

Development of a Sheep Model of Atrial Fibrillation for Preclinical Prosthetic Valve Testing

Andrew L. Rivard^{1,2}, Phillip T. Suwan³, Keyoumars Imaninaini⁴, Robert P. Gallegos⁵, Richard W. Bianco¹

¹Experimental Surgical Services, Department of Surgery, University of Minnesota, Minneapolis, MN, ²Department of Radiology, University of Florida, Gainesville, FL, ³University of Minnesota Medical School, University of Minnesota, Minneapolis, MN, ⁴Stem Cell Institute, McGuire Translational Research Facility, Minneapolis, MN, ⁵Division of Cardiac Surgery, Department of Surgery, Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA

Currently, prosthetic heart valve testing is performed on animal models with no underlying cardiovascular pathologies. Unfortunately, unforeseen adverse events may occur when heart valves tested in animals in normal sinus rhythm are implanted in patients suffering from arrhythmias. For example, the Medtronic Parallel valve functioned well in pre-clinical testing, but a high rate of thromboembolic complications appeared when the valve was placed in patients with atrial fibrillation (AF). Given the increasing number of patients afflicted with AF, an animal model of the disorder is needed to more accurately predict a valve's function in the clinical setting. Among methods available for inducing AF, electrophysiological pacing is the most practiced, but

the challenges associated with pacing have led to the development of alternative methods of inducing AF. These methods include gene transfer and a pharmacologic approach with acetylcholine and catecholamines. Finally, although stem cells have been widely investigated in terms of their therapeutic benefits, the use of their well-reported pro-arrhythmic behavior shows great promise for the development of an AF model in sheep. Such a model would have the potential for detecting adverse outcomes with mechanical heart valves before implantation in the clinical setting.

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Current Food and Drug Administration (FDA) regulations do not specify that prosthetic heart valves should undergo any type of in-vivo testing in animals with pre-existing cardiac pathology (1). However, since the report of the National Heart, Lung, and Blood Institute working group in 1997, there has been a growing movement to develop animal models for 'accelerated' or 'stressed' in-vivo valve testing (2). For example, Gregoric et al. used the high cardiac output of the bovine model for high-flow testing of a novel trileaflet mechanical valve (3). Another stressed animal model is that of atrial fibrillation (AF), although no study to date has used an AF model to evaluate new valve designs. Although under-utilization of the AF animal model is due primarily to the valve manufacturer's and investigator's focus on the mechanical properties of prosthetic valves and the surgical process of the replacement, there is also a clear absence of a well-established animal model.

Atrial fibrillation is a serious risk before and after mitral valve replacement (MVR), as the onset of AF marks a turning point in the progression of mitral valve disease. Indeed, there are consistently more patients in NYHA class III-IV heart failure with AF than in classes I and II before MVR (4). Furthermore, up to 33% of patients develop AF after MVR, and this may occur either early (within the first two postoperative weeks) or late (after two weeks). At present, there is active debate as to whether AF causes morbidity and mortality, or not. For example, Kuntze et al. concluded that, due to the need for lifelong anticoagulation, the risk of thromboembolism was minimal (5). On the other hand, Kernis et al. showed that AF was associated with morbidity secondary to stroke (13%) and congestive heart failure (CHF) (24%) despite anticoagulant treatment, and was independent of NYHA class, the type of surgery, coronary artery disease, coronary artery bypass grafting (CABG), or other cardiac risk factors (6).

It is not clear whether AF itself or frequently associated conditions that are difficult to separate from the arrhythmia (e.g., reduced left ventricular systolic or diastolic function, advanced age, hypertension, left ventricular hypertrophy) are responsible for the

Address for correspondence:
Richard W. Bianco, University of Minnesota, Department of Surgery, MMC 195, 420 Delaware Street SE, Minneapolis, MN 55455, USA
e-mail: bianc001@umn.edu

observed increase in morbidity. Furthermore, the pathogenesis and potential clinical consequences of AF (i.e., thromboembolism, CHF) are incompletely understood. It has been established, however, that AF leads to identifiable pathologic changes within the fibrillating left atrium. One of the primary consequences of a fibrillating left atrium is the increased risk of thromboembolic events (7). Specifically, the left atrial appendage (LAA) normally prevents stasis due to its high level of contractile activity. In the presence of AF, contractility of the LAA is greatly diminished; this in turn causes stasis, which is thought to increase the potential for thrombosis and subsequent thromboembolic events. In the setting of MVR, it is conceivable that the hemodynamic changes associated with AF may affect the flow profile of a prosthetic valve, allowing the valve itself to be more thrombogenic. Moreover, prosthetic valve implantation alone increases the risk of thromboembolic complications (2,8,9).

Currently, an estimated 2.2 million adults in the United States have AF, while further estimates suggest that, by the year 2050, a total of 5.6 million patients will have paroxysmal or permanent AF (10,11). Given the high prevalence of AF in patients who underwent MVR with the associated increased thromboembolic risk, the testing of prosthetic valves in an animal model of AF undoubtedly would provide additional information and may allow a better prediction of the thromboembolic risk of a particular valve in humans with AF (12-14).

The purpose of pre-clinical in-vivo testing is to evaluate the heart valve's performance characteristics in order to assess its safety profile prior to human use (15). This testing is carried out to establish the identity of any known and foreseeable hazards (16). If heart valves are not tested in animals under conditions as close to clinically possible, then unforeseen adverse events may occur after subsequent use in clinical trials. For example, the Medtronic Parallel valve functioned well in pre-clinical animal testing yet, despite the mechanical similarity to its predecessor, it was withdrawn from the market due to aggressive thromboembolus formation which was prevalent in patients with AF (17). This experience with the Parallel valve once again emphasizes the need for an animal model of AF, and highlights the under-appreciation of the 40-60% of patients who have AF and undergo mitral valve repair or replacement.

In order to reduce the potential morbidity associated with AF after MVR, focus should be centered on the treatment and prevention of AF after valve replacement, in addition to the prevention of adverse events - especially thromboembolism, which may be associated with a particular valve in the milieu of AF. Currently, a number of methods are employed to restore normal

sinus rhythm prior to MVR, with perhaps the most common being a radiofrequency (RF) ablative technique modeled after the Cox Maze procedure. Briefly, a percutaneous catheter is used to deliver an electrode to the left atrium, whereupon cells are targeted for ablation in such a way as to prevent the propagation of AF. Although this procedure is effective in some patients, as with other modalities of treating AF, some may experience recurrences of the condition. In order to better test and refine this method, a pre-clinical model that incorporates AF is critical. Once AF occurs, anti-arrhythmic agents as well as percutaneous, catheter-based techniques can be used to maintain sinus rhythm; however, the recurrence rate is substantial and any clinical benefit over anticoagulation and rate control is debatable. To prevent the specific risk associated with a particular valve in the milieu of AF, the development of an animal model to predict the likelihood of adverse clinical events associated with AF is paramount. Likewise, a clinical model of AF would be helpful to test preventive and treatment strategies for AF beyond that seen in patients with mitral valve disease. Such a model may be used in the testing and validation process of pharmacotherapy, atrioventricular (AV) nodal ablation, pulmonary vein isolation, pacing techniques, and ablative techniques (RF or cryotherapy) based upon the original Maze procedure, as well as in the development of new techniques. However, this review focuses mainly on AF induction - by a variety of methods, including atrial pacing, genetic manipulation and molecular intervention, stem cell therapy, and pharmacological administration - for use in mechanical prosthetic valve testing. In addition, potential new strategies are discussed that might hold promise, with specific attention focused on the well-established sheep model, which is also used for prosthetic valve implantation.

The development of a model

Previous research investigations have uncovered a number of cardiovascular structural and electrophysiological alterations associated with AF (18-21). The fibrillating heart has a shorter refractory period at the right atrial appendage (RAA), a shorter action potential duration, electrophysiological remodeling, and changes in gene expression (19,21). Myocardial modeling leading to atrial enlargement appears to be a direct result of AF. Alessie and colleagues have reported that AF induces atrial myocyte hypertrophy, glycogen accumulation, and intracellular organelle disruption (22); these changes result in a loss of myocardial tissue with a coexisting increase in fibrous tissue.

From a structural standpoint, the fibrillating left atrium is larger, has a relative stasis of blood (particularly

in the atrial appendage), and fails to provide the 'atrial kick' which comprises approximately 30% of ventricular filling. These characteristics also explain the increased thromboembolic risk and decreased cardiac output associated with AF. It is possible that the structural changes are due to the inability of the left atrium to properly contract, leading to an increased left atrial blood volume. Dilatation of the left atrium then occurs secondary to the increased volume, and this in turn leads to loss of the 'atrial kick' and subsequent pathophysiology.

Recognition of the structural and electrical pathologies associated with AF is important in developing a model that closely reflects the natural onset and progression of the disorder. The ideal AF model for testing prosthetic valves would reflect the physiologic and anatomic characteristics associated with the arrhythmia.

Risk of thromboemboli: Defining a sample size

One of the primary concerns of clinicians and prosthetic valve designers alike is the risk of thrombus formation associated with any new valve design. This is not a new concern, as studies documenting such risk following MVR surgery appeared as early as the 1970s and continue to the present day (8,23,24). In the only study of its kind (these studies would not be possible today due to the inherent lack of research subject protection), Stanford and colleagues reported in 1972 on a total of 64 MVRs using a Beall mechanical valve (25). Of the 64 patients, 36 received no anticoagulants post-operatively and, according to the authors, 'most' were in AF. During a follow up period of approximately 3.25 years, there was a 13.8% development of thromboemboli in the patients not receiving anticoagulant therapy. A limitation of these statistics was that the incidence of thromboemboli was based on symptoms and not the actual presence of thrombus; likewise, the presence of thrombus could be higher than was reported by Stanford et al. Thus, the thrombogenicity of the Beall mechanical valve may be underestimated in this case, thereby artificially creating a more stringent standard for prosthetic heart valve performance.

The development of thromboemboli is of primary concern when testing heart valves. The results gathered by Stanford et al. proved to be helpful in providing a baseline measurement to compare other heart valves in terms of this potential. Further, valve function with respect to thrombogenicity is tested strictly, since the subset of Stanford's data used was based on patients not receiving anticoagulant therapy. Based on these data, testing a valve for one year requires 117 sheep in AF. If more than 16 sheep were to develop

thromboemboli over the course of a year, then the valve would be considered more thrombogenic than the Beall low-profile valve.

Pacing

Rapid atrial pacing is the most practiced method of inducing AF for in-vivo investigation. Two primary means of pacing - intracardiac and transesophageal - have been developed. Although arguably the most straightforward method for the induction of AF, pacing is not without undesirable complications that limit the reproducibility of experiments using this method.

Intracardiac pacing, which may be subdivided into burst pacing and continuous pacing, is the most widely researched mechanism for the induction of AF in the sheep model, and in animal models overall. A hallmark study by Willems et al. demonstrated, in a sheep model, that rapid atrial stimulation led to a shortening of the atrial effective refractory period (26). This atrial electrical remodeling resulted in an increased vulnerability to AF. In this study, two different rapid atrial pacing protocols in a sheep model were used: intermittently burst-paced; and continuously paced. Using well-described procedures, electrodes were guided through the anterior transverse sinus and sutured into place on both atrial appendages and the lateral walls of the right and left atria.

The results indicated that, in the continuously paced group, 77% of the animals developed sustained AF (>1 h duration) compared to only 29% in the burst-paced group. Among the 20 animals, a mortality rate of 20% was shared equally by both groups. The causes of death included sudden death of unknown etiology, infection, loss of atrial capture and an electrode perforation (presumably of a major vessel, although this was not stated).

In a follow up study, Willems et al. utilized pacing in order to induce AF which provided insight into the use of pacing-induced electrical changes (27). Of the sheep used, 13 had pacemakers set to stimulate the atria at a rate of 600 beats per min continuously, while seven were modified to deliver bursts when the pacemaker detected sinus rhythm. These studies established that pacing consistently induces AF in the sheep model. Willems and colleagues reported several electrical changes taking place in the paced heart which included (but were not limited to) decreased sinus node function, an increased P-wave duration, and a shortening of the atrial effective refractory period. Yu and colleagues (28) concluded that the changes in cardiac electrical properties, as reported by Willems et al., were similar to those occurring in the fibrillating human heart. This opened up the possibility that the pathogenesis of AF leads to the atrial enlargement and elec-

trical remodeling found in the paced heart. However, further research is required to establish a link - or a lack thereof - between the pathology associated with the paced and fibrillating hearts.

The transesophageal approach to pacing represents another possible method to induce AF. This is used in humans and animal models, primarily in the detection and assessment of irregular cardiac rhythms and coronary artery disease (29). Kohl and colleagues used transesophageal electrocardiography in the diagnosis and stimulation of fetal sheep (30). In these studies, trocars were placed in 10 fetal sheep, between 87 and 103 days of gestation, using ultrasound guidance for the initial catheter until fetoscopic visualization could be established. Stimulation was attempted between the two electrodes recording the highest atrial amplitudes. Stimulus strength was varied between 10 and 150 mA and, if unsuccessful, stimulation was attempted using an alternate pair of electrodes. All sheep survived the procedure, and there were no signs of esophageal damage at the site of electrode placement. However, Kohl et al. found that this approach did not have long-lasting effects when used in the prevention of supraventricular tachycardia. Thus, it is unclear whether, using this approach, the induction of AF in a developed sheep would be long-lasting.

Although the goal was distinctly different from that of inducing AF, and the experiment was performed on sheep in utero, this study could be seen as one of the first ventures into the field of interventional pacing in a sheep model. This type of non-invasive approach is desirable when modifying the electrical and physiological behavior of the sheep heart. Support for a closer examination of the transesophageal method of pacing was provided by Schrickel et al., who found that the susceptibility and induction of the mouse heart into AF was greater with this method than with the more common intracardiac approach (31). While transesophageal pacing is far from a definitive conclusion with regards to its efficacy in the induction of AF in the sheep model, further studies are clearly warranted in order to determine the feasibility of such a model.

Recently, Au-Yeung et al. described an implantable system that would allow for the delivery of right atrial rapid pacing in order to induce AF in conscious animals (32). The implantable pacing device was designed to improve on past models developed by Wijffels et al. (20), Garratt and colleagues (33), and Todd et al. (34). In these previous models, the animals had been tethered in order to prevent movement that might cause displacement of the electrode leads but, since the device is implantable, the animals required neither sedation nor tethering to prevent electrode displacement. From an operational standpoint, the use of

an implantable device was also beneficial in reducing the likelihood of wound infection. Au-Yeung and colleagues hoped to further improve upon previous designs by creating a system that allowed for the induction of AF via rapid atrial pacing and continuous monitoring of a number of cardiac factors. The pacing device was used for 92 days in a sheep to test its *in vivo* characteristics. Chronic rapid atrial pacing was delivered on 36 non-consecutive days, and paroxysmal AF was noted on a total of 16 non-consecutive days. The system remained effective in studying AF and its induction for a period of three months.

Although this implantable system represented an improvement on past models, there were several issues to be addressed before it could be used to model AF. First, while the implantable model was advantageous in several ways, battery power was a limited resource. Given this, power usage had to be monitored meticulously to the extent that it may seem prohibitive to others attempting long-term studies. Second, the size of the device was significant, and the authors reported that this led to wound-healing complications. Finally, until it is established that the device is effective in producing sustained AF, its use in developing a model of chronic AF is limited.

Pacing, as a whole, has been proven to be a reliable method for inducing AF in a number of animal models. Developments have allowed AF induction in conscious animals with free mobility, which allows for an improved model of AF. Furthermore, various studies have shown that pacing not only in sheep but also in other animal models leads to myocardial (35) and electrical (26,32,36) changes which may be similar to those occurring in patients with AF. At present, further research is required to determine the degree of correlation between the electrical and myocardial properties of the paced heart and the fibrillating heart.

Pharmacological models

Recently, Sharifov et al. investigated the effects of beta-adrenergic and cholinergic stimulation and blockade on spontaneous AF in the intact dog heart (37). The administration of catecholamines and acetylcholine (ACh) perfused through the sinus node artery (SNA), either individually or in tandem, caused the induction of AF in 20 anesthetized open-chest dogs, without electrical stimulation of the atria. Isoproterenol (a non-selective beta-adrenergic agonist) and adrenaline (alpha- and beta-adrenergic agonist) induced AF in 21% (three of 14) and 17% (one of six) of dogs, respectively. Atropine treatment prevented catecholamine-mediated AF, indicating a critical role of cholinergic tone in these AF episodes.

Acetylcholine-mediated AF was facilitated by isopro-

terenol, which decreased the threshold ACh concentration required for AF induction and also increased the AF duration. Both, vagal stimulation and ACh application resulted in AF in experimental animals, without electrical stimulation of the atria (38). This method proved to become an established method of inducing AF successfully in dogs; furthermore, the duration of AF was increased with ACh mediated by isoproterenol perfused through the SNA.

The prolongation of AF by isoproterenol can be explained in several ways. First, it was shown that the effect of simultaneous sympathetic and vagal stimulation on right atrial refractoriness is not only additive but also synergistic (39,40). Simultaneous perfusion with isoproterenol and ACh may shorten the right atrial refractoriness. Despite a lower ACh concentration used to induce AF with isoproterenol, the average free wall of right atrium (AFCL) over the right atrium became somewhat smaller. Second, a high atrial rate induced by isoproterenol could also result in a shortening of atrial refractoriness, as observed after even a short period of rapid atrial pacing in dogs, thus facilitating ACh-mediated AF (41,42). This may also explain why the AFCL was decreased in the RAA, situated mainly outside of the SNA flow, and thus increased the accessibility of ACh (30). In addition, isoproterenol infused into the SNA could accumulate and affect the atrial myocardium. Differences in heart hemodynamics and other variables that can accompany beta-adrenergic stimulation might also be significant in promoting ACh-mediated AF. These data indicate that both autonomic systems contribute to AF initiation and maintenance; however, cholinergic stimulation is most likely the main factor for spontaneous AF initiation in this animal model, while adrenergic tone modulates the initiation and maintenance of cholinergically mediated AF. Interestingly, the effects of isoproterenol perfusion on ACh-mediated AF were reversible.

Subsequent full blockade of beta-adrenoreceptors by propranolol (1 mg/kg, intravenous) resulted in a shortening of AF duration and an increased threshold of ACh concentrations necessary for AF induction episodes (43). Thus, AF can be discontinued in animals during the course of a study, if deemed appropriate. This level of reversibility is not observed in the other methods of AF induction discussed herein.

Other studies conducted by Sharifov and colleagues tested the pharmacological approach on dogs, and established this to be a safe and effective method to induce AF (39,44). Using well-described methods, the SNA was cannulated and perfused with ACh to induce spontaneous AF. Acetylcholine induced AF in all dogs ($n = 20$), with a threshold ACh concentration of 2.8 ± 0.3 $\mu\text{mol/l}$. The AF onset was preceded by a slowing of the sinus rate. The AF paroxysms started ~ 10 s after perfu-

sion onset, and lasted for ~ 25 s. Acetylcholine-induced AF was modulated by background beta-adrenergic tone. With adrenergic tone enhanced by isoproterenol, ACh-induced AF occurred later after perfusion onset (~ 20 - 25 s) without the heart rate slowing, or at a faster rate than that before perfusion. The threshold ACh concentration for AF induction became significantly smaller, so that AF could be induced by ACh in concentrations ranging from 1 nmol/l to 1 $\mu\text{mol/l}$. Isoproterenol also resulted in significant AF prolongation. The inhibition of adrenergic tone by propranolol did not prevent ACh-mediated AF, but did result in an increased threshold of ACh necessary for AF induction and a shortened duration of AF paroxysms.

Although catecholamine infusion is often used in clinical practice to provoke paroxysmal AF, the present study by Sharifov is the first to demonstrate such an effect in intact animals without electrical stimulation of the atria. In this study, isoproterenol (1-2 or 10 $\mu\text{mol/l}$) facilitated both the initiation and maintenance of ACh-mediated AF, while propranolol (1 mg/kg) produced the opposite effects, allowing for the termination of AF if necessary. The pharmacological methods represent a non-invasive approach for the induction of AF. The efficacy of these techniques for modeling chronic AF has yet to be elucidated. Specifically, the question must be asked whether the maintenance of AF will require constant medication of the animals, and whether this method would induce AF in a sufficiently large population of sheep for it to be feasible for research purposes. Moreover, the efficacy of these techniques in a sheep model is incompletely understood. With respect to anatomical and physiological differences between the dog and sheep models, both animals have been studied extensively for inducing chronic AF. The sheep method is reproducible, less surgical trauma is involved, and animal survivability is high as the AF can be discontinued at any time.

Genetic models

Current research findings strongly support the concept that defective genes and genetic variation play significant roles in the development of AF (45). A review by Lai and colleagues outlined the growing number of genes thought to be involved in the pathogenesis of AF (46). Given the many associated genes, it is reasonable that genetic engineering might play a potential role in the development of an AF model. It is not the intent of this review to provide a complete discussion with regards to each of the genes and loci being studied as possibly contributing to AF; rather, the focus is to provide a background on those genes that have attracted the most attention with respect to their role in AF.

The first important advance in this direction has been the identification of a genetic locus for familial AF on chromosome 10q22-q24 by Brugada et al. (47) Three families from Catalonia, Spain, were studied and showed an autosomal dominant pattern of transmission and early onset of the arrhythmia (age from 1 to 45 years). Positional cloning and analyses of candidate genes to identify the disease gene are currently ongoing. Since the beginning of their studies, Brugada et al. have collected probands from more than 100 families with familial AF. Their findings have revealed that not all families were affected by the chromosome 10q22-q24 locus, which suggests that familial AF is a genetically heterogeneous disorder caused by more than one gene.

Chen et al. studied a family with hereditary persistent AF and identified an associated mutation (S140G) in the *KCNQ1* gene on chromosome 11p15.5 (48). DNA and amino acid sequencing of the *KCNQ1* missense mutation associated with affected members in the AF family was analyzed. DNA sequence analysis revealed an A-G substitution causing an S140G mutation in the S1 segment of *KCNQ1*. The argument for a genetic basis of AF is further strengthened by a series of recent discoveries describing the etiology of AF in humans. Briefly, research by Olson and colleagues has identified a *SCN5A* mutation associated with a number of cardiac arrhythmias, including AF (49). Separate studies conducted by Tsai et al. identified polymorphisms in the genes for the renin-angiotensin system that contribute to AF (50). These findings set the foundation for further research into the development of an AF model based on genetic manipulation, as well the development of an understanding of the molecular basis of AF that may be used in a therapeutic manner.

Although genetic manipulation would allow for the creation of animal lines that could be used reliably to induce AF, the demonstration of this approach remains theoretical, as the results of deliberately induced mutations in genes associated with human AF in an animal model remain speculative at the present time. A review of the current literature did not identify any investigators attempting to create a model using gene delivery. In fact, it would be suspected that this undertaking would require the ability to identify a gene capable of inducing AF under natural conditions, and the ability to modulate this gene in such a way as accurately to reproduce the arrhythmia. Furthermore, how the pathological characteristics of AF that have a lesser, or absent, genetic influence, would then be rectified has yet to be established.

While there remain many challenges to be overcome in the development of a genetic model, the potential benefits warrant that this be given consideration. When the loci of interest have been identified and a

technique is developed to modulate them correctly, this would provide a reproducible model of AF. It is also possible that this would reproduce the changes that occur in the myocardium to induce AF more accurately than electrophysiologic techniques, which seem less accurately to reproduce what occurs in the natural pathology of the disease.

Stem cell therapy

In clinical studies where stem cells have been used for cardiomyoplasty, the resultant arrhythmias have presented a significant barrier against the use of stem cells on a therapeutic basis (51,52). The discovery that stem-cell derived cardiomyocytes have an intrinsic arrhythmic potential leads to the question of whether stem cell therapy might serve as a basis for a model of AF. Indeed, whether this arrhythmic potential can be harnessed is one of the primary challenges that must be addressed if this modality is to be used in the development of an AF model.

In a recent study of myocardial repair, Zhang and colleagues showed that the intravenous injection of stem cells and bone marrow-stimulating cytokines improved cardiac function (51). While this result is of undoubted significance, of particular interest is the associated arrhythmias encountered during these studies. Additional research investigations were performed using mouse embryonic stem cells (ESC R1⁵) and embryonal carcinoma cells (ECC P 19⁶) in an in-vitro differentiation model. Cardiac myocytes were plated and isolated 12-15 days later using a well-delineated protocol. No later than five days after isolation, electrophysiological studies were performed on the cardiac myocytes using an Axopatch 200B amplifier and pCLAMP (Axon Instruments). Zhang showed that stem cells have a 'cardiac rhythm' that may allow for the development of an arrhythmia in in-vitro studies; however, the next step in the development of a stem cell model of AF requires that the arrhythmic properties of stem cells be demonstrated in vivo in an equally eloquent study. The potential for the induction of fibrillation in segregated cardiomyocytes suggests the possibility for adverse consequences when stem cells are used for cardiomyoplasty; however, it also suggests the potential for purposeful selection of the arrhythmic effect in the development of an AF model.

A review by Rosen and colleagues addresses the challenges of using stem cells as biological pacemakers (53). This is based on the knowledge that specific stem cell lines create impulses similar in nature to those of pacemaker cells. The study by Rosen et al. uncovered a notable challenge in using stem cells therapeutically that is also applicable to their use in the development of chronic AF. Although these authors are seeking to

maintain a normal sinus rhythm, they also had to address the issue of 'forcing' embryonic stem cells to follow a specific developmental pattern and to maintain that state. Interestingly, the authors acknowledged the fact that arrhythmogenicity has been reported in studies using isolated cardiomyocytes; however, they suggest that this phenomenon will dissipate if the ESC are allowed to differentiate into mature cardiomyocytes. This is based on the fact that mature mammalian myocytes also exhibit fibrillation when studied in culture or as single cells, whereas this potential is not observed in whole tissue.

Although Rosen suggests that the fibrillatory nature of stem cells does not pertain to the in-vivo heart, studies conducted by Menasche and colleagues suggest otherwise (54). A clinical study was conducted in 10 patients with left ventricular dysfunction following an infarction. Skeletal myoblasts were injected into the left ventricle in the area surrounding the scar and directly into the scar. Within 11 to 22 days of implantation, four patients had sustained monomorphic ventricular tachycardia (VT) with a right conduction delay pattern. This arrhythmia led to the implantation of an automatic internal cardioverter-defibrillator (AICD) and beta-blocker therapy. A skeletal myoblast injection into the atrium may elicit a similar response. When skeletal myoblasts are injected into an area of infarction, they develop contractile activity independent of the neighboring cardiomyocytes (55). It is possible that this independent electrical activity might propagate to the surrounding myocardium, thereby creating an arrhythmic potential. Although skeletal myoblasts do not have associated pacemaker activity, they may have the ability to induce a tachyarrhythmia.

While Menasche has used a skeletal myoblast injection to induce VT, it is equally plausible that a tumor cell line with contractile activity would also be a candidate for injection into the atrium. Claycomb et al. have developed a mouse-specific, cardiac muscle cell line that retains contractile activity (56). This cell line, termed HL-1, is derived from a culture of AT-1 cells and would have independent pacemaker activity. Unlike other cell lines, HL-1 is unique in that the cells maintain their differentiated cardiac phenotype. Given that HL-1 is a tumor cell line specific for a mouse, its compatibility in a sheep model is unknown. However, the concept of using a tumor cell line that retains contractile activity in order to induce AF is plausible.

One of the primary concerns with using HL-1 (or a similar tumor cell line) to induce AF is the uncertainty over the ability to control tumor development that would allow for careful evaluation of a heart valve under AF. In the case of the HL-1 tumor line, the control of gene expression, and also subsequent tumor growth, may be possible by using the Sleeping Beauty

(SB) transposon system (or a similar method) to down-regulate gene expression. Novel research by Chen et al. explores the use of SB transposons to decrease the expression of a gene associated with the progression of Huntington's disease, and it is possible that a similar system might be used for the regulation of HL-1 expression in an AF model (36). The administration of doxycycline, an antibiotic used in the regulation of gene expression, could be used for the activation of SB (57). Thus, doxycycline administration would activate SB and result in a decreased HL-1 expression. While this approach would be ideal, many questions remain regarding the logistics of such a technique. Research is needed to address questions regarding the feasibility of such an approach in addition to determining whether reduction in HL-1 expression would lead to a loss of either AF, tumor development, or both.

Finally, there also exists the possibility of selecting for cardiomyocytes with pacemaker potential and delivering these cells to the atrium. These cells would not have the tumorigenesis associated with the HL-1 cell line, but they would have the intrinsic pacemaker potential not observed in skeletal myoblasts. A substantial amount of information regarding the use of cardiomyocytes on a therapeutic basis has been produced, and recent investigations by Ruhparwar have suggested the possibility of cardiomyocytes acting as ectopic pacemakers in a canine model (58). The delivery of cardiomyocytes, selected for high levels of pacemaker activity, injected into the atrium could lead to AF.

The decision of which cell line to inject forms only part of the challenge, however, as stem cell injection into the atrial wall creates technical problems not seen when injecting into the markedly thicker ventricular wall. Although injection into the atrial wall is a possibility, another option to facilitate implantation might be to inject the cell line into a coronary vessel supplying the atrium. Given the association between the right atrium and the onset of AF, a viable option would be to target the branch to the sinoatrial node, originating from the right coronary artery, using a catheter-based approach. Another promising site for stem cell delivery would be the region surrounding the pulmonary veins. According to Cox, a premature atrial beat occurring within one of the pulmonary veins excites the left atrium in such a way that a series of macro-re-entrant circuits develops (59) that lead to fibrillation of the atria. Given that pulmonary vein triggers are responsible for a significant number of cases of AF, directing cells with pacemaker potential to these locations may propagate the formation of macro-re-entrant circuits and result in AF.

Because the occurrence of cardiac arrhythmia is highly unpredictable, this method requires long-term

follow up studies of cell transplant recipients that would seem to be essential for understanding the natural course of myoblast and stem cell-induced arrhythmogenesis. In addition, the feasibility of maintaining a persistently unstable myocardium after stem cell injection is unknown at this time. Despite these challenges, stem cells have the potential to provide a non-invasive method of AF that is both reproducible and reliable. The first step in the development of a stem cell model requires further research to elucidate the behavior of the cardiomyocytes when manipulated in isolation and modified *in vivo*.

Conclusions

The association between mitral valve disease and AF is well established (14). In cases of mitral valve disease, prosthetic valve implantation is often indicated. However, as a substantial proportion of patients undergoing valve replacement are also afflicted with AF, the hemodynamic properties of a mechanical valve are altered. Most concerning is the increased thromboembolic morbidity seen in patients with MVR and concurrent AF. Thus, an optimal model of pre-clinical heart valve testing should include animals with AF. Currently, testing is performed in animal models with no underlying cardiovascular pathology, but by testing the prosthetic valves in a fibrillating animal heart the clinical scenario of a patient requiring a prosthetic valve with concurrent AF is modeled more accurately. Herein are described the current and potential methods for AF induction in a pre-clinical model. Pacing techniques - the most established methods for AF induction - lead to undesirable atrial remodeling and the possibility of infection. Moreover, intracardiac insertion of the electrodes is an invasive procedure which causes significant change to the thoracic cavity. The transesophageal approach, although less invasive, has yet to be well established. Genetic manipulation to induce AF is still in its developmental stages, as the identification of genes associated with the onset of AF is ongoing. The pharmacological use of catecholamines and ACh may lead to AF, although questions pertaining to the dosing regimen and the proportion of animals that respond to this approach remain unanswered. Finally, there is the possibility of injecting stem cells to induce AF. When issues regarding which cell line should be injected, and which injection technique should be used, are resolved, this method shows great promise in the induction of AF, both consistently and non-invasively. In summary, future studies are warranted to define the optimal method for inducing AF in animal models for testing of novel mitral valves.

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