Nasal Spray of Neutralizing Monoclonal Antibody 35B5 Confers Potential Prophylaxis Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Variants of Concern (VOCs): A Small-scale Clinical Trial

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Running title: 35B5-nasal spray neutralizes SARS-CoV-2

ABSTRACT

Background. SARS-CoV-2 VOCs, especially the Delta and Omicron variants, have been reported to show significant resistance to approved neutralizing monoclonal antibodies (mAbs) and vaccines. We previously identified a mAb named 35B5 that harbors broad neutralization to SARS-CoV-2 VOCs. Herein, we explored the protection efficacy of a 35B5-based nasal spray against SARS-CoV-2 VOCs in a small-scale clinical trial.

Methods. We enrolled 30 healthy volunteers who were nasally administrated with the modified 35B5 formulation. At 12, 24, 48 and 72 hours after nasal spray, the neutralization efficacy of nasal mucosal samples was assayed with pseudoviruses coated with SARS-CoV-2 Spike protein of the wild-type (WT), Alpha, Beta, Delta, or Omicron variants.

Results. The nasal mucosal samples collected within 24 hours after nasal spray effectively neutralized SARS-CoV-2 VOCs (including Delta and Omicron). Meanwhile, the protection efficacy was 60% effective and 20% effective at 48 and 72 hours after nasal spray, respectively.

Conclusions. A single nasal spray of 35B5 formation conveys 24-hour effective protection against SARS-CoV-2 VOCs, including the Alpha, Beta, Delta, or Omicron variants. Thus, 35B5 nasal spray might be potential in strengthening SARS-CoV-2 prevention, especially in the high-risk population.

Keywords. COVID-19; SARS-CoV-2; variants of concern; antibody; nasal spray

INTRODUCTION

The novel coronavirus SARS-CoV-2 causes coronavirus disease 2019 (COVID-19) and has resulted in a global epidemic. Though a spectrum of COVID-19 vaccines has been investigated to control the epidemic [1], neutralizing mAb therapies targeting SARS-CoV-2, especially the Omicron (B.1.1.529) variant, are still of paramount importance due to 1) the Omicron variant is markedly resistant to current COVID-19 vaccines [2-5] and 2) the capacity of neutralizing mAbs to provide immediate protection for unvaccinated individuals and vaccine-responseless individuals.

Currently, neutralizing mAbs against SARS-CoV-2, including those approved for clinical use and being investigated in clinic trials, are mainly used through intravenous infusion [1]. However, antibody levels in the lung are 200-10,000 times lower than those in the serum after intravenous infusion [6, 7], which leads to suboptimal protection against respiratory viruses, such as SARS-CoV-2. Alternatively, intranasal delivery of neutralizing mAbs has been proved to be advantageous in preventing and treating respiratory viruses, exemplified by influenza virus [8] and respiratory syncytial virus [9, 10]. Indeed, nasal cavity is the first and major site of infection by SARS-CoV-2 [11, 12] and nasal delivery of anti-SARS-CoV-2 mAbs should prevent the transmission of SARS-CoV-2.

Our previous studies identified an array of human mAbs that target the

receptor-binding domain (RBD) protein of SARS-CoV-2 and neutralize SARS-CoV-2 [13-15]. Among them, one mAb named as 35B5 broadly and potently neutralizes WHO-stated SARS-CoV-2 VOCs, especially exhibiting the picomolar neutralizing efficacy to the Delta variant (B.1.617.2) [15] and the Omicron variant (B.1.1.529) [13]. Herein, we explored the effects of nasal spray of 35B5 mAb in protecting individuals from SARS-CoV-2 VOCs.

METHODS

Study Population

We enrolled two cohorts and each one contains 15 healthy volunteers in the study. All volunteers were provided with written informed consents. These volunteers were nasally administrated with modified 35B5 mAb formulation (1 mg/mL 35B5 mAb, diluted in 50% DPBS plus 50% glycerol) by a home-made nebulizer. For cohort 1, nasal sample of 12 hours was collected from one nasal cavity of each volunteer, while nasal sample of 24 hours was collected from another nasal cavity. For cohort 2, each nasal sample of a timepoint was collected from one individual. The nasal secretions were collected by the insertion of cotton swabs into nasal cavities for five minutes, then hydrating for ten minutes with 0.5 mL of PBS solution. The study received IRB approval at Chongqing Public Health Medical Center (2022-005-02-KY).

Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA plates (Thermo Fisher, 446469) were coated with 50 ng SARS-CoV-2 RBD protein (Sino Biological, 40592-V08H) in 100 µl PBS per well overnight. Then, the ELISA plates were incubated with blocking buffer (5% FBS + 0.1% Tween 20 in PBS) for 1 hour. Nasal mucosal samples (50 µL) were next added to each well and incubated for 1 hour. The ELISA plates were then washed with PBST (PBS + 0.1% Tween 20), incubated with HRP-conjugated goat anti-human IgG antibody (Bioss Biotech), washed with PBST and added with TMB (Beyotime). The ELISA plates were allowed to react with TMB for ~5 min and then stopped by 1 M H_2SO_4 stop buffer. The optical density (OD) value was determined at 450 nm. In each ELISA assay, a range of serially diluted original/unmodified 35B5 mAb (0.00064, 0.0032, 0.016, 0.08, 0.4, 2, 10 and 50 µg/mL; 5-fold dilution) was used as positive control.

SARS-CoV-2 Pseudovirus Neutralization Assay

SARS-CoV-2 pseudotype particles (0.01 MOI), including WT strain (SinoBiological, PSV001), the Alpha (B.1.1.7) strain (SinoBiological, PSV006), the Beta (B.1.351) strain (SinoBiological, PSV008), the Delta (B.1.617.2) strain (SinoBiological, PSV011) and the Omicron (B.1.1.529) strain (SinoBiological, PSV016), were pre-incubated with nasal mucosal samples for 1 hour at 37^{o}C. Then, hACE2-expressing HEK-293T (hACE2/293T) cells were incubated with the

mixtures overnight and then cultured with fresh media. At 48 hours after incubation, the luciferase activity of SARS-CoV-2 typed pseudovirus-infected hACE2/293T cells were determined by a luciferase reporter assay kit (Promega, E1910). Such SARS-CoV-2 pseudovirus neutralization assay has been proven highly consistent with the authentic SARS-CoV-2 neutralization assay [3, 13, 16, 17].

Statistics

The 35B5 concentrations of nasal mucosal samples of each individual were compared by the paired *t* test. The cutoff value in each pseudovirus neutralization assay was determined by the receiver operating characteristic curve analysis and was of the highest likelihood ratio. *P* values less than 0.05 were defied as statistically significant. GraphPad Prism version 6.0 software was used for statistical analysis.

RESULTS

The Mucosal Swabs Sampled Post Nasal Spray of 35B5 mAb Preserves Potent Neutralization Against SARS-CoV-2 VOCs within 24 Hours

We recruited 15 healthy volunteers (cohort 1; median age, 29 [range, 23-46] years), who have no history of SARS-CoV-2 infection and received COVID-19 vaccines (Supplementary table 1). Prior to antibody spray, nasal samples of all volunteers were collected as blank controls (Figure 1A). Then, volunteers were

intranasally administered with modified 35B5 mAb formulation (see details in METHODS) and were subsequently performed with nasal sampling at 12 hours and 24 hours after antibody spray (Figure 1A).

Firstly, we used IgG ELISA to detect 35B5 mAb in nasal mucosal samples of 3 different timepoints (pre-antibody spray, 12 hours and 24 hours). We found that mAbs specific for SARS-CoV-2 RBD were undetected in nasal mucosal samples of pre-antibody spray timepoint, suggesting no pre-existing anti-SARS-CoV-2 RBD mAbs in the nasal mucus of these volunteers who received COVID-19 vaccines (Figure 1B). Remarkably, abundant amounts of mAbs specific for SARS-CoV-2 RBD were observed in nasal mucosal samples of 12 hours and 24 hours after antibody spray (Figure 1B). Given no detectable anti-SARS-CoV-2 RBD mAbs in pre-antibody spray samples, nasal spray of 35B5 mAb acted as the sole contributor for the anti-SARS-CoV-2 RBD mAb-rich nasal mucus at 12 hours and 24 hours. We further observed that the 35B5 mAb concentration in nasal mucus varied in the range from 1 to 10 μg/mL (Figure 1B), which might be due to non-unified nasal administration. However, the 35B5 mAb concentrations were comparable between samples of 12 hours and samples of 24 hours (Figure 1B), which suggests that modified 35B5 mAbs were stably adhered to the nasal mucosal surface for at least 24 hours.

We next assessed the neutralizing activity of 35B5 mAb-rich nasal mucus

against SARS-CoV-2 variants by pseudovirus neutralization assays as previously described [18, 19]. Notably, we found that 100% of nasal mucosal samples from both 12 hours and 24 hours were able to protect hACE2/293T cells from being infected by SARS-CoV-2 pseudoviruses, including WT, B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta) and B.1.1.529 (Omicron) (Figure 1C-L). These findings echo the ultrapotent neutralizing activities of 35B5 mAb in treating authentic SARS-CoV-2-infected mice [15] and highlight the highly potent prophylactic effects of modified 35B5 mAb spray in protecting individuals from SARS-CoV-2 (especially the Omicron variant) infection.

Protection Efficacy of a Single Dosage of 35B5 Nasal Spray Wanes after 24 Hours

To further explore the protection duration of a single dosage of 35B5 mAb formation, we recruited another cohort of 15 healthy volunteers (cohort 2; median age, 28 [range, 26-31] years) (Supplementary table 1). These volunteers were nasally administrated with 35B5 mAb formation and the nasal mucosal samples were collected at 24, 48 or 72 hours (Figure 2A). As evaluated by the IgG ELISA assay, 35B5 mAb concentrations of 48-hour samples and 72-hour samples were 10-fold reduced and 100-fold reduced, respectively, compared to that of 24-hour samples (Figure 2B). Additionally, we also examined the neutralizing activities of these mucosal samples against the SARS-CoV-2 Delta and Omicron variants.

Consistently, we found that 24-hour samples show highly potent neutralizing capacity to the Delta and Omicron variants (Figure 2C-F). However, the frequency of protection dropped to 60% at 48 hours-post nasal spray and eventually descended to 20% at 72 hours-post nasal spray (Figure 2C-F). Thus, these findings suggest that a single dosage of 35B5 nasal spray conveys protection against SARS-CoV-2 within the first 24 hours and a second dosage of 35B5 nasal spray is needed for longer duration of protection.

DISCUSSION

Neutralizing mAbs convey immediate protection to individuals exposed to SARS-CoV-2. Our previous works identified a human mAb 35B5 that exhibits picomolar pan-neutralization against SARS-CoV-2 VOCs *in vitro* and *in vivo* [13, 15]. In this small-scale clinic trial, we show that nasal secretions of individuals nasally administrated with 35B5 mAb formulation within the first 24 hours convey efficient *in vitro* neutralization against SARS-CoV-2 VOCs, including the Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2) and Omicron (B.1.1.529) variants. To our knowledge, this is the first clinic study to report the prophylactic efficacy of neutralizing mAb against SARS-CoV-2 infection through nasal spray.

Nasal spray of neutralizing mAbs was recently reported to be of high efficacy to protect animals against SARS-CoV-2 [6, 20-22]. Indeed, nasal delivery of neutralizing mAbs mainly targets the airway [6], including nasal cavity and

lung, and was approved to be more effective than systemic application (e.g., intraperitoneal) in curtailing SARS-CoV-2 load and ameliorating lung pathology [22]. In addition, nasal delivery of neutralizing mAbs was also suggested to be superior to systemic application in protecting animals against influenza [8] and respiratory syncytial virus [9, 10]. Herein, we found that nasal spray of 35B5 antibody generates enriched antibodies in the nasal mucosa, thus conveying potent prophylactic protection against SARS-CoV-2 by nasal spray of neutralizing mAbs in a clinic setting. Further studies are needed to explore whether the nasal spray of 35B5 antibody could efficiently delivery antibodies to lung of volunteers, as reported in the animal study [6], and thus further support the usage of 35B5 nasal spray in clinical practice.

The portal of entry for SARS-CoV-2 is the respiratory tract and the lung is the primary organ for pathogenesis. IgM and IgA are mucosal antibodies that defend against respiratory viruses locally. Remarkably, nasal delivery of an IgM mAb conferred higher respiratory protection than the IgG1 counterpart for protection against SARS-CoV-2 in mice [6]. Given the high prophylactic efficacy of nasal spray of 35B5 IgG1 mAb in the study, the effects of nasal spray of engineered IgM- or IgA-typed 35B5 mAb warrant further investigation.

The Omicron (B.1.1.529) variant is a novel VOC identified in December 2021 and has rapidly increased in frequency worldwide [23], attributing to its

remarkable escape from current COVID-19 vaccines and therapeutic neutralizing mAbs approved for clinical use [2-5]. In the study, we found that the nasal secretions of individuals administrated with the modified 35B5 mAb formulation efficiently neutralize the Omicron variant *in vitro* and thus highlights the modified 35B5 mAb formulation as an emergency medical countermeasure in coping with the Omicron variant.

Given the fact that some studies indicate higher SARS-CoV-2 titers in the throat/saliva than nasopharyngeal swabs and throat/saliva was suggested to be more sensitive than the corresponding nasopharyngeal swabs for SARS-CoV-2 detection [24-27], we assume that a delivery of 35B5 mAb to the respiratory tract via both the nose and mouth could convey a reinforced protection against SARS-CoV-2 infection.

There are some limitations in our study. One limitation is the relatively small population size, even though we got consistent results in two cohorts. Another limitation is the lack of functional validation of 35B5 nasal spray with the authentic SARS-CoV-2 inhibition assays or mouse experiments, which need further investigations. In addition, we found that the effective protection duration of one single dosage of 35B5 nasal spray only cover the first 24 hours, which requires daily inhalation in clinical practice. More strategies, such as modifying the initial antibody concentration or optimizing the formulation for extended

duration of antibody at nasal mucosa, are needed to improve the protection duration of 35B5 nasal spray in the future.

In conclusion, we showed the first clinical evidence that nasal spray of modified neutralizing mAb may efficiently protect individuals from SARS-CoV-2, including WT, B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta) and B.1.1.529 (Omicron) variants. We advise to take nasal spray of neutralizing mAb as a strategy to strengthen COVID-19 prevention, especially for the high-risk population.

Notes

Author contributions. Y.L., S.Y., Y.Y., Z.P. and X.Y. performed the experiments. S.Y., Z.L., L.H., J.T., Q.W., S.L. and Q.T. assisted in recruiting the healthy participants. L.G., J.Z., Y.W., Y.H., L.X., Q.H. and B.Z. helped to discuss the results; X.C. designed the study, analyzed the data and drafted the paper with L.Y. and Y.C.; and L.Y. and Y.C. supervised the study.

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Potential conflicts of interest. The patent of 35B5 has been licensed (reported by L.Y.).

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Figure legends

Figure 1. The mucosal swabs sampled post nasal spray of 35B5 mAb preserves potent neutralization against SARS-CoV-2 VOCs within 24 hours. A, schematic diagram. B, the 35B5 mAb concentrations in nasal mucosal samples at different timepoints as determined by ELISA assays. C-L, neutralization of nasal mucosal samples against SARS-CoV-2 WT (C and D), Alpha (E and F), Beta (G and H), Delta (I and J) and Omicron (K and L) as determined by pseudovirus neutralization assays. Nasal secretions from individuals with luciferase value under cutoff line were considered as protective. The cutoff value in each pseudovirus-neutralizing function assay was determined by the receiver operating characteristic curve analysis and was of the highest likelihood ratio to distinguish samples of positive controls (pre-spray) and samples of negative controls (no virus). Error bars in (C, E, G, I, K) indicate SD.

Figure 2. Protection efficacy of a single dosage of 35B5 nasal spray wanes after 24 hours. A, schematic diagram. B, the 35B5 mAb concentrations in nasal mucosal samples at different timepoints as determined by ELISA assays. C-F, neutralization of nasal mucosal samples against SARS-CoV-2 Delta (C and D) and Omicron (E and F) as determined by pseudovirus neutralization assays. Nasal secretions from individuals with luciferase value under cutoff line were considered as protective. The cutoff value in each pseudovirus-neutralizing

function assay was determined by the receiver operating characteristic curve analysis and was of the highest likelihood ratio to distinguish samples of positive controls (pre-spray) and samples of negative controls (no virus). Error bars in B indicate SEM and error bars in (C, E) indicate SD.

Figure 1

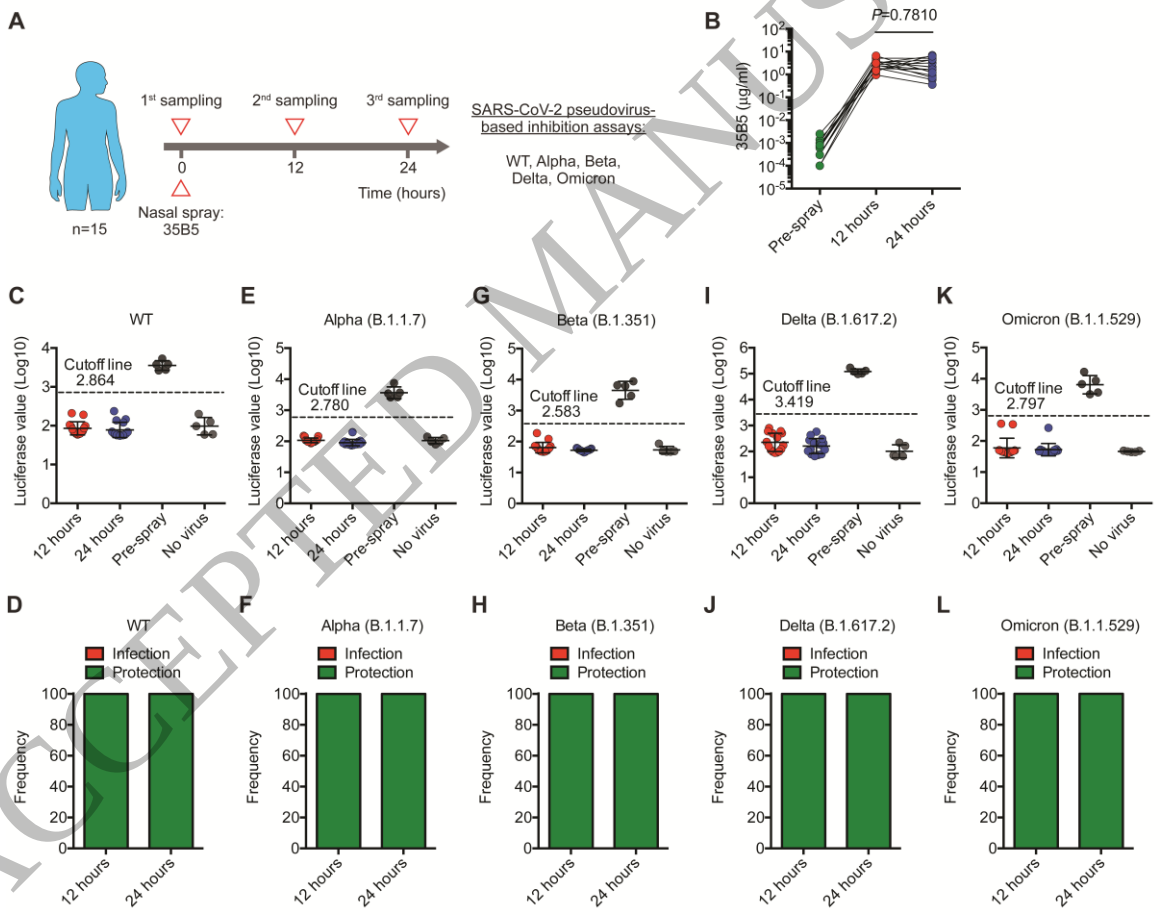

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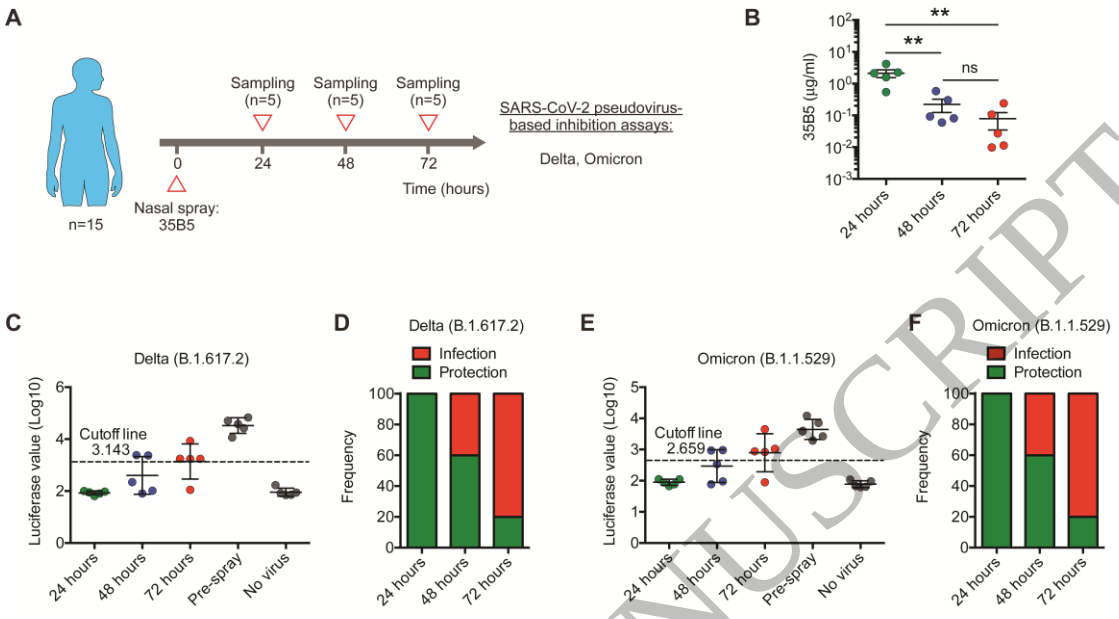
Figure 2



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