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Comparison of SARS-CoV-2 variant lethality in human angiotensin-converting enzyme 2 transgenic mice

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ABSTRACT

This study compared the lethality of severe acute respiratory syndrome coronavirus 2 variants belonging to the S, V, L, G, GH, and GR clades using K18-human angiotensin-converting enzyme 2 heterozygous mice. To estimate the 50% lethal dose (LD₅₀) of each variant, increasing viral loads (10⁰–10⁴ plaque-forming units [PFU]) were administered intranasally. Mouse weight and survival were monitored for 14 days. The LD₅₀ of the GH and GR clades was significantly lower than that of other clades at 50 PFU. These findings suggest that the GH and GR clades, which are prevalent worldwide, are more virulent than the other clades.

Abbreviations
SARS-CoV-2  severe acute respiratory syndrome coronavirus 2
hACE2  human angiotensin-converting enzyme 2
KDCA  Korea Disease Control and Prevention Agency
PFU  plaque-forming unit
LD₅₀  50% lethal dose

SARS-CoV-2 variants were obtained from the National Culture Collection for Pathogens of the Korea Disease Control and Prevention Agency (KDCA). Their genetic information was collected from the GenBank database: MW466791.1 (S clade: A), MW466795.1 (L clade: B), MW466797.1 (V clade: B), MW466798.1 (GR clade: B.1.1), MW466799.1 (G clade: B.1), and MW466800.1 (GH clade: B.1.497). Each virus was propagated in Vero-E6 cells, and the aliquots were stored at −80°C until use. The viruses used in this experiment were collected at the third passage.

The growth properties and plaque morphologies of SARS-CoV-2 variants in Vero-E6 cells were investigated. No significant difference was observed between the growth kinetics of the six clades (Fig. 1A). The G clade formed larger plaques than the other clades in Vero-E6 cells (Fig. 1B). As the loss of furin-cleavage site (PRRAR) of spike protein has been reported in Vero-E6 cultured SARS-CoV-2, which results in attenuated viral pathogenesis (Johnson et al., 2021; Lau et al., 2020), the spike gene sequences of the viruses were analyzed, and there were no deletion of furin-cleavage site on the spike protein of each virus (Fig. 1C).

All animal experiments were approved by the Institutional Animal Care and Use Committee of KDCA (Approval No. KCDC-088-20-2A) and performed under the Guidelines for the Care and Use of Laboratory Animals of Korea National Institute of Health. Six-week-old male transgenic K18-hACE2 mice were purchased from The Jackson

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Laboratory (Bar Harbor, ME, USA). For intranasal infection, K18-hACE2 mice were anesthetized with tiletamine/zolazepam and xylazine combination injected intraperitoneally. Subsequently, each virus was administered intranasally in decreasing 10-fold concentrations (from $10^4$ to $10^0$ plaque-forming units [PFU]) to groups of four or five mice, and a back titration was performed to check the virus titers. The infected mice were monitored daily for 14 days for weight loss and mortality. When the body weight decreased to 75% of the initial body weight, the mice were anesthetized and humanely euthanized. The 50% lethal dose (LD$_{50}$) was determined by assessing the number of dead and live mice on day 14, as previously described (Ramakrishnan, 2016).

Mice infected with L, G, V, and S clades at $10^4$ PFU started losing weight four days after inoculation until they exhibited a reduction of 25% of body weight or died (Fig. 2A). However, the weight reduction in GR and GH groups at the same PFU was observed one day earlier than in the other clade groups. All the mice infected with $10^4$ PFU of SARS-CoV-2 died within eight or nine days (Fig. 2B). The titers of LD$_{50}$ were calculated as 501, 316, 200, 125, 50, and 50 PFU for the L, G, V, S, GR, and GH clades, respectively (Table 1). In conclusion, the GR and GH clades were at least 2–10 times more lethal than the other clades.
Previous studies exploring SARS-CoV-2 infection in transgenic K-18-hACE2 mice only used high or low doses of the D614 type virus, and thus, the exact LD$_{50}$ was not measured (Yinda et al., 2021; Golden et al., 2020). Herein, the viral replication fitness and lethality of six SARS-CoV-2 clades were evaluated in Vero-E6 cells and transgenic K-18-hACE2 male mice, respectively. No differences in virus growth were observed in Vero-E6 cells. Plaque morphology of G clade was larger than other five clades. This finding is consistent with recent findings suggesting that compared with the original D614 virus, the SARS-CoV-2 spike protein substitution D614G variant enhances viral replication and infectivity in the human lung epithelial cell line Calu-3 or in primary human upper airway tissues but not in Vero-E6 cells (Plante et al., 2020). However, the D614G variant did not result in more severe pathogenicity in hamsters and patients (Lorenzo-Redondo et al., 2020). Hence, further studies using infectious cDNA clones with specific amino acid changes are required to evaluate whether the GR (N-G204R substitution) or GH clade (NS3-Q57H substitution) may result in increased infectiousness and transmissibility, as well as to determine the virus variant-associated disease severity using in vivo models (Plante et al., 2020).

This is the first study that compares the pathogenicity of the different SARS-CoV-2 clades identified in the ongoing pandemic. However, the use of only male heterozygous hACE2 mice was a limiting factor, as there is a possibility of differences in SARS-CoV-2 pathogenicity and...
lethality with sex. Therefore, future animal studies should include both male and female mice. Nevertheless, the current findings will be valuable for basic research on the pathogenicity of emerging SARS-CoV-2 variants, including the mink variant, B.1.1.7, and B.1.351, and will be useful in preclinical studies for the efficacy evaluation of SARS-CoV-2 vaccine candidates and therapeutic agents.

CRediT authorship contribution statement

Tae-Young Lee: Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. Hansaem Lee: . Nayoung Kim: . Pyeonghwa Jeon: . Jun-Won Kim: . Hee-Young Lim: Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. Jeong-Sun Yang: Visualization, Writing – review & editing. Kyung-Chang Kim: . Joo-Yeon Lee: Visualization, Writing – review & editing, Conceptualization, Supervision, Funding acquisition.

Table 1
SARS-CoV-2 variants lethal dose in K18-hACE2 transgenic mice.

| Virus clades | Challenge dose (PFU/mouse) | No. of deaths/No. of challenged mice(%) | LD50(PFU)* |
|--------------|-----------------------------|----------------------------------------|------------|
| L            | 10^4 10^5 10^6 10^7 10^8   | 5/5 (100) 3/5 (60) 1/5 (20) 0/5 (0)     | 501        |
| G            | 10^4 10^5 10^6 10^7 10^8   | 5/5 (100) 3/5 (60) 1/5 (20) 1/5 (20) 0/4 (0) | 316        |
| V            | 10^4 10^5 10^6 10^7 10^8   | 5/5 (100) 4/5 (80) 1/5 (20) 1/5 (20) 0/4 (0) | 200        |
| S            | 10^4 10^5 10^6 10^7 10^8   | 4/4 (100) 5/5 (100) 1/5 (20) 1/5 (20) 0/4 (0) | 125        |
| GR           | 10^4 10^5 10^6 10^7 10^8   | 2/5 (40) 0/4 (0)                          | 50         |
| GH           | 10^4 10^5 10^6 10^7 10^8   | 2/5 (40) 0/4 (0)                          | 50         |

* LD50 values were calculated from the data shown in Fig. 2 and using the method described in Ramakrishnan (2016).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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