Phase I dose-escalation study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of an inhaled recombinant human ACE2

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Shareable abstract (@ERSpublications)
Application of aerosolised APN01 (soluble recombinant human ACE2) is safe and well tolerated. By achieving a high local concentration in the lungs and relatively low levels of systemic bioavailability, inhaled APN01 presents a promising therapeutic option. https://bit.ly/48jrfg4

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Abstract

Background APN01 is a soluble recombinant human angiotensin-converting enzyme 2 (rhACE2), a key player in the renin–aldosterone–angiotensin system (RAAS). In clinical studies, APN01 was administered intravenously only, so far. The aim of this study (ClinicalTrials.gov: NCT05065645) was to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of inhaled APN01.

Methods This was a phase I, double-blind, placebo-controlled, dose-escalation study. Inhalation was conducted *via* a nebuliser over 15 min in three single ascending dose (SAD) cohorts (n=24) and two multiple ascending dose (MAD) cohorts (n=16: every 12 h for 7 days). Doses in the SAD cohort were 1.25, 2.5 and 5 mg·mL^{-1}; doses in the MAD cohort were 2.5 and 5 mg·mL^{-1}. Safety (including adverse events (AEs), laboratory findings and lung function results), PK and PD data were assessed.

Results In the SAD and MAD cohorts, treatment-related AEs were slightly more frequent in the active treatment group than in the placebo group. AEs were mild to moderate, with no dose-limiting toxicities. No clinically relevant changes in lung function and laboratory results were observed. The mean maximum observed plasma concentration (C_{max}) values after single and multiple doses of 5 mg·mL^{-1} APN01 were 1.88 and 6.61 ng·mL^{-1}, respectively. Among the PD variables, significance was found for ACE2 and angiotensin 1–5.

Conclusions The application of aerosolised APN01 is safe and well tolerated after single and multiple doses. By achieving a high local concentration in the lungs and low systemic bioavailability, inhaled rhACE2 may present a therapeutic option in ACE2-related diseases.

Introduction

Angiotensin-converting enzyme 2 (ACE2) is a key host protein by which severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters cells. The level of ACE2 expression in the lung is hypothesised to correlate with an increased risk of severe infection and complications in coronavirus disease 2019 (COVID-19) [1]. The disease ranges in severity from asymptomatic or mild forms to severe and critical cases characterised by life-threatening end-organ failure, including acute respiratory distress syndrome (ARDS) and multiorgan failure [2].

APN01 is a soluble recombinant form of human ACE2 (rhACE2) that has been developed as a potential therapy for COVID-19. APN01 is designed to mimic ACE2 within the body and thus to hinder SARS-CoV-2 from binding to the ACE2 receptor, preventing it from infecting cells. At the same time, APN01 is designed to downregulate the renin–aldosterone–angiotensin system (RAAS) to help prevent inflammation and organ injury.

Competitive binding by exogenous rhACE2 may block viral entry, thereby decreasing viral replication in ACE2-expressing organs and protecting the lungs and distal organs from injury induced by SARS-CoV-2 [3]. The protection provided by rhACE2 showed dose-dependent efficacy *in vivo* [4]. Studies in cell culture and multiple organoids have shown that soluble ACE2 decreases viral replication and neutralises viral infection of VeroE6 cells and human lung epithelial cells [3, 5]. Furthermore, ACE2 cleaves angiotensin (Ang) II of the RAAS into Ang 1–7. Elevated levels of Ang II are associated with vasoconstriction, inflammation, fibrosis, vascular leak and sodium absorption. Ang 1–7 is known to act as a counter-regulatory peptide in the RAAS axis, and is associated with vasodilation, anti-proliferation, anti-inflammation and reduced vascular leak [6, 7]. The RAAS controls important functions such as blood pressure, volume and electrolytes, and affects the function of most organs. Target indications of rhACE2 are diseases and conditions with an imbalance of the RAAS, insufficient natural ACE2 activity and pathologically elevated Ang II levels, such as a variety of lung, cardiovascular, liver and kidney diseases. Therapeutic application of rhACE2 may restore the balance of the RAAS by degrading Ang II and simultaneously generating Ang I [1, 4, 5, 8–10].

The intravenous formulation of APN01 has been studied in six phase I and phase II studies for different indications, including patients with ARDS and pulmonary arterial hypertension (PAH) [11–13].

The clinical pharmacology and mechanisms of action of ACE2 in COVID-19 (*i.e.* binding to the SARS-CoV-2 spike protein, minimising organ damage and decreasing viral replication) have been studied in several phase I and II clinical studies and a study in cell cultures and human blood vessel organoids [5]. Findings are further supported by a case report of the first course of treatment with APN01 in a patient with severe COVID-19 [14]. In this patient, a 7-day treatment with APN01 (0.4 $mg \cdot kg^{-1}$ by *i.v.* infusion twice daily) was well tolerated and associated with a rapid decrease in viral load, a marked increase in neutralising antibody titres, a reduction of inflammatory markers and a substantial improvement of the patient's clinical condition.

Topical use of APN01 might achieve higher concentration at the target site of action and might lead to even better activity. However, pulmonary drug delivery is complex because the respiratory tract has evolved defence mechanisms to keep inhaled drug particles out of the lungs and to remove or inactivate them once deposited. In addition to these mechanical, chemical and immunological barriers, pulmonary drug delivery is adversely affected by the behavioural barriers of poor adherence and poor inhaler technique [8]. Thus, this study evaluated the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of single ascending doses (SAD) and multiple ascending doses (MAD) of inhaled APN01 administered *via* a nebuliser.

Methods

Ethics

The study was registered at ClinicalTrials.gov with identifier number NCT05065645 and approved by the responsible ethics committee (Medical University of Vienna; 1784/2021) and the competent authority. All study participants were provided with oral and written information about the study and signed an informed consent form.

Study design

This first-in-human clinical study of aerosolised rhACE2 was conducted as a randomised, double-blind, placebo-controlled study to assess the safety, tolerability, PK and PD of inhaled APN01 in healthy subjects after SAD and MAD [11, 15]. As this was the first trial to test aerosolised APN01 inhalation in humans, the study used a gradual ascending-dose design taking into account safety margins between the no observed adverse effect level (NOAEL) dose in non-human primate studies and the proposed clinical doses. The starting dose was 8 mL of 1.25 $mg \cdot mL^{-1}$ APN01 inhalation solution (10 mg in total, or ~0.17 $mg \cdot kg^{-1}$ APN01 in a 60 kg human). The Principal Investigator and a Data Safety Monitoring Board (DSMB) were responsible for reviewing safety data at regular, predefined intervals (figure 1).

Main study objectives

The primary objective of the study was to evaluate the safety and tolerability of SAD and MAD of inhaled APN01 when administered *via* a jet nebuliser in healthy subjects. Secondary objectives comprised the

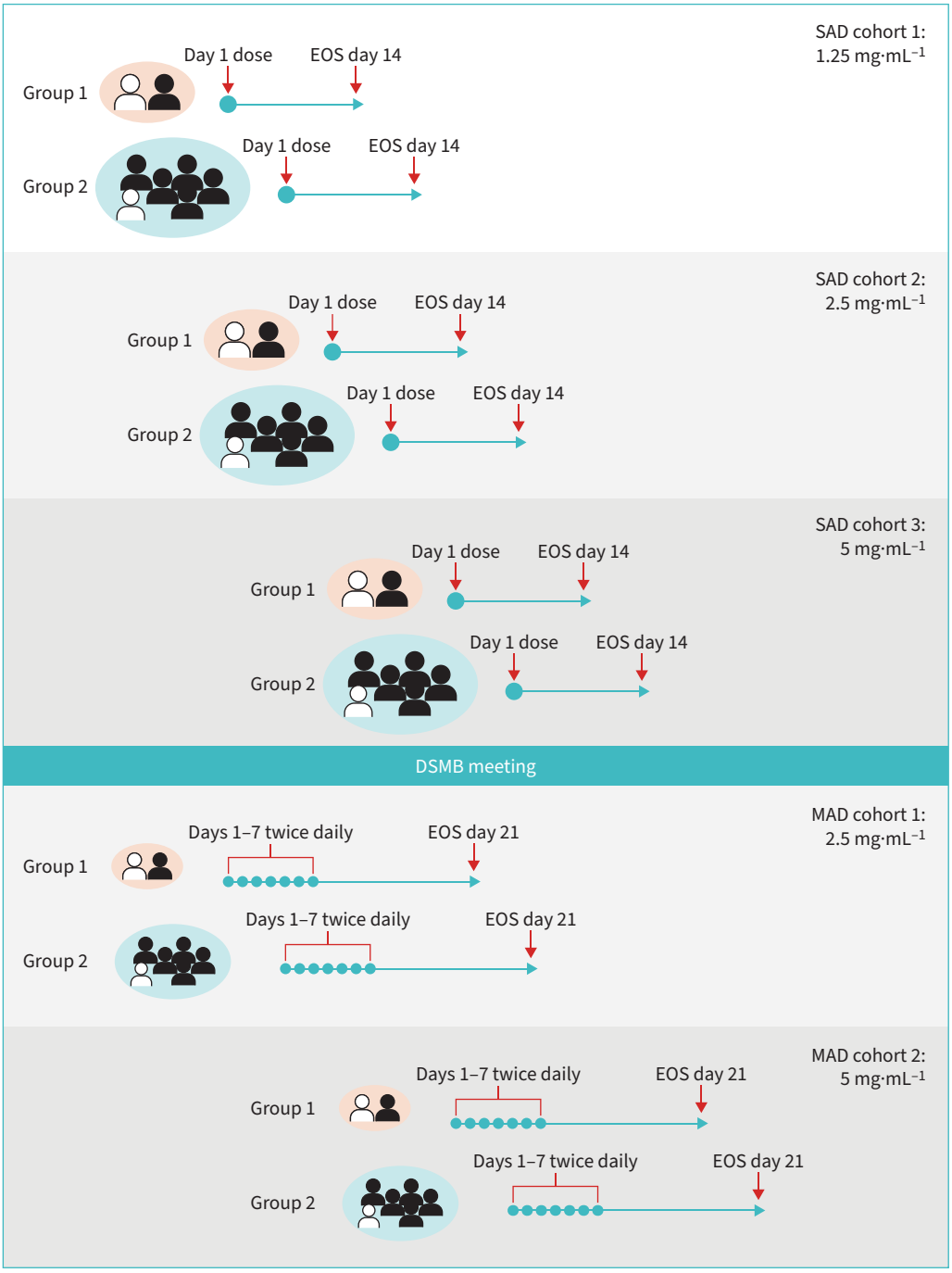

FIGURE 1 Aerosolised APN01 in healthy subjects: study design with single ascending dose (SAD) and multiple ascending dose (MAD) cohorts. DSMB: Data Safety Monitoring Board; EOS: end of study.

evaluation of the maximum tolerated dose of inhaled APN01 when administered as a single dose and multiple doses *via* a nebuliser, PK, effect of inhaled APN01 on key PD biomarkers of the RAAS and potential immunogenicity of inhaled APN01.

Sample size justification

No formal sample size calculation was performed. The number of subjects per MAD cohort was n=8 (n=2 placebo and n=6 treated with APN01, with a sentinel of the first two subjects randomised 1:1 to be treated with APN01 or placebo), which is considered sufficient to detect relevant safety signals of drugs. A total of 40 subjects (n=24 in SAD and n=16 in MAD) were included in the analysis (figure 1).

Study treatments

Active treatment was APN01 inhalation, whereas matching placebo consisted of sterile, 0.9% sodium chloride inhalation. Undiluted APN01 solution 5 mg·mL^{-1}, 4.0 mL, was presented as a sterile liquid formulation in 10 mL clear glass, stoppered vials. The drug was supplied as a single-use vial and was not formulated with a preservative. For dilution of APN01 in support of study arms requiring product concentrations of 1.25 and 2.5 mg·mL^{-1}, sterile diluent solution presented in 10 mL clear glass, stoppered vials was used. Study medication was prepared by unblinded pharmacists and delivered to the blinded study team. APN01 or placebo inhalation solution was administered with a PARI LC SPRINT (PARI Respiratory Equipment, Midlothian, VA, USA) nebuliser cup driven by a PARI BOY compressor over 15 min at 25 psi.

Overview of the main tests or experiments

After the screening visit (1–21 days prior to treatment) eligible subjects (for inclusion and exclusion criteria and details on experiments, see supplementary material) were randomised on day 1 for the SAD and MAD (inhalation every 12 h for 7 days) cohorts (as shown in figure 1). Study drug or placebo with 8 mL fill volume was prepared in the nebuliser. Doses in the SAD cohort were 1.25, 2.5 and 5 mg·mL^{-1}; doses in the MAD cohort were 2.5 and 5 mg·mL^{-1}.

Safety assessments and stopping criteria

Safety including adverse event (AE) assessment, vital signs, laboratory tests and repeated spirometry, as well as blood draws for PK and PD data were continuously assessed. A detailed description is provided in the supplementary material.

Stopping criteria were defined as two or more of the subjects in one and the same cohort developing AEs of Grade >2 or one subject developing AEs of Grade >3 (as defined in Common Terminology Criteria for Adverse Events version 5.0) (for further information, see supplementary material).

Vital signs and electrocardiography

Vital signs assessment (blood pressure, heart rate, body temperature, respiratory rate and peripheral oxygen saturation) was performed at various time-points: pre-dose, and 1, 4 and 12 h post-dose for SAD cohorts, and for MAD groups on dosing days at pre-dose, 1, 2, 4, and 8 h post-dose after the morning dose, and pre-dose and 1 h after the evening dose.

Electrocardiography was performed at pre-dose (in the morning of dosing day) and 2 h post-dose. For MAD cohorts electrocardiography was assessed every other dosing day at 2 h post-dosing.

Spirometry, plethysmography and fractional exhaled nitric oxide

At screening, for the MAD safety and end-of-study (EOS) visit assessment of the lung function parameters, forced expiratory volume in 1 s (FEV_1), peak expiratory flow (PEF), forced vital capacity (FVC) and FEV_1/FVC ratio were measured with a spirometer (MicroLab ML3500 MK8; Vyaire Medical, Mettawa, IL, USA) using established reference values. Additional parameters such as total lung capacity (TLC), residual volume (RV), relative residual volume (RV%TLC), diffusing capacity of the lung for carbon monoxide (D_{LCO}), carbon monoxide diffusion capacity (K_{CO}) and effective airway resistance (R_{eff}) were determined by body plethysmography. Fractional exhaled nitric oxide (F_{ENO}) was measured using an off-line nitric oxide analyser (NIOX VERO airway monitor; Circassia, Oxford, UK). Spirometry assessments on study days and safety visits were performed with AioCare (Vyaire Medical). Normal, intermediate and high F_{ENO} levels were categorised as: low/normal <25 ppb, intermediate 25–50 ppb or high >50 ppb. F_{ENO} measurement at screening could be repeated once if abnormal.

Spirometry for the SAD groups was performed at pre-dose and immediately after completion of inhalation, as well as 2, 4, 8, 12 and 48 h after dosing, and for the MAD groups on dosing days at pre-dose, 1 and 6 h after the morning dose and at 1 h after the evening dose. On day 7, additional measurements were taken at 2 and 4 h, and on day 8, 9 and 14.

Blood draws

Blood for safety assessments (haematology, coagulation and clinical chemistry) was collected at screening, on every other dosing day in the morning and EOS visit.

Blood draws for APN01 plasma PK assessments and determination of changes in relevant circulating biomarkers of the RAAS system over time (*i.e.* PD assessments) were collected for the SAD groups at pre-dose (in the morning of the dosing day) time-points baseline, 0.5, 2, 4, 8 h and after dosing on day 1,

24 h (day 2) and 48 h (day 3). On MAD dosing days, PK and PD blood samples were taken before the morning and evening dose. On days 4 and 7, additional PK blood samples were taken at 2 h±15 min, 4 h ±15 min and 8 h±15 min after the morning dose, and on day 8, 9 and 14. A detailed description of PK parameters is provided in the supplementary material.

Assessment of PD parameters aimed to provide knowledge on the main mode of action of rhACE, *i.e.* the cleavage of Ang II/Ang I into Ang 1–7, which is suggested to lead to the beneficial therapeutic effects.

Statistical methods

Demographic, safety, PK and PD data were listed and tabulated by descriptive statistics as appropriate. PK data were also displayed graphically. Summary tables and figures were presented for each cohort. Categorical variables (*e.g.* AEs) were summarised by treatment. ANCOVA was calculated for PD measurements of the MAD dataset. The level of significance was taken at 5%. Pearson's correlation coefficients for pairwise comparisons among the six PD end-points were estimated pairwise.

Where appropriate, 95% confidence intervals were calculated. Statistical tests were performed for explorative purposes only.

DSMB meeting

Before enrolment of subjects in the MAD cohort, data from the SAD cohorts were provided to the DSMB for review. After review of the safety results from all SAD cohorts, the DSMB was responsible for making a recommendation whether or not to continue enrolment as designed in the study protocol.

Results

Demographics, baseline values and treatment compliance

The study population comprised 19 males and 21 females, all Caucasian, mean±SD age 29±7 years in the SAD cohorts and 26±5 years in the MAD cohorts. All eligibility criteria were met. All of the study's safety investigations were initially carried out at screening, without any relevant or clinically significant findings. All subjects received all doses as per protocol correctly, with the single exception of a subject who at one dosing received active treatment in error instead of placebo. However, this was considered by including the subject in all relevant analyses. Treatment compliance was assessed based on the difference in the weight of the nebuliser with investigational medicinal product before and after inhalation (maximum difference 6.0 g). The average amount of APN01 solution inhaled was comparable across all SAD cohorts (between 3.6 and 4.5 g). Moreover, dosing in the MAD cohorts remained at a consistent level throughout the 7 days of treatment (between 3.2 and 3.5 g). A summary of the safety population and allocation to study treatment is provided in the supplementary material.

Safety

In total, 103 AEs were reported. In the SAD cohorts, 16 out of 24 subjects (67%) experienced 28 AEs; in the MAD cohorts, 11 out of 16 subjects (69%) experienced 75 AEs. Thus, the proportion of subjects affected by AEs was almost the same, despite the far greater number of administrations of APN01 in the MAD regimens (n=14) than in the SAD regimens (n=1). Most AEs were mild; the rest were moderate. Importantly, there were no AEs above Grade 2. None of the AEs were serious or led to a subject's withdrawal from the study (summary statistics of AEs are given in the supplementary material).

In the SAD groups, numbers of treatment-related AEs in the verum-treated cohorts were slightly greater than in the placebo-treated cohort, but no pattern of events or of affected system organ classes (SOCs) was discernible (table 1). In the MAD cohorts, there was a dose-related trend to higher incidence of AEs at the higher dosing levels. The following AEs were most clearly associated with the subjects having received the highest dosage of APN01: rhinitis, cough, throat irritation and dysphonia (table 1). In particular, rhinitis was reported by 25% of the verum-treated subjects, with some experiencing several episodes during the treatment period. Other events with less clear trends were nasal congestion, pharyngeal erythema and respiratory tract irritation. Further AEs with greater incidence in the higher-dose cohort were migraine, dizziness and a decrease in pulmonary function.

Overall, most of the 103 AEs were of Grade 1 (mild, n=82). 21 AEs were of Grade 2 (moderate).

Electrocardiography in the SAD cohorts gave "normal" results throughout the study. In the MAD cohorts the results suggest an increase in Bazett corrected QT interval (QTcB), but this rise did not constitute a safety signal. One MAD subject and two SAD subjects showed QTcB values between 450 and 480 ms; all other QTcB values were below this range.

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TABLE 1 Adverse events (AEs) by system organ class (SOC) for single ascending dose (SAD) and multiple ascending dose (MAD) cohorts

	SAD								MAD					
	APN01 1.25 mg·mL^{-1} (n=6)		APN01 2.5 mg·mL^{-1} (n=6)		APN01 5 mg·mL^{-1} (n=6)		Placebo (n=6)		APN01 2.5 mg·mL^{-1} (n=6)		APN01 5 mg·mL^{-1} (n=6)		Placebo (n=6)	
	AEs	Subjects	AEs	Subjects	AEs	Subjects	AEs	Subjects	AEs	Subjects	AEs	Subjects	AEs	Subjects
Any SOC	8	4	9	5	7	4	4	3	21	4	40	4	14	3
Ear and labyrinth disorders			1	1					6	1				
Gastrointestinal disorders	2	2	1	1			1	1	2	2	4	1	1	1
General disorders and administration site conditions													1	1
Infections and infestations					1	1			1	1			1	1
Injury, poisoning and procedural complications					1	1			1	1			1	1
Deviations in the pulmonary function challenge test			4	3	2	1	1	1	1	1	2	1		
Musculoskeletal and connective tissue disorders	1	1									4	3	1	1
Nervous system disorders	1	1	1	1	1	1	1	1					1	1
Reproductive system and breast disorders													1	1
Respiratory, thoracic and mediastinal disorders	4	3	2	1	1	1	1	1	2	1	5	3	5	3
Skin and subcutaneous tissue disorders					1	1							1	1

Data are presented as n.

Analysis of vital signs, haematological, chemistry, coagulation and urinalytical results revealed no trends or individual changes. The physical examination revealed several abnormalities, but none that appeared plausibly associated with the inhalation of APN01. In spirometry, a single clinically significant spirometric value was reported; however, the subject received placebo. Body plethysmography and F_{ENO} revealed no noteworthy changes.

Pharmacokinetics

All but one SAD subject of the highest dose group showed APN01 plasma concentrations below the limit of quantification. A single inhaled dose of 5 mg·mL^{-1} APN01 resulted in a mean maximum observed plasma concentration (C_{max}) of 1.88 ng·mL^{-1}, whereas multiple doses almost quadrupled the mean C_{max} to 6.61 ng·mL^{-1} (figure 2 and table 2).

Pharmacodynamics

PD analyses, among others, determined concentrations of the Ang system effector peptides Ang I (Ang 1–10), Ang II (Ang 1–8), aldosterone, Ang 1–7 and Ang 1–5 (table 3). In the highest dose SAD cohort (5 mg·mL^{-1}), the mean ACE2 enzymatic activity was determined by concentrations of the Ang 1–7 metabolite. Ang 1–7 levels increased from baseline (<1 nmol Ang 1–7·L^{-1}·h^{-1}) to a maximum of 2.7 nmol Ang 1–7·L^{-1}·h^{-1} at 24 h. In the highest dose MAD cohort, the peak ACE2 activity level of 7.1 nmol Ang 1–7·L^{-1}·h^{-1} was observed on day 7, with levels decreasing afterwards until day 14. Accordingly, mean Ang 1–5 levels also increased from baseline values of ~3.75 pmol·L^{-1} to a maximum of 17.2 pmol·L^{-1} on day 9 in the highest dose MAD cohort. For all other PD variables, APN01 effects were less consistent across all SAD and MAD cohorts. These results highlight the potential therapeutic effect of inhaled APN01 to modulate the ACE2/Ang 1–7 "alternative" RAAS axis.

Immunogenicity

Inhalation of rhACE2 did not trigger any immune response following single or repeated dosage in the current study; anti-APN antibodies were not found in any of the study subjects. Thus, there was no evidence for immunogenicity of APN01 when inhaled.

Discussion

In this phase I study, safety and tolerability of APN01, a formulation for inhalation of soluble rhACE2, was assessed in healthy male and female subjects. Inhalation of multiple APN01 doses up to 5 mg·mL^{-1} for 7 days in healthy subjects was safe and well tolerated.

The RAAS is a central and complex system that plays a key role in the pathophysiology of various diseases, such as ARDS, PAH, heart and renal disease, and in COVID-19. The enzymatic reaction most effectively catalysed by ACE2 is the degradation of Ang II (Ang 1–8) to generate the heptapeptide Ang 1–7. Ang 1–8 is known to exert vasoconstrictive, proliferative and pro-inflammatory actions after binding to the Ang II type 1 receptor. On the contrary, Ang 1–7 functions as an antagonist of Ang 1–8, with vasodilatory, antiproliferative, antiangiogenic and anti-inflammatory properties known to be the

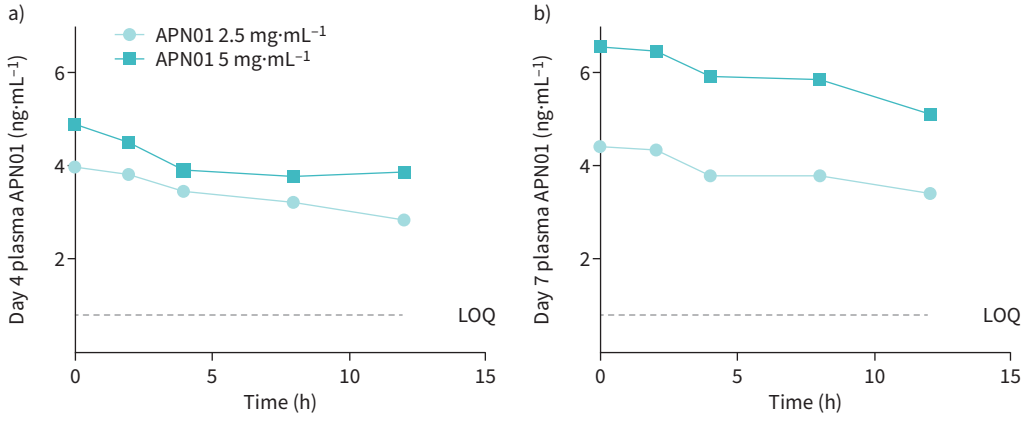

FIGURE 2 APN01 plasma concentration–time plots for the two multiple ascending dose cohorts at a) day 4 and b) day 7. LOQ: limit of quantification.

TABLE 2 Multiple ascending dose pharmacokinetic parameters in both APN01 dose groups at day 4 and day 7

	APN01 2.5 mg·mL^{-1}	APN01 5 mg·mL^{-1}
Day 4		
t_{max} (h)	1.21±1.40	2.64±3.42
C_{max} (ng·mL^{-1})	3.99±2.12	4.87±3.04
$AUC_{12\,h}$ (ng·h·mL^{-1})	40.3±24.7	47.7±31.2
AUC_{all} (ng·h·mL^{-1})	40.3±24.7	47.7±31.2
MRT_{last} (h)	5.50±0.22	5.85±0.52
Day 7		
t_{max} (h)	1.36±1.99	0.38±0.94
C_{max} (ng·mL^{-1})	4.49±2.72	6.61±4.54
$AUC_{12\,h}$ (ng·h·mL^{-1})	46.2±28.1	70.3±49.5
AUC_{all} (ng·h·mL^{-1})	46.2±28.1	70.3±49.5
MRT_{last} (h)	5.66±0.09	5.71±0.11

Data are presented as mean±SD. t_{max}: time of maximum observed plasma concentration; C_{max}: maximum observed plasma concentration; AUC: area under the plasma concentration *versus* time curve; MRT: mean residence time. See supplementary material for further details.

"alternative" RAAS axis [16]. In healthy volunteers a single *i.v.* dose of 0.8 mg·kg^{-1} APN01 was well tolerated, while a single *i.v.* infusion (3–6 min) of 0.8 mg·kg^{-1} APN01 caused a robust and sustained reduction in Ang II and a corresponding increase in Ang 1–7 and Ang 1–5, confirming the pharmacological activity of APN01 *in vivo* [17]. In COVID-19 patients, 0.4 mg·kg^{-1} *i.v.* APN01 administered twice daily for 7 days was found to be safe and well tolerated, with the proportion of patients affected by any AE lower in the APN01 group than in the placebo group (49% *versus* 62% of patients) [17].

No dose-limiting toxicity was observed in the current study, so it can be concluded that ∼8 mL of an APN01 5 mg·mL^{-1} solution inhaled twice daily for 7 days, with each inhalation session lasting for 15 min, is safe and well tolerated (table 1).

The analysis of AEs in the SAD cohorts revealed that 67% of subjects experienced 28 AEs; in the MAD cohorts 69% experienced 75 AEs. This indicates the absence of any dose relationship of AEs overall, despite the far greater number of administrations of APN01 in the MAD group (n=14) than in the SAD group (n=1) (table 1). There were no fatal, life-threatening or severe AEs, and there were also no serious AEs. No dose relationship of the moderate AEs was observed.

In the SAD cohorts, numbers of treatment-related AEs in the verum-treated group were slightly greater than in the placebo-treated group, but no pattern of events or of affected SOCs was discernible. In the MAD cohorts, there was a dose-related trend to a higher incidence of AEs at the higher dosing levels. The most common AEs, associated with the highest dosage of APN01, were rhinitis, cough, throat irritation and dysphonia. In particular, rhinitis affected 25% of verum-treated subjects, but none of the

TABLE 3 Pharmacodynamic analysis: statistically significant associations revealed by ANCOVA (multiple ascending dose; day 7)

	Pearson's ρ	p-value
ln AUC–ACE2	0.911 (0.686–0.977)	<0.001
Ang II (1–8)–Ang I (1–10)	0.870 (0.658–0.954)	<0.001
Ang II (1–8)–Ang 1–5	0.684 (0.285–0.881)	0.003
Ang II (1–8)–aldosterone	0.522 (0.035–0.808)	0.038
Ang I (1–10)–Ang 1–5	0.673 (0.266–0.876)	0.004
Ang I (1–5)–aldosterone	0.638 (0.208–0.861)	0.008

Correlations between pharmacokinetic and pharmacodynamic parameters were investigated (with 95% confidence interval lower and upper bound in parentheses) and significances (p<0.05) were noted. ln: natural logarithm; AUC: area under the plasma concentration *versus* time curve; ACE: angiotensin-converting enzyme 2; Ang: angiotensin.

placebo-treated subjects, with some experiencing multiple episodes during the treatment period. Thus, rhinitis may be causally related to APN01 inhalation.

Of the other safety-relevant observations, vital signs, electrocardiography, haematological, clinico-chemical, coagulation and urinalytical tests, physical examination, spirometry, body plethysmography and F_{ENO} all showed no relevant trends for either the SAD or the MAD regimen. Of note, a decrease in D_{LCO} and an increase in F_{ENO} were reported in seven (23%) verum-treated subjects and two (20%) placebo-treated subjects. However, these changes in D_{LCO} and F_{ENO} were not associated with clinical signs and were deemed not clinically relevant.

Immunogenicity, an indirect but important safety criterion, was investigated by detection of anti-APN antibodies. None were found in any of the study subjects. Thus, this study adduced no evidence for immunogenicity of inhaled APN01 solution under the conditions of the study.

Assessment of plasma drug concentrations after inhalation can be used as a surrogate for monitoring lung exposure [18]. All but one SAD subject of the 5 $mg \cdot mL^{-1}$ dose group showed APN01 plasma concentrations below the limit of quantification, indicating a very low systemic bioavailability following a single dose of inhaled APN01.

Quantification of APN01 plasma concentrations following a single *i.v.* dose of 0.8 $mg \cdot kg^{-1}$ APN01 revealed a mean C_{max} of 11.3 $\mu g \cdot mL^{-1}$ and a mean area under the plasma concentration *versus* time curve over the entire study period of 20.0 $\mu g \cdot h \cdot mL^{-1}$ (table 2). In this study, a single inhaled dose of APN01 5 $mg \cdot mL^{-1}$ resulted in a mean C_{max} of 1.88 $ng \cdot mL^{-1}$, a plasma concentration >5000 times lower than that obtained following a single *i.v.* dose of 0.8 $mg \cdot kg^{-1}$ APN01. Furthermore, although we observed greater C_{max} for the high-dose cohorts, the value did not suggest dose proportionality. High variability of PK parameters was observed among subjects within individual dose groups. The plasma half-life of *i.v.* administered APN01 in humans is ~7–9 h; median plasma half-life of inhaled APN01 was found to be 41 h in beagle dogs [17]. In the present study, the mean plasma half-live values found were between 35 and 52 h in the MAD cohorts. The longer elimination half-life for inhaled APN01 is expected to result in greater steady-state accumulation during repeated dosing. In fact, multiple doses of 5 $mg \cdot mL^{-1}$ APN01 increased C_{max} 4 times to ~6.61 $ng \cdot mL^{-1}$ (mean value).

Regarding PD variables, in a previous study, a single *i.v.* infusion (3–6 min) of 0.8 $mg \cdot kg^{-1}$ APN01 caused a robust and sustained reduction in Ang II and a corresponding increase in Ang 1–7 and Ang 1–5, confirming the pharmacological activity of APN01 *in vivo* [17]. In this study, only Ang 1–5 levels increased consistently from baseline values in all SAD and MAD cohorts (table 3). Ang 1–7 levels increased from baseline (<1 nmol Ang 1–7$\cdot L^{-1} \cdot h^{-1}$) to a maximum of 2.7 nmol Ang 1–7$\cdot L^{-1} \cdot h^{-1}$ at 24 h. In the highest dose MAD cohort, the peak ACE2 activity level of 7.1 nmol Ang 1–7$\cdot L^{-1} \cdot h^{-1}$ was observed on day 7, with levels decreasing afterwards until day 14. This increase in ACE2 enzymatic activity is consistent with APN01 being a soluble recombinant form of human ACE2 and demonstrates APN01's systemic bioactivity. For all other PD variables, significance was found for SAD only. However, this may also be due to excessive variability in bioavailability from subject to subject (especially in the MAD cohorts), partly caused by the high dose fluctuations. Furthermore, the weak systemic pharmacological activity may be explained by the low systemic bioavailability observed after APN01 inhalation.

Taken together, these results point to low systemic bioavailability and bioactivity following single or repeated doses of inhaled APN01. These findings also corroborate the highly favourable safety profile of the drug. By achieving a high local concentration in the lungs and relatively low levels of systemic bioavailability, inhaled APN01 may present a high therapeutic index. Whether this safe APN01 dose range reaches a therapeutically relevant level in the lungs needs to be addressed in future clinical studies.

Conclusions

All results in this phase I study suggest a favourable safety profile of APN01 inhalation, as expected for a naturally occurring ACE2 protein and based on the broad-spectrum safety profile of *i.v.* administered APN01 gathered from previous clinical trials conducted to date. PD analyses confirmed that rhACE2 possesses enzymatic activity to mediate the degradation of Ang 1–8 to Ang 1–7 when inhaled. This may lead to a shift in the balance in the RAAS system from a vasoconstrictive, pro-inflammatory to a vasodilatory, anti-inflammatory state, underpinning the therapeutic potential of APN01 in various acute and chronic diseases and syndromes. Therefore, the results of the present study conducted in healthy subjects imply that it is justified to continue the investigation of inhaled APN01 as a potential treatment

option in diseases where disbalanced RAAS plays a critical pathophysiological role, such as ARDS, PAH and various others.

Provenance: Submitted article, peer reviewed.

Ethics statement: This first-in-human clinical study of aerosolised rhACE2 was conducted as a randomised, double-blind, placebo-controlled study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of inhaled APN01 in healthy subjects after ascending single doses and ascending multiple doses. This study was fully approved by the Ethics Committee of the Medical University of Vienna.

The study was registered at ClinicalTrials.gov with identifier number NCT05065645. Individual de-identified data will be shared upon request (by e-mail to the corresponding author).

Conflict of interest: This work was supported by Apeiron Biologics and conducted as contract research at the Medical University of Vienna, Department of Pulmology and Department of Clinical Pharmacology. The funder did not have any role in the execution of the study or interpretation of data. The academic authors declare no conflict of interest.

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