Swine models to study genetic diseases

David K. Meyerholz, DVM, MS, PhD
Diplomate, ACVP & ACVM

Human diseases can be caused by multiple etiologies including those of genetic origin. Monogenic diseases are caused by alterations in the expression of a solitary gene and often characterized by notable heritability patterns. Polygenic diseases are influenced by multiple genes that can contribute to susceptibility of a phenotype. Study of genetic diseases were enhanced by the development of genetically engineered mice (GEM) decades ago. However, GEM did not serve as optimal models for some genetic conditions due to several features including poor to absent phenotype. The development of somatic cell nuclear transfer (SCNT) techniques allowed for the initial development of genetically engineering swine and other non-murine species. In 2008, the first genetically modified swine (GES) model of a human genetic disease was reported for the condition called cystic fibrosis. This model demonstrated that GES could be created and produce a phenotype similar to the human condition. Since then, numerous swine models of monogenic and polygenic diseases have been developed for study. Swine have been selected as investigational models for several similarities to humans including: size, lifespan, anatomy, physiology, metabolism, etc. In this session, select conditions will be highlighted to exemplify how these GES models have helped advance understanding and treatments for genetic diseases.

Select bibliography of GES models and their impact on select diseases

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Concurrent Session: Pigs as a Translational Model
Tuesday, November 15, 2022; between 8:00 AM and 12:00 PM (40 min)

Dr. Nicole Kirchhof, DACVP

Medtronic, PLC

Pathology of Select Large Animal Models in Medical Device Research

Part 1: Introduction

Medical devices are now an omnipresent part of modern medical care. The locations of device implants combined with their eventual applications appear limitless, and their mode of therapy delivery is broad and will most certainly expand to human diseases in ways we have yet to realize. Preclinical testing is an important element in the development and approval of medical devices. It is often the culmination of very fast-paced technological innovation or improvement. Preclinical studies are performed primarily in large animal species and are sandwiched between bench tests to ensure engineering performance and device longevity, and clinical trials to confirm safety and ensure efficacy. Preclinical testing is a scientific necessity and a regulatory requirement to reveal if a device has the anticipated biological and physiological effects. Well-executed and well-documented preclinical research will accelerate the approval process when it provides sound scientific evidence integrated as safety data to substantiate a claim. Overall, the use of preclinical animal models is currently essential to assess the safety of medical devices.

Besides the physiologic and pathologic challenges in extrapolating animal data to humans (Zilla et al., 2007), the device world is prone to carelessly use the term “animal models”, thus instilling the belief that there is a repository of animal *disease* models that can be used during device development (Schomberg et al., 2016). Current animal models used in device research or regulatory submissions are imperfect anatomical and physiological proxies to the human species. They are predominantly simple surgery

models, with the main intent of the animal recipient being of adequate size to accommodate human-tailored devices. Animal testing is expensive and time consuming and, while necessary, still is touted as ineffective as the animal models utilized do not replicate the complex physiology that influences disease (Schomberg et al, 2016). Therefore, project leaders should prudently complete their risk assessment before conducting and explaining animal studies (U.S. Food and Drug Administration 2015, Durfee and Iaizzo, 2016).

The US Food and Drug Administration has accepted data from swine for biocompatibility testing and the functioning (i.e., safety) of implanted devices in a variety of organs and systems (Swindle et al. 2012, Kirchhof, 2019). There are no strict regulatory guidelines for the species or even the number of animals to be used to reach approval. If the researchers have established beforehand that an animal study is necessary, regulatory bodies expect that a scientific justification will be provided for animal species and number. One approach is to deploy animal species that were previously utilized in support of a very similar, successfully approved device. Another approach is that a preclinical study was conducted in a certain species and the results were reported in peer-reviewed literature. Overall, the animal species and its physiological attributes should provide “a test system that offers a best attempt at simulating the clinical setting” (U.S. Food and Drug Administration 2015, Gad and Schuh, 2018). In addition, preclinical safety or efficacy recommendations for devices are usually based on low animal numbers, abbreviated implant-duration times, and on implant data from healthy animals. A comprehensive understanding of the benefits and of the limitations of different species and the setting the study was conducted in will be of unquestionable importance as well.

When preclinical testing is specifically aimed at implanted or temporarily inserted medical devices, the following main factors need to be considered in regard to the animal model: similarities to human anatomy and physiology, comparable organ sizes allowing treatment with or implantation of actual size medical devices as they are intended for human clinical implant (i.e., the final finished product with the actual clinical design), reproducibility based on low genetic variation among individuals, but also

understanding of unavoidable background lesions and long-term healing effects. Besides dogs and sheep, swine are the major large animal species used in the preclinical testing of medical devices in our facility.

Juvenile farm swine are the default model for nonsurvival surgical training classes (Swindle et al., 2012). The haptics of performing surgery on animals cannot be replaced by mannequins or simulators, so the pig or pig tissues are commonly used in training for interventional catheter techniques, complex trauma procedures, and endoscopic procedures. Apart from implanter training, also prototype devices are widely tested in farm pigs via acute nonsurvival procedures. In regard to survival studies that extent beyond a few weeks, the farm pig's rapid growth rate usually precludes its participation not only as their handling at body weights beyond 100kg is a challenge but also that this rapid growth impacts the sizing ratio to the device, in particular for implantable cardiac or vascular types. Fully grown miniature swine offer the same physiological advantages as the farm pig model, but at the equivalent maturity they are much smaller and therefore, they are the preferred selection for chronic studies. The most widely used miniature swine breed in our facility is the Yucatan minipig. Although several different strains of this breed are available from various vendors, their phenotype, and disposition remains relatively consistent (Nunoya et al., 2007). One disadvantage is their cost: since they are specifically bred for research in relatively small numbers, the cost per animal is typically 3 times higher than for a regular farm swine and 1.5 to 2 times higher than a purpose-bred hound dog for research.

In general, there is low incidence of naturally occurring pathologies described in pigs that can be applied as actual disease models. First, human intervention by way of selective breeding has eliminated genes that increase disease susceptibility. Then, the majority of the domestic farm pigs are slaughtered at a young age (< 6 months old), precluding the detection of late onset diseases such as cancer (Gutierrez et al, 2015). Genetically engineered pigs are vital for gaining a proper understanding of disease mechanisms (Perleberg, 2018), but have yet to find their way into medical device innovation, testing, and approval (Schomberg et al., 2016).

Part 2: Organ-specific medical devices in major large animal models

Amongst others, the most common medical devices tested in pigs or minipigs in our facilities are implanted coronary stents (Nakazawa et al, 2008, Perkins, 2010, Perkins and Rippy, 2019), abdominal aortic aneurysm (AAA) grafts (Zilla et al., 2007), prosthetic heart valves (Tellez et al, 2017), abdominal meshes (Keating et al, 2019), and leadless cardiac pacing devices. Also, neurovascular devices (Spangler and Katzman, 2019) as well as temporarily inserted energy-based devices for renal denervation (Sakakura et al., 2014), or for cardiac (Stoffregen et al., 2019, Stewart et al., 2021), lung (Sebek et al., 2020), and liver ablations (Wang et al., 2020) are usually tested in pigs.

Sheep are preferentially used for the long-term implantation of subcutaneous electronic devices (cardiac- or neurostimulators) with or without absorbable antibacterial envelopes (Virmani et al., in preparation) as well as for the testing of complex lead systems designed for deep-brain stimulation (Cramer et al., 2019), or brain catheters that are used in focal laser ablation in the brain. For these types of studies, sheep are preferred to pigs based on their favorable skull anatomy. Sheep have been the historical model for prosthetic mitral-valve research (Schoen et al., 1994) as adolescent animals show a detrimental tendency for leaflet-calcification similar to that observed in children. These animals continue being used for catheter-delivered sutureless mitral valves (Vahl et al., 2019). Skin implants in the ovine candidates are preferred to the porcine or canine model as sheep are respectively less prone for “pocket infection” and can accommodate more concurrently implanted devices over their larger body surface. Likewise, the sheep heart is also used for leadless cardiac pacing devices (Vatterott et al., 2022).

Dogs were the historical model for developing and refining cardiac leads for pacing and/or defibrillation (including MRI-safety) but are being replaced by pigs or sheep unless the project has matured towards the need of regulatory submission via a pivotal GLP study.

In this session, select devices and their pathology assessments will be shown to demonstrate the principal approach of device pathology (Friedemann et al., 2019;

Rousselle et al., 2019; Stanley et al., 2019). Without exaggeration, pathology data does provide the most relevant measure of the local device healing and its systemic consequences. This information is essential in determining the overall impact, both positive and negative, of a medical device on the body. “Preparing and processing medical device implants for evaluation is a relatively high-risk and high-dollar process in which studies get made and endpoints can be lost with no second chance” (Rousselle and Wicks, 2008).

In closing, large animal models and in particular the pig are currently center-stage in bringing incrementally improved or completely novel medical devices to market. At what time and to what degree computer modeling or human “digital twins” will substitute a living animal in preclinical research, or whether genetic swine models of human disease will have matured into a practical and validated translational platform for testing device efficacy and safety cannot be predicted. Lastly, this review is incomplete as it does not touch on devices that are currently evaluated by device pathologist within their much wider application spectrum (e.g., bone, special senses, peripheral nerve, reproductive organs, other), or preclinical research that involves rodents, rabbits, or other large animals.

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