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ANIMAL MODELS FOR THE TREATMENT OF HUMAN DISEASES – A REVIEW

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Abstract

Currently, studies conducted on animal models provide new insight into the aetiology and course of various pathological conditions in humans. Due to the cause of disease, animal models can be divided into two categories: natural models, such as nude mice, and induced models, such as transgenic pigs. This article discusses several examples of animal models and their contributions to the development of effective therapies for human diseases such as muscular dystrophy, cystic fibrosis, and atherosclerosis. In addition, we presented the latest news on gene therapy, xenotransplantation, and drug trials in humans and animals.

Key words: animal models, natural models, induced models, transgenic models, xenotransplantation, human trials

Currently, animal models of human diseases are essential tools for expanding the knowledge about the aetiology, course and treatment of human disorders. An animal model can be defined as a living organism in which normative biology or behaviour can be studied, in which spontaneous or induced pathological processes can be investigated, or in which the phenomenon in one or more aspects resembles the same phenomenon in humans or other species of animal (Khorramizadeh and Saadat, 2020). The history of the use of animal models in biomedical research dates to ancient times. The earliest studies were conducted by Erasistratus of Alexandria, in the years 302–258 BC, who investigated heart and respiratory system function in pigs. Since then, considerable progress in this branch of medical research has been made, and particular breakthroughs have occurred in the second half of the 20th century. During this period, research on animal models enabled the elucidation of the pathogenesis of diseases such as cystic fibrosis, rheumatoid arthritis and spongiform encephalopathies. Not all species are suitable as models for the investigation of pathophysiological processes. The ideal animal models for general use in research should mimic human disease as closely as possible, be easily accessible and inexpensive to maintain, live long enough to obtain meaningful results, be properly sized to provide multiple samples, be easily handled by most investigators, be suitable for the study and fit available animal housing facilities (Mukherjee et al., 2022).

Depending on the cause of the pathological condition, animal models may be grouped into two categories: natural and induced models. The disease occurs spontaneously in natural models. Most natural models involve mouse and rat models; however, a wide range of mutants of different species have been reported. Induced models are healthy animals in which pathological processes or diseases result due to experimental modifications. Modifications used to create induced models may be broken down into the following groups: genetic modifications (knockout, transgenesis), surgical modifications, chemical modifications and modifications induced by an abnormal diet (Khorramizadeh and Saadat, 2020).

Natural models

For several years, animal models caused by spontaneous mutations have been used to study the relationship between cause and effect in pathophysiological processes. An animal model widely used for biomedical research is the so-called athymic nude mouse, whose phenotype and functional defects are caused by spontaneous mutation of the *foxn1* gene. The discovery of this animal model in 1962 was a breakthrough in cancer research (Sundberg and Rice, 2023).

Another naturally occurring model is the *mottled* mouse model, which carries mutations in the *atp7a* gene and is an animal model of Menkes disease. Menkes disease is an X-linked lethal disorder leading to defects in copper metabolism. Most patients die before the third year of life due to progressive neurodegeneration and connective tissue abnormalities (Kochanowska et al., 2008). There are at least 35 different mutations in the *mottled* locus, resulting in a wide spectrum of neurological and connective tissue defects. Most of these mutations arise spontaneously. The treatment strategies under-

taken in mice play a key role in developing therapies for patients with Menkes disease. Therapy for both animals and humans involves injecting copper. In humans, copper is combined with D-penicillin, vitamin E or C and histidine. The supply of copper should be initiated before the clinical manifestations of the disease occur, ideally within the first two months of life. According to various reports, improvements in psychomotor development and delays in the neurodegenerative process were observed in treated patients (Lenartowicz et al., 2015).

Two naturally occurring animal models for Duchenne muscular dystrophy have been described: *mdx* mice and golden retriever muscular dystrophy (GRMD) dogs. An abnormal phenotype in *mdx* mice is caused by a single point mutation in exon 23 of the dystrophin gene, which leads to a premature stop codon. In turn, the GMRD dog carries a point mutation in the splice acceptor site in intron 6 of the orthologue X-linked dystrophin gene, resulting in the absence of the protein in the muscles (Gaina and Popa, 2021). Generally, there are no current therapeutic strategies available for DMD patients. The application of corticosteroids such as prednisone to DMD patients results in only partial improvements in muscle strength and function. Animal models of Duchenne dystrophy are widely used to develop gene therapy strategies. Gene therapy involving the injection of an adenoviral vector expressing a synthetic utrophin into the muscles of newborn GMRD dogs has been introduced. Utrophin is a dystrophin homologue with a comparable structure. In muscles in which transgenic utrophin is efficiently expressed, reduced fibrosis and increased expression of dystrophin-associated proteins (DAPs) have been observed. In the muscles of DMD patients, DAP expression is considerably decreased. These findings demonstrated that intramuscular injections of mini-utrophin can compensate for a lack of dystrophin in muscles, leading to improvement of the GMRD phenotype (Kinter et al., 2014). Further experiments were conducted to investigate the influence of intramuscular injection of the AAV1-cMinidys vector (adeno-associated virus (AAV) vector carrying the miniaturized dystrophin gene) on the phenotype of *mdx* mice. Intramuscular injection of the AAV1-cMinidys vector into *mdx* mice reduced fibrosis and myofiber central nucleation. The results revealed the therapeutic impact of minidystrophin on pathological defects in the muscles of *mdx* mice (Manini et al., 2021).

By selective breeding techniques, some type 1 diabetes symptoms have been detected in nonobese diabetic (NOD) mice and biobred (BB) rats. Type 1 diabetes is caused by the autoimmune destruction of insulin-producing B cells within the pancreatic islets of Langerhans and elevates glucose levels in the blood and urine. Unfortunately, therapeutic strategies effective in animals, including insulin and nicotinamide administration, have yielded disappointing results in humans (Kottaisamy et al., 2021). On the other hand, rodent models are useful in studies on the influence of specific viruses on the course of disease phenotypes. For example, infection with the mouse hepatitis virus reduces the probability of diabetes in NOD mice, whereas infection with the Kilham rat virus increases the risk of disease in BB rats (Mine et al., 2020).

An example of a natural pig model is a swine with a spontaneously occurring, dominant mutation in the *COL10A1* gene resulting in a dwarf phenotype. Genetic, biochemical, and histological research conducted on dwarf pigs indicated that these animals are useful models of Schmid metaphysis chondrodysplasia (SMCD) (Zhang et al., 2023).

Induced models

Since it is possible to induce specific pathological processes relatively rapidly, induced models are widely used in various studies. Scientists researching induced models should consider that the cause of symptoms in animal models is often different from the aetiology of the corresponding disease in humans. Therefore, treatment therapies effective in induced models often do not yield satisfactory results in human trials.

Knockout models

Due to improvements in genetic engineering in recent years, genetically modified animals are the most used materials in biomedical research. A genetically modified model is an animal in which a new gene is inserted and integrated into the genome (transgenesis) or an endogenous gene has been inactivated through homologous recombination (knockout). Inactivation of the gene may involve one or more tissues or all body tissues. The first knockout mouse was generated in 1989 by Sir Martin Evans, who, along with Mario Capecchi and Oliver Smithies, was awarded the Nobel Prize in Medicine in November 2007 for his pioneering achievement. Currently, knockout mice are indispensable tools for investigating the mechanisms of human diseases.

In 1992, the first cystic fibrosis (CF) mouse model was generated (Dorin et al., 1992). CF is a fatal genetic disease involving impaired ion transport across several epithelial tissues. Gastrointestinal complications of the disease are observed first in neonates; however, pulmonary complications are the cause of mortality among patients. Two strategies are used to create mouse models of cystic fibrosis. The first involves the disruption of the CFTR gene in embryonic stem cells. The result of this strategy is undetectable mRNA and CFTR protein levels. The second is based on the insertion method, which disrupts the endogenous CFTR gene. In this case, the CFTR mRNA level decreased. This finding is significant because there is a strong correlation between mRNA levels and mortality in animals (McCarron et al., 2021). There are many similarities between murine models and patients with cystic fibrosis, for instance, changes in respiratory intestinal epithelial electrophysiology, intestinal pathology, osteoporosis, and pancreatic failure. Murine models of CF are useful tools for developing new treatment strategies and improving patient quality of life. PTC124 is a crucial drug for developing human trials for genetic disorders caused by nonsense mutations. PTC124

permits ribosomes to read through premature stop codons in mRNA. As a result, functional proteins are produced. A novel mouse model with gut-specific expression of a human CFTR-G542X transgene was created. Oral or intravenous administration of PTC124 effectively suppressed the hCFTR-G542X nonsense mutation in vivo and promoted the expression of functional CFTR (Crawford al., 2021). In 10% of patients, cystic fibrosis is caused by a nonsense mutation that prevents the formation of full-length functional CFTR protein. Currently, PTC124 therapy is successfully used to treat this group of CF patients (Sharma et al., 2020).

The p53 protein is a crucial tumour suppressor involved in preventing cancer development. In humans, p53 mutations induce Li–Fraumeni syndrome, which is associated with susceptibility to various tumours, such as breast carcinomas, brain tumours, and bone sarcomas. Studies examining the influence of small doses of vitamin E (VE) in the maternal diet on the postnatal tumorigenesis of embryos have been conducted. VE-exposed p53 +/- offspring are characterized by a decreased risk of spontaneous postnatal tumorigenesis. The survival of these mice was twice as high as that of control mice (Chen et al., 2009).

Knockout mouse models are also widely used in neurological research. Dopamine active transporter (DAT) knockout (DAT-KO) mice exhibit distinct alterations in dopamine homeostasis. Dopamine is a neurotransmitter responsible for the regulation of emotions and locomotor activity. The dopamine transporter (DAT) is a protein involved in the reuptake of dopamine into presynaptic structures. DAT-KO mice may be a plausible model for many diseases associated with compromised dopamine transduction in humans, such as hyperactivity syndrome with impaired attention in children (ADHD). Treatment applied in murine models of ADHD demonstrated that hyperactivity caused by high extracellular levels of dopamine can be lowered by drugs that activate the serotonergic system. Moreover, antagonists of the 5-HT2A serotonin receptor reduce the behavioural deficits of DAT-KO mice, suggesting that specific antagonists of the 5-HT2A receptor may be efficient in ADHD treatment (Leo and Gainetdinov, 2013).

Transgenic animals

Transgenic animals are those in which a segment of foreign DNA is incorporated into their genome. Currently, the most common method of transgenic animal production is CRISPR/Cas9 technology (clustered regularly interspaced short palindromic repeats/Cas9). Because of their availability, the ease of breeding and the short reproductive cycle, transgenic mice are crucial for studying gene expression and causes of pathological conditions in humans. However, larger transgenic animals can be used both as bioreactors and as models for some specific human diseases.

Due to the short lactation period, transgenic rabbits are valuable bioreactors and are used to produce therapeutic proteins in milk, such as C1-esterase inhibitor (Ruconest®) for hereditary angioedema in 2010 (EU) and 2014 (USA), Factor VIIa (Sevenfact®) for haemophilia A or B and inhibitors in 2020 (USA) (Matsuhisa et al., 2020), human α-glucosidase (Sariyatun et al., 2021), and human nerve growth factor (hNGF-b) (Zeng et al., 2022). Furthermore, transgenic rabbits are useful for identifying diseases such as atherosclerosis, cancer, AIDS, hypertrophic cardiomyopathy, and diabetes mellitus. To date, transgenic rabbits expressing human apolipoproteins (apo(a), apoA-I, apoB, apoE2, and apoE3), hepatic lipase (HL), lecithin cholesterol acyltransferase (LCAT), lipoprotein lipase (LPL), 15-lipoxygenase (15-LO), and matrix metalloproteinase-12 (MMP-12)) have been generated (Matsuhisa et al., 2020). Moreover, human transgenes (apoA-I, LCAT, apo(a), and LPL) have been incorporated into the genome of Watanabe heritable hyperlipidaemic (WHHL) rabbits, which are deficient in low-density lipoprotein (LDL) receptors. Studies carried out on transgenic rabbits have led to an understanding of the mechanisms underlying lipoprotein metabolism and atherosclerosis (Matsuhisa et al., 2020).

Due to their anatomical, physiological and genetic resemblance to humans, pigs are beneficial models for biomedical research. Transgenic pigs can be used both as models of human diseases and as organ donors for xenotransplantation. A porcine retinitis pigmentosa (RP) model was generated for the first time in transgenic pigs. RP patients suffer from photoreceptor loss and night blindness. Transgenic pigs that express a mutated rhodopsin gene have many phenotypes in common with humans. Therapeutic strategies valuable for human patients are being developed.

The high resemblance between the cardiovascular systems of humans and pigs makes the pig an ideal model for studying human cardiovascular diseases. For instance, transgenic pigs overexpressing nitric oxide synthase (eNOS) have provided new insight into regulating muscle metabolism and the cardiorespiratory system (Dawson et al., 2017).

Another field of biomedical research involving transgenic pigs is the production of organs and tissues for xenotransplantation. In 2022, approximately 150 000 successful allotransplantations were performed worldwide. On the other hand, the shortage of human organ donors is a worldwide concern. There is a discrepancy between the demand and availability of human organs, and researchers are investigating alternative sources of organs for transplantation. Because the size of organs and their physiological capacity are almost identical to those of humans, xenografts from domesticated pigs are the best alternative. Several obstacles must be overcome for successful xenotransplantation, such as xenograft rejection or the transmission of pathogens from the animal donor to the human recipient (e.g., porcine endogenous retroviruses (PERVs)). The humanization of pigs by genetic modifications minimizes the risk of serious rejection. The first step in obtaining humanized xenografts was the production of genetically modified pigs

defective in the α1,3-galactosyltransferase gene (Dai et al., 2002; Phelps et al., 2003; Tanihara et al., 2020). Another swine valuable for xenotransplantology is pigs in which human α1,2-fucosyltransferase and GGTA1 (α1,3-galactosyltransferase) are knocked out, which may be advantageous for overcoming the immunological barriers between pigs and humans. These studies resulted in the generation of a line of genetically modified pigs (including homozygous individuals) (Jura et al., 2006; Wiater et al., 2021). Further studies have shown that perfusion of an isolated transgenic pig heart causes a delay in acute phase rejection (Skarysz and Bochenek, 2006).

In recent years, researchers have successfully transplanted kidneys from pigs into rhesus monkeys, with the transplants functioning for 435 days. In addition, pig hearts transplanted into baboons that still had functioning hearts survived for 945 days. However, in the latter case, the transplanted heart was not essential to the recipient's life. To date, life-supporting pig-to-baboon transplants have been performed for 197 days (Knosalla, 2018). Another step forward in xenotransplantation was achieved by the production of transgenic pigs with multiple system gene modifications: PERV/GGTA1/CMAH/B4GALNT2/hCD46/hCD55/hCD59/hTHBD/hTFPI/hCD39/hB2M/HLA-E/hCD47, which resulted in inactivation of porcine endogenous retrovirus, deletion of the α-Gal epitope and inactivation of the complement system, regulation of the coagulation system, inhibition of NK cell-mediated xenograft rejection and inhibition of macrophage-mediated xenograft rejection (Yue et al., 2021). Moreover, in 2020, the FDA approved the first-of-its-kind intentional genomic alteration of domestic pigs for potential therapeutic uses in human food. Alterations in the genome of modified pigs are intended to eliminate alpha-gal sugars on the surface of their cells. Therefore, GalSafe pigs could be used as a source of medical products, such as the blood-thinning drug heparin, which is free of detectable alpha-gal sugars. The use of tissues and organs from GalSafe pigs could address the issue of immune rejection in patients receiving xenotransplants, as alpha-gal sugar is believed to be a cause of rejection in patients (https://www.fda.gov/news-events/press-announcements/fda-approves-first-its-kind-intentional-genomic-alteration-line-domestic-pigs-both-human-food).

Recently, it was reported that surgeons at NYU Langone Health had transplanted pig kidneys – with only one genetic modification (GGTA1 knockout) and without experimental medications or devices – and replaced the function of a human kidney for at least 32 days without being rejected (https://nyulangone.org/news/pig-kidney-xenotransplantation-performing-optimally-after-32-days-human-body). Moreover, it was recently reported that researchers have coaxed human stem cells to form early-stage human kidneys in pigs – the first time a human organ has been produced in another animal. Advances, stem cell researchers say, could bring pig-grown human organs closer to reality, offering a new solution for many people on waitlists for transplants (Wang et al., 2023).

Surgical induced models

Two examples of surgically induced models are ovariectomized rats (OVX rats) and murine models of ischaemic stroke. In OVX rats, ovariectomy significantly modifies collagen type II, leading to articular cartilage erosion. Moreover, the application of exogenous oestrogen or selective oestrogen receptor modulators (SERMs) to OVX rats inhibited the progression of these processes. Thus, OVX rats may be a valuable tool for identifying appropriate treatments for postmenopausal osteoarthritis (OA) (Pang et al., 2023).

Stroke is one of the three most common causes of death among adults. Most strokes (approximately 87%) are the result of ischaemia. Global cerebral ischaemia can be induced by cardiac arrest (via ventricular fibrillation) or mechanical compression of the blood vessels in the neck or inside the chest. Focal cerebral ischaemia may be caused by injecting a blood clot or a synthetic material such as collagen, silicone, or silver microspheres, leading to intravascular blood clot formation. The recombinant tissue plasminogen activator (rt-PA) alteplase is the only approved therapy for acute ischaemic stroke. Among more than 700 drugs that are effective in animal stroke models as new free-radical tapering agents, NXY-059 has yielded satisfactory results in human treatment (Zeng et al., 2023).

Chemically induced models

Parkinson's disease (PD) is a neurodegenerative disease affecting 1% of people aged over 55 years. Substances that inactivate the catecholaminergic system are used to induce symptoms of Parkinson's disease in mice. The Raserpine-induced model is useful for studying the therapeutic effects of dopamine replacement agents such as L-DOPA and dopamine receptor agonists. An MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced model is crucial for understanding the role of mitochondrial dysfunction in Parkinson's disease and provides potential neuroprotective therapies, such as drug treatments and dietary alterations (Konnova et al., 2018).

The fat diet and administration of aloxan to Yucatan minipigs have led to the development of a diabetic dyslipidaemia model. Attempts to study the impact of atorvastatin on atherogenesis and diabetes in a diabetic swine model have been undertaken. Atavistic is a cholesterol-lowering drug. These experiments indicated that atorvastatin reduces total and very low-density lipoprotein (VLDL1) triglyceride levels and prevents atherosclerosis. In contrast, atorvasin has a minimal influence on the dyslipidaemic profile or LDL/HDL (low-density lipoprotein/high-density lipoprotein) ratio. The diabetic dyslipidaemic Yucatan minipigs also allowed the exploration of microvascular disease. It is a pathological condition associated with diabetes mellitus that leads to the destruction of small arterial branches. Studies on diabetic Yucatan pigs have demonstrated that these animals have thicker retinal capillary basement membranes than nondiabetic pigs. This model can be a valuable tool for understanding the mechanisms underlying diabetic retinopathy (Curtasu et al., 2019).

Table 1. Animal models for the treatment of human diseases

Animal model	Model type	Modifications	Human disease/application
Mouse – *athymic nude mice*	natural	*foxn1* mutation	cancer
Mouse – *mottled mice*	natural	*atp7a* mutation	Menkes disease
Mouse – *mdx mice*	natural	single-point mutation in exon 23 of the dystrophin gene	Duchenne muscular dystrophy
Dog – *golden retriever muscular dystrophy (GRMD) dog*	natural	point mutation in the splice acceptor site in intron 6 of the orthologue X-linked dystrophin gene	Duchenne muscular dystrophy
Mouse – *nonobese diabetic (NOD) mouse*	natural	type 1 diabetes symptoms	type 1 diabetes
Rat – *bio breeding (BB) rat*	natural	type 1 diabetes symptoms	type 1 diabetes
Pig – *dwarf pig*	natural	dominant mutation in the *COL10A1* gene	Schmid metaphyseal chondrodysplasia (SMCD)
Mouse – *cystic fibrosis mouse model*	knockout	disruption of *CFTR* gene	cystic fibrosis (CF)
Mouse – *p53 mice*	knockout	p53 deficiency	wide variety of tumours such as breast carcinomas, brain tumours, bone sarcomas
Mouse – *DAT-KO (dopamine active transporter knockout) mice*	knockout	*dopamine* decreased level	attention-deficit/hyperactivity disorder (ADHD)
Rabbit – *Watanabe heritable hyperlipidaemic (WHHL) rabbits*	incorporation	human transgenes (apoA-I, LCAT, apo(a), LPL)	lipoprotein metabolism dysfunction and atherosclerosis
Pig – *retinitis pigmentosa (RP) pig*	incorporation	mutated rhodopsin gene	retinitis pigmentosa (RP)
Pig – *overexpression of nitric oxide synthase (eNOS)*	incorporation	nitric oxide synthase (eNOS) gene	muscle metabolism and the cardio-respiratory system diseases
Pig – *α1,3-galactosyltransferase pig*	knockout	deficiency of the Galα1,3Gal (Gal) oligosaccharide	organs xenotransplantation
Pig – *human α1,2-fucosyltransferase pig*	incorporation	human α1,2-fucosyltransferase overexpression	organs xenotransplantation
Pig – *PERV/GGTA1/CMAH/B4GALNT2/hCD46/hCD55/hCD59/hTHBD/hTFPI/hCD39/hB2M/HLA-E/hCD47*- multiple system gene modifications	knockout incorporation	inactivation of porcine endogenous retrovirus, deletion of α-Gal epitope and inactivation of complement system, regulation of coagulation system, inhibition of NK cell-mediated xenograft rejection and inhibition of macrophage-mediated xenograft rejection	organs xenotransplantation
Rat – *ovariectomized (OVX) rats*	surgical	ovariectomy causes a significant modification of collagen type II leading to articular cartilage erosion	postmenopausal osteoarthritis (OA).
Mice – *induced symptoms of Parkinson's disease*	chemically induced	Raserpine induced	Parkinson's disease
Pigs – *Yucatan minipigs*	chemically induced	fat diet and administration of aloxan	diabetic dyslipidaemia – type 2 diabetes microvascular disease – diabetic retinopathy
Pigs – *Yucatan minipigs*	diet	overfed	obesity and insulin resistance

Recently, a model for childhood obesity and insulin resistance was generated without chemical induction. Four-month-old Yucatan pigs were maintained on a Western-style diet until adulthood. These overfed pigs may be crucial for studying the relationship between a fat diet and mechanisms of obesity development (Lee et al., 2012).

Scientists have been using animals to help model human diseases for over one hundred years. Animal models are the best tools for understanding the aetiology and course of a specific human disease. This information is critical for furthering our understanding of disease mechanisms, discovering novel targets for treatment, and translational research. The availability of many standardized strains of model mice has allowed the development of effective therapies. However, mice lack the genetic diversity found in human populations, and consequently, results obtained from mouse models may not fully apply to humans. Therefore, natural, induced or genetically modified animals of other species, such as rats, rabbits, sheep, pigs or dogs, are used in this type of research. However, due to breed diversity, these models are less standardized than mouse models. This sometimes translates into discrepancies in the results obtained. It seems that artificial intelligence (AI), which is increasingly used in various areas of human activity, especially in science, will shortly allow the development of better research models that are helpful in human disease treatment. Perhaps AI will completely replace animal models.

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