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REVIEW ARTICLE

Albino Wistar Rat Models in Cardiovascular Disease Research

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ABSTRACT

Cardiovascular disease research is one of the heavily concentrated areas in biomedical sciences as these group of disorders affecting the heart and blood vessels are the leading cause of mortality and morbidity worldwide. Research on cardiovascular disease should therefore focus on distinct etiological variables involving genetic and epigenetic factors, different pathophysiological pathways used in disease circumstances, and responses to various therapeutic approaches. Such research outcomes are provided through pre-clinical and clinical trials for future consideration. Such conclusions are equally based on in vitro and in vivo experiments. To explore the in vivo research on cardiovascular illnesses, animal models are used. Therefore, choosing an appropriate animal model is a crucial component of any biological investigation. A combination of in vitro and in vivo experiments in several animal models can produce high-quality research output, even when no one animal model is sufficient to produce a scientific notion about the pathophysiology, aetiology, and therapeutic response of the illness condition. Rats are a commonly used animal model in cardiovascular research, and several rat strains are now readily available, created specifically for research. Effectively aiding in this process are transgenic animals. In order to conduct cardiovascular disease research, a variety of rat strains are currently accessible. A researcher must choose a good rat strain by taking into account the type of research being conducted, the availability of the animal, the cost-effectiveness of the study, and other criteria. The albino Wistar rat is a popular model animal for cardiovascular research, and this review article will focus on the settings under which it performs well and the responses it displays.

Key words: Cardiovascular disease, Albino Wistar rats, Animal model

INTRODUCTION

According to the World Health Organization, cardiovascular illnesses are the leading cause of death worldwide, accounting for 17.9 million fatalities per year. Changes in diet and lifestyle, as well as hypertension, diabetes mellitus, and other metabolic syndromes are thought to be the primary etiological factors contributing to an increased incidence of cardiovascular disease. Incidence of cardiovascular disease is also significantly influenced by hereditary factors, which may involve one or more genes. Despite the rarity of monogenic cardiovascular diseases (cardiovascular disorders caused by a single gene), many prevalent cardiovascular diseases can be triggered by such gene involvements. Monogenic cardiovascular illnesses can be compared to genetic disorders that cause hyperlipidemia. Complex cardiovascular disease features are underpinned by a number of risk variables with fewer genes involved as well as gene-environment interactions. In order to establish the pathophysiological mechanisms underlying a cardiovascular disease, developing cardiovascular disease research thus becomes complex and calls for gene expression and gene polymorphism studies in addition to clinical and molecular diagnostic studies. [(1) (2)]. Additional efforts are being given to the cardiovascular disease research.

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Animal experiment studies are widely accepted research approach to understand the pathophysiology of human diseases and the efficacy of therapeutic interventions [3]. However, preclinical studies are experiencing increased challenges with reproducibility due to experimental research employing animal models. [4] [5] and as a result, the results cannot be translated to humans. Systematic assessments of animal research are crucial for making it useful and relevant, just as experimental planning and execution. [6]

CARDIOVASCULAR DISEASE RESEARCH USING ANIMAL MODELS

Animal models are used in cardiovascular disease research to examine disease aetiology mechanisms and the prognosis of conditions following treatment interventions. [7]. An excellent animal model for cardiovascular disease research should have comparable metabolic-signal transduction pathways and disease features similar to those found in humans in the end stage. [8]. Larger animal models, such as dogs, pigs, sheep, and non-human primates, are also being employed in cardiovascular disease research even though rodents are the most frequently used animal species. These animals share similar clinical traits with humans. Larger animal models are less popular due to the limits of complex genetic modification and expensive feeding expenditures. [9].

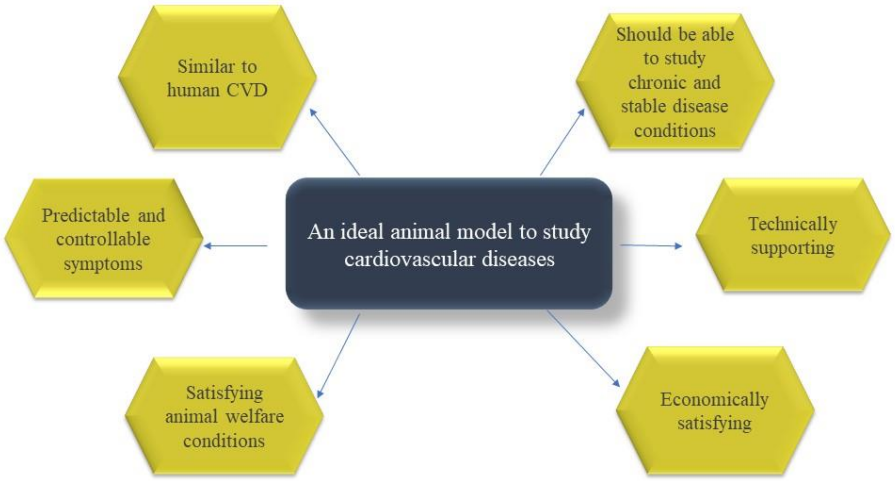

Figure 1 [7]

The selection of an animal model for a particular area of biomedical study depends on how closely the animal model resembles the pathophysiology or physiological state of humans. Not all clinical or biological conditions can be accurately simulated by all animal models now in use. The size, price, and lifetime of the animal should be taken into account while selecting those animal models that almost reflect human disease conditions. Given that a poor choice of the animal model will produce poor findings for the research, it is also important to consider the animal's availability and the researcher's convenience. [10]. Studies conducted using many animal models can have a better outcome for a particular study subject because, generally speaking, an animal model does not perfectly replicate all of the human cardiovascular disease conditions. [11]. Table 1 lists a number of

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animal models mostly used in cardiovascular disease research. Apart from all these conventional cardiovascular disease models, researchers are trying to find alternative models (Example: Zebra fish) to give better scientific values similar to humans and also for replacing mammalian models [12].

Atherothrombotic diseases animal models	Mouse models Rabbit models Porcine models
Abdominal Aortic Aneurysms models	Rat models Mouse models Rabbit models Porcine models
Heart failure models	Rat models Mouse models Large animal models

Table 1 Showing different animal models use in CVD research [13].

RAT MODELS IN CARDIOVASCULAR DISEASE STUDIES

Rat models are considerably less expensive animal models because they provide high sample sizes quickly, making them commonly employed in studies of cardiovascular disease. Rat models are an excellent animal model for cardiovascular research since they can be handled more easily than large animal models and genetic manipulation experiments can be carried out using recognised gene sequencing techniques. Rat models are used for researching long-term pharmaceutical therapies even though they differ significantly from human settings in a number of ways. [14] [15]. Studying human cardiovascular disease rat models has its limitations in many aspects, like slow reproduction of the disease, lacking neurohormonal and other homeostasis mechanism against the disease pathogenesis as in humans and showing other pathophysiological changes which do not ordinarily shown by humans. These limitations are in fact to be considered while extrapolating the research outcome to translate to human disease conditions.[16]

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Rat models can be outbred stocks or inbred strains and can be chosen based on the research type. There are different rat models used in Medical and biological research including several inbred rat strains, Sprague Dawley, Long-Evans, albino Wistar, Fischer 344, Lister black hooded rats etc. Outbreeding produces genetically diverse groups of rats and this group can represent a human population. On other hand, inbreeding produces genetically identical rat groups and can be used in studies not involving genetic diversity as a factor [17] [18]. Studies done on different rat strains shows different cardiac function and the choice of the rat strain is therefore important in which clinical condition is being studied [19].

Among all the rat models, albino rats are the widely preferred laboratory models worldwide and more than hundred albino rat strains are available now [20]. In this review, we are focusing on the most commonly used animal model in cardiovascular research- the albino Wistar rats.

ALBINO WISTAR RAT (*Rattus norvegicus*) MODELS IN CARDIOVASCULAR DISEASE RESEARCH

Albino Wistar rats are outbred and most commonly used laboratory rats suitable as human population models. Albino Wistar rats were first developed in Wistar institute for laboratory research purposes in 1906 [21]. The Wistar rat strain have substrains, including both inbred and outbred rats. All these outbred stocks and inbred strains show considerable variations in the pathophysiology and hence establishing a prior knowledge on the type of rat models used is important in the outcome of the research [22]. Age, sex, body weight, developmental stage, strain etc. are animal characteristics to be fixed prior to the experimental design [23].

Figure 2: Albino Wistar rat (© GlobalP)

The cardiovascular system anatomy of Wistar rats (described by Halpern,1957) show a dual blood supply system supplying the heart, including coronary arteries and extracardiac systems. Extracardiac system comprises of cardio-mediastinal arteries, internal mammary vessel branches or subclavian vessels. The ventricles and intraventricular septum are supplied with right and left coronary arteries whereas the right and left atria are supplied by arteries on the right side and the atrial septum is supplied by right coronary artery. Left atrium can also be supplied with arteries on the left side. Presence of right and left superior vena cava is a striking feature [24]. Moreover, albino Wistar rats show considerable anatomical variations on the male and female heart surface. Mostly male Wistar rats are preferred over females because of the smaller and shorter sizes of female hearts. Comparing to the male Wistar rat's heart female Wistar rat has less stretched, shorter and lighter heart [25]. Wistar rats' circulatory systems differ significantly from those of humans in a number of ways, therefore when using Wistar rats as experimental models for cardiovascular illness, researchers should take all of these differences into account. Even though there is no precise method to correctly measure a rat's age, researchers can often establish age by a few basic factors.

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When comparing human age to that of a laboratory rat, it may be said that one human year is about equivalent to a rat that is two weeks old, given that the typical lifespan of a laboratory rat is three years. This relative age may fluctuate depending on the stage of life, so a researcher should ascertain the age of the experimental rats before the inquiry and should also take into account the parameters being studied as a function of age. [26] [27]. Due to the important role that age plays in the occurrence of cardiovascular disease, albino rats of various ages should be taken into consideration when examining age-related changes. Mature rat groups are gaining more body weight and adipose tissue lipid content. Albino rats can be used as mature models for research at the age of twelve months, as adult models between the ages of six and eight months, and as young adult models at the age of two months. Wistar rats gain weight as they age, achieve a stable weight by six months of age, and then begin to put on weight by twelve months of age. [28]. Careful correlation of the parameter analyzing or the experimental set up with age of the rats used is a necessary factor in making a conclusion.

REFERENCE RANGE OF BIOCHEMICAL AND HEMATOLOGICAL ANALYTES

The interpretation of biochemical and hematological parameters of experimental models has essentiality of reference ranges. R Vigneshwar et al., 2021 studied all such parameter in albino Wistar rats of 4 months old and made reference intervals for both male and female rat models. Table 2 lists all the biochemical and hematological reference values of albino Wistar rat models taken from the article titled “Sex- specific reference intervals for Wistar albino rats: hematology and clinical biochemistry “, aimed to generate a database for usually estimating biochemical and hematological analytes [29].

Table 2: Biochemical and hematological parameters (29)

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Biochemical & Haematological analytes	Albino Wistar rats	
	Male	Female
WBC (x 10^3 mm^3)	3.7-5.8	2.5-3.6
RBC (x$10^6$$mm^3$)	6.1-8.5	5.1-8.1
Hb (g/dl)	11.8-16.2	10.7-17.7
HCT (%)	32.6-46.2	27.3-48.4
PLT (x 10^3 mm^3)	315-512	330-540
Lymphocytes %	54.9-65.3	59.8-73.9
Monocytes %	7.0-8.3	5.6-7.3
Neutrophils %	23.4-40.5	20.5-37.5
Eosinophils %	0.3-3.4	0.5-4.5
Basophils%	0-0.8	0-0.8
Glucose (mg/dl)	90-180	82-170
Total protein (g/dl)	5.1-7.6	5.2-8.2
Total cholesterol (mg/dl)	60-100	62-104
TG (mg/dl)	32 -78	26-65
HDL-C (mg/dl)	36-54	37-68
LDL-C (mg/dl)	15-35	12-27
BUN (mg/dl)	12-20	13-24
Creatinine (mg/dl)	0.3-0.6	0.3-0.6
ALT (IU/L)	24-67	29-72
AST (IU/L)	55-98	62-126
Calcium (mg/dl)	7.5-10.5	7.4-9.0
Phosphorus (mg/dl)	3.5-7.5	3.8-8.0
Sodium (mEq/L)	130-148	140-155
Potassium (mEq/L)	4.2-7.8	4.0-7.5

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ECG ANALYSIS IN WISTAR RATS

Electrocardiographic (ECG) analysis and assessment of change in ECG parameters are inevitable part of any cardiovascular disease research. ECG analysis is

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generally used to assess the heart rate, alteration of which has direct role in the functioning of cardiovascular system, to detect the presence of an ischemic injury, behavioural responses, response to drug metabolisms and to understand the cardio electric mechanisms. Therefore, electrocardiogram recording in Wistar rat models is important in the validation of any cardiovascular research output. As ECG of rats have almost close resemblance to that of human ECG patters, that can be an accepted parameter pointing human pathological conditions. P D Arini et al,2018 on comparing with the temporal technique and by evaluating delineation based on the wavelet transform Wistar rat electrocardiogram database (WR_{DB}), proposed the Wavelet transform based ECG delineator (WT_D) based protocol for the assessment of ECG in Wistar rat models [30]. Though the electrocardiographic recording can be done simply in Wistar rat models, analysing the ECG results seem a difficult task as the experimental set up, age, anaesthesia etc. can have significant role in the result. Wistar rats show increase in heart rate (HR) as the age progresses from birth to 4 months of age and shows a decrease in post pubertal models. The type of anaesthesia given to rats is also a factor determining the heart rate [31]. However, standard parameters for ECG wave delineation in Wistar rat models have to be established as it is difficult to delineate Wistar rat ECG waves. The absence or low Q-wave in Wistar rats makes a difficulty and ST wave absence makes difficulty in positioning QRS end and J-point [32]

ARRHYTHMIA MODELS

Disruption of the orderly excitation and relaxation of myocardium results in arrhythmic conditions [33]. Arrhythmia studies in Wistar rat models by checking heart rate (HR) shows it has significant relationship with LD cycle, showing high HR during night and low during day time. Male rat models show increase in HR when exposed to intermittent hypoxic condition on compared to female models lacking HR increase [34]. Apneic episodes result in arrhythmia condition was studied in Wistar rat models, showed much reduction in heart rate and electrical stability of the heart. Disorders of impulse formation and conduction (prolonged PQ interval) and refractory period enlarged dispersion (prolonged QT interval) causing ventricular arrhythmia depends on the duration of apneic episodes. Arrhythmia conditions are also affected by restored pulmonary ventilation following apneic episodes and LD cycles play role in this part also, where the dark cycle exerts anti-arrhythmogenic and light cycle exerts pro-arrhythmogenic effects. This ventricular arrythmia susceptibility also differ at different time of the day. Thus, LD cycles play a pivotal role in myocardial vulnerability to ventricular arrhythmia [35] [36] [37]. This change in vulnerability of the heart to ventricular arrhythmic condition is mainly associated with the electrophysiological properties of the myocardium. In humans, the change in this electrophysiological property is primarily regulated by the autonomic nervous system and in rat models it largely depends on K^+ ion gradients and ventricular arrhythmia vulnerability is associated with decreased serum K^+ ion concentration [38] [39].

ATHEROSCLEROSIS AND MYOCARDIAL INFARCTION (MI) MODELS

High fat fed albino Wistar rats followed by intraperitoneal VD3 injection, are ideal for studying coronary atherosclerosis lesion models [40] or high cholesterol fed for 21 days can also be chosen for atherosclerotic models [41]. Isoproterenol (synthetic β -adrenergic drug) in low doses (3mg/kg body weight) is ideal for creating cardiac hypertrophic model study [42]) which give insight to atherosclerosis induced Myocardial infarction studies.

Morphological studies on cardiac toxicity in albino Wistar rats show increase in the weight of heart due to increased metabolic activities [43]. Oxidative stress plays a crucial role in the

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pathogenesis of myocardial infarction. The antioxidant defence mechanisms studied in female Wistar rats shows different muscle cells has difference in the preference of antioxidant systems activation against oxidative stress generated. On comparing with the skeletal muscles, heart muscle shows much increase in GSH and CAT action than SOD activation. Thus, when there is a change in free radicals and ROS production, different defence mechanisms play roles differently to the change in redox potential [44].

Isoproterenol (ISO), a synthetic β -adrenergic agonist, is the widely used chemical for inducing myocardial infarction model. Coronary ligation model is a widely used method to induce myocardial infarction in rats having the risk of increased mortality rate of the animals during the procedure. Isoproterenol is therefore validated for its use in inducing myocardial infarction model in Wistar rats [45]. Isoproterenol induces cardiac hypertrophy by generating oxidative stress, calcium overload, inflammatory and immune responses. The isoproterenol induced myocardial infarction model has close resemblance with human myocardial infarction [46]. The different doses of ISO administration will result in different cardiac output and by designing the dosage of ISO administration different cardiovascular disease models can be generated using Wistar rats. When low doses of ISO (0.3-6 mg/kg body weight) are administered, cardiac hypertrophy resulted. Administrating the low dose for less than 7 days will result in physiological hypertrophy, characterized by a number of small necrotic foci and swollen aggregation of mitochondria in the cardiomyocytes. On other hand, administrating the same low dosage for a prolonged period will result in pathological hypertrophy, characterized by aggregate formation of swollen mitochondria with disrupted cristae, enlarged myofibrils and loss of membrane integrity. Medium dose of ISO (10-85mg/kg body weight) administration result in change in energy metabolism at the cellular level, characterized by low oxygen consumption, reduced ATP production and mitochondrial membrane potential. Structural changes in the cardiomyocytes shows swelling of mitochondria, reduction in the surface area of the inner mitochondrial membrane and sarcoplasmic reticulum tubules. High doses of ISO (85-300mg/kg body weight) produce changes in cardiomyocytes similar to human myocardial infarction (MI) and hence high dose of ISO induced albino Wistar rat models is an ideal choice for MI studies [47]. Cardiac remodeling is one of the challenges in the prognosis of cardiovascular disease treatment, as it is associated with changes in cell-molecular-interstitial structures and gene expression, eventually leading to a gross change in the heart structure [48]. Thus, studies in rat models showed ventricular remodeling after a myocardial injury and the extent of which is directly related to the extent of the myocardial injury, usually induced by ISO administration in rats [49].

Myocardial infarction model of albino Wistar rats can also be produced by adrenalin administration, the catecholamine inducing MI rat models by increasing oxidative stress and following cellular mechanisms [50]. Oxidative and nitrative stress induced cellular damage exerted by adrenalin in albino Wistar rat models through damaging proteins, lipids and DNA structure and followed by increased concentrations of nitro-tyrosine derivatives, AGP, Hp and decreased serum concentrations of albumin [51].

Doxorubicin (DOX), an anthracycline drug used as a chemotherapeutic agent in the treatment of cancers also used as an agent inducing cardiotoxicity in Wistar rat models [52]. Dox induces cardiotoxicity by forming iron- anthracycline complexes, generating free radicals and leading to redox imbalance and corresponding lipid peroxidation, loss of membrane integrity, necrotic effects etc. in a dose depended manner [53] [54]. Doxorubicin can be used as a cytotoxic agent in albino Wistar rat inducing dilatated cardiomyopathy (DCM) models. The sufficient dose

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required to produce DCM rat models was proved as 1mg/ kg weight of the rat taken twice in a week for six weeks [55].

On comparing the weight loss, infarct sizes and survival rates of AMI induced albino Wistar rat strains purchased from different suppliers, it was showing considerable variations in these factors studied. It proves rats supplied by different vendors may have difference in response to AMI inducing factors [56].

HEART FAILURE (HF) MODELS

Isoproterenol (ISO) is widely used to induce left ventricular remodeling and heart failure models of albino Wistar rats. An ISO induced heart failure model produced by administrating 150mg/kg body weight of ISO in two consecutive days will generate HF symptoms after 15 days of last dose taken [57]. Alternatively, doxorubicin (DOX) can also be used to induce HF models, 2.5mg/kg of DOX administered intraperitoneally for two weeks will induce cardiomyopathy and HF models [58]. HF models can be characterized by increased lipid peroxidation, reduced cardiac output, cardiac hypertrophy, apoptosis, reduced antioxidant defense mechanisms, histopathological changes, inflammatory cytokine responses, serum cardiac markers etc.

STROKE MODELS

Stroke is one of the serious issues among all the cardiovascular diseases, causing death and acquired disabilities, majorly in elder population. Cerebral circulatory occlusion is the major reason behind cerebral stroke, causing brain infarction. Rat model in stroke has the advantage of having small brain size, making it easier to perform the fixation techniques, to assess other physiological parameters and having almost similar physiological aspects of human stroke [59]. Male albino Wistar rat strain can be used as middle cerebral artery occlusion (MCAO) and reperfusion models, where MCAO in animal models almost resemble human ischemic stroke. Permanent or transient induction of MCAO (Koizumi J et al. 1986) can be done in albino Wistar rat models through incision of the right common carotid artery and blocking the origin of MCA [60] [61] . Inducing MCAO with suture generally may last for 60 minutes, 90 minutes, 120 minutes or may be permanent in rat models. The MCAO also has the advantage of producing large infarct volume, reproducibility and ease in handling the procedure with a short time span. Endothelin-1 (ET-1), a long-lasting vasoconstrictive peptide inducing focal stroke albino Wistar rat models. ET-1 administration produces ischemic lesion in dose dependent manner [62] and focal stroke albino Wistar rat models are also useful. Alternatively, thromboembolic clot models are produced. The common carotid artery is temporarily occluded by injecting fibrin rich emboli into the internal carotid artery [63]. Common carotid artery thrombosis (CCAT) can be induced by photochemically in Wistar rats [64].

CONCLUSION

Albino Wistar rats are the widely used and preferred experimental rat models in cardiovascular disease research. Some special features of these rats make them suitable for experiments in biomedical sciences. Short breeding time, smaller size, cost effectiveness, ease in maintenance and availability of the animal are the attractive features of albino Wistar rats. Even though in vivo studies using a single animal model is not completely reliable to generalize human cardiovascular disease pathogenesis, albino Wistar rats are close resemblance to human disease conditions. Established facts on anatomical and pathophysiological variations of albino Wistar rats and humans will help to understand and correlate the research findings further, and will help to make conclusions and reproducible research outputs. Making database on most accepted experimental protocols and reference parameters of this experimental animal model will be helpful to make standardised research outputs and to reduce publication bias.

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