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Respiratory delivery of passive immunotherapies for SARS-CoV-2 prophylaxis and therapy

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ABSTRACT

Convalescent plasma has been extensively tested during the COVID-19 pandemic as a transfusion product. Similarly, monoclonal antibodies have been largely administered either intravenously or intramuscularly. Nevertheless, when used against a respiratory pathogen, respiratory delivery is preferable to maximize the amount of antibody that reaches the entry door in order to prevent sustained viral multiplication. In this narrative review, we review the different types of inhalation device and summarize evidence from animal models and early clinical trials supporting the respiratory delivery (for either prophylactic or therapeutic purposes) of convalescent plasma or monoclonal antibodies (either full antibodies, single-chain variable fragments, or camelid-derived monoclonal heavy-chain only antibodies). Preliminary evidences from animal models suggest similar safety and noninferior efficacy, but efficacy evaluation from clinical trials is still limited.

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Introduction

While the mucosal (especially nasal) route is being increasingly investigated for COVID-19 vaccination, very few studies have reported intranasal (i.n.) delivery of passive immunotherapies. Respiratory delivery presents less logistical burden, offering a needle- and nurse-free delivery, requiring lower antibody doses (which facilitates scaling up of deployment), enabling self-administration at home, and not requiring refrigeration (which is relevant in low-and-middle-income countries).^{1,2}

Intranasal delivery of passive immunotherapies against human pathogens has been scarcely used in the past. In the setting of influenza virus (including highly pathogenic avian influenza), administration of broadly-neutralizing IgG^{3,4} or IgA⁵ through i.n. and aerosol routes in mice showed higher prophylactic protection as compared to systemic routes, and controlled the advanced stage infection with a much-reduced dose. All of this translates into higher chances of viral clearance and lower chances of treatment-emergent resistance.⁶ In comparison to parenteral treatment, inhalation of IgG's gave better protection and a greater therapeutic response in pneumonia models using the influenza virus.^{7,8} Nasal delivery of viral vectors encoding anti-influenza IgG has also been tested successfully in mice.⁹ More limited experiences have been reported for monkeypox virus¹⁰ and respiratory syncytial virus.¹¹ Again, no clinical study has been run for those pathogens.

All the monoclonal antibodies (mAb) targeting the SARS-CoV-2 Spike protein and emergency-use authorized since the beginning of the COVID-19 pandemic have been of the IgG₁ subtype: the levels of IgG antibodies after intravenous infusion are 200–500 times lower in the mucosal compartments than in serum.⁴ This results in the need for administration of high

doses of IgG (i.e., up to 8 g) to achieve antiviral activity in respiratory tissues. Despite such high doses, the antiviral effect is still marginal in the respiratory tract compared to the placebo controls.⁵ On the contrary IgM¹² and IgA₁¹³ assemble into multimers via the J-chain, facilitating mucosal transcytosis, and can be nebulized.¹⁴ Of interest, IgA represents the majority of SARS-CoV-2-neutralizing antibodies *in vivo*.¹⁵

Inhalation devices

In addition to drop inoculation, three different types of inhalation devices are commonly used (Figure 1).¹⁶ Nebulizers (jet, ultrasonic, and vibrating mesh) use a liquid solution of the medicine to produce aerosol droplets and are used to deliver them to airways and lungs: they are cheap but prolonged storage of proteins in liquid solutions causes deamination, hydrolysis, and aggregation,^{17–19} which can be minimized by the use of surfactant.¹⁸ Human immunoglobulin preparations were successfully deposited into the airways as well as the alveoli of treated lungs when nebulized with eFlow[®] in both rats and non-human primates (NHP) studies.¹⁴

Dry powder inhalers (DPIs) are propellant-free devices that deliver dry powder (solid) aerosol to the lungs, which comes with shelf-life advantages. Unfortunately, pure antibody solutions significantly agglomerate during spray drying,²⁰ requiring excipients such as trehalose or Tween 20 surfactant.^{21–23} The recent phase 1 double-blind, randomized clinical trial (RCT) on inhaled IL-13 mAb supports the development of inhaled antibodies as a viable future therapy option for parenteral mAbs. VR942 (an anti-IL-13 mAb) represents a first-in-class for DPI-delivered mAbs.²⁴

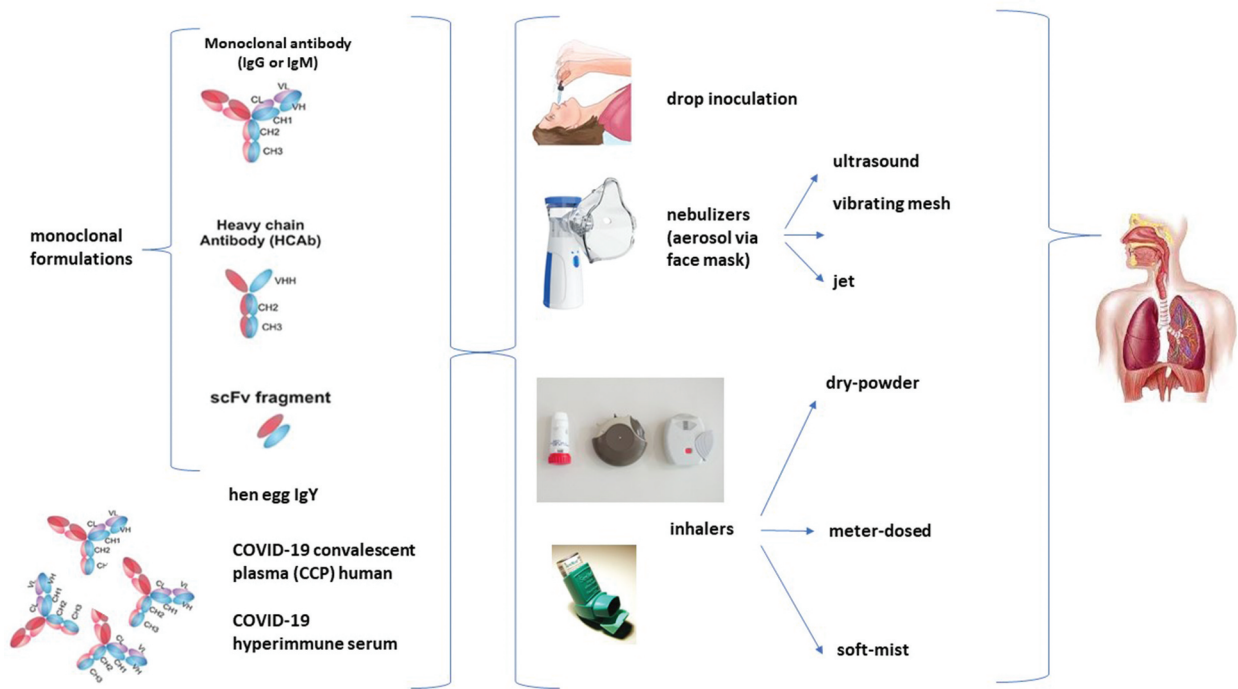


Figure 1. Potential approaches for respiratory delivery of passive immunotherapeutics against COVID-19.

Finally, metered-dose inhalers (MDIs) create fixed-dose aerosols²⁵ but come with the limitation of requiring lipophilic propellers, which hydrophilic biologics cannot solve,²⁶ thus limiting their dose range.^{27,28}

Search strategy

On June 20, 2023, we searched PubMed for English-language literature published after December 1, 2019, using the query “SARS-CoV-2 AND (intranasal OR nasal OR inhaled OR aerosol OR spray OR inhalable) AND (“monoclonal antibody” OR “convalescent plasma” or “immunoglobulins” or “nanobodies” or “scFv”).”

Results

Monoclonal antibodies (mAb)

Human mAb

Most studies reported protective efficacy of single dose administered via nasal inoculation within a few hours after SARS-CoV-2 challenge in K18-hACE2 mice (IgM-14,²⁹ F61 and H121³⁰) or golden (Syrian) hamster model (STI-2020 and STI-5041,³¹ 1249A8 ± 1213H7,³² 58G6³³). A few studies also tested prophylactic models in mice (IgM-14,²⁹ DZIF-10c³⁴).

While doses were in the range of 2–5 mg/kg of body weight, the lowest dose was achieved using an IgM (0.044 mg/kg of IgM-14), a mAb engineered from COV2-14 which persisted 168 h in the lungs.²⁹

Delivery modes alternative to drop inoculation have been also investigated in animal models. At a single 5 mg/kg nebulized dose, the peak HB27 concentration in mice’s pulmonary epithelial lining fluid reached 857.8 µg/mL, 670-fold higher than the PRNT₉₀ value of 1.28 µg/mL, and was maintained

above PRNT₉₀ over 240 h.³⁵ Dual administration of WKS13 mAb in particles below 5 mm (by 2-fluid nozzle) or above 10 mm (by ultrasonic nozzle) to the lung and nasal cavity of hamsters, respectively, was effective in offering prophylactic protection against the SARS-CoV-2 Delta variant.³⁶

Only two clinical trials have been reported so far. In a phase 1 clinical trial, a single nasal spray of 35B5 conveyed 24-h effective *in vitro* protection against SARS-CoV-2 VOCs, including the Alpha, Beta, Delta, or Omicron variants in 30 healthy volunteers.³⁷ In a single-blind placebo-controlled RCT of post-exposure prophylaxis in healthy adult workers, the anti-Spike mAb SA58 reduced laboratory-confirmed symptomatic COVID-19 from 2.8 to 1.04 per 100 person-days (61% efficacy).³⁸

Lumen Bioscience has recently reported exploiting its spirulina-based oral delivery platform for i.n. delivery of its anti-Spike mAb cocktail LMN-301.³⁹ The edible photosynthetic cyanobacterium *Arthrospira platensis* (spirulina) can be engineered to express the mAbs and dry spirulina can be used to encapsulate the mAbs.^{40,41}

Single chain variable fragments (scFv)

Being devoid of immunoglobulin Fc, scFv antibodies are not at risk for inducing antibody-dependent enhancement (ADE) of viral infection, and because of their rapid production at large scale in non-mammalian expression systems, they could be developed at a relatively lower cost compared with mAbs. Moreover, because of their smaller size (about 28 kDa vs 150 kDa of mAbs) and lower complexity (no glycosylation), they are particularly stable and suitable for topical use in non-hospital settings. For example, the first human scFv antibody, brolicizumab, was approved in 2019 for topical treatment of exudative age-related macular degeneration by the FDA, thus paving the regulatory

pathway for this class of molecules,⁴² while the anti-CD3 mAb foralumab has been used i.n. in COVID-19.⁴³ I.n. administration (37 µg/nostril) of scFv76 2 h before and 4 h after infection, completely blocked infection by luciferase-expressing D614G S-pseudotyped virus in hACE2-expressing transgenic mice, as deduced by the loss of luminescent signal; the same was true when 35 µg/nostril were administered to hamsters 2 h before and once daily for the 2 consecutive days post-infection.⁴⁴ Furthermore, nebulized scFv76 antibodies can be effectively delivered to the lung down to the alveolar space, as shown by immunohistochemistry.

Camelid-derived monoclonal heavy-chain only antibodies (HcAbs)

Camelids (camels, dromedaries, alpacas and llamas) produce antibodies devoid of light chains⁴⁵ and highly thermostable, named heavy-chain only antibodies (HcAbs), and weighing approximately 90 kDa. The variable domain on each heavy chain (VHH or nanobody, 15 kDa) is longer, acting like an extended hand, able to reach buried sites the short-handed classical antibodies can't.⁴⁶ However, being nanobodies of camelid origin, they require sophisticated humanization procedures to avoid immunogenic responses, potentially hampering their full development: e.g., nanobodies can be humanized by fusing them to human Fc regions.

Small, single-domain VHH nanobodies, specific for SARS-CoV-2, were proposed for respiratory delivery as an alternative to systemic mAbs: e.g., 50 µg of PiN-21 treated infection in Syrian hamsters when administered immediately or 6 h post-infection via aerosol generated with a vibrating mesh nebulizers.⁴⁷ A bispecific nanobody dimer (2-3-Fc), manufactured by fusing aSA3-Fc to aRBD-2, a previously identified broad-spectrum nanobody targeting an RBD epitope distinct from aSA3, when prophylactically inoculated with nasal drops drastically reduced viral RNA loads and completely eliminated infectious Omicron particles in the trachea and lungs of hamsters at three low doses of 0.5 mg/kg, 2-3-Fc.⁴⁸ Nasal drop inoculation of K-874A, effectively inhibited SARS-CoV-2 titers in the infected lungs of Syrian hamsters without causing weight loss or cytokine induction.⁴⁹ I.n. drop inoculation of alpaca-derived Nb22 exhibited protection against the SARS-CoV-2 Delta variant in post-exposure prophylaxis (PEP) and pre-exposure prophylaxis (PrEP). Of note, i.n. Nb22 also demonstrated high efficacy against the SARS-CoV-2 Delta for 7 days when administered as single-dose prophylaxis, and exhibited long-lasting retention in the respiratory system for >1 month when administered by four doses.⁵⁰

VHH-Fc ABS-VIR-001 (a trispecific VHH fused to the Fc of human IgG₁), thermostable at 45°C for up to 4 weeks, prevented SARS-CoV-2 infection and death when provided as an i.n. drop inoculation prophylaxis in a humanized ACE-2 mouse model. In addition, ABS-VIR-001 post-exposure treatment was shown to greatly reduce viral loads by as much as 50-fold.⁵¹ In mice-adapted COVID-19 severe model, intranasal drop inoculation of three alpaca-derived nanobodies fused to Fcγ₁ (aVHH-11-Fc, aVHH-13-Fc and aVHH-14-Fc) effectively protected mice from lethal challenges with Delta, BA.1 and BA.2, and reduced viral loads in both the upper and lower respiratory tracts.⁵²

Polyclonal immunoglobulins

No study has been reported yet deploying i.n. the two most common human polyclonal immunoglobulin formulations, namely standard IVIG and hyperimmune serum.

We instead have studies on polyclonal immunoglobulins generated in commercial hens (IgY), which are concentrated in their eggs to 50–100 mg/egg within 2–3 weeks after vaccination (the yearly yield per hen can be escalated up to 5 times higher when using specific-pathogen-free (SPF) hens). IgY antibodies have shown good thermal stability and can be stored stably in a liquid state at 2–8°C for 6 months, or at room temperature for 2 weeks.² Strikingly, egg production has already been scaled up, making low-cost and large-scale IgY antibodies feasible. Nasal drop inoculation of broadly protective IgY antibody against SARS-CoV-2 showed a residence time of 2–4 h⁵³ and conferred significant protection against SARS-CoV-2 infection in hACE2-transgenic mice,⁵⁴ rats⁵⁵ and hamsters.⁵⁶ A double-blind, placebo-controlled, phase 1 RCT (NCT04567810) evaluating single-ascending and multiple doses of anti-SARS-CoV-2 RBD IgY administered i.n. for 14 days in 48 healthy adults also demonstrated an excellent safety and tolerability profile, and no evidence of systemic absorption.⁵⁵

COVID-19 convalescent plasma (CCP)

Wouters *et al* tested i.n. drop inoculation of 25 µL CCP/nostril (i.e., 0.01–0.06 mg anti-RBD antibodies/kg) in wild-type Syrian golden hamsters exposed the day after to infected littermates. Animals received CCP for 4 more days after challenge and were sacrificed on the last day: the authors found that 40% of CCP treated hamsters were fully protected and 40% had significantly reduced viral loads. The effect was dose-dependent because high-titer CCP from a vaccinated donor was more effective than low-titer CCP from a donation prior to vaccine rollout. I.n. administration of human CCP resulted in a reactive (immune) response in hamster lungs, whose contribution compared to viral neutralization could not be discerned.⁵⁷

Notably, respiratory delivery of human CCP for therapeutic purposes has not been attempted so far.

Discussion

Passive immunotherapies are not the only protein biologics considered for COVID-19. Other biologics considered for respiratory delivery include ACE2 decoys⁵⁸ and exosomes from convalescent human immune cells,⁵⁹ but passive immunotherapies remain the most tested and reliable antiviral.

The preliminary findings we reviewed here support clinical safety and efficacy of passive immunotherapies delivered to the respiratory tract. Overall, preliminary findings reviewed in this manuscript support that intranasal delivery of passive immunotherapies is as safe as intravenous or intramuscular administration. Blood derivatives other than immunoglobulins have been administered mucosally in the past. E.g., human platelet lysates are used to treat conjunctivitis and keratitis due to either Sjogren syndrome or graft-versus-host disease. As

such, excellent safety is expected from i.n. administration of passive immunotherapeutics. In regard to efficacy, clinical trials are still very limited in number:^{37,38,55} given the widespread availability of oral small-molecule antivirals, proving superior efficacy at preventing disease progression in head-to-head RCTs will likely be needed to implement further development by pharma manufacturers. Discriminating the relative contribution to viral eradication of the local inflammatory reaction against xenogeneic human proteins that are invariably observed in immunocompetent animal models will provide informative insights.

As per any antiviral approach, delivery of a prophylactic regimen as opposed to a therapeutic regimen comes with obvious differences. In particular, modification of mAb half-life (as achieved, e.g., for the Evusheld™ mAb cocktail⁶⁰ has not been attempted so far during intranasal delivery: even if they would be engineered, mucus clearance is likely to remain a major barrier to the actual half-life of mAbs delivered in the respiratory tract. As such pre-exposure prophylaxis with respiratory delivery is likely to require repeated and very frequent administrations. Although intranasal delivery comes with a much comfortable administration than other parenteral routes, is the authors' opinion that intranasal delivery of passive immunotherapeutics will likely only have a role as for post-exposure prophylaxis or early treatment of COVID-19 outpatients. In particular, it could prove useful for the frail immunocompromised patients who cannot tolerate the side effects or the pharmacokinetic interactions of small-molecule antivirals.

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