

RESEARCH ARTICLE

Pharmacokinetic profiles of a SARS-COV-2 neutralizing antibody BD-604 in cynomolgus monkeys

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Abstract

Background: Currently, severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) has spread worldwide as a severe pandemic and effective therapeutic medications are urgently needed. As reported previously, BD-604 is a fully human monoclonal antibody with strong *in vitro* and *in vivo* neutralizing activity to SARS-COV-2.

Objective: The purpose of this study was to characterize the pharmacokinetic properties of BD-604 in cynomolgus monkeys.

Methods: To analyze the concentration of BD-604 in cynomolgus monkey serum, an ELISA assay was established, and a systemic validation was performed including accuracy and precision, dilution linearity and hook effect, selectivity, specificity, stability, and parallelism tests. Then, six naïve cynomolgus monkeys (3/sex) were administered BD-604 at a single dose of 10 mg/kg via intravenous infusion (60 min). Blood samples were collected at various time points (0–672 h) and analyzed for serum concentrations of BD-604.

Results: The data from validation experiments assure the reproducibility and reliability of the established ELISA assay. Then, the validated method was used to measure BD-604 concentration in cynomolgus monkey serum. The pharmacokinetics parameters including terminal half-life ($t_{1/2}$), peak serum concentration (C_{max}), area under curve from time zero to last timepoint or infinity (AUC_{last}/AUC_{inf}), apparent volume of distribution (V_z), clearance rate (CL), and mean residence time (MRT) were calculated and reported. BD-604 showed no marked sex differences at the dose of 10 mg/kg when comparing the AUC_{0-last} and C_{max} between female and male cynomolgus monkeys.

Conclusion: In cynomolgus monkeys, BD-604 possesses pharmacokinetic properties similar to natural IgGs.

KEYWORDS

BD-604, monkey, monoclonal antibody, pharmacokinetics, SARS-COV-2

1 | INTRODUCTION

Currently, severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) has led to a global severe pandemic. SARS-COV-2 is a novel coronavirus that can cause a life-threatening disease known as

Coronavirus Disease 2019 (COVID-19), and effective therapeutic medications are urgently needed.

Though convalescent patients' plasma had been widely used in the clinic for the management of COVID-19 patients, it is difficult to obtain enough plasma for the treatment of a large patient

population. To solve this problem, scientists and researchers contribute their efforts to discover and validate monoclonal antibodies with neutralizing activity. These antibodies possess potent and specific binding to spike protein of SARS-COV-2 and block the infection of viruses. At the same time, they could be easily produced on a large scale. As a payback of the efforts, many neutralizing antibodies have been discovered and validated to be effective in preclinical and clinical studies,¹⁻⁷ and some of them have been granted for Emergent Use Authorization by FDA. These antibodies include Regeneron's cocktail therapy casirivimab (formerly known as REGN10933) plus imdevimab (formerly REGN10987), Lilly's bamlanivimab (formerly LY-CoV555) alone and in combination with etesevimab (formerly LY-CoV016).

BD-604 is a monoclonal antibody discovered and identified by high-throughput single-cell sequencing of convalescent patients' B cells.^{8,9} The *in vitro* and *in vivo* studies revealed its potent neutralizing activity to SARS-CoV-2.⁹ In this study, we determined and reported pharmacokinetic profiles of BD-604 in cynomolgus monkeys.

2 | MATERIALS AND METHODS

2.1 | Reagents

BD-604 was *in vitro* expressed using CHO-K1 cells based on the sequences determined in the previous studies.⁹ The stock solution was stored at $\leq -60^{\circ}\text{C}$. SARS-CoV-2 spike protein (RBD, His Tagged, Cat. No. 40592-V08H) was purchased from Sino Biologics and stored at $\leq -20^{\circ}\text{C}$. Goat anti-Human IgG-HRP (Cat. No. 2040-05) was purchased from Southern Biotech and stored at $2-8^{\circ}\text{C}$. Pooled monkey serum (PMS) was from Guangxi Qianyan Biotechnology and stored at $\leq -60^{\circ}\text{C}$. Human IgG was purchased from Beijing Solarbio Life Sciences (Cat. No. SP001).

2.2 | Animals and treatments

Six (3/sex, 2.08–4.08 kg) naïve cynomolgus monkeys supplied by Guangxi Xionsen Primate Laboratory Animal Breeding and Development Co., Ltd., were used in this study. Each animal was given a collar marking and written on the cage card. The room was controlled and monitored for relative humidity (40% to 70%) and temperature (18° to 26°C). The room was on a 12-h light/dark cycle. Fresh drinking water was available to all animals, *ad libitum*. Animals were fed with approximately 200 g per animal daily of certified animal diet from a certified vendor (Beijing Keao Xieli Feed Co. Ltd.). In addition, animals received fruits daily as nutritional enrichment (around 50 g/day/animal).

Animals were weighed prior to dose administration on the dosing day to calculate the actual dose volume. All animals received a single 1-h intravenous infusion of BD-604 from posterior limbs.

2.3 | Clinical observations

Cage-side observations for the general health condition and appearance of animals were performed before and after dosing and at each time point of sample collection. Unusual observations were recorded throughout the duration of the study.

2.4 | PK sampling and analysis

Approximately 1.0-ml blood was collected at each time point (pre-dose, 1.0833 h, 1.5 h, 2 h, 3 h, 5 h, 9 h, 25 h, 49 h, 73 h, 97 h, 121 h, 169 h, 241 h, 337 h, 505 h, 673 h post-beginning of infusion) via peripheral vessels from each study animal. All blood samples were collected into commercially available non-anticoagulant centrifuge tubes. Samples were centrifuged at 4°C for 10 min at $3000 \times g$. The samples were then aliquoted and stored at -60°C or lower until bioanalytical analysis was performed.

Serum concentrations of BD-604 were determined by using a validated ELISA method with an assay range of 10–300 ng/ml. Briefly, SARS-CoV-2 Spike protein (RBD, His Tagged, 0.5 $\mu\text{g}/\text{ml}$, 100 $\mu\text{l}/\text{well}$) diluted in coating buffer (Sigma, Cat. No. SRE0034) was coated onto a microplate and incubated at $2-8^{\circ}\text{C}$ for overnight. After washing with Dulbecco's phosphate-buffered saline (DPBS) supplied with 0.05% Tween-20 (PBST), the microplate was blocked with PBST containing 3% bovine serum albumin (BSA) at 25°C for 2 h. After washing again with PBST, samples were diluted (1:100) with 1% BSA-containing PBST and pipetted into the wells (100 $\mu\text{l}/\text{well}$). If the samples needed to be further diluted, diluted the samples with PMS first to appropriate concentrations and then diluted with 1% BSA-containing PBST at 1:100. After the incubation on a shaker (350 rpm) at 25°C for 2 h, any BD-604 present would be bound by the coating antibody. After washing away any unbound substances, a detection antibody (Goat Anti-Human IgG-HRP) was added into well to bind with BD-604. The microplate was incubated on a shaker (350 rpm) at 25°C for 1 hour. Following a wash to remove any unbound detection antibody, a TMB substrate solution (Surmodics Inc.) was added to the well (100 $\mu\text{l}/\text{well}$), and the color was developed at 25°C for 10 min. Finally, the color development was stopped and the intensity of the color was measured on a plate-reader (Molecular Devices, SpectraMax M5).

The serum concentrations of BD-604 in study animals were subjected to a non-compartmental pharmacokinetic analysis by using the WinNonlin software (version 8.0, Mountain View, CA).

2.5 | Validation of assay method

2.5.1 | Calibration standards

BD-604 stock was diluted with PMS to obtain a series of samples for calibration standards (10, 20, 30, 50, 100, 150, and 300 ng/ml).

2.5.2 | Selectivity

The matrix effect of the assay was investigated by analyzing 10 lots of individual monkey serum. Individual monkey serum was tested with un-spiking and spiking of BD-604 at 10 ng/ml (LLOQ) and 240 ng/ml (HQC).

2.5.3 | Specificity

To evaluate the specificity of the method, PMS was used to obtain following samples: 2400 ng/ml human IgG, 240 ng/ml human IgG, human IgG 240 ng/ml + BD-604 240 ng/ml, human IgG 240 ng/ml + BD-604 25 ng/ml, human IgG 2400 ng/ml + BD-604 240 ng/ml, human IgG 2400 ng/ml + BD-604 25 ng/ml. Each sample was prepared for five sets.

2.5.4 | Accuracy and precision

BD-604 stock was diluted with PMS to obtain following quality control (QC) samples: ULOQ (300 ng/ml), HQC (240 ng/ml), MQC (80 ng/ml), LQC (25 ng/ml), and LLOQ (10 ng/ml). Each sample was tested in triplicate. These QC samples were used for evaluating intra- and inter-assay accuracy as well as precision of the method during six accuracy and precision runs by two analysts in 2 days.

2.5.5 | Dilution linearity and hook effect

The dilution scheme is designed to obtain hook effect samples above ULOQ level and dilution linearity samples falling within the assay range. Hook effect samples were prepared by spiking BD-604 in PMS (HK-1, 200 µg/ml; HK-2, 20 µg/ml; HK-3, 2 µg/ml). To evaluate dilution linearity, hook effect samples were diluted with PBST supplied with 1% BSA at 1:100, and then the samples were diluted with designated dilution factors (HK-1, 1:2500; HK-2, 1:250; HK-3, 1:25 and 1:10). Five sets of dilution linearity and hook effect samples were analyzed.

2.5.6 | Solution stability

Bench-top (room temperature for 4 and 24 h), freeze-thawed ($\leq -60^{\circ}\text{C}$ and room temperature, 3 cycles), and long-term stability ($\leq -60^{\circ}\text{C}$, 33 days) samples were prepared by spiking BD-604 in PMS at the concentration of 240 ng/ml (STB-H, equal to HQC) and 25 ng/ml (STB-L, equal to LQC). Three sets of stability samples at a given storage condition at each level were included in an analytical run.

2.5.7 | Parallelism

Total three incurred samples with BD-604 were evaluated for parallelism. Concentrations of all diluted samples fell within assay range, and back-calculated concentrations were used for precision evaluation. The samples were diluted with PBST supplied with 1% BSA at 1:100, and then the samples were diluted with designated dilution factors.

2.6 | Data collection and analysis

OD values were collected by using Softmax Pro Software (6.5.1) and introduced into Watson LIMS (7.5) system. Microsoft Office Excel was used to calculate mean values, standard deviation (SD), coefficient of variation (CV, %), bias (%), and total error (%).

3 | RESULTS

In this section, firstly, we are going to present validation data of assay method used for measurements of BD-604 concentrations in monkey serum samples. All the data met the acceptance criteria as required by International Conference on Harmonization (ICH) and U.S. Food and Drug Administration (FDA) Guidelines on Bioanalytical Method Validation.^{10,11} Subsequently, six cynomolgus monkeys were treated with BD-604. Serum samples were collected and analyzed for BD-604 concentrations with validated method.

TABLE 1 Validation of standard curves used for determination of serum concentrations of BD-604

	Measured concentrations (mean \pm SD, ng/ml)	Range of bias (%)	CV (%)
10 ng/ml	10.10 \pm 0.16	-0.70-4.50	1.59
20 ng/ml	20.00 \pm 0.23	-2.35-1.15	1.14
30 ng/ml	29.92 \pm 0.50	-1.67-3.77	1.67
50 ng/ml	49.44 \pm 0.60	-3.30-0.26	1.22
100 ng/ml	100.06 \pm 1.08	-2.20-2.07	1.08
150 ng/ml	152.88 \pm 2.22	-0.35-4.03	1.45
300 ng/ml	297.00 \pm 2.83	-2.84-0.1	0.95

Abbreviations: CV, coefficient variance; SD, standard deviation.

Finally, pharmacokinetic parameters were calculated as described in Section 2 and presented below.

3.1 | Validation of assay method

3.1.1 | Calibration standards

Ten sets of standards at concentrations of 10–300 ng/ml had been tested for method validation. The bias (%) of all samples fell within a range of –3.30%–4.50% (Table 1) and met the preset acceptance criteria ($\pm 25\%$ for LLOQ and ULOQ and $\pm 20\%$ for other samples).

3.1.2 | Selectivity

Ten lots of individual monkey serum was tested with un-spiking and spiking of BD-604 at 10 ng/ml (LLOQ) and 240 ng/ml (HQC). The data showed that bias (%) of all samples met preset

acceptance criteria ($\pm 25\%$ for LLOQ and $\pm 20\%$ for HQC) as shown in Figure 1.

3.1.3 | Specificity

Five sets of samples were used to determine the specificity of the assay method. As shown in Figure 2, the method showed excellent specificity and met preset acceptance criteria ($\pm 20\%$ for all samples). The presence of hIgG did not interfere with the determination of BD-604 in monkey serum.

3.1.4 | Accuracy and precision

The following samples had been used for evaluating intra- and inter-assay accuracy as well as precision of the method: ULOQ (300 ng/ml), HQC (240 ng/ml), MQC (80 ng/ml), LQC (25 ng/mL), and LLOQ (10 ng/ml). The data had been summarized in Table 2, and all results met preset acceptance criteria (accuracy and precision, $\pm 25\%$ for ULOQ/LLOQ, and $\pm 20\%$ for others; total error, $\pm 40\%$ for ULOQ/LLOQ and $\pm 30\%$ for others).

3.1.5 | Dilution linearity and hook effect

Dilution linearity and hook effect were determined, and the data were presented in Figure 3. No obvious hook effect was observed even when the concentration of BD-604 was up to 200 $\mu\text{g/ml}$, suggesting that preset acceptance criteria was met ($\pm 20\%$ for all samples).

3.1.6 | Solution stability

The stability of BD-604 in serum samples was investigated under various conditions, and the data were listed in Table 3. All results met preset acceptance criteria ($\pm 20\%$ for all samples).

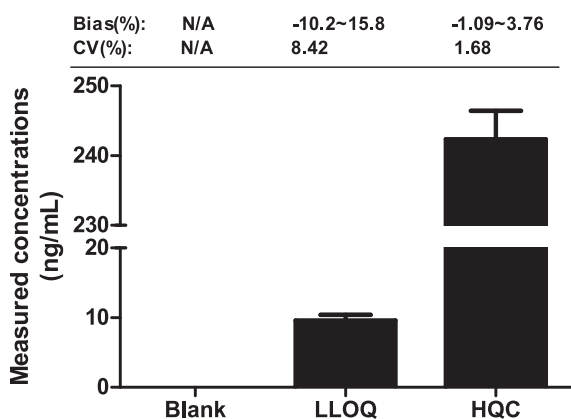


FIGURE 1 Selectivity of the assay method used for determination of serum concentrations of BD-604. CV, coefficient variance; HQC, high quality control, 240 ng/ml; LLOQ, lower limit of quantification, 10 ng/ml; N/A, not available

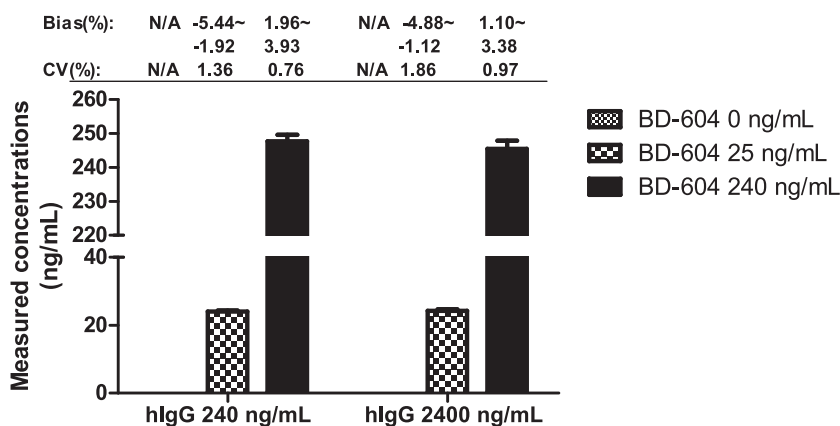


FIGURE 2 Specificity of the assay method used for determination of serum concentrations of BD-604. CV, coefficient of variance; N/A, not available

TABLE 2 Intra-assay and inter-assay accuracy and precision of the assay method used for determination of serum concentrations of BD-604

		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Inter-assay
LLOQ (10 ng/ml)	Measured (ng/ml)	9.73 ± 0.13	10.15 ± 0.20	10.44 ± 0.10	9.61 ± 0.13	9.69 ± 0.26	9.90 ± 0.17	9.92 ± 0.33
	CV (%)	1.37	1.98	0.91	1.36	2.63	1.68	3.35
	Bias (%)	-2.73	1.50	4.40	-3.90	-3.07	-0.97	-0.79
	TE (%)	-	-	-	-	-	-	4.15
LQC (25 ng/ml)	Measured (ng/ml)	24.49 ± 0.02	26.17 ± 0.42	25.98 ± 0.27	24.21 ± 0.30	23.92 ± 0.14	24.34 ± 0.52	24.85 ± 0.95
	CV (%)	0.08	1.62	1.04	1.25	0.57	2.12	3.82
	Bias (%)	-2.03	4.69	3.92	-3.15	-4.31	-2.64	-0.58
	TE (%)	-	-	-	-	-	-	4.40
MQC (80 ng/ml)	Measured (ng/ml)	77.54 ± 0.21	80.90 ± 1.16	81.43 ± 0.35	77.04 ± 0.85	76.33 ± 0.78	76.26 ± 0.91	78.25 ± 2.27
	CV (%)	0.26	1.44	0.43	1.10	1.02	1.19	2.90
	Bias (%)	-3.07	1.13	1.79	-3.70	-4.59	-4.68	-2.19
	TE (%)	-	-	-	-	-	-	5.09
HQC (240 ng/ml)	Measured (ng/ml)	234.84 ± 0.59	240.53 ± 2.23	243.94 ± 4.03	226.73 ± 4.46	224.72 ± 1.76	221.85 ± 3.42	232.10 ± 8.86
	CV (%)	0.25	0.93	1.65	1.96	0.78	1.54	3.82
	Bias (%)	-2.15	0.22	1.64	-5.53	-6.37	-7.56	-3.29
	TE (%)	-	-	-	-	-	-	7.11
ULOQ (300 ng/ml)	Measured (ng/ml)	281.93 ± 1.60	289.11 ± 2.25	286.67 ± 5.97	285.79 ± 1.68	284.57 ± 11.86	272.79 ± 3.63	283.48 ± 7.26
	CV (%)	0.57	0.78	2.08	0.59	4.17	1.33	2.56
	Bias (%)	-6.02	-3.63	-4.44	-4.74	-5.14	-9.07	-5.51
	TE (%)	-	-	-	-	-	-	8.07

Abbreviations: CV, coefficient variance; TE, total error.

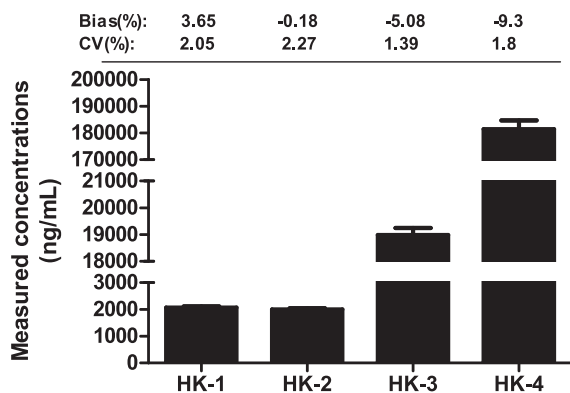


FIGURE 3 Dilution linearity and hook effect of the assay method used for determination of serum concentrations of BD-604. HK-1, 2000 ng/ml of BD-604 with a dilution factor of 10. HK-2, 2000 ng/ml of BD-604 with a dilution factor of 25. HK-3, 20,000 ng/ml of BD-604 with a dilution factor of 250. HK-4, 200,000 ng/ml of BD-604 with a dilution factor of 2500

3.1.7 | Parallelism

Parallelism of the assay method was evaluated with three incurred samples, and excellent parallelism was demonstrated as shown in Table 4.

3.2 | Pharmacokinetic analysis

No mortality or morbidity was observed throughout the study. No BD-604 related changes in hematology or serum chemistry were noted (data not shown here).

Following single IV infusion administration of BD-604 at 10 mg/kg in male and female monkeys, mean serum concentrations of BD-604 were shown in Figure 4 and pharmacokinetic parameters were listed in Table 5. Generally, BD-604 showed pharmacokinetic profiles similar to natural IgG1 with a half-life of approximately 13.91 days (333.93 h), which was similar to previous reports.¹² The C_{max} was around 301.10 μ g/ml and at the end of the study (673 h), and mean serum concentration of BD-604 was 31.43 μ g/ml. At the same time, BD-604 showed no marked sex differences at the dose level when comparing AUC_{0-last} (ratio, 0.984) and C_{max} (ratio, 0.939) in female and male cynomolgus monkeys.

4 | DISCUSSION

In this study, we established and validated an ELISA method for determination of BD-604 in monkey serum. The method demonstrated excellent sensitivity, specificity, selectivity, accuracy and

TABLE 3 Solution stability of BD-604 in monkey serum

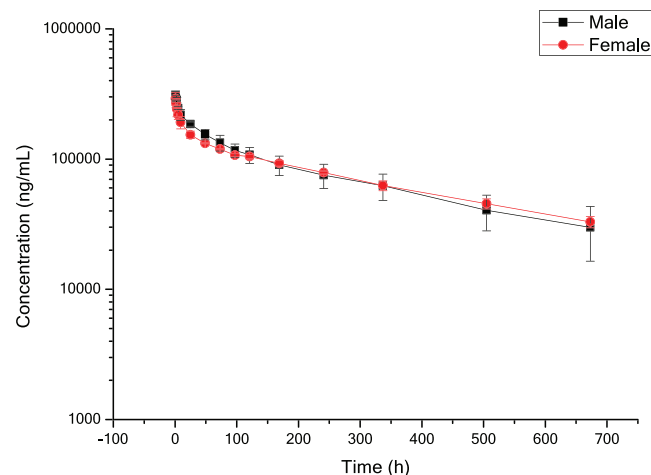
		0 h	Room temperature		Freeze-thawed 3 cycles	-60°C 33 days
			4 h	24 h		
STB-L, 25 ng/ml	Measured (ng/ml)	26.54 ± 0.50	26.94 ± 0.26	25.55 ± 0.82	24.73 ± 0.35	23.33 ± 0.39
	CV (%)	1.88	0.98	3.19	1.40	1.68
	Bias (%)	6.16	7.75	2.19	-1.09	-6.67
STB-H, 240 ng/ml	Measured (ng/ml)	247.93 ± 2.35	242.47 ± 8.50	232.13 ± 3.00	229.46 ± 2.50	231.28 ± 7.82
	CV (%)	0.95	3.51	1.29	1.09	3.38
	Bias (%)	3.30	1.03	-3.28	-4.39	-3.63

Abbreviations: CV, coefficient variance.

	Dilution factor			Mean ± SD (ng/ml)	CV (%)
	500	1000	2000		
1-2-169 h	83,706.89	82,438.13	79,092.13	81,745.72 ± 2384.03	2.92
1-4-169 h	87,985.06	88,314.71	84,281.56	86,860.44 ± 2239.45	2.58
1-5-169 h	88,220.25	85,472.97	84,101.11	85,931.44 ± 2097.49	2.44

Abbreviations: CV, coefficient variance. SD, standard deviation.

Note: 1-2-169 h, 1-4-169 h, and 1-5-169 h represent three samples from three individual monkeys after treatment with BD-604 for 169 h.

TABLE 4 Parallelism of the assay method used for determination of serum concentrations of BD-604**FIGURE 4** Mean serum concentration profiles of BD-604 in male and female cynomolgus monkeys following single intravenous infusion of BD-604 at 10 mg/kg [Colour figure can be viewed at wileyonlinelibrary.com]

precision, dilution linearity, and parallelism (Tables 1 and 2 and 4 and Figures 1–3). Taking tested dilution factor and range of standards into the consideration, this method is enough for determination of BD-604 at high concentrations such as ~300 µg/ml (~C_{max}). For long-term stability (Table 3), we stored BD-604 samples at ≤-60°C for 33 days, which is enough to cover the duration of both PK sampling (0–673 h) and following sample analysis/reanalysis.

Since the validation studies met all the preset acceptance criteria, the method was used to analyze BD-604 concentrations in monkey serum samples. As shown in Table 5 and Figure 4, the data suggested that BD-604 possesses similar pharmacokinetic profiles to natural IgGs. At the same time, no sex difference was observed between male and female monkeys. Mean V_z and CL were 73.08 ml/kg and 0.16 ml/h/kg, respectively, leading to long MRT (235.99 h) and $t_{1/2}$ (333.93 h). As a result, mean AUC_{last} and AUC_{inf} reached 50189.67 h*µg/ml and 65893.9 h*µg/ml, respectively. Even at the end of the study (673 h), mean serum concentration of BD-604 was still over 30 µg/ml. These data suggested that a single dose of BD-604 at 10 mg/kg has the capability to provide long-term protection against SARS-COV-2 infection.

As shown previously, BD-604 strongly binds to viral protein with K_D value of 1.8×10^{-10} M and potently neutralizes the infection of SARS-COV-2 pseudovirus with IC_{50} value of 5 ng/ml.⁹ In this study, we could see the serum concentrations of BD-604, even at 673 h post infusion, are far beyond IC_{50} we observed in *in vitro* virus neutralization assays.

Since lung is the most important target organ of SARS-COV-2 infection, the biodistribution of monoclonal antibodies in lungs is taken into consideration. According to published publications, lung biodistribution of post-exposure prophylactic monoclonal antibodies ranges from 0.5% to 15%.^{13–16} Here, we assume that the concentration of BD-604 in lung interstitial fluid is also 0.5–15% of its serum concentration. Based on this hypothesis, even at 673 h post

TABLE 5 Mean pharmacokinetic parameters of BD-604 in male and female cynomolgus monkeys following single intravenous infusion of BD-604 at 10 mg/kg

		$t_{1/2}$ h	C_{max} μg/ml	AUC_{last} h*μg/ml	AUC_{inf} h*μg/ml	V_z ml/kg	CL ml/h/kg	MRT h
Male	Mean	306.56	310.55	50582.26	64764.34	68.29	0.16	227.32
	SD	82.48	23.42	7821.99	15868.45	10.46	0.04	24.92
	CV(%)	26.90	7.54	15.46	24.50	15.32	24.70	10.96
Female	Mean	361.29	291.65	49,797.07	67,023.45	77.86	0.15	244.66
	SD	22.71	32.07	1923.58	3863.82	5.00	0.01	7.48
	CV(%)	6.29	11.00	3.86	5.76	6.43	5.80	3.06
Ratio	-	-	0.939	0.984	-	-	-	-

Note: AUC_{last}/AUC_{inf} , area under curve from time zero to last quantifiable concentration or infinity; CL, clearance rate; C_{max} , peak serum concentration; CV, coefficient variance; MRT, mean residence time; Ratio, mean (female)/mean (male); SD, standard deviation; $t_{1/2}$, half-life; V_z , apparent volume of distribution during the terminal phase.

infusion, the concentration of BD-604 in lung interstitial fluid is supposed to be 150–4500 ng/ml. Still, this concentration range is far beyond IC_{50} (>30-fold) we observed in *in vitro* virus neutralization assays. This data suggested that BD-604 at a dose of 10 mg/kg may be enough for the prevention or treatment of SARS-CoV-2 infection.

Generally, this study showed that BD-604, a potent neutralizing antibody to SARS-CoV-2, is well tolerated in cynomolgus monkeys and possesses pharmacokinetic properties similar to natural IgGs.

5 | CONCLUSION

In this study, we established a validated assay method to measure BD-604 concentrations in monkey serum samples. The pharmacokinetic analysis indicated that BD-604, a potent neutralizing antibody to SARS-CoV-2, possesses long half-life as other IgGs does and has a capability to provide a long-term protection against SARS-CoV-2 infection. These data provide us pharmacokinetic clues for experimental design, including dose selection and dosing frequency, of further pre-clinical and clinical studies. At the same time, based on the data, we are expecting to see a long-term anti-virus effect of BD-604 in clinical trials, as we observed in pre-clinical studies.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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