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Preclinical screening techniques used in pharmacological evaluation of new drug for COVID-19 treatment

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Abstract
A Novel Coronavirus is a Severe Acute Respiratory Syndrome Coronavirus 2 also known as SARS-CoV-2 appeared in Wuhan, Hubei Province, China, in December 2019 causing a syndrome termed by World Health Organization in February 11, 2020 and recognized as pandemic on March 11, 2020. It is basically a virus-induced inflammatory disease of airways and lungs that causes severe respiratory issues. The virus has characteristics such as long incubation period, rapid transmission and strong pathogenicity. As humans do not have pre-existing immunity to SARS- CoV-2 there was urgent need to develop therapeutic techniques for SARS-CoV-2, it evoked tremendous investigative effort mainly for the clinical studies, choosing right animals for testing animal models. Studies mentioned include comparison of existing drugs, animal models, newly developed models for COVID-19 also includes testing of safety background for predisposing chronic diseases and development of new treatment and prevention measures. They summarize clinical trial research and discuss new strategies to be used in multiple trials aiming to increase the reliability of results which may in turn help in developing better COVID-19 clinical treatment practices.

Keywords: COVID 19, preclinical screening techniques, animal model, SARS-COV-2

Introduction
Coronavirus disease 2019 (COVID-19) is a highly contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Coronavirus is an enveloped virus with a single stranded RNA genomes (One of the largest known genomes in RNA viruses) these viruses arise through cross-species transmission and may cause fatal diseases in humans. Sequence Analysis and epidemiological investigations suggests the original source is probably from animal reservoirs most likely bats that infected Humans, the role of animals as reservoirs is yet to be explored [2]. The virus has the characteristics of rapid transmission, long incubation period and strong pathogenicity, and has spread all over the world. Therefore, it is of great significance to select appropriate animal models for antiviral drug development and therapeutic effect evaluation, Clinical and preclinical studies being conducted in order to test models that have been developed for Sars-Cov-2/ COVID-19 these aim to increase the reliability of these results that might help in shaping the development of clinical treatment practices for COVID-19, also the main features of the disease are described but they do hamper the clinical trials, in order to know the targeted preventions and develop reliable treatments comparison of existing drugs testing their safety including the background of predisposing chronic diseases is being done. The focus being mainly on the mechanisms and animal models of SARS-CoV-2 infection as they provide a theoretical basis for understanding the pathogenesis of COVID19 and the prevention and treatment of the disease. The identification of animal reservoirs plays a crucial role in effective disease control [2, 3, 4]. Studies reviewed in this article includes information regarding preclinical studies on COVID-19, comparing current animal models of SARS-CoV-2.

Preclinical screening techniques/animals models used in COVID- 19 treatment
Mouse
The repurposing of many antiinflammatory or antiviral drugs has also, as yet, failed to show significant impact on disease. Therefore, there is an urgent need to improve our understanding of SARS-CoV-2 infection and pathogenesis and develop new therapeutic and preventative treatment strategies.
One approach to identify novel disease mechanisms and therapeutic targets has been large-scale screening in preclinical small animal models, particularly mice.

SARS-CoV-2 cell entry is mediated by binding of the viral S (Spike) protein to hACE2 (human angiotensin-converting enzyme 2) that is broadly expressed in the respiratory, but also the nasal and gastrointestinal, epithelium. Unfortunately, mouse ACE2 shows limited binding to the SARS-CoV-2 S protein, meaning commercially available wild-type (WT) inbred mouse strains are not useful in the study of SARS-CoV-2 infection. In this issue of the Journal, Han and colleagues describe a rapid mouse model of SARS-CoV-2 infection utilizing a recombinant human Ad5-hACE2 (adenovirus type 5–expressing hACE2) that results in hACE2 expression and supports SARS-CoV-2 replication in the lower respiratory tract of WT mice [5].

Clinical signs and symptoms characterization: For clinical monitoring and survival studies, mice could be infected with SARS-CoV-2 including both lab-adapted strains and natural isolates with several pathogenic potentials. For virologic, histopathologic, and immunologic studies, mice would be observed to see whether they are manifesting overt clinical signs as scored by severity scores: weight loss, ruffling of fur, ataxia, inability to eat/drink, lethargy, signs of distress such as dyspnea, rales, tachypnea, and dehydration. Diabetes, obesity, and hypertension are major risk factors for the development of severe COVID-19 [6].

Studies in non-human primates (viral model)

Rhesus macaques (n = 10), cynomolgus (n = 3), and African Green model (n = 1) and common marmoset (n = 1) were assessed as models for COVID-19. SARS-CoV-2 strains, dose, and route of inoculation were different across studies.

Different doses of virus inoculum were compared in a single study and showed that viral load in the upper and lower respiratory tract, fever, weight loss, respiratory distress, and mortality were comparable regardless of the doses except for mild transient neutropenia and lymphopenia in the high dose group. In contrast, the route of administration resulted in different pathological response as the intratracheal route elicited severe interstitial pneumonia, as compared with mild interstitial pneumonia and no pneumonia from the intra conjunctival and intragastric routes, respectively. The animals were euthanized at different time-points post-inoculation ranging from 3 to 33 days [7].

hACE2 Mice Developed Interstitial Pneumonia upon SARS-CoV-2 Infection

Pathological Changes and Inflammatory Response in SARS-CoV-2-Infected hACE2 Mice

A. H&E staining analysis showed inflammatory cell infiltration (yellow arrow), alveolar septal thickening, focal haemorrhage (green arrow), and distinctive vascular system injury (blue arrow) in hACE2 mice. Right panel, semiquantitative analysis of the H&E-stained lung sections.
B. IHC staining analysis for neutrophils (Neu^+) and macrophages ($CD68^+$) in SARS-CoV-2 infected hACE2 mice. Right panel, semiquantitative analysis of the neutrophil and macrophage counts. Statistical significance was analyzed by unpaired Student's t tests. $*p < 0.05$.

In comparison with SARS-CoV-2-infected WT mice, H&E staining showed that both young and aged hACE2 mice developed interstitial pneumonia characterized with inflammatory cell infiltration, alveolar septal thickening, and distinctive vascular system injury.

More lesions of alveolar epithelial cells and focal haemorrhage were observed in the aged mice. In comparison with WT mice, IHC staining analysis showed SARS-CoV-2 infection induced more neutrophil (Neu^+) and macrophage ($CD68^+$) infiltration in the aged hACE2 mice.

Immunofluorescence co-staining showed SARS-CoV-2 directly infected $CD68^+$ macrophages in the lung, resulting in significant apoptosis ($C\text{-}Casp3^+$) in the aged hACE2 mice.

Luminex cytokine analysis showed that SARS-CoV-2 infection led to elevated cytokine production including Eotaxin, G-CSF, IFN-g, IL-9, and MIP-1b in aged hACE2 mice, but it had a weaker response in young mice [8].

Syrian hamster model

Syrian hamsters (Mesorectums auratus) are small mammals that have been used as models for infection with respiratory viruses, including SARS-CoV, influenza virus and adenovirus. In silico comparison of the ACE2 sequence of humans-known to interact with the receptor-binding domain of the SARS-CoV-2 spike glycoprotein-with that of hamsters suggested that Syrian hamsters might be susceptible to infection with SARS-CoV-2. Upon experimental intranasal infection, Syrian hamsters show mild-to-moderate disease with progressive weight loss that starts very early after infection (days 1-2 after inoculation). All hamsters that have been challenged by different groups and with different SARS-CoV-2 isolates consistently showed signs of respiratory distress, including laboured breathing. Additional signs of morbidity included lethargy, ruffled fur and a hunched posture. After two weeks of infection, hamsters typically recovered. Of particular interest is the fact that infection with SARS-CoV-2 in hamsters reflects some of the demographic differences of COVID-19 in humans. Thus, aged hamsters and male hamsters seem to develop a more severe dis- ease than young and female hamsters, respectively [9].

Ferret

Ferrets (Mustela putorius furo) have been shown to be a highly valuable model for testing the pathogenicity and transmission of human respiratory viruses, including influenza virus and respiratory syncytial virus44,45. It is thus not surprising that the ferret model has been investigated for studies of the pathogenesis of COVID-19 and SARS-CoV-2 transmission. Despite the use of different isolates of SARS-CoV-2, the results have been notably consistent across all laboratories [9].

Recently, ferret ACE2 was shown to contain the critical residues required for binding by SARS-CoV-2 RBD. The animals were susceptible to SARS-CoV-2 infection with viral replication in the URT, however, only low levels of virus were detected in lungs. Viral loads in the lung and nasal turbinate peaked at 4 dpi with clearance by 8 and 12 dpi, respectively. While infectious virus was not detected outside the respiratory tract, viral RNA was found in the intestine, saliva, urine, rectal swabs and faeces up to 8dpi. This suggests that SARS-CoV-2 preferentially replicates in the URT of ferrets. In line with this, disease is mild with reduced activity and occasional cough from days 2–6. In most studies, elevated body temperatures are observed from

2 dpi, returning to baseline by day 8 with no change in body weight. Shi *et al*., reported only 1 out of 3 ferrets developed fever and loss of appetite following intranasal inoculation with different SARS-CoV-2 isolates, suggesting isolate variability and dose may alter disease outcomes. Limited studies have examined immune responses to SARS-CoV-2 in ferrets. Intranasal infection induced serum neutralizing antibodies [10].

Conclusion

There are some animal model that investigators can use to explore important aspect of covid 19, this animal model helps to establish safety and efficacy of potential therapeutic agent and vaccine.

In this article we summerize preclinical screening techniques used in multiple trails aiming to increase the reliability of result which may in turn help in developing better covid-19 clinical treatment practice.

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