Original Article

An Overview on Animal Models to Boost Preclinical Research in COVID 19

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Abstract

The current review was undertaken by collecting data through a thorough literature search in PubMed and Google Scholar in order to identify the relevant English-language articles published on animal models for COVID-19 research. The data was obtained from articles published between 2019 and 2021. After a comprehensive review of various available articles, this review outlines the available animal models for COVID-19 and their key roles in elucidating the pathogenesis, disease transmission, and host response to the SARS-CoV-2 virus. Reports from various preclinical trials have shown the use of several animal models such as mice, hamsters, ferrets, and non-human primates (NHPs) for studying the SARS-CoV-2 infection. However, availability of an ideal animal model would help researchers in assessing the efficacy of investigational drugs before they enter clinical trials. No single animal model delineates the totality of pathogenesis or predicts interventional responses faithfully as in humans. Therefore, defining animal models and their use is deemed as a prerequisite for conducting comparative studies of vaccines and therapeutics' efficacy so that the most promising ones advance to the next phase.

Keywords: COVID-19, SARS- CoV-2, Animal models, Preclinical studies, vaccines and drug evaluation

INTRODUCTION:

Coronavirus disease 2019 (COVID-19) is caused by the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is an emerging respiratory infection caused by the infection of a novel coronavirus in humans during late 2019 (first detected in Hubei province, China).[1] The ongoing spread of this virus may potentially bring major challenges to worldwide health systems and detrimental consequences to the global economy and financial markets if not controlled effectively.[2] Multiple clinical trials are currently underway for prevention or intervention in the disease progression.[3] Simultaneously, it is equally essential to undertake basic research on SARS-CoV-2 in order to support efficient development of therapeutic agents.[4] Animal models are essential tools for studying infectious diseases and they help researchers not only to understand the pathogenesis and mechanisms of SARS-CoV-2 disease biology but also to elucidate the aspects of pharmacology, toxicology, and immunology of therapeutic agents and vaccine strategies.[5] No single animal model captures the totality of pathogenesis or predicts interventional responses as faithfully as human models. Defining animal models and their uses is deemed a prerequisite for conducting comparative studies of vaccines and therapeutics' efficacy so that the most promising ones advance to the next phase.[6]

With this objective, the current study aims to synthesize data from various databases and search engines for reviewing and analysing the information on various available animal models for COVID-19. The information included in this review article aims to provide a strong intellectual framework for evaluating the investigational drugs and promising vaccine candidates and also boost preclinical research on COVID-19.

LITERATURE SEARCH METHODOLOGY

A literature review of relevant articles published in English till December 2021 was conducted using the PubMed and Google Scholar databases. Search parameters included Coronavirus, SARS-CoV-2, COVID-19, Preclinical trials for Covid 19, Animal models for Covid 19, Evaluation of drugs, new drugs, and vaccine development for Covid 19. Review articles and preclinical studies on COVID-19 were also considered for the purpose of this review. Additional articles were obtained from the citations of the cross-referenced articles.

Need for animal models for Covid 19

There is an urgent and pressing need for the development of an animal model to study the infection and to test vaccines or new forms of therapies, as well as to understand the molecular mechanisms of COVID-19. Animal models mimic the disease pattern in animals as seen in human infection and it is through these animal models that SARS-CoV-2 was reported to infect humans through the angiotensin-converting enzyme - 2 (ACE-2) receptors.[7] Therefore, animals having ACE-2 receptors and that are closely similar to human beings can act as a model for various studies on SARS-CoV-2. ACE-2 represents the main receptor for SARS-CoV-2 entry into cells whereas the lungs and bronchi are the main targets for viral infection (Figure 1).[8] However, the heart, kidneys, liver, brain, gastrointestinal tract, and upper respiratory tract have also been shown to be affected in COVID-19 infection.[8] Researchers have postulated that some amino acid sequences present in ACE-2, considered as critical residues, are more important than the total number of similar amino acids when comparing animal models to the human model and these critical residues have a direct influence on the susceptibility to infection.[9] Another factor that directly impacts the ACE-2 affinity and differs from one species to another is the tissue distribution of ACE2 together with **Transmembrane serine protease 2** (TMPRSS2) and furin.[9] The TMPRSS2 cellular serine protease and the furin proprotein convertase act as cofactors for this binding, which can be considered as potential targets for the action of inhibitory drugs and.[9]

Animals Used for Developing Animal Models for Covid 19

Studies on COVID-19 have reported that ferrets, monkeys, mice, hamsters, civet cats, camelids, and rabbits can all be considered as animal models for coronavirus infection.[10-17] Larger animal models, specifically the non-human primates (NHPs) models such as Rhesus Macaques, Cynomolgus macaque, and African green monkeys, are traditionally considered the most translational models to humans.[6] NHPs bear close similarities to human genetic, neurological, cognitive, physiological, reproductive, anatomical, and immunological systems.[6] Their size and longevity make them excellent models for studying the disease pathogenesis and also allow for repeated sampling and imaging in vivo for longitudinal studies. However, their limited supply in the face of numerous drug and vaccine candidates make them an even more precious resource.[6] It is therefore imperative to prioritize the agents to be tested by demonstrating tolerability and efficacy in smaller mammalian models like mice, hamsters, and ferrets (currently the small animal species of choice).[6]

Mouse Models

Small animal models are widely used to study emerging viruses but often need to be genetically modified or adapting the virus for different species in order for the virus to be susceptible and this holds good for the SARS-CoV-2 infection as well.[7] Mice have several advantages over other experimental animal models with regards to their small size, low cost, rapid breeding for reaching large group numbers, and availability of research tools. However, laboratory mouse models have shown to be non-responsive to the SARS-CoV-2 infection due to a lack of the viral entry receptor hACE2.[18,19,20] Viral spike protein doesn't bind to the murine ACE-2 effectively due to amino acid differences in the ACE-2 receptors between mice and humans.[20]

Several approaches have been employed to increase the susceptibility to SARS-CoV-2 infection in laboratory mice. The first approach was to use an adapted virus method in which multiple passages/or selected mutations in the virus made the mice more susceptible to infection.[21] The second approach was to express the human ACE-2 gene (hACE2), either by viral transduction or by using genetic engineering tools.[21]

Mouse-Adapted SARS-CoV-2 model

Standard inbred mouse strains such as WT BALB/c mice, C57BL/6 mice can be made susceptible to the SARS-CoV-2 infection by serially passage of the virus in the respiratory tracts of young BALB/c mice.[22] This has led to the generation of a mouse-adapted SARS- CoV strain (MA15) which was lethal to the mice 3 days post infection (dpi).[22] The infection resulted in high viral titers in the lungs from 1 dpi, followed by viremia and diffusion to extra-pulmonary sites with significant lymphopenia, neutrophilia, mild and focal pneumonitis, and necrotic cellular debris in the airways and alveoli being observed.[22] Nevertheless, these adapted mouse strains can be used for developing neutralizing antibodies against the spike protein, pseudo viral vaccine candidates, and other antiviral drugs.[23]

Transgenic mouse model expressing hACE2:

Genetic alteration of the lab mouse has shown to help in viral binding to mouse ACE-2, expression of human ACE-2 under the influence of a variety of tissue-specific promoters, and the transfection of mice with human ACE-2 cDNA using viral vectors.[24] Given the similarity between SARS-CoV-2 to SARS-Co V in its use of ACE-2 as an entry receptor, several research teams have evaluated transgenic mice expressing hACE2 under the control of HFH1/FOXJ1, HFH4, K18, and mouse ACE2 promoters.[25-30] These ACE-2 transgenic mouse models could potentially be useful in studying the SARSCoV-2 replication in lungs and its subsequent pathogenesis. However, SARS-CoV-2 infection in some transgenic mouse models have led to neuro-invasion with high viral replication in the brain, which may consequently be related to its high lethality. [26-29]

K18 Promoter-hACE2 Transgenic Mouse Model

K18-hACE2 mice, when treated with doses of SARS-Co V (2.3 9 104 PFU) were observed to develop severe lung damage and neuronal damage to the CNS. The transgenic mice exhibited viral replication in the lungs, weight loss, pathological lung inflammation, and followed by mortality at 4 dpi. However, SARS-CoV-2 at 105 TCID50 caused weight loss, evoked antibody responses, and developed histological evidence of lung inflammation in K18-hACE2 transgenic mice in a dependent manner with interstitial congestion, inflammatory exudate, epithelial damage, etc. [25,31] These mouse models provide a stringent test for vaccine and therapeutic efficacy and may be useful for pathogenic studies.

HFH4/FOXJ1 Promoter-hACE2 Transgenic Mouse Model

Earlier studies have shown that transgenic mice over expressing hACE2 under the control of HFH4/FOXJ1 promoters were susceptible to SARS-CoV-2 infection.[26] Other studies exhibited that HFH4-hACE2 infected mice had detectable viral titers in the lung, eyes, brain, and heart, suggesting that the virus may have had additional tissue tropisms following the initial lung infection.[25,26] Most infected HFH4-hACE2 mice had minimal weight loss over 7 dpi. However, mice that later became moribund showed significant weight loss from day 4 and significant lymphopenia and neutrophilia in peripheral blood on day 6, which was indicative of severe human disease. [32,33] Lung histology showed initial macrophage and lymphocyte infiltration and fibrin exudation from 1 dpi, which steadily progressed to severe pneumonia, blockage of terminal bronchioles, extensive fibroplasia, and alveolar necrosis by day 7.[26]

Murine mAce2 Promoter-hACE2 Transgenic Mouse Model

The first live SARS-CoV-2 infection model used was the transgenic mice expressing hACE2 under the control of the mACE2 promoter. [25,34] The mice exhibited significant weight loss over a 14-day infection period and high viral lung titers of 1–5 dpi. Histological lung examination revealed moderate interstitial pneumonia, infiltration of lymphocytes, mucus accumulation, and desquamation of bronchial epithelial cells from day 3.[25] There were no detectable viral titers or pathology in other tissues or organs, except for day 1 in the intestine, suggesting that infection was localized almost exclusively to the lungs.

Viral Transduction of hACE2 Using Adenoviral systems

Transduction of the BALB/c mice with adenovirus containing hACE2 (AdV-hACE2) was observed to lead to a stable hACE2 expression in the lungs from 10hrs post-transfection.[35] SARS-CoV-2 infected AdVhACE2 mice exhibited ~10% weight loss over 8 days of infection coupled with high viral lung titers, and modest viral titers in the heart, brain, liver, and spleen. Histological examination also revealed extensive neutrophil accumulation in perivascular and alveolar locations, and vascular congestion.[24] This model can be potentially suitable for researching on drug therapy and antibody testing.[24]

Murine mAce2 Exon 2-hACE2 Knock in Mouse using g CRISPR-Cas9 technology

In a past study, the hACE2 cDNA was inserted into Exon 2 of the mAce2 gene using the CRISPR Cas-9 gene editing technology to disrupt the mAce2 gene expression and drive the expression of hACE2 under the control of the mAce2 promoter.[35] In the lungs, hACE2 was expressed in the CC10+ Clara cells in the airways as well as a subpopulation of surfactant protein C positive alveolar type II cells. High viral loads of SARS-CoV2 were evident in the lung, trachea, and brain (despite low hACE2 expression) on intranasal infection.[35] Young inoculated animals did not display obvious clinical symptoms but developed interstitial pneumonia and vascular system injury. More severe disease was seen in aged mice population expressing the hACE2. These mice exhibited marked weight loss, prolonged viral shedding (including from feces), and severe pneumonia accompanied by stronger cytokine responses. Intragastric inoculation of SARS-CoV-2 induced productive infection and pulmonary disease.[35]

Besides this, a few knock-out mouse models have been developed to understand the pathology of SARS-CoV-2. Amongst them, ACE-/- knockout mice can potentially be used to study the effects of Angiotensin conversion enzyme during acute lung injury study.[36] Using TMPRSS2-/-knockout mice, the role of TMPRSS2 during SARS-CoV entry into cells can be researched upon for developing new drugs against SARS-CoV-2 infections.[37] Humanized DPP4 mice and STAT-1-/-knockout mice, having susceptibility to coronavirus infection and used in prior studies as a model for MERS, can possess the potential to help in SARS-CoV-2 research as well.[38,39]

Syrian hamster model

Syrian hamsters demonstrate clinical features, viral kinetics, histopathological changes, and immune responses that closely mimic the mild to the moderate disease that have been described in human COVID-19 patients.[40-42] Golden Syrian hamsters' (Mesocricetus auratus) ACE 2 receptor binds to the SARS-CoV-2 spike protein, rendering this species highly susceptible to infection with doses as low as 5 TCID50.[43] In this form of the non-lethal disease, the clinical symptoms included rapid breathing, decreased activity, and weight loss that was most severe by 6-dpi. Airway involvement was evident, with histopathology evidencing progression from initial exudative phase of diffuse alveolar damage and extensive apoptosis to the later proliferative phase of tissue repair. Micro-CT analysis of the infected hamsters revealed severe lung injury with the degree of lung abnormalities related to the infection dose. High-dose SARS-CoV-2 infection was observed to lead to severe weight loss and partial mortality while older hamsters appeared to exhibit more pronounced and consistent weight loss. [44,45] Other findings included intestinal mucosal inflammation, degenerative changes, and lymphoid necrosis. There was a marked activation of the innate immune response, with high levels of chemokines/cytokines induced by the infection.[42] Evidence of transmission of COVID-19 from infected hamsters to uninfected cage mates was suggestive of the utility of the model for studying transmission mechanisms.[46]

Ferret models

Ferrets have been considered as good models for studying respiratory diseases, as the physiology of their lungs and airways are close to that of humans.[47] Unlike rodents, ferrets cough and possess a sneeze reflex, making them a particularly useful model in the study of disease transmission. Ferrets have been observed to exhibit lethargy and visually show appetite loss following infection with SARS-CoV-2 via the intranasal route, but the disease does not progress to acute respiratory disease and the animals recover from the infection.[48] Virus shedding from the upper respiratory tract (nasal washes, saliva) can persist for up to 21 dpi with the length of shedding appearing to be dependent on the initial viral challenge dose and can be intermittent after 14 days. Mild multifocal bronchopneumonia was observed in early post-infection (day 3 in animals receiving 4 to 6 logs of the virus). Fever has been reported in some studies, but neither coughing nor dyspnoea have been observed. [48,49] Ferrets re-challenged after 28 dpi appear to be completely protected against re-infection.[50] Studies performed in ferrets till date strongly indicate that experimental SARS-CoV-2 infection results in a predominantly upper-respiratory-tract infection in these animals.[50]

Non-human primates

Some of the Non-human primates which have been used for COVID-19 research are rhesus macaques, cynomolgus macaques, and African green monkeys.[51] NHPs physiologically and phylogenetically mimic humans, which have made them the gold standard for the study of emerging viruses.[52]

Rhesus macaques (*M. mulatta*) infected with SARS CoV-2 developed a moderate, non-lethal shedding disease phenotype with few to no clinical manifestations such as reduced appetite, mild dehydration, tachypnoea, piloerection, and dyspnoea.[53] The virus was detected in the nasal, throat, rectal, and ocular swabs and in bronchoalveolar lavage (BALs) through median tissue culture infectious dose (TCID50) beginning at approximately 2dpi, peaking around 4/5dpi, and decreasing after 6 dpi.[53] When reported, fever was mild and transient beginning shortly after 2 dpi and resolving within 2 or 3 days.[53] Bodyweight loss was found in mild infection along with transient drops in weight followed by complete recovery.[53] Transient leucocytosis, neutrophilia, monocytosis, and lymphopenia were also reported.[53,55] Imaging (radiographs or PET/CT) confirmed that the rhesus macaques were infected, with infiltrates and ground-glass appearances in radiographs, which began early after exposure (2 or 3dpi) and resolved by 10–14 dpi.[53,55] Anecdotal evidence suggested that older rhesus macaques developed chronic infection in which infiltrates persisted throughout the study.[56]

Cynomolgus macaques (*M. fascicularis*), when exposed to SARS-CoV2, became infected but exhibited no overt clinical signs of disease.[57] Virus shedding from the upper respiratory tract occurred, peaking early at 1dpi in young animals and 4 dpi in aged (15–20 years) animals, then decreased rapidly but with intermittent detection up to 10dpi.[57] Overall, higher levels of virus shedding were measured in aged animals than in younger animals.[57] They developed mild to moderate lung abnormalities and macroscopic lesions in the lungs including alveolar and bronchiolar epithelial necrosis, alveolar edema, hyaline membrane formation, and accumulation of immune cells.[58,59] While the infection was self-limiting, the disease in cynomolgus macaques does mirror many aspects of human COVID-19 and could potentially be utilized to test preventative and therapeutic strategies.[57]

African green monkeys (AGMs) exposed to SARS-CoV-2 as young adults displayed a mild, non-lethal shedding disease phenotype that included few to no clinical observations.[60] If any clinical observations, such as fever, were reported, they were typically transient and mild with no serious manifestations. [60,61] Bodyweight findings were also generally unremarkable. Clinical chemistry and haematology revealed mild and transient shifts in leukocyte populations, mild thrombocytopenia, and selected liver enzymes.[61] A measure of acute inflammation, CRP was elevated during early stages of the infection. Imaging (radiographs or PET/CT) confirmed that AGMs were infected with the presence of infiltrates and ground-glass appearances in radiographs beginning early after 2 or 3 dpi and resolving by 10dpi.[61] PET/CT images also corroborated the radiographical findings.[61]

CONCLUSION

There is a dire need for the development of animal models to promote preclinical research in COVID-19. Animal models are required to define the cause and effects, and to elucidate and validate the pathophysiology and transmission processes that can be translated into human studies. However, there is no clear preferred model for studying the SARS CoV-2 infection as the clinical signs, recovery, and transmission vary between and within species. Each animal model seems to have its own merits and demerits, and careful consideration is required before the selection of animal models. Genetically engineered mice, mice infected with modified adenoviruses, mice changed with CRISPR, and WT mice infected with mouse-adapted viruses will probably be the most commonly employed models due to their ease of handling and cheaper prices. They'd also be employed again because they mimic human lung inflammation, histopathology, and pneumonia characteristics. However, researchers using these mouse strains should be careful in interpreting the data obtained. Studies performed on transgenic mice can provide a proof of concept for understanding the viral pathogenesis. The review of past studies show that hamsters are ideal for studying the replication of mild SARS-CoV-2 infections seen in humans, as well as analysing the virus's host defence response. They may also be utilized to better understand the SARS-CoV-2 pathogenesis and to test vaccines and antiviral medicines. Ferrets on the other hand can be appropriate models for studying disease transmission and lung infections. SARS-CoV-2 infection in non-human primates, particularly the rhesus macaque, is most similar to that seen in humans, and therefore could be a useful model for testing vaccinations and medication efficacy.

All the animal models available as of date are invaluable scientific resources for researchers to elucidate the preclinical data on investigational products to prevent and control COVID-19. However, these resources on animal models need to be clearer and validated by many more studies to understand the disease pattern as seen in humans. Standardization of animal models is critical for future research on COVID-19 for comparing different medications and vaccine candidates, as well as for developing appropriate animal models for drug testing and testing vaccine efficacy/potential.

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