Progress in Pediatric Cardiology 35 (2013) 91–94

Contents lists available at ScienceDirect

Progress in Pediatric Cardiology

journal homepage: www.elsevier.com/locate/ppedcard

Animal models for bench to bedside translation of regenerative cardiac constructs

Rachael W. Quinn *

Cardiac Regenerative Surgery Research Laboratories, Cardiology and Cardiac Surgery of The Ward Family Heart Center, Children's Mercy Hospital, Kansas City, MO, United States

ARTICLE INFO

Keywords:
Cardiovascular system
Heart valve prosthesis
Disease models
Animal
Heart

ABSTRACT

Investigators seeking to select an animal model for use in preclinical research studies prior to FDA submission for allograft heart valves have several types to choose from. Dogs, pigs, cattle, primates and sheep have all served as successful medium and large animal models, and their murine counterparts have also been integral in the advancement of replacement heart valve research. While the national and international regulatory bodies have not specified a universal animal model for use in preclinical research studies, the ovine and porcine models have become the frontrunners within the peer-reviewed literature. Sheep are an excellent model of bioprosthetic valve calcification, with a robust mineralization response that mimics observations of human clinical disease progression, while swine are the model of choice for valves which pose a risk of thrombotic events. In the rapidly advancing field of tissue engineered cardiovascular products, dogs, primates, rodents, sheep and pigs are all valuable models for the study of scaffold remodeling and recellularization. Once an animal model has been chosen, investigators are recommended to consult with the FDA via submission of a Request for Designation in order to identify the type of product and the appropriate Center to which the product should be submitted. Additionally, the investigator should outline a risk analysis plan that categorizes the failure modes of the product, as well as design an in vivo pre-clinical safety and performance study that will capture data applicable to the assessment of the failure modes enumerated in the risk analysis.

1. Introduction

A critical step bridging the gap between the laboratory and the clinic is the pivotal in vivo animal study demonstrating safety and performance of the investigational device. For both early stage discovery and confirmatory preclinical chronic animal studies to test replacement heart valves, the peer reviewed literature reports several useful animal models which can be used alone or in combination to assess the safety and performance of implanted valves. It is the goal of this review to give a summary of the animal models that have been most often used in replacement heart valve research followed by an analysis of the applicability of various previously validated or novel alternative animal models for pre-clinical testing prior to regulatory submission, with special focus on the special requirements and constraints for modeling a tissue engineered heart valve (TEHV).

2. Animal Modeling in Pre-clinical Research

Historically, dogs, pigs, baboons, calves, sheep and goats have all been used for heart valve research, but not all have remained favorable models for numerous reasons, including ethics, size, husbandry and physiologic complications of the models. Currently, sheep retain the majority share of in vivo replacement heart valve research, followed by pigs and calves [1]. While rodents also remain an important component of pre-clinical research, especially for evaluating the effects of anti-calcification treatments on tissue valves, their usefulness lies mainly as an inexpensive interim model prior to initiating large animal studies [2] and are not suitable as actual orthotopic valve replacement survival models.

2.1. Canine

Dogs have a long history in cardiovascular research, and have been used successfully in the study of replacement heart valves, with easy husbandry, small size and limited somatic growth (10–30 kg mature size) making them an appealing model [1,3,4]. However, their small size precludes their use in testing larger clinical versions of the valves, and coupled with reports of an increased incidence of sepsis and thrombosis, inconsistent bioprosthetic valve calcification, and significant ethical considerations, canine research has decreased significantly in recent years [4–9]. Dogs continue to be utilized in tissue engineered cardiovascular products research, however, mostly serving as animal models for testing scaffold feasibility, recellularization, and graft patency [4].

2.2. Bovine

Aside from the obvious drawback of rapid and massive somatic growth resulting in large mature size and difficult surgical husbandry

* Tel.: +1 816 234 3519.
E-mail address: rwquinn@cmh.edu.

1058-9813/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ppedcard.2013.08.001

obstacles, calves have been found to be an excellent model for heart valve research. In bovids, bioprosthetic valves are rapidly calcified in a manner consistent with human clinical observations. The larger size of the animal allows for the implantation of valves on the upper end of clinical usefulness [10,11]. However, the rapid growth of calves can lead to the early development of relative valvular stenosis and/or paravalvular leakage, and the species is also susceptible to a higher-than-average rate of congenital atrial septal defects [1,3,11]. Additionally, the naturally greater cardiac output of the growing calf has not been shown to accurately mimic human conditions, unless the intent is to model potential valve patient–prosthesis mismatch, which can be observed as the calf outgrows its valve [1].

2.3. Porcine

Swine have a long history as a surgical research and training species and are increasingly utilized for pharmaceutical toxicology testing [12]. As a cardiovascular disease model, there are well-documented advantages and human similarities, especially of coronary anatomy [13]. Thromboembolism is a major cause of mechanical valve dysfunction, often with catastrophic effects [14], and swine have been found to be an excellent model for capturing valve-related thrombosis and testing blood-materials interactions [1,15,16]. In bioprosthetic valves, however, the primary failure mode is calcification, which is enhanced and accelerated in children [14]. While both glutaraldehyde-fixed bioprosthetic and decellularized bioprosthetic valves implanted in swine have been shown to calcify [17,18], the degradation rates are found to be slower than in other species. Additionally, the usefulness of swine as a model for testing bioprosthetic valves is overshadowed by the difficulties in surgical husbandry and somatic growth. Cardiopulmonary bypass is poorly tolerated by pigs [19–21] and difficulty in maintaining anticoagulation can lead to increased hemorrhagic complications [22]. The rapid somatic growth of pigs, which hampers peripheral venous access and creates husbandry difficulties [3] can also lead to increased perivalvular leakage, exacerbating the restricted valve motion that occurs due to extensive fibrous sheathing of valve leaflets [22].

2.4. Primates

Non-human primates are uncommon in heart valve research, but have been shown to be a viable model, especially the baboon [23–25]. The advantages of the baboon model include their similarity to humans in cardiac anatomy, physiology, valve dimensions and immunology, as well as their ability to tolerate valve replacement surgery and associated cardiopulmonary bypass [25,26], which has been further explored in this volume of Progress in Pediatric Cardiology. Additionally, primates are useful for testing the effectiveness of decellularization techniques developed for human tissue, especially with regards to xenogenic tissue valves with potential transplant antigenicity, as with porcine-derived valves [27]. Xenograft tissues of non-primate mammals (e.g. porcine), prosimians and New World monkeys contain the α-gal epitope (Galα1-3Galβ1-4GlcNAc-R or Galα1-3Galβ1-3GlcNAc-R), to which humans (and apes and Old World monkeys e.g. baboons) produce innate antibodies (the anti-Gal antibody) [28]. The interaction between the α-gal epitopes and the anti-Gal antibodies trigger hyperacute rejection, leading to rapid inflammatory responses and accelerated valve failure [27,28]. The usefulness of the baboon model to determine the relative inflammatory potential of biological and bioprosthetic heart valves is tempered, however, by the fact that the model does not display a satisfactory calcification response [29]. Additionally, the specialized housing and management required, the ethical quandary associated with primate research and the expense of the model limits the likelihood that it will be widely utilized, despite the potential advantage of the data [3].

2.5. Ovine

Sheep are the classic animal model for mimicking the pattern of human bioprosthetic valve calcification [30,31], with prominent calcific deposits developing the in valve cusps and conduit walls in as early as three weeks post-implant [32] and valve calcium content increasing in valve tissue within days of implant [33]. The calcification response of sheep is age-dependent, with juvenile sheep (≤20 weeks of age at implant) showing overall greater calcification potential as compared to older animals (e.g. adolescent sheep, ≤11 months old) [33]. However, the level of calcification in older animals does not preclude their use, as they are still a sensitive model of calcification as compared to other species [33], and may be a useful model for testing larger valve prostheses. Juvenile sheep have similar heart rates, blood pressure, cardiac output and intracardiac pressure to humans, and tolerate anesthesia and cardiopulmonary bypass well [3,30,31,34]. Additionally, sheep have similar blood profiles and platelet aggregation as compared to humans [35], provide fewer challenges for husbandry and surgical management and are excellent subjects for assessing valve functional performance via transesophageal echocardiography [36,37]. There appear to be no specific advantages for goats over sheep and there is far less prior validated research published in that species.

While sheep appear to be the preferred model for biological and bioprosthetic valve evaluation, challenges do arise when studying larger sized valves, due to the inherent anatomical differences between humans and sheep. Sheep have a relatively shorter ascending aorta and more proximal brachiocephalic branches as compared to humans, which can be problematic for valves with higher profiles. Similarly, the anatomic location of the coronary arteries can hinder adequate coronary perfusion during aortic valve replacement and the size and position of larger aortic valves can interfere with the anterior leaflet of the mitral valve.

2.6. Small Mammals

Rabbits and rodents have been used for years for both subcutaneous and non-orthotopic implant studies. Due to their small size, easier husbandry and lower cost as compared to large animal models, rats, mice and rabbits are attractive models for testing the effectiveness of tissue anticalcification agents, along with their mechanisms of action, dose–response relationships and toxicity [32]. Subcutaneous implantation of bioprosthetic heart valve material in rats and rabbits reveals that the calcification response to the material is similar to that observed in human clinical cases and large animal (sheep, calf) models but occurs in a fraction of the time (weeks instead of months) [10,14]. Indeed, rats and rabbits have been shown to be adept at calcifying subcutaneously implanted tissue, with greater rates of calcium accumulation than bovine or avian models [38]. Small mammal models are currently being used to evaluate tissue engineered vascular constructs, although their small size limits the extent to which such vessels can be tested [4]. A significant advantage to small mammals, specifically rats and mice, however, is the ability to purchase various strains of animals that have been bred or genetically engineered to serve as specific disease models (e.g. atherosclerotic, targeted mutation/gene knockouts) [4].

While small mammal subcutaneous implant models are an important step in the development of bioprosthetic valves (i.e. the testing of anticalcification agents), it has been strongly recommended that the results be confirmed using both in vitro testing (e.g. accelerated wear testing) and in a large animal model [32]. Because subcutaneous implant models do not provide adequate contact with the blood, nor do they approximate the varied stresses of the circulatory system, they do not provide adequate representation of the dynamic environment into which the valve is intended to be placed [2,11,32,39,40]. Indeed, it is staunchly urged that replacement heart valves be implanted orthotopically in a large animal model so that the valves can be evaluated under similar parameters to those under which it will function