Development of a nasal spray containing a novel human recombinant antibody for SARS-CoV-2 therapy

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Summary

A novel human recombinant antibody for prophylactic treatment against SARS-CoV-2 was formulated in a nasal solution comprising chitosan as mucoadhesive polymer. Two levels of protein concentration have been assessed and formulations loaded into Aptar VP3 nasal pump. The formulations produced showed values of pH (6.2-6.3) and osmolality (414 and 421 mosm/kg) suitable to prevent precipitation of the antibody in the final solution and for nasal administration. Assay of the protein after formulation manufacturing showed a lower dimeric fraction than the reference standard and hydrodynamic diameter of the final formulations was also comparable to the unprocessed antibody solution (10 nm). Zeta-potential values were higher than 25 mV, indicating colloidal stability against aggregation due to charge stabilization for the formulations obtained. Spray performance did not evidence any difference between protein levels in the final formulations when combined with VP3 nasal pump. Particularly, droplet size distribution (mean volume diameter of 55.13 µm for the low dose formulation and 57.21 µm for the high dose), spray pattern and plume geometry resulted to be applicable for nasal delivery. Finally, for both solutions sprayed antibody content was within 75-125% of the target delivered dose with a very low variability on ten consecutive shots (5%). Future studies will assess the formulations stability under refrigerated and ambient storage conditions of the combination product and of the antibody comprised in the formulation, whereas *in vivo* studies will define pharmacokinetics and pharmacodynamics profile of these final formulations.

Key Message

The possibility to deliver to the nose a novel human antibody for prophylactic treatment against SARS-CoV-2 employing Aptar VP3 pump was assessed. Spray performance of the formulations manufactured was characterized and no protein agglomeration was observed in the formulations and after spraying, indicating favourable results in applying this system for delivery of antibodies to the nose.

Introduction

Diomics Corporation isolated and identified a recombinant immunoglobulin G clone (IgG) potent enough to neutralize SARS-CoV-2 virus particles with 98% effectiveness[1]. The administration of a long-lasting antibody nasal product as prophylactic treatment may, therefore, positively impact the SARS-CoV-2 infection patterns and its severity[1]. In order to achieve a long lasting protection through the administration of a nasal product, a mucoadhesive polymer should be employed during formulation development.

Generally, mucoadhesive polymers are able to form hydrogen bonds, possess charged groups, have a high molecular weight, chain flexibility and present surface energy properties that favour spreading into mucus layers[2].

Chitosan is a linear polysaccharide, positively charged at physiological pH. Its structure allows in determining different interaction with mucin present in the nose, such as hydrogen bond and electrostatic interactions. Chitosan-mucin interaction has been proven *in-vitro* and *in-vivo* and its mucoadhesive properties has been exploited in order to construct the right platform to achieve the long-lasting effect[3,4]. Although some concerns can be raised in terms of toxicity, several studies reported that the exposure of animal nasal mucosa to chitosan did not determine any significant change in the mucosa cell morphology compared to the control[5,6].

The aim of this study was to investigate the possibility to develop a formulation suitable to deliver a recombinant antibody (Ab) in the nasal cavity for prophylactic treatment of SARS-CoV-2 employing

Aptar VP3 nasal pump. This approach is very interesting in the treatment of viral infections and innovative compared to classical intravenous administration of antibodies; presenting, however, challenges particularly related to protein aggregation due to shear stress.

Experimental Methods

In order to screen formulation feasibility for different delivered doses of the Ab two formulations were manufactured at a high and low protein concentration. The Ab solution supplied by Diomics Corporation (USA) was concentrated from 8 mg/mL up to 40 mg/mL employing Amicon® Ultra 15 mL 30 kDa MWCO tubes (Merck Sigma-Aldrich, USA) by centrifugation of the tubes at 4000 rpm for a total of 25 minutes and Ab concentration quantified by size exclusion chromatography (SEC) coupled with Diode Array Detector (DAD). The final formulations were manufactured by mixing together the Ab at two different final levels (20 and 2 mg/mL) in 100 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Merck Sigma-Aldrich, USA), 100 mM of sodium chloride (Merck Sigma-Aldrich, USA), 50 mM of sodium acetate (Merck SigmaAldrich, USA) in Milli-Q water purified by reverse osmosis (Merck Millipore, USA) at pH 6.0, a preservative, benzalkonium chloride (Novo Nordisk Pharmatech A/S, DK), chitosan as mucoadhesive polymer and a surfactant, polysorbate 80, to prevent Ab aggregation (Merk Sigma-Aldrich, US).

Hydrodynamic diameter and Zeta potential of the Ab in the final formulations was determined with Malvern Zetasizer Nano (Malvern Panalytical, UK). pH (SevenCompact™ pH meter, Mettler Toledo, UK) and osmolality (Löser Messtachnik, DE) were measured as well in order to assess they were suitable for nasal delivery and to prevent protein precipitation.

The formulations prepared were then loaded into the Aptar VP3 system (Aptar Pharma, USA), a commonly used multi-dose spray pump. Formulation-device combination characterized in terms of droplet size distribution (DSD) by Spraytec (Malvern Panalytical, UK), spray pattern (SP) and plume geometry (PG, Oxford Laser, UK) and spray content uniformity (SCU) for ten consecutive shots employing SEC assay method to measure protein quantification and aggregation.

Results and Discussion

Monomeric and dimeric peaks of the Ab solution were identified after the supplied Ab solution was concentrated to 40 mg/mL. The area percentage of the dimeric peak was lower than the reference (0.65% vs 1.32%), indicating that the process performed did not determine any aggregation of the protein (Figure 1). After formulations manufacturing, Ab concentration quantified was closer to the target and the area percentage of the dimeric peak was similar to the value collected after the concentration step: 0.80% for the low dose formulation and 0.87% for the high dose one.

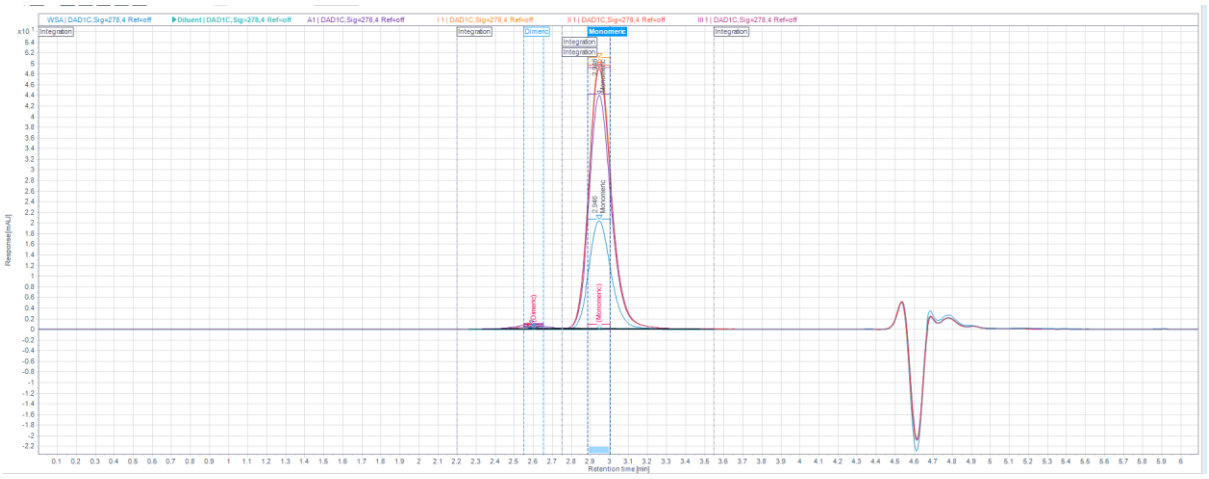

Figure 1. SEC spectrum of concentrated Ab solutions.

Hydrodynamic diameter of the protein in the two formulations was about 10 nm, comparable with the diameter of the unprocessed antibody solution. Zeta-potential was also measured for the two formulations comprising different concentrations of Ab (26.98 and 25.20 mV). Both of them reported a value higher than 25 mV, indicating a moderately stability against aggregation due to charge stabilization[7].

pH for both formulations was slightly acidic (pH 6.2-6.3), usually recommended for nasal formulations. This was similar to the pH of the original buffer used as vehicle for the final formulation (pH 6.0), therefore it was expected to preserve the protein stability in the formulation. pH value obtained was also in a range suitable for nasal delivery. Moreover, the values of pH collected were within the upper limit (2.4-6.3) of pH, which have been observed to promote the interaction between chitosan and mucin[8].

Osmolality measurements obtained for both formulations were inside the range of optimal values for nasal delivery (290-500 mosm/kg[9]): 414 mosm/kg for the low dose formulation and 421 mosm/kg for the high dose one.

After loading the two formulations in Aptar VP3 pump, spray emitted was characterized for DSD, SP and PG (Table 1). DSD was comparable between the two formulations and mean volume diameter ($D_{v,50}$) was higher than 50 µm (55.13 µm for the low dose formulation and 57.21 µm for the high dose one), indicating a more likely deposition in the anterior region of the nose[10].

Like for DSD, also for SP and PG no differences were observed between the two Ab concentrations employed in the final formulation. However, a different plume was noticed when comparing SP and PG of a blank solution (just excipients) and the two final formulations, possibly due to the different composition and higher viscosity (Figure 2).

Average droplets diameter of final formulations was lower than the one collected for blank solution (Dmin about 4 cm and Dmax about 5 cm). This may indicate a full cone plume for formulations comprising the antibody instead of a flatter spray when the IgG was not present in the blank solution. On the other side, Plume Geometry (PG) was comparable with blank solution in terms of plume length (13 cm), but plume angle was slightly lower for both of the formulations compared to the blank solution (52°), indicating probably again a full cone plume.

Table 1. Average values and residual standard deviation (%, in parentheses) of spray performance (n=3). Droplet size distribution (DSD) and spray pattern (SP, n=3).

Formulation	DSD				SP			
	$D_{v,10}$ (µm)	$D_{v,50}$ (µm)	$D_{v,90}$ (µm)	Span	Dmin (cm)	Dmax (cm)	Ovality ratio	Area (cm^2)
low dose	23.8 (14.4)	55.1 (9.7)	131.1 (8.2)	2.0 (12.6)	2.6 (4.9)	3.7 (8.3)	0.7 (8.7)	7.5 (10.4)
high dose	24.7 (10.5)	57.2 (11.6)	141.3 (20.2)	2.0 (19.6)	2.4 (5.2)	3.2 (3.7)	0.8 (2.9)	6.2 (8.6)

Finally, SCU was 122.22% of the expected dose base on protein concentration for one shot of the low dose and 124.59% of the target dose for the high dose formulation, with a relative standard deviation of 5% for both of them. No protein agglomeration was observed for the dose emitted, which should have maintained its structure and activity when administered in the nose. This will be further assessed.

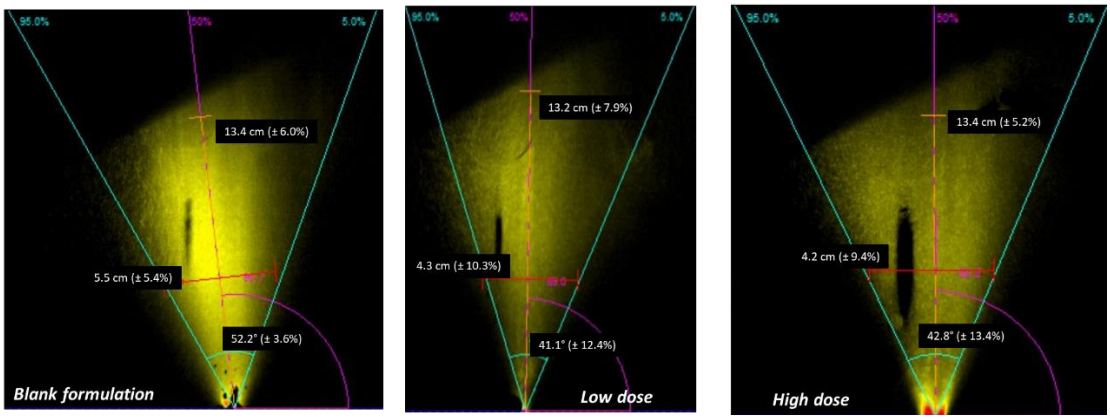

Figure 1. Plume geometry for blank, low dose and high dose formulations.

Conclusion

This study focused on the development of a nasal formulation comprising a novel antibody for SARS-CoV-2 prophylactic treatment. The possibility to deliver to the nose the antibody employing an Aptar VP3 pump was assessed. Two formulations were manufactured at two levels of protein concentration (20 and 2 mg/mL). These proved to be suitable for nasal delivery in terms of pH, droplet size and osmolality. Moreover, no protein agglomeration was observed in the two formulations prepared and after spraying with Aptar VP3 pump, indicating favourable results in applying this system for delivery of antibodies to the nose. The next step of the development will be the assessment of the stability under refrigerated and ambient storage conditions of the combination product and of the antibody comprised in the formulation. Moreover, *in vivo* animal studies will allow to determine pharmacokinetics and pharmacodynamics of these two formulations.

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