Title: The *in vitro* and *in vivo* potency of CT-P59 against Delta and its associated variants of SARS-CoV-2

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Highlights

- CT-P59 exerts the antiviral effect on authentic Delta, Epsilon and Kappa variants in cell-based experiments.
- CT-P59 showed neutralizing potency against variants including Delta, Epsilon, Kappa, L452R, T478K and P681H pseudovirus variants.
- The administration of clinically relevant dose of CT-P59 showed *in vivo* protection against Delta variants in animal challenge experiment.

Abstract

The Delta variant originally from India is rapidly spreading across the world and causes to resurge infections of SARS-CoV-2. We previously reported that CT-P59 presented its *in vivo* potency against Beta and Gamma variants, despite its reduced activity in cell experiments. Yet, it remains uncertain to exert the antiviral effect of CT-P59 on the Delta and its associated variants (L452R). To tackle this question, we carried out cell tests and animal study. CT-P59 showed reduced antiviral activity but enabled neutralization against Delta, Epsilon, and Kappa variants in cells. In line with *in vitro* results, the mouse challenge experiment with the Delta variant substantiated *in vivo* potency of CT-P59 showing symptom remission and virus abrogation in the respiratory tract. Collectively, cell and animal studies showed that CT-P59 is effective against the Delta variant infection, hinting that CT-P59 has therapeutic potency for patients infected with Delta and its associated variants.

Keywords

SARS-CoV-2 virus; Delta, Indian variant; CT-P59; Regdanvimab; Therapeutic antibody

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1. Introduction

New lineage B.1.617 of SARS-CoV-2 is considered to cause a steep increase in infection cases and death in India. Concerning transmissibility and pathogenicity, WHO (World Health Organization) designated Kappa (B.1.617.1) as Variants of Interest (VOI) and Delta (B.1.617.2) as Variants of Concern (VOC), respectively. In particular, highly transmissible Delta variants rapidly spread and become dominant in India and other countries [1, 2]. Also, the global dominant Alpha variants were replaced by Delta variants, even in most vaccinated countries such as the United Kingdom and the United States [3, 4].

The B.1.617 variant (L452R/E484Q/Δ681/Δ1072) has three main sub-lineages; B.1.617.1 (E154K/L452R/E484Q/P681R/Q1071H), B.1.617.2 (T19R/G142D/Δ156-157/R158G/L452R/T478K/P681R/D950N) and B.1.617.3 (T19R/Δ156-157/R158G/L452R/E484K/P681R/D950N) [5, 6]. The Delta variant has RBD (Receptor Binding Domain) mutations in the spike protein which may contribute to increased infectivity, pathogenicity and immune evasion, resulting in re-infection or resistance to vaccine-elicited antibody and therapeutic antibodies [7-9]. One of Delta-associated variants, B.1.427 (L452R) and B.1.429 (S13I/W152C/L452R) also known as the Epsilon have L452R mutation, causing reduction in antibody neutralization [10-12]. Delta and Kappa variants have a P681R mutation at furin cleavage sites (681PRRAR/S686). Recent studies showed that P681R enhances spike cleavage and fusion for viral entry, suggesting augmented transmissibility and pathogenicity [13, 14]. In addition, Mexican variant, B.1.1.519 (T478K/P681H/T732A) has common mutation sites with Delta variant at T478 and P681, in common with Alpha variant (Δ69-70/Δ144/N501Y/A570D/P681H/T716I/S982A/D1118H) [15]. T478K was a previously predominant variant in Mexico and located at the interface of RBD and ACE2 (Angiotensin-Converting Enzyme 2) interaction,

thereby potentially impacting viral infection [16]. Thus, it is important to assess the impact of mutations within the spike on COVID-19 therapeutics.

The CT-P59 human monoclonal antibody (Regdanvimab) specifically binds to spike of SARS-CoV-2, blocking interaction with ACE2 for viral entry. We have demonstrated that CT-P59 has high potency against the original SARS-CoV-2 in *in vitro* and *in vivo* studies and also showed *in vitro* and *in vivo* potency against B.1.351 and P.1 [17-19]. However, it remains uncertain whether CT-P59 has the therapeutic effect on Delta and its associated variants. To this end, we examined the sensitivity to monoclonal CT-P59 against Delta, Epsilon and Kappa variants by cell assays (plaque reduction neutralization test and pseudovirus assay), and animal test using Delta-infected mice.

2. Materials and methods

2.1. Viruses

Clinical isolates, Delta (hCoV-19/Korea/KDCA5439/2021), Epsilon (hCoV-19/Korea/KDCA1792/2020, and hCoV-19/Korea/KDCA1793/2020), Kappa (hCoV-19/Korea/KDCA2950/2021) were obtained through National Culture Collection for Pathogens. Pseudoviruses for Delta (L452R/T478K/P681R), Epsilon (S13I/W152C/L452R), Kappa (L452R/E484Q/P681R), L452R, T478K, and P681H were produced as previously described [18].

2.2. Biolayer interferometry (BLI)

Binding affinity of CT-P59 to wild type and mutant SARS-CoV-2 RBDs were evaluated by the Octet QK^e system (ForteBio). Mutant RBDs (L452R/T478K, and L452R) were purchased from Sino Biological [17, 19].

2.3. Plaque Reduction Neutralization Test (PRNT)

PRNT for authentic SARS-CoV-2 variants were carried out as previously described [19]. Briefly, pre-incubated mixture of variant and CT-P59 was inoculated to VeroE6 cells. After incubation, the neutralization of CT-P59 against each variant was determined as the half-maximal inhibitory concentration (IC₅₀) using GraphPad Prism6 software.

2.4. Pseudovirus assay

Luciferase-based pseudovirus assay was performed as previously described [18]. In brief, Mutant pseudoviruses were generated by PCR-based mutagenesis and produced by transiently transfection. Serially diluted antibodies mixed with pseudoviruses. The mixture inoculated ACE2-HEK293T cells, and incubated for 72 h. Neutralization was assessed by luciferase measurement and IC₅₀ calculation

2.5. Animal experiments

8-week-old female human ACE2 transgenic mice, tg(K18-ACE2)2Prlnn, were purchased from The Jackson Laboratory. Mice were housed in a certified A/BSL-3 facility at the KDCA. All procedures were approved by the Institutional Animal Care and Use Committee at KDCA (No. KDCA-IACUC-21-026).

2.6. Mouse study

For intranasal infection, mice (n=8 for virus only group as a placebo group and n=9/group for each CT-P59 treatment group) were humanely euthanized by intraperitoneal (IP) injection with a mix of 30 mg/kg Zoletil® (Virbac) and 10 mg/kg Rompun® (Bayer). Subsequently, mice were intranasally inoculated with 1x10⁴ PFU of Delta variant in a 30 µL volume. CT-P59 was administered via a single IP injection at dose levels of 5, 20, 40 and 80 mg/kg (equivalent to

human doses of 2.5, 10, 20 and 40 mg/kg, based on exposure to mAb from the PK studies) at 8 h post-inoculation. The negative control (n=5) and virus only group mice were administered with formulation buffer via the same route. The body weight and mortality were monitored daily until 6 dpi (days post-inoculation) and lung tissues were collected at scheduled necropsy at 3 dpi (n=3 for the negative control group, n=4 for the virus only group and n=5 for each treatment group) and 6 dpi (n=2 for the negative control group and n=4 for virus only and each treatment group, respectively) for virus quantitation. Animals with 25% body weight loss were euthanized and excluded from statistical analysis.

2.7. Virus quantitation

For the SARS-CoV-2 titration from the respiratory tract, mice were euthanized on 3 and 6 dpi, and lungs were harvested. The lung tissues were homogenized in 1 mL of DMEM media with bead-based homogenizer (Bertin Technologies). The lung supernatant samples were 10-fold serially diluted in DMEM and inoculated to Vero E6 cells in a 24-well plate. After incubation for 1 h, the inoculum was removed and covered with agar-overlay media including 0.5% (w/v) agarose (Lonza) and 2% FBS in MEM. Following incubation at 37°C for 2 days, the cells were fixed with 4% (v/v) paraformaldehyde solution, and stained with 0.2% crystal violet (Sigma). The number of plaques per well was counted and analyzed.

3. Results

3.1. *In vitro* potency of CT-P59 against Delta and its associated variants in cells

To investigate the therapeutic efficacy of CT-P59 against Delta and Epsilon variants, we first evaluated the binding affinity of CT-P59 against mutant RBDs (L452R/T478K or L452R) by using BLI (Bio-Layer interferometry). The equilibrium dissociation constant (K_D) of CT-P59 against RBD mutant proteins of Delta or Epsilon variant was reduced by 9 ~ 10-fold compared

to that against wild-type RBD (Supplementary Table 1). Next, we carried out two types of *in vitro* assays with live or pseudotyped viruses to assess the susceptibility of Delta and its associated variants to CT-P59. CT-P59 showed reduced susceptibility against Delta, Epsilon and Kappa variants with IC₅₀ of 1,237, 365.6~499.5 and 161.5 ng/mL compared to that against wild type (6.76 ng/mL) in PRNT. Approx. 183-, 54~74-, 24-fold reduced susceptibility against Delta, Epsilon and Kappa variants were observed compared to wild type SARS-CoV-2, respectively (Table 2 and Supplementary Fig. 1A). In addition, CT-P59 showed approx. 98-, 31-, 50-fold reduced neutralizing activity against Delta (L452R/T478K/P681R), Epsilon (S13I/W152C/L452R) and Kappa (L452R/E484Q/P681R) pseudotyped virus with an IC₅₀ of 21.52 ng/mL, 6.819 ng/mL and 11.08 ng/ml compared to that of D614G pseudovirus (0.219 ng/mL), respectively (Table 2 and Supplementary Fig. 1B). Thus, we found that CT-P59 can neutralize Delta and Epsilon variants despite reduced binding affinity and antiviral ability in *in vitro* experiments. We also tested whether single mutants (L452R, T478K, and P681H) impact neutralization of CT-P59. CT-P59 was less susceptible to L452R (13.22 ng/mL), but retained its own neutralizing effect against T478K (0.213 ng/mL), and P681H (0.378 ng/mL) (Table 2 and Supplementary Fig. 1B).

3.2. *In vivo* potency of CT-P59 against Delta variant in mice

To evaluate whether CT-P59 has an ability to lower viral burden against Delta variant in *in vivo* setting, mice were treated with clinically relevant dosages in consideration of the 40 mg/kg of clinical dose of CT-P59. The dose of 80 mg/kg is considered as the equivalent exposure in clinical study (data not shown), and lower doses of 5 and 20 mg/kg were selected to investigate the lower exposure in the clinical situation.

Virus only (placebo) group were severely affected by Delta variant, showing the survival rate of 75% at 5 dpi and 0% at 6 dpi, respectively. In contrast, there was no lethality in all CT-P59

treatment groups (Fig. 1 A). CT-P59 treatment also ameliorated the weight loss of the mice infected with B.1.617.2 variants. 5 and 20 mg/kg CT-P59 treatment groups showed the delay in weight loss until 6 dpi and 40 and 80 mg/kg treatment showed statistically significant difference compared to the virus only group at 4 dpi and 5 dpi, respectively. Importantly, all CT-P59 treatment group maintained their body weight until 6 dpi, which was statistically comparable with negative control. Mean body weight loss of 5, 20, 40 and 80 mg/kg treatment groups were 14.3, 13.2, 4.6 and 5.7% at 5 dpi, respectively. In contrast, virus only group showed an average 16.3% of weight loss at 4 dpi, and eventually showed an average of 22.7% decrease for three mice while a single euthanized mouse excluded from statistical analysis at 5 dpi (Fig. 1 B).

To examine viral loads in the lower respiratory tract, we performed plaque assay with viruses from lung tissues. CT-P59 significantly abrogated the virus replication in lungs. In the virus only group, the mean virus titer in lung tissues reached 4.1 log₁₀ PFU/mL at 3 dpi and declined to 2.3 log₁₀ PFU/mL at 6 dpi. In contrast, all CT-P59 treatment showed substantial reduction in viral titers at 3 dpi and 6 dpi. At 3 dpi, infectious viral titers were 3.1 and 3.3 log reduced in 5 and 40 mg/kg groups, respectively, while complete reduction was observed in 20 and 80 mg/kg groups. At 6 dpi, no viral titers were detected in all CT P59 treatment (Fig 1 C and D).

4. Discussion

Here, we tested and confirmed *in vitro* neutralizing ability of CT-P59 against Delta, Epsilon, and Kappa variants in two different cell-based assays which are generally used to evaluate the antiviral effect of therapeutic agents. In general, it's said that PRNT is a gold standard method to assess antivirals, since it is a cell-based assay with authentic viruses to see all the step in

viral life cycle from viral entry to release, and re-infection [20]. Pseudovirus assay is broadly used since it is more convenient and known to correlate with PRNT. The advantages over PRNT are to generate mutants you want, simply detect the antiviral activities by measuring luciferase and GFP reporter, and more accessible than Bio-Safety Level 3 Laboratory. However, it's limited to determine the *in vivo* antiviral efficacy of therapeutics since IC₅₀ calculated from cell-based assay indicates its antiviral ability and it's not reflect the effectiveness of treatment dosage and *in vivo* environments.

Like CT-P59, Sotrovimab is a monoclonal antibody which obtained the Emergency Use Authorization for the treatment of COVID-19, demonstrating the effectiveness of 500 mg dosage in clinical trial with 100 ng/mL of IC₅₀ against wild type virus [21]. Also, Sotrovimab retains susceptibility against currently circulating variants such as Alpha, Beta, Gamma, Epsilon, and Kappa, in contrast to Bamlanivimab or Etesevimab [22, 23]. Regdanviamb is very potent antibody having 6 ng/mL of IC₅₀ against wild type virus, which is 16.7-fold stronger than Sotrovimab [19]. Moreover, the clinical dosage of Regdanviamb come to 2,400 mg for 60 kg adult, which is 4.8-fold higher than Sotrovimab. The therapeutic ability of Regdanviamb could come to at least 80-fold (16.7 x 4.8) over that of Sotrovimab.

In accordance with *in vitro* and *in vivo* potency against Beta and Gamma variants [17, 18], CT-P59 showed reduced viral loads and clinical symptoms in Delta-infected mice. Recently, Chen *et al.*, showed that a monoclonal antibody with partial loss of *in vitro* neutralizing activity confers the *in vivo* protections from variant infections in mice comparably [23]. This notion was supported by our results, suggesting that clinical dosage of CT-P59 could overcome a certain level of reduction in *in vitro* antiviral activity, as long as it is not a complete loss in neutralizing activity.

In addition, the biodistribution of monoclonal antibody to target sites *i.e.*, respiratory tracts for COVID-19 is important to translate the *in vitro* antiviral activity to the *in vivo* efficacy in terms of pharmacokinetics of patients [24]. Given that a lung to serum concentration ratio is 15% in development of Casirivimab/Imdevimab, and Sotrovimab for the treatment of COVID-19, lung concentrations during the treatment are predicted with the *in vitro* EC₉₀ to provide the rationale for clinical dosing recommendations [25-27]. CT-P59 is predicted to achieve sufficient lung epithelial lining fluid concentrations above the IC₉₀ values against Delta and its associated variants during the treatment period. Hence, this biodistribution of CT-P59 supports that expected sufficient concentration in lung could compensate for reduced activity of CT-P59 against Delta and its associated variants in real world. Importantly, the finding that CT-P59 ameliorated clinical symptoms in Delta-infected mice [28] supports effectiveness of CT-P59 in patients by protection from progress into severe COVID-19. To obtain conclusive correlation between animal and human therapeutic effectiveness, it needs to be investigated in patients infected with the delta variant.

Conclusion

In accord with previous studies with Beta and Gamma variants, this study revealed that CT-P59 retained reduced activity to Delta, Epsilon, and Kappa variants in cells, and showed *in vivo* substantial protection in Delta variant-infected mice, suggesting that the use of CT-P59 could be a curable option to COVID-19 patients infected with these variants and potential variants including identical mutation sites.

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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Figure/Table Legends

Table 1. Mutations in spike protein of variants

SARS-CoV-2 Variant	Origin	S1								S2	
		N-Terminal Domain				Receptor Binding Domain				Furin Cleavage Site	-
Delta (B.1.617.2)	India	T19R	G142D	Δ156-157	R158G	L452R	T478K	-	D614G	P681R	D950N
Epsilon (B.1.427)	US	-	-	-	-	L452R	-	-	D614G	-	-
Epsilon (B.1.429)	US	S13I	G142D	-	W152C	L452R	-	-	D614G	-	-

Kappa (B.1.617.1)	India	-	-	E154K	-	L452R	-	E484Q	D614G	P681R	Q1071H
B.1.1.519	Mexico	-	-	-	-	-	T478K	-	D614G	P681H	T732A

Table 2. Neutralization effect of CT-P59 against variant live viruses and pseudoviruses

Viruses		IC ₅₀ (ng/mL)	Fold change (reduction)
Live virus	Delta (B.1.617.2)	1237 (WT: 6.76)	182.99
	Epsilon (B.1.427)	499.5 (WT: 6.76)	73.89
	Epsilon (B.1.429)	365.6 (WT: 6.76)	54.08
	Kappa (B.1.617.1)	161.5 (WT: 6.76)	23.89
Pseudovirus	Delta (L452R/T478K/P681R)	21.52 (D614G: 0.219)	98.26
	Epsilon (S13I/W152C/L452R)	6.819 (D614G: 0.219)	31.14
	Kappa (L452R/E484Q/P681R)	11.08 (D614G: 0.219)	50.59
	L452R	13.22 (D614: 0.378)	34.97
	T478K	0.213 (D614G: 0.219)	0.97
	P681H	0.378 (D614: 0.378)	1.00

A

B

Figure 1. *In vivo* efficacy of clinical relevant dose of CT-P59 against Delta variant in mouse model.

ACE2 mice were challenged with B.1.617.2 variants. Negative control (NC) indicates naïve mice with virus. Vehicle and 5, 20, 40 and 80 mg/kg of CT-P59 were administered intraperitoneally 8 h after virus inoculation. Body weights and survival rates were monitored daily (A, B). 25% or higher of weight loss was considered to be dead. Five and four animals each were euthanized for virus titration at 3 dpi and 6 dpi, respectively. The virus titers from lung tissue was measured (C, D) using plaque assay. Alphabets and asterisks indicates statistical significance between the virus only and each treatment group as determined by one-way ANOVA followed by a Dunnett's post-hos test. *a* denotes statistically significant difference ($p < 0.05$) between virus only and each treatment group (5, 20, 40 and 80 mg/kg). *b* denotes statistically significant difference ($p < 0.05$) between virus only and 40 mg/kg. *c* denotes statistically significant difference ($p < 0.05$) between virus only and each treatment group (40 and 80 mg/kg). *** indicates $P < 0.001$, and **** indicates $P < 0.0001$ between virus only and each treatment group.

Supplementary Material

Supplementary Table 1. Binding affinity between CT-P59 and variant RBD proteins

Ligand ¹	Analyte	K _D (M)	Fold change (reduction)
L452R/T478K	CT-P59	5.41E-10 (WT: 5.56E-11)	10
L452R	CT-P59	4.82E-10 (WT: 5.10E-11)	9

¹RBD (wild-type) was prepared in-house and mutant RBDs were purchased from Sino Biological.

A

B

Supplementary figure 1. *In vitro* susceptibility test of CT-P59 against Delta, Epsilon, and Kappa variants.

Two fold-serially diluted CT-P59 were incubated with various SARS-CoV-2 variants such as Delta (B.1.617.2), Epsilon (B.1.427/429), Kappa (B.1.617.1). Each antibody-virus mixture was added to VeroE6 cells. After 2–3 days of incubation, the neutralization activity was evaluated by counting plaques (A). Serially diluted CT-P59 was premixed with indicated variant pseudoviruses followed by inoculation to ACE2-expressing HEK293T cells. Luciferase activity was measured to calculate %Neutralization of CT-P59.