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Mini-pigs as replacement for non-rodent species

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Abstract

Swine is the optimal model species for investigation of a large number of human diseases and have made valuable contributions to almost every field of human medicine. Similarities in the cardiovascular, urogenital, integument, skeletal and digestive systems of swine to humans have contributed to increased use of pigs in research. Swine offer additional advantages over other species by having a renal anatomy and function very similar to human. Studies have lead to the development of a highly warranted vaccine for various diseases using swine as model. In animal models, whole cell vaccination resulted in hypersensitivity reactions, so new strategies are devised. The first immunogenic molecule described was the major outer membrane protein and this molecule has been studied in great detail as a candidate vaccine. Even though complete protection was not obtained, reduced shedding was observed and vaccine trials in SPF pigs as animal models using naked DNA as a vaccine resulted in stimulation of both the humoral and the cellular immune responses indicating progress in vaccine development. This model is also used in pharmacokinetic studies, evaluating ADME, interspecies variations in CYP subfamily. Further, because of their similarities with humans, pig tissues and organs are currently being used and studied as human xenografts with the potential for increased use in this area in the near future.

Key words : Minipig, xenografts, non- rodent, pharmacokinetic

Introduction

The main purpose of using animals in biomedical research is to ensure suitable animal models for human related validation of valuable research information gathered from experimentation by using such animals and to compare biological phenomena between species (Swindle *et al.* 1986; Vodicka *et al.* 2005). Traditional mammalian models in biomedical research include rats, mice, Guinea pigs, hamsters and rabbits. Genetically engineered, inbred, mutant and hybrid strains of mice have enabled scientists to study discrete genetic anomalies, hereditary or spontaneous diseases homologous to the human condition. Information about the usefulness of rodent and other small animal models to biomedical research is voluminous. Although the rodent and primate species have well-defined genetic backgrounds and are economically suited to most research settings, larger domestic animals are proving to be valid research models because of their anatomical and physiological similarity to humans since they have similar cardiovascular structures, multiparous nature, omnivorous habits and are of useful size. Also, the genome of most domestic species is well characterized. Miniature pig is used in wide range of research fields, such as medicine and pharmacology, because of its small size, the possibility of breeding it under minimum environmental controls and the physiology that is potentially similar to that of human (Vodicka *et al.* 2005; Uchida *et al.* 2001). Researchers continually identify or develop new animal models to evaluate pathogenic mechanisms, diagnostic and therapeutic procedures, nutrition and metabolic disease and the efficacy of a novel drug development. Animal models for large animal and ruminant research are also required to conduct studies on diseases in such animals.

Swine

Pigs are kept in almost every part of the world to produce meat for human consumption. Their ancestor is the European wild boar (*Sus scrofa*) crossed with pigs of East Asian origin (*Sus vittatus*). Wild and domesticated pigs will readily mate, thereby producing fertile off springs. The University of Wisconsin attempted to raise disease free pigs as early as 1943 and again in 1950 (Abelth, 1962). Swine have been useful for biomedical research for the past four decades. The same applies to miniature pigs, which are bred to serve as laboratory animals primarily for biomedical research. Characteristics that make them particularly useful for this purpose are similarities in structure and function between pigs and human beings regarding size, feeding pattern, digestive physiology, dietary habits, kidney structure and function, pulmonary vascular bed structure, coronary artery distribution, respiratory rate, tidal volume and social behavior. Pig provides an adoptable model to evaluate acute and chronic exposure to xenobiotics such as alcohol, caffeine, tobacco, food additives and environmental pollutants. Severe shortage of organs and tissues for transplantation in patients with severe organ failure has encouraged the consideration of interspecies xenotransplantation (Yamada et al. 2005). Pig is generally regarded as donor because of its likeliness in its size, physiology and availability. Pigs harbor relatively few diseases which could be transmitted to human patients. Due to advanced genetic engineering, genetically modified pigs ameliorate consequences of the graft rejection from human side.

Miniature breeds currently used in Biomedical Research:

Hormel Miniature – Sinclair (Skavlen *et al.* 1986; Svendsen, 2006; Wang *et al.* 2007) - First developed at Hormel Institute at the University of Minnesota in 1949. Primary cross between Guinea hogs from Alabama and wild boars from Catalina island.

Hanford Miniature (Smith *et al.* 1990; Svendsen, 2006) - Initially developed at the Hanford Laboratory (later known as Battelle Northwest Research Laboratories) in Richland, Washington in 1958.

Goettingen Miniature (Bollen and Ellegaard, 1997; Svendsen, 2006) - Developed in 1980 at the University of Goettingen in West Germany from a cross between a Minnesota miniature and a small Vietnamese pig.

Ohmini - Developed in Japan in 1945 from crosses of Manchurian pigs with Hampshire, Duroc domestic, and Hormel miniature. They are black with course hair coat exceptionally long ears and wrinkled skin. Average one year old weight is 35 kg.

Lee Sung - Developed in 1975 at the University of Taiwan from Taiwanese small-ear pigs. Average one year weight is 30 kg.

Yucatan Miniature (Smith *et al.* 1990; Svendsen, 2006) - Native of Southern Mexico, Costa Rico and other areas of Latin America.

Indiana University School of Medicine and Purdue University Scientists have the only research and large scale breeding colony of Ossabaw pigs in the world that are certified to express the metabolic syndrome phenotype and heart disease.

Minipigs in Biomedical Research

Minipigs are already playing a significant role as laboratory animals in the areas of physiology, pharmacology, toxicology, radiology, surgery, organ transplantation, traumatology, pathology, embryology, gastroenterology, nephrology and pediatrics (Tetsuo *et al.* 2007). Both domestic and miniature swine have been used extensively in cardiovascular research. The pig has been used extensively for studies concerning the metabolism of fats and carbohydrates as studies in obesity, diabetes mellitus, atherosclerosis and hypertension. Swine have also been useful in elucidating the relationship between exercise and cardiopulmonary function. Due to size and anatomic and physiologic similarities, swine have been used extensively in surgical research.

Swine are animal species in which cardiovascular anatomy and physiology resembles human beings (Hughes, 1986; Swindle *et al.* 1986). There are many similarities; size and distribution of the arteries, hemodynamics and similarities in capillaries (Smith *et al.* 1990). Anatomy of coronary vessels resembles that of human beings making pig as an excellent model for study of regional myocardial ischemia.

The heart to body size ratio of 25-30 kg pigs is identical to that of humans. They have platelet coagulation system related more closely to humans than most other species. Pigs are one of the animals that develop atherosclerosis naturally and have a high incidence of end artery lesions. The severity of atherosclerosis can be increased by feeding diets high in fat and cholesterol. Atherosclerotic plaques observed in domestic and miniature pigs possess close resemblance to human conditions (Spurlock and Gabler, 2008). The combination of an atherogenic diet and arterial injury in pigs create a lesion typical of human atherosclerosis than diet alone. Pigs are considered as model of choice for studying effects of luminal injury of coronary artery, neointimal thickness resulting from vascular injury is directly proportional to the depth of the artery injury (Hamburger *et al*. 1991).

In view of more morphological and physiological similarities between human and porcine skin compared to other laboratory animal species, the mini pig is considered to be preferred model to evaluate the safety profile of dermally applied xenobiotics (Mahl *et al.* 2006). Techniques in plastic and reconstructive surgery have been developed using porcine models due to the similarities in skin anatomy and wound healing. Stem cells derived from epidermis of the miniature pigs used as a cell therapy for skin repair and as a model for squamous carcinoma of the head and neck. Asymmetric mitosis allows them to produce one daughter cell with the properties of stem cells (self-renewal) and a second cell with characteristics of progenitor cells, or transit amplifying cells, which proliferate quickly but with a limited number of mitotic divisions. Porcine epidermal stem cells, located in the bulge region of the outer root sheath of hair follicles, migrate *in vitro* from hair sheaths and because they are resistant to anoikis (detachment induced apoptosis); survive in non-adhesive conditions to form spheroids (Motlik *et al*. 2007). Due to easy monitoring of external skin changes, pigs has been extensively used in dermal studies, evaluation of both local and systemic toxicity and dermal absorption through skin flap research and also in *In vivo* wound healing and dermal irritation by topical drugs. It is also used in wound induction and testing efficacy of wound healing material. It is also used as a laboratory model in photodermatological research where there will be sunburn cells formation by UV-radiation (Rausch *et al*. 2003).

Since the male accessory sex glands and urethra of swine are similar in size and structure to the human male and the fallopian tube is similar to the human female, these organs have been used to develop microsurgical techniques for human genitourinary tract disorders (Sampaio *et al.* 1998).

Miniature pigs have been introduced as a model in pharmacology and toxicology, because of scientific, economic and ethical reasons (Bollen and Ellegaard, 1997). This model is also used in pharmacokinetic studies, evaluating ADME, Interspecies variations in CYP subfamily in non-clinical drug delivery systems, bio-availability, bio-distribution and bio-equivalence for new drugs. Miniature pigs become a popular substitute for the traditional non-rodent species although little information is available on its P450 system (Skaanild and Friis, 1997; Pavel *et al.* 2001). Cytochrome P450 (CYP) of the 3A family (CYP3A) has been detected in mini pig liver microsomes by immunochemical screening (Western blotting), revealing bands that co-migrate with human CYP3A4 and 3A5 (Anzenbacher *et al.* 1998). Appropriate animal model relevant to human metabolism is crucial. Dogs for CYP2D, monkeys for CYP 2C, minipigs for CYP3A which are the main human liver enzymes involved in biotransformation where CYP2A, CYP2C and CYP3A do have similar N-terminal sequence (Skaanild and Friis, 1997; Pavel *et al.* 2001). It has become obvious that minipigs can be used for all routes of administration, and in many cases are preferable to dogs or primates for metabolic or pharmacological reasons. Their use in general toxicology testing employing the continuous intravenous infusion, dermal or inhalation route has been described in detail in the literature. Background data on toxicological endpoints (ophthalmology, clinical pathology, ECG, organ weight, histopathology and reproduction parameters) have been well-established allowing studies to be interpreted (Kvetina *et al.* 1999). There are advantages over the traditional non-rodent species in relation to the ethical difficulties of use of animals in biomedical research. Consequently, there are scientific, economic and sociological reasons that make minipigs good toxicological and pharmacological models (Svendsen, 2006).

The oral maxillofacial region of miniature pigs is similar to that of humans in anatomy, development, physiology, pathophysiology, and disease occurrence. As the size of jawbone is as thick as human beings mini pigs are used as models in dental surgery and as disease models. Adult stem cell originated from pulp show that new dental materials can be created and implanted (Wang *et al*. 2007).

Improved methods in pharmaceutical, anesthesia administration and the effects of malignant hyperthermia are of considerable importance in evaluation of pharmacogenetic disease of humans and pigs which is possible using swine models. Pigs are born with a similar degree of maturity and have similar growth patterns to human newborns. For this reason, newborn swine have been used to develop a variety of techniques employed today in pediatric surgery and neonatal care. Gnotobiotic piglets serve as a useful animal model for studies of human rotavirus infections, including

disease pathogenesis and immunity Daisy *et al.* 2005). An advantage of piglets over laboratory animal models is their prolonged susceptibility to human rotavirus-induced disease, permitting cross protection studies and for the analysis of active immunity. These studies thus have established basic parameters related to immune protection in the piglet model of human rotavirus-induced disease, verifying the usefulness of this model to examine new strategies for the design and improvement of human rotavirus vaccines.

Xenotransplantation, in particular the transplantation of pig cells, tissues and organs into human recipients (Takeuchi et al. 2005). The current trend in xenotransplantation research is to overcome the challenges of hyper acute, acute vascular and T-cell-mediated rejection with hopes of developing a clinically feasible xenotransplant program (Fiane and Mollnes, 1999; Niemann and Rath, 2001). Previously, xenotransplantation programs were expected to involve the transplantation of primate organs into humans. Significant ethical issues were raised, including the appropriateness of using, engaging sociable primates for the tissues or an organ. The realities of establishing a clinical xenotransplant program quickly shifted attention to other animals as sources of organs. *In vitro* production of embryos, generation of identical multiples, and transgenic pigs useful for xenotransplantation (Niemann and Rath, 2001). It was found that Old World monkeys, baboons, and chimpanzees were in too short supply to provide a reasonable source of organs (Ye *et al.* 1994). Focus quickly shifted to the pig, an animal that has been bred for food for centuries, with millions slaughtered each year for food (Dehoux and Gianello, 2007).

Conclusion

Swine are considered to be optimal model species for investigation of a large number of human diseases. Pigs have a six layer, diffuse epitheliochorial placentation, which resists transmission of agents *in utero*, subsequently the young ones are less likely to acquire maternally derived diseases and do well under human foster care. Swine are the optimal model species for investigation of a large number of human diseases and have made valuable contributions to almost every field of human medicine. Swine share anatomic and physiologic characteristics with humans that make them ideal models for research. In addition, the anatomy and physiology make pig organs likely candidates for xenotransplantation. Similarities with humans, pig tissues and organs are currently being used and studied as human xenografts. Hence they can be utilized as alternative model to non-rodent species in near future.

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