolonged action potential duration in a reverse rate-dependent manner[44]. To further complicate matters, not only do the channel kinetics of I_{Kr} and I_{Ks} vary by species, but the relative concentrations of I_{Kr} and I_{Ks} vary between species as well. For example, very high levels of I_{Ks} are reported to be present in guinea pig ventricle while little to no functional I_{Ks} appears in rabbit ventricle, yet I_{Kr} blockers display reverse-rate dependence in both species[43,60-62]. This is clearly inconsistent with the I_{Ks} accumulation theory. Other studies showed that azimilide causes reverse rate-dependent APD prolongation in canine ventricular myocytes at drug concentrations that inhibit I_{Kr} and I_{Ks} to similar extents[45]. More recent studies have suggested that I_{Ks} may play little role in canine ventricular muscle or purkinje fibre action potentials under normal conditions[46]. These observations suggest that either the mechanism of reverse rate-dependence in dogs is different from that in guinea pigs, and/or that the 'I_{Ks} accumulation' theory provides, at best, a partial explanation for the phenomenon of reverse rate-dependence.

Despite this, the combination potassium channel block experiments described here are being pursued based on the 'I_{Ks} accumulation' theory. We believe that the I_{Ks} accumulation theory does offer at least a partial explanation for the observed phenomenon of reverse rate-dependence because of the observations that I_{Ks} blockers, and drugs which block both I_{Kr} and I_{Ks} display much less reverse rate dependence than I_{Kr} blockers alone[43,153]. Additionally, prior data from Fiset et al. indicate that the electrophysiologic effects of I_{Kr} blocker can be modulated by the addition of an I_{Ks} blocker[152]. Studies with azimilide, a single drug with class III action which blocks both the I_{Kr} and I_{Ks} channels, also indicate that the combined block of I_{Kr} and I_{Ks} is likely to display less reverse rate-dependence in than is present with an I_{Kr} blocker alone[153]. We believed that combining the I_{Kr} blocker dofetilide (fig. XIV) with the I_{Ks} blocker HMR 1556 (fig. XV) will produce APD prolongation equal to or greater than either drug alone in a manner with is less reverse rate-dependent than is seen with an I_{Kr} blocker alone.

Dofetilide [1-(4-methanesulfonamidophenoxy)2-[N-(4-methanesulfonamidophene thyl)-N- methylamine]ethane] is a relatively new antiarrhythmic agent first described in the early 1990's. The original papers described a compound that prolonged action potential duration in *in vitro* preparations of canine ventricular muscle and purkinje fibres, as well as guinea pig papillary muscle, in a dose-dependent manner, while resting membrane potential, amplitude and maximum upstroke velocity of action potentials were unaffected[47]. *In vivo* dofetilide is also described as a negative chronotrope and a positive inotrope[48]. This same paper also introduces the fact that not all species respond to dofetilide in the same manner, in that the noted effects are seen in guinea pigs, but none are observed in rats. Dofetilide has repeatedly been described as prolonging action

potential duration in a reverse rate-dependent fashion[49,50,51,52] in several species including rats, guinea pigs, rabbits, dogs, and humans, and it is this reverse rate-dependence that poses the most problems with regards to the clinical use of dofetilide.

HMR 1556 was selected as the I_{Ks} blocker for use in these experiments. HMR 1556 has been demonstrated to be a potent, selective blocker of the I_{Ks} channel, in *in vitro* preparations using cloned channels[53-55] as well guinea pig and canine atrial and ventricular myocytes[56-58]. HMR 1556 has also been demonstrated to block the I_{Ks} channel *in vivo* in conscious dogs[59]. Like the earlier I_{Ks} blocker, the chromanol 293B, HMR 1556 is neutral with respect to rate-dependence[53], and demonstrated greater potency and greater selectivity for I_{Ks} than the chromanol 293B[58]. The role of I_{Ks} in large animals, including humans is still under investigation. The administration of HMR 1556 alone to conscious dogs does not appear to prolong refractoriness or repolarization, but this may be due to the slower channel kinetics of I_{Kr} relative to I_{Ks} in this species[59]. If HMR 1556 is combined with an I_{Kr} blocker such as dofetilide we believe it may be possible to prolong refractoriness and repolarization in a non- or reduced rate-dependent manner. Prior data from Fiset et al. indicate that the electrophysiologic effects of I_{Kr} blocker can be modulated by the addition of an I_{Ks} blocker[152]. Studies with azimilide, a single drug with class III action which blocks both the I_{Kr} and I_{Ks} channels, also indicate that the combined block of I_{Kr} and I_{Ks} is likely to display less reverse rate-dependence in than is present with an I_{Kr} blocker alone[153].

Figures

The Chemical Structure of Dofetilide

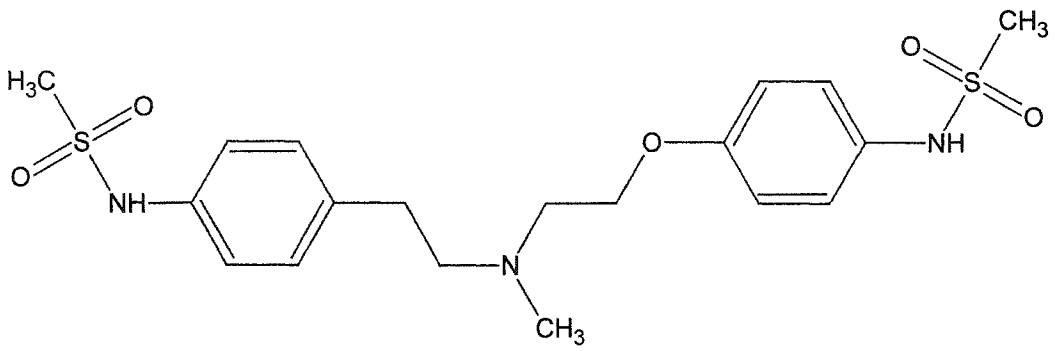


Figure XIV. The chemical structure of dofetilide

The Chemical Structure of HMR 1556

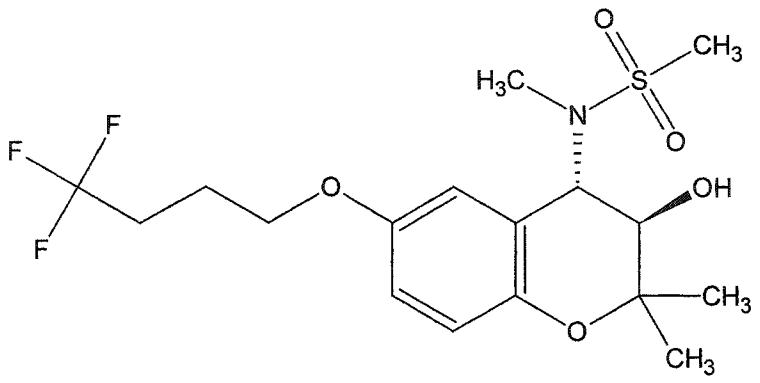


Figure XV. The chemical structure of HMR 1556

EXPERIMENTAL DESIGN, PROCEDURE, AND METHODS OF APPROACH

EXPERIMENTAL MODEL

A pig model was chosen for this study. It was decided that a large animal model would be more appropriate than a small animal model given that smaller animals have much higher basal heart rates, and that the two most common small animal models used for antiarrhythmic drug research, the guinea pig and the rabbit, are known to have very different relative amounts of I_{Kr} and I_{Ks} than is known to be present in humans[43,60]. The most common large animal model used in antiarrhythmic drug research is the dog. Unfortunately, the cost of procuring dogs for research at the time that this study was begun made the use of dogs impossible. After much investigation it was decided that pigs would be used. Pigs are currently the standard model used for CPR research[73,74,87,89,93,96]. Pigs are also the most likely candidate in the search for donor cardiac tissue for xenotransplantation to humans[114-116]. Additionally, dofetilide and azimilide have both been administered to pigs and have been observed to prolong right and left atrial ERP at doses of 0.0010 mg/kg and 5 mg/kg respectively[169]. Based on the above evidence it is reasonable to assume that the anatomy and physiology of the pig heart bears enough resemblance to that of the human heart that it would be an appropriate model for this line of investigation.

General Methods

Male Pigs (20-30 kg) were anesthetized using Ketamine (20 mg/kg) and Xylazine (2 mg/kg) as a sedative, Thiopental (8mg/kg) for induction, and following intubation received an inhalation mixture of 50:50 oxygen:nitrous oxide with 2% isoflurane. All pigs also received oxymorphone (1.5 mg/10 kg) for analgesia. The anaesthetic regimen

differs from that previously described[73,75] in order to better simulate an out-of-hospital cardiac arrest. Pentobarbital has been demonstrated to block sodium channels in human ventricular myocytes[124]. Based on this evidence, pentobarbital anaesthesia is not appropriate. The chosen anaesthetic regimen listed above uses drugs that are known to cause minimal cardiac effects. Isoflurane causes a slight depression of the maximum rate of rise of action potential upstroke, but has minimal effects on intraventricular conduction[126]. Pigs were ventilated at a rate and volume adjusted to keep blood pH between 7.35 and 7.45. Blood gasses were monitored every hour. Body temperature was kept at 38°C using a recirculating water blanket. Normal saline was infused at a rate of 2-4 mL/kg/hr to prevent volume depletion.

Once properly anaesthetized, the right and left femoral veins and arteries were isolated and canulated, as will the right external jugular vein. Catheter sheaths were inserted into the right femoral artery and vein for the introduction of 7 French monophasic action potential (MAP) catheters. Arterial blood pressure was monitored from the left femoral artery, and canulation of the left femoral vein allowed for i.v. administration of drug and fluids. Electrodes were attached to all four limbs for surface ECG recordings (leads I, II, III). Three limb leads of the surface electrocardiogram, unipolar electrograms from the endocardial MAP catheter, endocardial MAP electrograms, and blood pressure were amplified using a custom-made amplifier (Cartesian Labs, Toronto, ON) and recorded using a custom-made computer software program, (Electrophysiological Recording System – Acqui2, Cartesian Labs, Toronto, ON). The MAP signals are DC coupled and filtered with a 500 Hz low pass filter, the unipolar electrograms are filtered at 0.05 – 500 Hz.

Specific Methods

Electrophysiological Measurements

Ventricular effective refractory period (VERP) was measured to the nearest 2 msec by the incremental extrastimulus technique, delivering an S2 stimulus at twice diastolic pacing threshold following a 50 beat pacing train at cycle lengths of 400, 300, 250, and 200 msec from the RV endocardial monophasic action potential (MAP) catheter. Cardiac refractoriness was determined at numerous cycle lengths to assess the presence or lack of reverse rate-dependence of any of the drugs under investigation. MAPs was measured using an Ag-AgCl electrode connected to a DC coupled amplifier. All signals were displayed on a monitor with a customized multi-channel digital recording system, and archived on compact disc.

Electrical restitution characterizes the dependence of action potential duration (APD) on the preceding diastolic interval, which is evident in changes in APD accompanied by changes in rate. Restitution kinetics plays a significant role in the initiation of ventricular arrhythmias, particularly ventricular fibrillation. Dynamic restitution, that is, electrical restitution measured at multiple cycle lengths during steady-state pacing rather than single beat pacing, was determined by measuring MAP duration at 90% repolarization (MAP90), after a 50 beat pacing train and 10 second pause at each of the following cycle lengths: from 400 to 350 in 50 msec steps, and from 350 to the fastest follow frequency (FFF) (defined as the shortest cycle length of right ventricular endocardial pacing at twice diastolic threshold which results in 1:1 ventricular capture with continuous pacing) in 10 msec steps. Global conduction velocity was estimated from

the total QRS duration measured during ventricular pacing at cycle lengths of 400, 300 msec and the FFF.

Study Drug Administration

A series of doses of dofetilide (fig. XIV) and HMR 1556 (fig. XV) were administered. The effects of combining drugs was studied by administering a range of doses of each drug combined with the dose of the other producing the half-maximal effect on action potential duration (dofetilide dose range: 2.5 μg/kg – 50 μg/kg; HMR 1556: 1 mg/kg – 20 mg/kg). Dose-response curves will be modeled as "Effect=Ae^{x}", and was to be generated after administration of each drug alone, and in combination, with respect to DFT, VFCL, VERP, and MAPD90.

RESULTS

Prior to commencing the experiments proper, a series of feasibility studies were performed in order to be certain that the model was stable over time and to obtain a clear understanding of the behaviour of all relevant drugs and diluents in the pig model.

The pig model of dynamic restitution was found to be stable over time as seen in table 17 and figure XVI. The changes in effective refractory period (ERP) observed at various paced cycle lengths ranging between 400 ms and 200 ms before and after a 5 cc normal saline bolus were no greater than 6% or 10 ms. This was performed on two animals.

Once temporal stability of the model was confirmed, dofetilide was administered to pigs in various doses ranging from 2 μg/kg to 32 μg/kg to determine an appropriate dose. Two important observations were made. First, that the ERP prolongation was, minimal except at the highest dose delivered, not dose-dependent, and not consistent within one animal; for example, at dose 8 μg/kg the ERP prolongation at various cycle lengths appears almost random. Prolongation is not greatest at either the longest or shortest cycle length, but in the middle. Additionally, the magnitude of the prolongation does not trend towards being greater at longer or shorter cycle lengths, nor does it trend towards being greater at a mid point. This can be seen in the data presented in Table 18 and figure XVII. Second, that dofetilide did not generate the expected reverse-rate dependant pattern (figure XVIII).

The I_{Ks} blocker HMR 1556 is not water-soluble. We were advised by Dr. Uwe Gerlach, one of the researchers from Aventis involved in the discovery of HMR 1556, who supplied us with the drug for this study, that in order to administer this compound intravenously it must first be dissolved in enough dimethyl sulfoxide (DMSO) to just dissolve the HMR 1556 (1 part DMSO). This solution must then be dissolved in a volume of polyethylene glycol (PEG) equal to 3 times the volume of DMSO (3 parts PEG). This solution can then be dissolved in the desired volume of normal saline. We found that in order to dissolve 25 mg HMR 1556 (dose 1 mg/kg for a 25 kg pig) we required 1.5 ml DMSO and 4.5 ml PEG. Prior to testing the I_{Ks} blocker, the DMSO-PEG vehicle was evaluated for any potential electrophysiological effects in the pig model. This DMSO-PEG vehicle was found to cause profound shortening of ERP, shortening of 30-56% at the minimum required dose, and 30-38% at a DMSO-PEG dose 1/3 of the required dose as seen in table 19 and figure XIX. One animal was tested at each dose. Because of the effects observed with the vehicle, the HMR 1556 was never administered.

Based on the above observations that dofetilide did not produce a dose dependent prolongation of ERP and did not exhibit reverse rate-dependence in pigs, combined with the observation that the minimum required amount of the DMSO-PEG solvent produced ERP changes opposite in direction, and of greater magnitude than could be expected from the I_{Ks} blocker HMR 1556, it was determined that the objectives of the experiments could not be achieved in the pig model and the combination potassium channel block experiments were terminated.

Tables and Figures

Effect of a 5 cc Saline Bolus on the Effective Refractory Period

Control 1			
PCL msec	ERP pre msec	ERP post msec	% change Pre vs. post
400	222	212	-4.5
350	202	204	1.0
300	182	186	2.2
280	178	182	2.2
260	164	174	6.1
240	162	164	1.2
220	152	156	2.6
200	144	144	0.0

Control 2			
PCL msec	ERP pre msec	ERP post msec	% change Pre vs. post
400	254	244	-3.9
350	234	224	-4.3
300	206	206	0.0
280	194	202	4.1
260	184	184	0.0
240	178	174	-2.2
220	172	170	-1.2

Table 17 – Stability of Effective Refractory Period (ERP) at various paced cycle lengths (PCL) before and 20 minutes after a 5 cc saline bolus demonstrating temporal stability of the pig model of dynamic restitution. The change in effective refractory period at any given cycle length is no greater than 6.1% relative to baseline. n=2. PCL=paced cycle length, ERP pre=effective refractory period before drug administration, ERP post=effective refractory period after drug administration.

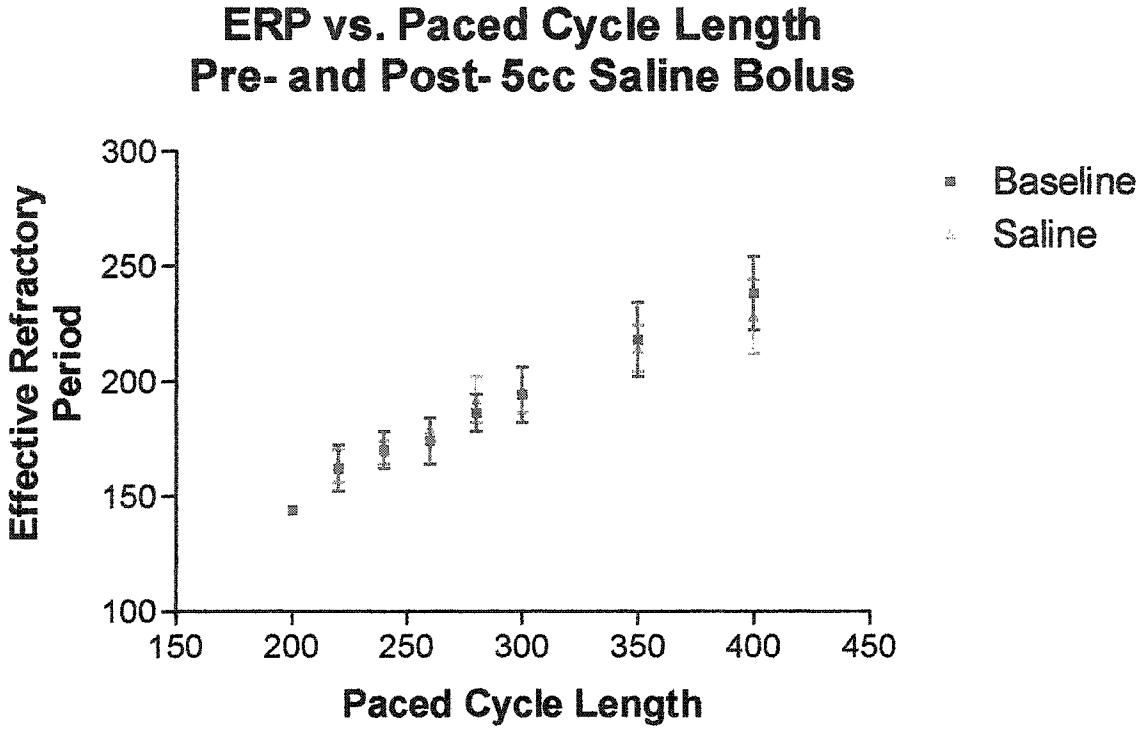


Figure XVI – Effective refractory period before and 20 minutes after 5cc saline bolus demonstrating temporal stability of pig model. There was no significant difference between the ERP as measured at baseline compared to the ERP measured after a 5 cc saline bolus followed by a 20-minute equilibration period. n=2

Effect of various doses of Dofetilide on the Effective Refractory Period

2ug/kg			
PCL	ERP pre	ERP post	% change
msec	msec	msec	Pre vs. post
400	152	164	7.9
350	144	152	5.6
300	142	142	0.0
280	136	134	-1.5
260	134	134	0.0
240	132	130	-1.5
220	126	128	1.6
200	122	122	0.0

4ug/kg			
PCL	ERP pre	ERP post	% change
msec	msec	msec	Pre vs. post
400	236	234	-0.8
350	222	228	2.7
300	204	204	0.0
280	194	198	2.1
260	182	186	2.2
240	172	182	5.8
220	166	174	4.8
200	154	168	9.1

8ug/kg			
PCL	ERP pre	ERP post	% change
msec	msec	msec	Pre vs. post
400	192	202	5.2
350	182	194	6.6
300	168	184	9.5
280	162	176	8.6
260	156	168	7.7
240	148	162	9.5
220	142	158	11.3
200	138	146	5.8

16ug/kg			
PCL	ERP pre	ERP post	% change
msec	msec	msec	Pre vs. post
400	224	224	0.0
350	214	222	3.7
300	198	202	2.0
280	190	194	2.1
260	184	182	-1.1
240	176	174	-1.1
220	168	170	1.2
200	160	158	-1.3

32ug/kg			
PCL	ERP pre	ERP post	% change
msec	msec	msec	Pre vs. post
400	174	250	43.7
350	168	242	44.0
300	158	214	35.4
280	154	214	39.0
260	144	196	36.1
240	138	184	33.3
220	134	178	32.8
200	128	170	32.8

Table 18 – Changes in effective refractory period (ERP) at various paced cycle lengths (PCL) after administration of various doses of dofetilide. Note that increasing the administration of dofetilide to pigs does not result in consistent prolongation of effective refractory period, and that any prolongation of ERP that is present does not appear to be dose-related except at the highest dose of 32 μg/kg. n=1 for each dose. PCL=paced cycle length, ERP pre=effective refractory period prior to drug administration, ERP post=effective refractory period after drug administration.

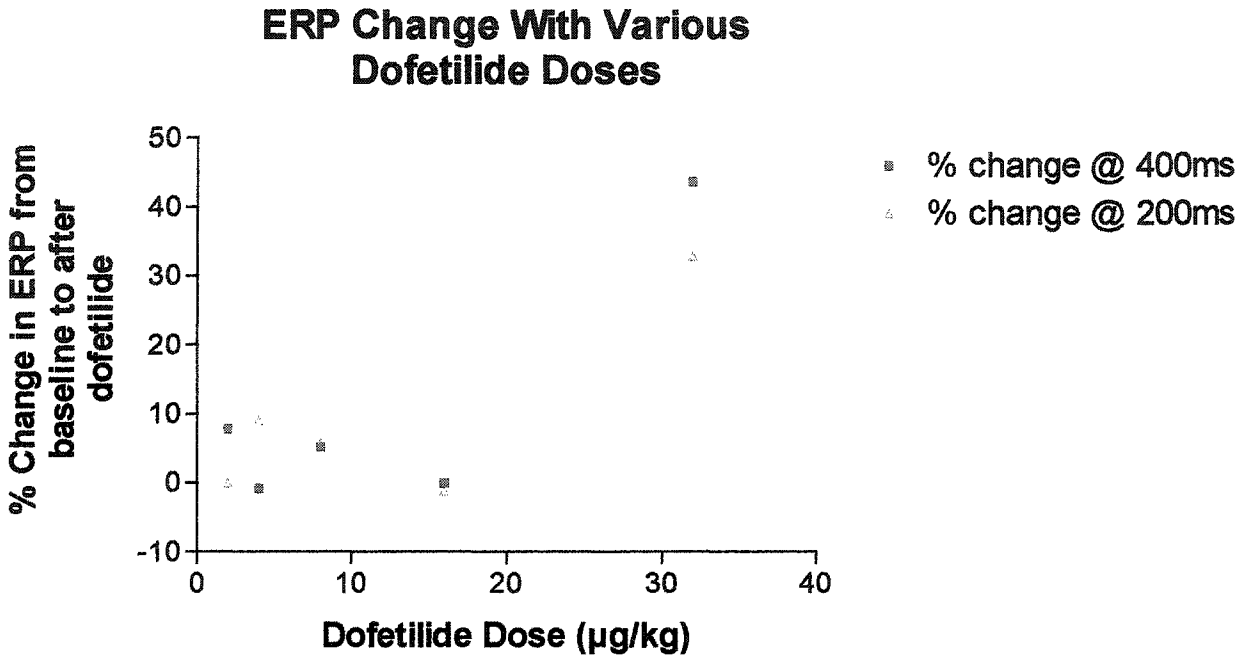


Figure XVII – Dofetilide-induced effective refractory period (ERP) prolongation is not consistent, and does not correlate with administered dose. n=1 for each dose.

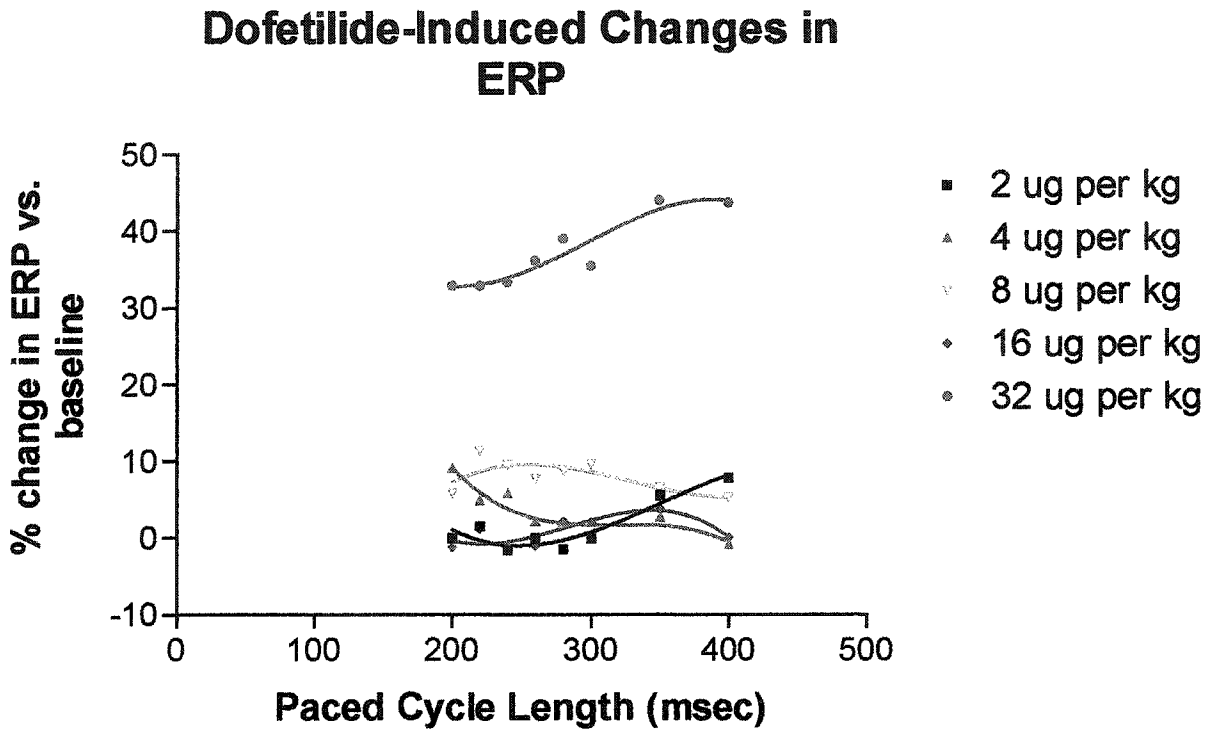


Figure XVIII – Dofetilide failed to display reverse-rate dependence at any of the doses tested. The trend lines were fitted using a third-order polynomial equation. n=1 for each dose.

Effect of 2 Doses of the PEG-DMSO Vehicle on the Effective Refractory Period

PEG(1.5 ml) -DMSO(0.5 ml)			
PCL	ERP pre	ERP post	% change
msec	msec	msec	
400	222	138	-37.8
350	214	142	-33.6
300	202	134	-33.7
280	192	132	-31.3
260	182	126	-30.8
240	174	118	-32.2
220	164	116	-29.3
200	160	112	-30.0

PEG(4.5 ml) -DMSO (1.5 ml)			
PCL	ERP pre	ERP post	% change
msec	msec	msec	
400	226	100	-55.8
350	222	130	-41.4
300	212	136	-35.8
280	208	132	-36.5
260	198	132	-33.3
240	190	132	-30.5
220	180	126	-30.0
200	174	122	-29.9

Table 19 – Polyethelene Glycol – Dimethyl Sulfoxide (PEG-DMSO) vehicle for intravenous delivery of HMR 1556 has profound effective refractory period (ERP) shortening effects measured at various paced cycle lengths (PCL). Due to the magnitude of this effect, even if HMR 1556 were found to have an ERP prolonging effect, the net effect would be neutral at best, thus clinically useless. Additionally, it would be difficult to separate the effects of the drug from the effects of the vehicle, and impossible to determine the effects the drug would have without the presence of the vehicle. The table on the left is from a pig given 0.5 ml DMSO and 1.5 ml PEG, insufficient vehicle to dissolve the necessary dose of HMR 1556. The table on the right is data from a pig given 1.5 ml DMSO and 4.5 ml PEG, sufficient vehicle to dissolve the necessary dose of HMR 1556

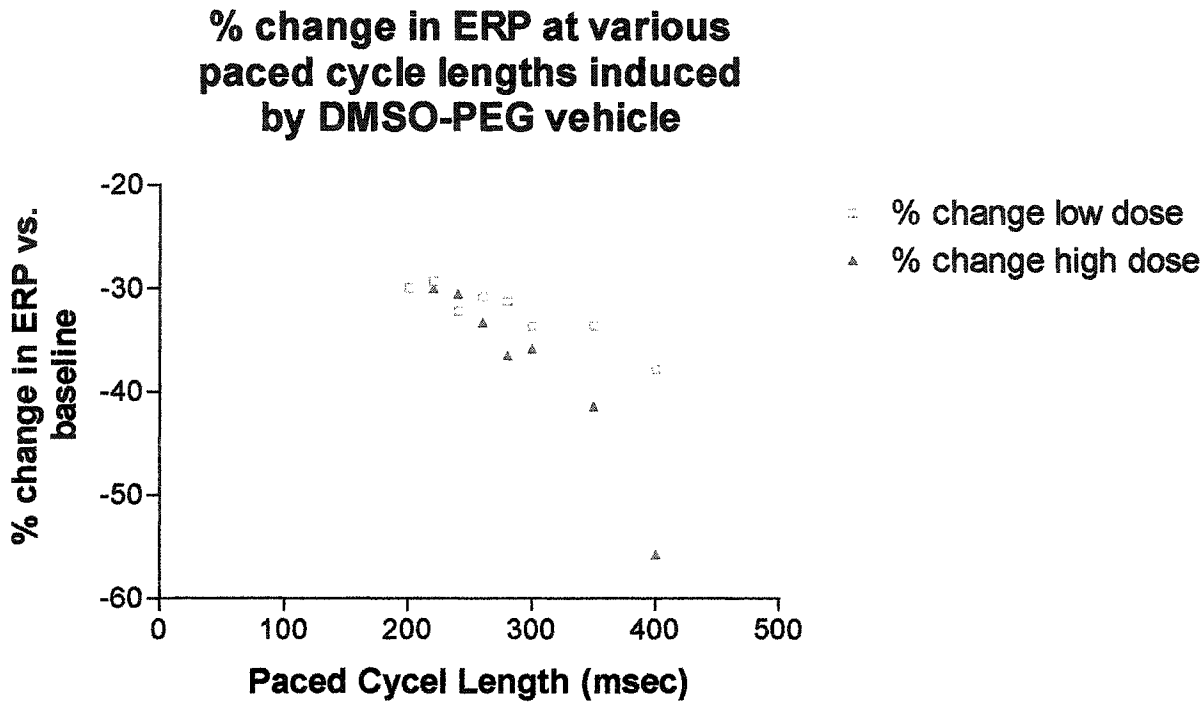


Figure XIX – Effective refractory period (ERP) shortening at various paced cycle lengths caused by the administration of 2 different doses of Dimethyl Sulfoxide-Polyethelene Glycol (DMSO-PEG) vehicle necessary to administer HMR 1556 intravenously. The low dose plot represents a dose of 0.5 ml DMSO and 1.5 ml PEG, a dose insufficient to dissolve the necessary amount of HMR 1556. The high dose plot represents a dose of 1.5 ml DMSO and 4.5 ml PEG, the lowest dose sufficient to dissolve the necessary amount of HMR 1556.

DISCUSSION

Principal findings

The principal findings of this study were twofold; firstly, that dofetilide does not cause a dose dependent prolongation of action potential duration and effective refractory period in a reverse rate-dependent manner in pigs, and secondly, that the DMSO-PEG diluent needed for the administration of HMR 1556 has profound electrophysiological effects on pigs, effects opposite those of class III antiarrhythmic action.

Strengths and Weaknesses of the Study

This study was designed to investigate if combining an I_{Ks} blocker with an I_{Kr} blocker could result in prolongation of refractoriness equal to that achieved with I_{Kr} blockers alone with less reverse rate-dependence than is exhibited with an I_{Kr} blocker alone. The greatest single weakness of this study is the choice of model. Pigs have never before been used as a model for the investigation of antiarrhythmic drugs. Additionally, there has been no work done characterizing the presence and activity of membrane ion channels in porcine cardiac cells. Because of this lack of data in the scientific literature, the choice of a pig model was a calculated risk. I_{Kr} is present to a degree in every species investigated, including rabbits, guinea pigs, dogs, and humans[11-27], so it is reasonable to assume that it is present in pigs as well. Dofetilide has also prolonged APD in a dose-dependent manner, and displayed reverse rate-dependence in all the above noted species, to greater or lesser degrees[50,51, 136-138], so it was reasonable to assume that the same would be true in pigs.

The I_{Ks} blocker HMR 1556 has been administered to both dogs[56] and guinea pigs (unpublished results from this lab) and the profound electrophysiologic effects of the DMSO/PEG diluent observed in this pig model was not observed in either the dog or guinea pig model. This would suggest that there are very important differences in the way that various species react to the same drug.

With the information available there was every reason to believe that this study should have generated positive results. Unfortunately this study demonstrates that, despite the best planning, and appropriate background investigation prior to commencing a new study, information that is unknown is as important as information that is known with regards to the possible success of any study.

Strengths and Weaknesses in Relation to Other Studies

This study departed from established animal models for electrophysiological research. The results from this study demonstrate that departing from established and well characterized models without fully understanding the chosen model has inherent dangers. Previous studies using established animal models for electrophysiological and antiarrhythmic drug research have established that dofetilide does block the I_{Kr} channel, does cause a dose-dependent prolongation of APD, and does so in a reverse rate-dependent manner. Since none of these could be demonstrated in this study it suggests strongly that the pig model is inappropriate for this particular type of study.

Unanswered Questions and Future Research

This study leaves essentially all the questions it attempted to address unanswered. Because of the unpredicted problems with the model this study was unable to address the

questions it was designed to, such as, can the combination of I_{Kr} and I_{Ks} blockers prolong APD and ERP as effectively as either drug alone, and, can the combination of I_{Kr} and I_{Ks} blockers cause APD and ERP prolongation with less reverse rate-dependence than is observed with either drug alone. These questions require further investigation in animal species whose electrophysiology is well understood and are established models for this type of study. If this study were to be repeated I believe it would be wise to use a different I_{Kr} blocker, such as d,l-sotalol. Although d,l-sotalol is also a β-blocker[154,155], the use of a different I_{Kr} blocker would help elucidate whether or not the results observed here are unique to dofetilide in pigs, or occur with all I_{Kr} blockers. I also think a different I_{Ks} blocker should be chosen, such as indapamide, which does not require DMSO-PEG as a diluent to be administered[152].

Additionally the problems observed with the model raise additional questions about the electrophysiology of the pig heart. The cellular and sub-cellular electrophysiology of the pig heart should be investigated and characterized before studies of this nature are begun using a pig model. Further study of the electrophysiology of the pig heart would also appear to be a pertinent line of investigation for researchers interested in xenotransplantation of pig cardiac tissue to human recipients.