

Drug Development and Industrial Pharmacy

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iddi20

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To cite this article: Martin M. Sailer, Melanie Köllmer, Beatrice Masson, Fabio Fais, Ilja P. Hohenfeld, Michael E. Herbig, Assen K. Koitschev & Sven Becker (2023): Nasal residence time and rheological properties of a new bentonite-based thixotropic gel emulsion nasal spray – AM-301, Drug Development and Industrial Pharmacy, DOI: 10.1080/03639045.2023.2183724

To link to this article: https://doi.org/10.1080/03639045.2023.2183724

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Published online: 28 Feb 2023.

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DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY
https://doi.org/10.1080/03639045.2023.2183724

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RESEARCH ARTICLE

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Nasal residence time and rheological properties of a new bentonite-based thixotropic gel emulsion nasal spray – AM-301

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ABSTRACT

Objective: The present work provides characterization of rheological properties of a new bentonite-based thixotropic gel emulsion nasal spray (AM-301), its nasal residence time, distribution, safety and tolerability.
Significance: The nasal epithelium is a portal of entry for allergens and primary infection by airborne pathogens. Non-pharmacological interventions, which enhance physical and biological barriers, protect against allergens and pathogens without drug-related side effects. AM-301 has shown promising efficacy and safety in the nasal epithelium against viruses (*in vitro*) and pollen (clinical).
Methods: Technical part (i) spray characterization was performed with a validated droplet size distribution method; evaluation of the rheological properties of the formulation was performed by a validated amplitude sweep method and a validated oscillation, rotation, oscillation; Clinical part (ii) nasal and oropharyngeal endoscopy were used to provide a semi-quantitative evaluation of distribution and residence time of fluorescein-labelled AM-301 in the nose and oropharynx of healthy volunteers; (iii) tolerability and safety.
Results: (i) The non-Newtonian rheological properties of the formulation allow AM-301 to be sprayed and then to revert to a gel to prevent run-off from the nasal cavity; (ii) the formulation remains on the inferior turbinate, septum and oropharynx of volunteers for up to 210 min and on the middle turbinate for up to 60 min; two nasal sprays provide no substantial benefit over a single application with regards to coverage or retention; (iii) the spray is well tolerated
Conclusions: Single dose spray delivery of AM-301 provides extended coverage of the nasal mucosa up to the inferior turbinates.

ARTICLE HISTORY

Received 17 May 2022
Revised 13 February 2023
Accepted 18 February 2023

KEYWORDS

Nose; nasal spray; medical device; mucosal deposition; nasal residence time; bentonite; thixotropic; rhinoscopy; viscoelastic properties; rheology; disitribution

Introduction

The nose is the first physical and immunological barrier against pathogens and allergens directed at the upper and lower respiratory tract. Indeed, it works as a filter for the incoming air and protects the structures of the airway tract: ciliated epithelium with mucus-producing goblet cells and mucosal immune responses activated by the nasal and nasopharynx-associated lymphoid tissue [1,2]. In addition to the physical and immunological barrier provided by the mucus, the respiratory tract is protected by the mechanical action of the cilia, which move ('beat') in a coordinated fashion, pushing pathogens and allergens from the upper and lower airways toward the oropharynx, where they are either expectorated or swallowed [3–5]. The combined action of the mucus and cilia is called mucociliary clearance and is essential in preventing pathogens from coming into direct contact with epithelial cells and alveoli [6–10].

A new nasal spray medical device (AM-301, marketed as Bentrio®) was designed to help the natural protective role of the nose. AM-301 is a bentonite-based thixotropic gel emulsion which coats the nasal epithelium, acting as an additional mucus layer to trap or bind pathogens and allergens for expulsion by mucociliary clearance. This proposed mechanism of action is supported by previous *in vitro* studies showing the virus-binding properties of bentonite [11–14], and also by our recent observations of the positive protective action of AM-301 against SARS-CoV-2 infection in human airway epithelial cells [15] in which viral titers were significantly reduced. It could also be demonstrated for allergic rhinitis in an open-label trial [16] where the protective duration of AM-301 in reducing allergic nasal symptoms was shown to last around 210 min. The present work is another step forward in understanding how AM-301 interacts with the nasal environment, considering in particular its distribution in the nose and nasal residence. Indeed, a challenge with the intranasal delivery of medications and formulations is the role of the mucociliary clearance in removing them quickly from the nasal mucosa.

Many intranasal formulations show a clearance half-life of about 15 min [17]. An important requirement for an effective nasal administration is nasal residence time which may be increased by two different methods: (i) mucoadhesion; (ii) viscosity enhancers. Mucoadhesion requires the addition of polymers to the formulation to alter the viscosity, rheology and ciliary beating frequency. The second approach involves the use of viscosity enhancers to alter the intrinsic physical properties of the formulation without

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impairing the ciliary beating frequency and the physiological features of the nasal environment [18,19]. In particular, *in vitro* cytotoxicity assays performed in one of our previous studies showed that AM-301 slightly reduces the ciliary beating frequency (by about 1 Hz) when applied on human nasal epithelial inserts. However, this effect seems to be due to the viscosity of the preparation rather than to the bentonite per se [15]. AM-301 has been prepared following this approach and contains only excipients and inert ingredients listed in the Inactive Ingredient Database of the US Food and Drug Administration (FDA), 'generally recognized as safe' or approved as food additives [20].

Interindividual anatomical differences in nasal structures, due to variables such as age, gender, ethnicity, pathologies, etc., are important considerations in the assessment of nasal deposition mechanisms of new formulations. Previous studies have tested the nasal distribution of sprays, without the direct involvement of human volunteers, by reproducing human nasal anatomy using 3D-printed nasal cavity replicas based on patients' CT scans [21]. Despite such nasal casts being useful in showing the distribution of nasal spray in the nasal turbinates [21], we decided to perform the present study in 8 healthy volunteers. This was to observe not only the distribution and residence time of AM-301 in nasal turbinates, but also in other structures (i.e. olfactory cleft and septum) in addition to the oropharyngeal cavity. This is particularly important considering that the formulation used in the present study is intended to bind airborne viruses and allergens coming into contact with the nasal mucosa and also the oropharyngeal cavity. AM-301 can be visualized on rhinoscopy and oropharyngeal endoscopy examinations by adding a fluorescent dye (fluorescein 0.1%) to the formulation, thus enabling nasal residence time to be measured and intranasal distribution assessed. This technique has been used in previous studies to measure fluorescein clearance in patients with sinonasal disease [22] and to assess fluorescein-labeled (stained) medications [23]. A fluorescent dyed saline spray served as control as described by Bitter [24].

The aim of the current study was to further characterize AM-301 and to complement clinical outcomes from various other studies performed with AM-301. The behavior of the AM-301 formulation at physiological temperature and with fluorescein dye was evaluated. Furthermore, the distribution of AM-301 in the human nose after administration of one versus two sprays per nostril and its subsequent clearance to the oropharynx was also compared.

Material and methods

Technical section includes 'Spray characterization' and 'Rheological characterization.'

Spray characterization

Droplet Size Distribution (DSD) measurements were performed on AM-301 [25] (ingredients: purified water, glycerides, mixed decanoyl and octanoyl, propylene glycol, bentonite, glyceryl mono and dipalmitostearate, xanthan gum, disodium EDTA, mannitol, citric acid, butylated hydroxyanisole) and fluorescein-labeled AM-301 medical devices (batch CH102, the same batch tested during the clinical part of the study). DSD was measured by laser diffraction using a Granulometer Spraytec (Malvern Panalytical Ltd., Malvern, UK) provided with a 750 mm lens. DSD was determined 3 cm from the nozzle of the device. Briefly, after priming the device with 6 actuations, 3 measurements per device were carried out as follow: the device was shaken up and down in an upright position at least 6 times and actuated once. These steps were repeated twice, waiting at least 5 s between actuations. DSD was characterized by Dx(10), Dx(50), Dx(90), Span values and percentage of droplets lower than 10 μm.

Spray Pattern measurements were performed on AM-301 medical device (batch CH102). Spray pattern was measured on a white sheet of paper positioned 3 cm from the nozzle of the device. Briefly, after priming the AM-301 device with 6 actuations, 2 measurements per device were carried out as follows: the device was shaken up and down in an upright position at least 6 times and actuated once onto the surface of the white paper. These steps were repeated once, waiting at least 5 s after the first actuation. Immediately after each actuation, the outline of the blot formed by the spray on the paper was circled with a pencil. The characteristic dimensions Dmin (minimum diameter of the spray) and Dmax (maximum diameter of the spray) were measured and the ovality ratio (Dmax/Dmin) was calculated.

Rheological characterization

General setup

All samples were analyzed with an MCR 102 rotational rheometer (Anton Paar GmbH, Graz, Austria). The plate-plate measuring system PP25 (25 mm diameter plate) was used for the amplitude sweeps and oscillation, rotation, oscillation (ORO)-tests. Both of these methods were validated. Prior to conducting the experiments, the amplitude sweep and the ORO-test were analyzed for precision ($N = 8$ measurements/one operator) and intermediate precision ($N = 8$ measurements/2 different operators) to assess the suitability of the methods for the analysis of the nasal spray formulations containing a smectite clay (Veegum K) and xanthan gum as thickeners. The relative standard deviation (RSD) for the parameter complex modulus (derived from amplitude sweeps) was 9.12% during the repeatability test and 17.06% for the intermediate precision test (data derived from validation report; not included here). The ORO test showed a RSD of 15.92% for the repeatability test and displayed an RSD of 14.84% for the intermediate precision test of the parameter structure regeneration after 240 s (data derived from validation report; not included here). Both rheological methods passed the acceptance criteria. An RSD below 30% was set as acceptance criteria prior to the rheological method validation experiment to take into account the variability of the internal structure of a semisolid formulation with two thickeners. A parallel plate measuring system with a 50 mm diameter (PP50) was used for the temperature sweeps. Temperature was maintained at 25 °C *via* the Peltier P-PTD200/Air or adjusted accordingly using the heating hood H-PTD200 (temperature sweeps, modified amplitude sweeps at 34 °C, modified ORO-tests with structure recovery at 34 °C) (Anton Paar GmbH, Graz, Austria). The measuring position was adjusted to 1 mm. Data collection and evaluation was performed with RheoCompass™ software (Anton Paar GmbH, Graz, Austria). The formulation vials were shaken up and down for 5 s to mimic the handling of AM-301 before use. Rheological evaluation was performed immediately after shaking the vials.

Amplitude sweep method

The amplitude sweep was conducted at a constant circular frequency of 10 s^{-1} and a strain ranging from 0.01% to 1000%. The test was run at 25 °C (original AS) or at 34 °C to mimic the temperature in the nasal cavity (modified AS).

ORO-test method

The ORO-test comprises three steps: oscillation at low deformation values within the linear viscoelastic region to mimic resting behavior; rotation at a high shear rate to simulate structure decomposition; another oscillation step at low deformation values to evaluate structure regeneration. Initially, a strain of 0.02% and a circular frequency of 10 rad/s was applied for 240 s, a rotation step at a shear rate of 1000 s^{-1} followed for 2.5 s and finally a strain at 0.02% and a circular frequency of 10 rad/s was applied for 244 s. The structure regeneration (percentage of recovered G′ related to the last G′ value of the first measuring step) was evaluated 240 s after shear stress cessation. The temperature was maintained at 25 °C during all three measuring segments (original ORO) or increased to 34 °C in the third measuring segment (modified ORO) to mimic structure recovery in the nasal cavity. The heating from 25 °C to 34 °C was accomplished in around 53 s after starting segment 3 (recovery step).

Temperature sweep method

Temperature sweeps, where circular frequency and shear deformation are kept constant while temperature is varied, were conducted to investigate the impact of increasing temperatures on material structure. Temperature sweeps were performed at a constant strain of 0.01% and a circular frequency of 10 rad/s at a heating and cooling rate of 2 °C/min.

Clinical section

Examinations were performed at the Department of Otorhinolaryngology, Head and Neck Surgery of the University Medical Center of the Eberhard-Karls University Tübingen, Germany. Investigations were performed according to the Declaration of Helsinki on Biomedical Studies involving human subjects. The study design was approved by the local ethics committee (EC reference: 258/2021MPG1). The trial was registered in the German Clinical Trial Register (DRKS00025692) and with EUDAMED (CIV-21-03-036105). As this study involved a class I medical device, it was granted a formal approval waiver by the Federal Institute of Drugs and Medical Devices. All study subjects provided written informed consent, including consent for publication of the trial data in a scientific journal.

Subject population

The main inclusion and exclusion criteria of the study were the following: Eight healthy male volunteers aged between 18 and 50 years and with a BMI between 18.0 and 25.0 kg/m^2 were invited to participate in the study *via* a message through a study portal for clinical trials of the Eberhard-Karls University Tübingen. Subjects with a clinically relevant nasal obstruction or pathology precluding effective and/or efficient intranasal delivery (determined by nasal cavity endoscopy) were excluded. Other exclusion criteria were any nasal treatments within 30 days prior to study start (including nasal sprays, nasal drops and saline washes), any ongoing upper respiratory tract infection, rhinitis (symptomatic) or asthma, any clinically relevant medical history or concomitant medication, history of severe or abnormal drug reaction, known sensitivity to fluorescein or any constituents of AM-301, or concomitant or prior treatment with another investigational medicinal product or device within 30 days prior to the study start.

Medical devices

AM-301 (Bentrio; batch CH102) was used for subjects in Groups A and B. The device was administered by a trained investigator. Saline nasal spray (Olynth® Salin Nasenspray, Lösung, Johnson and Johnson GmbH, Neuss, Germany; batch 10D07;) was used as comparator for subjects in Group C. Both AM-301 and Saline nasal sprays delivered 0.14 ml per actuation.

Main study endpoints

The primary endpoint of the study was to assess the nasal residence time of AM-301 (one or two administrations per nostril) defined as time during which the product is visible in different structures of the nose, starting from its application until its disappearance from the middle and inferior turbinates. Distribution of fluorescein-dyed product within nasal cavity was observed until up to 240 min, or until its complete disappearance. The secondary endpoint was to observe the time interval from the application of the product in the nose to its appearance in the oropharynx. As safety endpoints, the occurrence of adverse events, changes in vital signs, TNSS and global rating of overall tolerability were also evaluated by both the volunteers and the investigators during the entire testing period.

Study assessments

Medical history was recorded for all subjects. An otorhinolaryngological examination was performed before device administration which included endoscopic inspection of the mouth and oropharynx, especially of the posterior pharyngeal wall, followed by an endoscopic anterior and posterior rhinoscopy (all 0°-endoscope, type 7230AA serial number SN1214FR, Karl Storz SE & Co. KG, Tuttlingen, Germany). All examinations were stored in 4K-quality on a Storz-AIDA system (KARL STORZ AIDA®, TP1045381, Karl Storz SE & Co. KG, Tuttlingen, Germany) for later off-line evaluation. Fluorescein-labeled AM-301 (Group A, $n = 4$: one spray to each nostril; Group B, $n = 4$: two sprays per nostril) or fluorescein-labeled saline spray (Group C, $n = 4$: 1 spray per nostril) was then administered. Group C includes the same 4 subjects as group A, receiving saline spray around 3.5 months after AM-301 administration.

To visualize the fluorescein a light source with a fluorescein filter on the endoscope was used (product number 20100032).

Fluorescence of middle and inferior turbinates, septum and olfactory cleft was semi-quantitatively measured on both sides intranasally and in the oropharynx at 1, 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min after administration. Distribution of stained test product within the nasal cavity was graded as 'intense = 3,' 'moderate = 2,' 'minimal = 1' or 'absent = 0.' Intense = strong fluorescence; Moderate = good fluorescence compared to non-stained tissue areas; Minimal = slight fluorescence in comparison with the non-stained tissue areas.

Oropharyngeal endoscopy was performed every 5 min after test product administration until it was first visible in the oropharynx. Thereafter the presence of test product in the oropharynx was assessed at every rhinoscopy up to 240 min, or until the last rhinoscopy was performed. Evaluation was either 'yes − 1' fluorescence visible in oropharynx or 'no − 0' fluorescence no longer visible in the oropharynx.

Appearance of potential nasal symptoms were collected using the total nasal symptom score (TNSS), a four-item questionnaire that investigates nasal congestion, rhinorrhea, nasal itching, and sneezing [26]. Each symptom is scored on a 4-point severity scale (0 = none; 1 = mild; 2 = moderate; 3 = severe), and TNSS is the

sum of each score, with a maximum value of 12. The questionnaire items and the overall scale was explained to subjects before the first assessment. The TNSS was collected prior to the first spray administration and at 10, 20, 30, 60 min, then every 30 min until up to 240 min, or until the last rhinoscopy was performed.

Subjects' vital signs were monitored by noninvasive blood pressure and heart rate recordings and subjects were asked about any adverse events. Investigators and subjects completed a global rating of overall tolerability at the end of testing.

A nasal rinse and mouth wash were performed at the end of the assessment day if required.

Fluorescein-labeling

AM-301 formulation was stained with fluorescein by a trained pharmacist at the study site's pharmacy the same day prior to administration. Briefly, the pump was removed from the spray bottle and 0.2 ml Fluorescein-sodium 10% (Fluorescein Alcon® 10% Injektionslösung, Alcon, Freiburg, Germany) was added with a syringe to a 20 ml bottle of nasal spray formulation. The formulation was then manually stirred for 5 min and a new nasal spray pump Advanced Preservative Free (APF), Aptar Pharma, Eigeltingen, Germany) was mounted using a manual assembly press.

The Olynth saline spray device containing 15 ml saline solution was stained with 0.15 ml Fluorescein-sodium 10% following the above procedure.

Statistical analysis and graphical representation

No statistical analyses were planned for the clinical part of this study. Data were analyzed descriptively showing mean values with relative standard deviation (RSD) for spray characterization and mean values with standard deviation (SD) for rheological characterization and clinical evaluations. For graphs of nasal residence time and TNSS presentation it is presented in mean values with standard error (SEM). Graphs were created using GraphPad Prism software.

Results

The results of the technical (Part 1) and clinical (Part 2) characterization are presented in three main sections. The first two sections present the technical characterization of AM-301: Section 'Spray characterization' describes the impact of fluorescein on droplet size distribution and spray pattern and 3.2, 'Rheological properties' describes the rheological characterization of the AM-301 formulation; the clinical part, Section 'Clinical data' presents demographics, nasal residence time and safety of the formulation.

Spray characterization

Impact of fluorescein on the droplet size distribution

Dx(10), Dx(50), Dx(90) and span values reported in Table 1 allow comparison of the formulation with and without fluorescein label. Data demonstrate that the addition of fluorescein may have some impact on DSD, however this does not significantly impact the product in terms of nasal regional deposition as, essentially, with a Dv50 $>$ 50 μm there would be no difference between anterior, middle, posterior or olfactory region deposition [27] The deposition of the product for both formulations is localized in the nasal cavity (i.e. the vast majority of the droplets are larger than 10 microns), the Dx(10) mean values are around 100–150 μm and the Dx50 values around 200–300 μm.

Spray pattern

Spray pattern testing is a classic test in the evaluation of the performance of a nasal spray pump besides being a readout useful to verify batch to batch consistency of a combination product characteristics depending on normal formulation and pump manufacturing variability. Data shown in Table 2 were obtained from the formulation without fluorescein and are as expected for typical nasal sprays, i.e. with ovality between 1.0 and 1.5 and diameters generally between ∼1.2 cm and ∼3.5 cm [28].

Rheological properties

Impact of fluorescein on viscoelastic properties and on structure regeneration

Values of the complex modulus (G*) and the storage modulus G′ at the flow point (G'_{FP}) are derived from amplitude sweeps that are applied to describe the viscoelastic properties of the evaluated samples. The amplitude sweep results are displayed as a diagram with strain (or shear stress) plotted on the x-axis and storage modulus G′ and loss modulus G″ plotted on the y-axis (see Figure 1(a)). The storage modulus G′ is a measure of the energy stored in the material and characterizes the elastic or solid-like behavior of the sample. The loss modulus G″ represents the lost energy per cycle of sinusoidal deformation and represents the viscous or fluid-like behavior of a material. The complex modulus G* refers to the vectorial sum of G′ and G″ in the linear viscoelastic region (structure of the material is still intact and strain-independent) and represents the total viscoelastic behavior of the formulation. The flow point is the intercept of storage and loss moduli (see Figure 1(a)). After passing the flow point, the formulation shows more viscous behavior and flows. Complex moduli and flow points were slightly lower with the addition of 0.1% fluorescein (Sigma Aldrich, Oakville, Ont., Canada) but were still within the same order of magnitude (Figure 2(a,b)). The ORO-test provides information on the extent to which a thixotropic material recovers its structure after being exposed to a (stronger) shear stress in a certain timeframe. In the current ORO-test, a shear rate of 1000 s^{-1} was chosen to mimic spraying from the nasal spray container. The rheological tests show that the addition of up to 0.1% fluorescein to a development batch of AM-301 did not affect structure regeneration of the formulation (Figure 2(c)).

Viscoelastic properties at 34 °C

Amplitude sweeps were conducted at 25 °C and 34 °C to assess the impact of higher nasal cavity temperatures (∼34 °C) on the viscoelastic structure of the nasal spray. At both temperatures, the evaluated AM-301 batch showed a predominance of elastic behavior (G′ $>$ G″) (Figure 1(a)). At both temperatures, the formulation displayed a critical strain of $\gamma = 0.07\%$ at the end of the linear viscoelastic (LVE) region (Figure 1(b)). Below the critical strain, the viscoelastic structure of the formulation is intact. However, complex moduli were lower at 34 °C (509.33 Pa vs. 848.67 Pa at 25 °C) indicating a moderately lower structural rigidity at higher temperatures (Figure 1(c)). Storage moduli and shear stresses at the flow point were circa 2-fold lower when the amplitude sweep was conducted at 34 °C (Figure 1(d,e)). Results indicate that the viscoelastic properties of the AM-301 formulation are moderately lower in the nasal cavity.

Table 1. The comparison between AM-301 formulation with and without fluorescein by DSD analysis (batch CH102).

Batch	Unit ID number	Spray ID number	Dx(10) µm	Dx(50) µm	Dx(90) µm	Span	% <10 µm
AM-301 batch	1	1	114	207	403	1.4	0.0
#CH102 – without fluorescein	1	2	194	433	1278	2.5	0.0
	1	3	217	582	1645	2.5	0.0
	2	1	136	230	377	1.0	0.0
	2	2	125	226	1581	6.4	0.0
	2	3	133	234	415	1.2	0.1
	Average	–	153	319	950	2.5	0.0
	RSD	–	27%	48%	65%	81%	N/A
AM-301 batch	1	1	93	191	1532	7.5	0.2
#CH102 – with fluorescein	1	2	96	198	1653	7.9	0.2
	1	3	89	179	1199	6.2	0.2
	2	1	102	192	355	1.3	0.2
	2	2	100	204	1642	7.6	0.2
	2	3	87	175	1192	6.3	0.2
	3	1	106	212	1652	7.3	0.2
	3	2	97	205	1697	7.8	0.2
	3	3	118	227	1581	6.4	0.2
	Average	–	99	198	1389	6.5	0.2
	RSD	–	10%	8%	31%	32%	N/A

For AM-301 batch #CH102 – without fluorescein 2 units were used ($n = 2$), identified as 'Unit ID number' and each unit was actuated three times ($n = 3$, intra-unit variability), identified as 'Spray ID number.' The same result layout applies to AM-301 batch #CH102 – with fluorescein except that the measurement was performed on 3 units ($n = 3$). RSD: relative standard deviation.

Table 2. The spray pattern characterization of the AM-301 formulation without fluorescein (batch CH102), 2 units were used ($n = 2$), identified as 'Unit ID number' and each unit was actuated three times ($n = 3$, intra-unit variability), identified as 'Spray ID number.'

Batch	Unit ID number	Spray ID number	D_{max} (cm)	D_{min} (cm)	Ovality Ratio
CH102 – without fluorescein	1	1	3.3	3.1	1.1
		2	3.3	3.1	1.1
	2	1	3.2	3.1	1.0
		2	3.2	3.0	1.1
	Mean		3.3	3.1	1.1

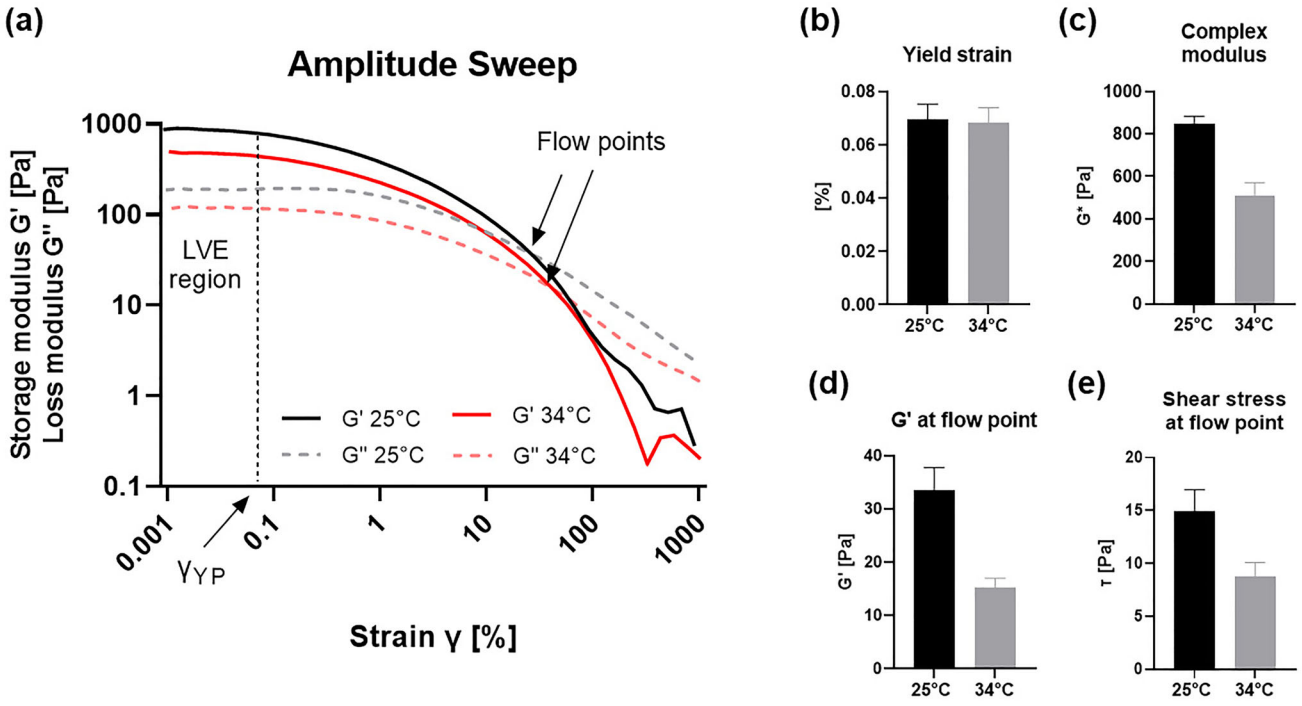


Figure 1. Representative amplitude sweeps of an AM-301 batch conducted at 25 °C and 34 °C (a) and viscoelastic properties at both temperatures (b–e). Results in bar graphs are expressed as mean values with standard deviations ($n = 3$ measurements).

Structure regeneration at 34 °C

Two hundred forty seconds after stress application, the average structure regeneration of AM-301 nasal spray was 87.80% (Figure 3(a)). When the recovery step was conducted at 34 °C, which mimics the temperature in the nasal cavity after spray application, the average structure regeneration was 55.70% (Figure 3(b)). Thus, structure regeneration of AM-301 nasal spray was slightly slower at higher temperatures but its overall capacity for structure regeneration remained. Immediately after cessation of the shear stress (second 'O' step), the observed structure was again more gel- or

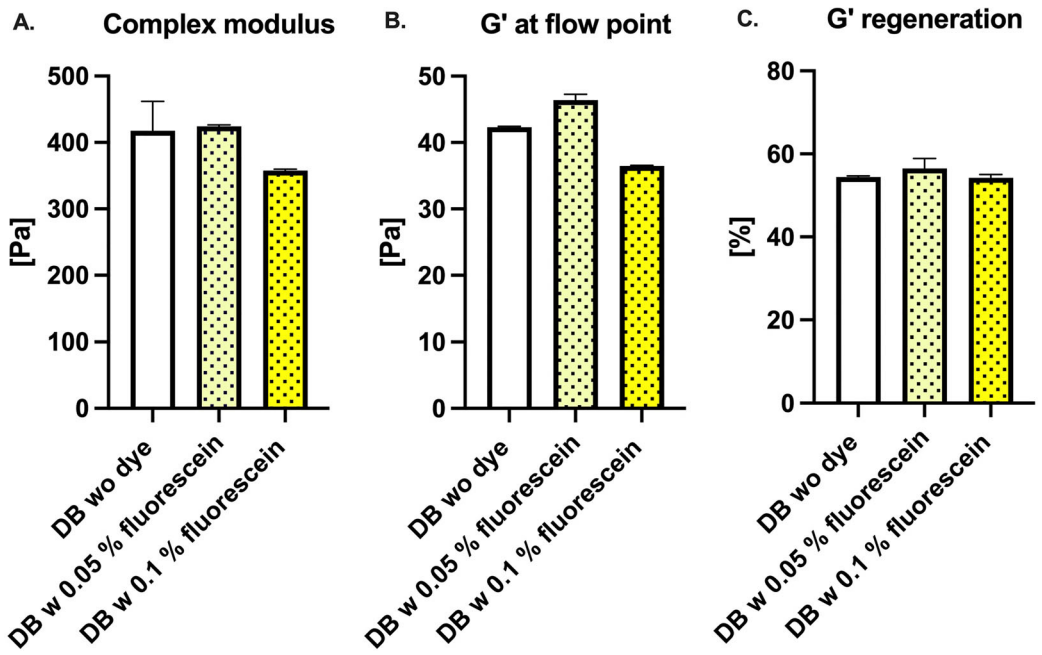


Figure 2. Impact of fluorescein on viscoelastic properties (G* & G′ at flow point) and structure regeneration of an AM-301 development batch (DB). Results are expressed as mean values with standard deviations ($n = 2$ measurements), method validated: intermediate precision 14.84% RSD%, repeatability 15.92% RSD%. (a) Complex modulus. (b) G′ at flow point. (c) G′ regeneration.

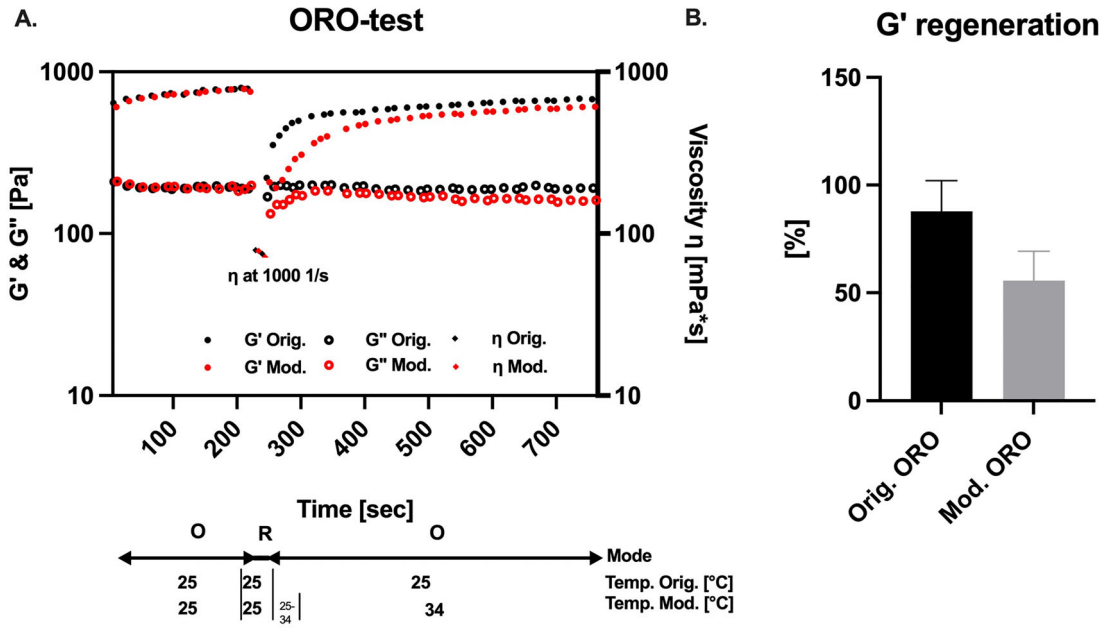


Figure 3. Representative ORO-tests of an AM-301 batch display structure regeneration at 25 °C (Original Test) and at 34 °C (Modified Test). Results from the bar graph are expressed as mean values with standard deviations ($n = 3$ measurements). (a) ORO-test. (b) G′ regeneration.

solid-like with G′ > G″ indicating structure recovery. Due to the decrease of moduli at higher temperatures (see amplitude sweeps), it is likely that higher regeneration values might have been obtained if all segments of the ORO-test had been set to 34 °C. However, this would have been less representative of the actual spraying process whereby the formulation is kept in the spray bottle at room temperature before use.

Temperature sweeps

Temperature sweeps were conducted to investigate the impact of temperature on the material structure of AM-301 nasal spray. When heated from 25 °C (room temperature) to 34 °C (nasal cavity, according to Kecks et al.), complex viscosity decreased by 42%, storage modulus decreased by 45% and loss modulus decreased by 28% which indicated a decrease in the viscoelastic structure and temperature-dependent behavior of AM-301 (Figure 4(a,b)). However, there was no crossover of storage and loss modulus. Thus, when heated from 5 °C to 40 °C the structure-building ingredients did not melt (Figure 4(a)). When cooled from 40 °C to 5 °C, complex viscosity of the nasal spray increased again, and was even higher than the initial complex viscosity before heating, indicating that structure rebuilding occurred after exposure to higher temperatures (Figure 4(b)).

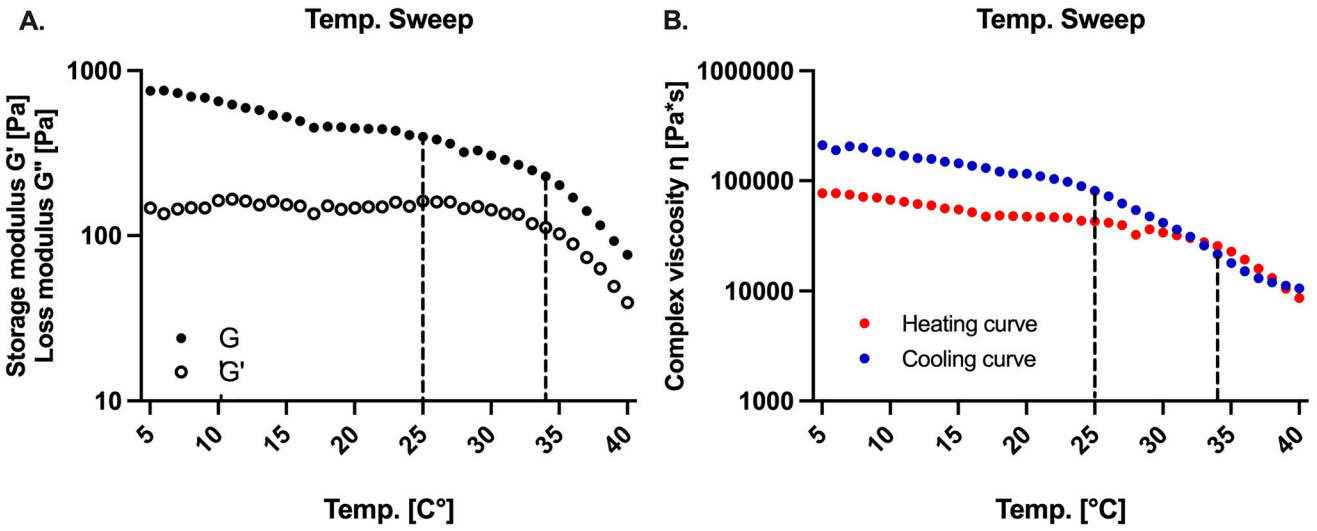


Figure 4. Representative temperature sweep of a batch of AM-301. Temperature-dependence of storage and loss moduli (a) and complex viscosity (b) is displayed.

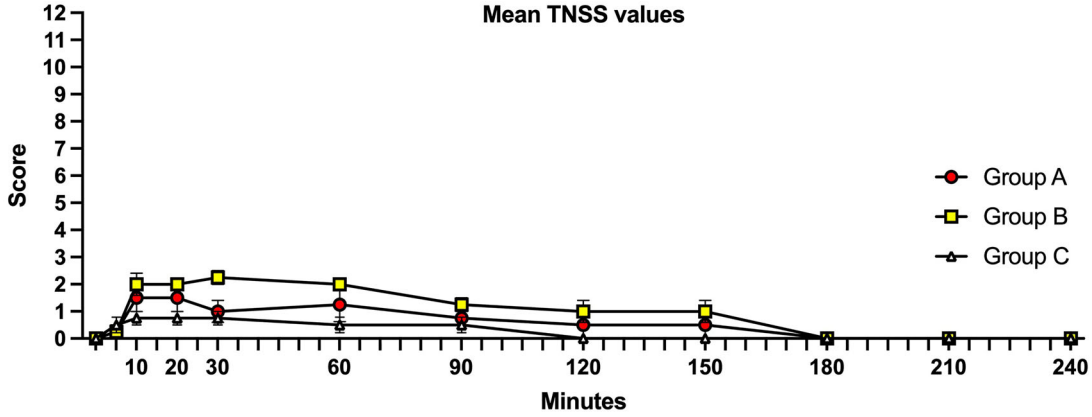


Figure 5. Values represent the mean TNSS ± SEM ($N = 4$/group), including nasal obstruction, runny nose, itching of nose and sneezing. Group A: subjects received 1 AM-301 spray/nostril (0.14 ml); Group B: subjects received 2 AM-301 sprays/nostril (0.28 ml); Group C: subjects received 1 Saline control spray/nostril (0.14 ml).

Clinical data

Subject disposition

Eight male volunteers were recruited to the study with a mean age of 24.5 ± 2.8 years. The first four subjects were assigned to Group A and the next four to Group B. Subjects from Group C, control group, were rescreened from Group A and were examined again around 3.5 months later. All subjects completed the study.

Nasal residence time

Nasal residence time was defined as the time from application of the device to the disappearance of the fluorescein-stained test product from the middle and inferior turbinates. Distribution was evaluated up to 240 min, or until no more stained test product was visible following application. In addition, the septum and olfactory cleft were evaluated. Nasal residence time averaged 210 min in Group A, 180 min in Group B and 60 min in Group C. Figure 6 shows that AM-301 reached the septum and the middle and inferior turbinates. Intense staining (strong fluorescence) was visible on average up to 10 min in the middle turbinates and septum, and up to 20 min in the inferior turbinates. Thereafter fluorescence faded with different kinetics in different structures (Figure 6). Coverage of the middle turbinates was more intense when two AM-301 sprays were applied per nostril (Figure 6(A)). In contrast, coverage of the inferior turbinates did not vary between Group A or Group B subjects, or between 1 or 2 sprays per nostril of AM-301 (Figure 6(B)). There was no fluorescence in the olfactory cleft in any group (Figure 6(D)). When the stained saline control was applied (1 spray per nostril as in Group A) the septum and inferior turbinates showed intense staining for 5–10 min, which then faded quickly. Fluorescence disappeared from the nose within 60 min with saline, in contrast to AM-301, where fluorescence was detected for up to 210 min. Coverage of the middle turbinates showed the highest variability between subjects and nostrils. The duration of staining varied between the right and left nostril for some subjects. Representative pictures (Figure 7) extracted from anterior rhinoscopy videos show the presence of fluorescence for up to 240 min in Group A (AM-301) and for about 120 min in Group C (saline control).

Fluorescence in the oropharynx

The clearance of the formulation from the nose and its appearance and residence in the oropharynx were also observed. Fluorescence was visible and broadly distributed in the

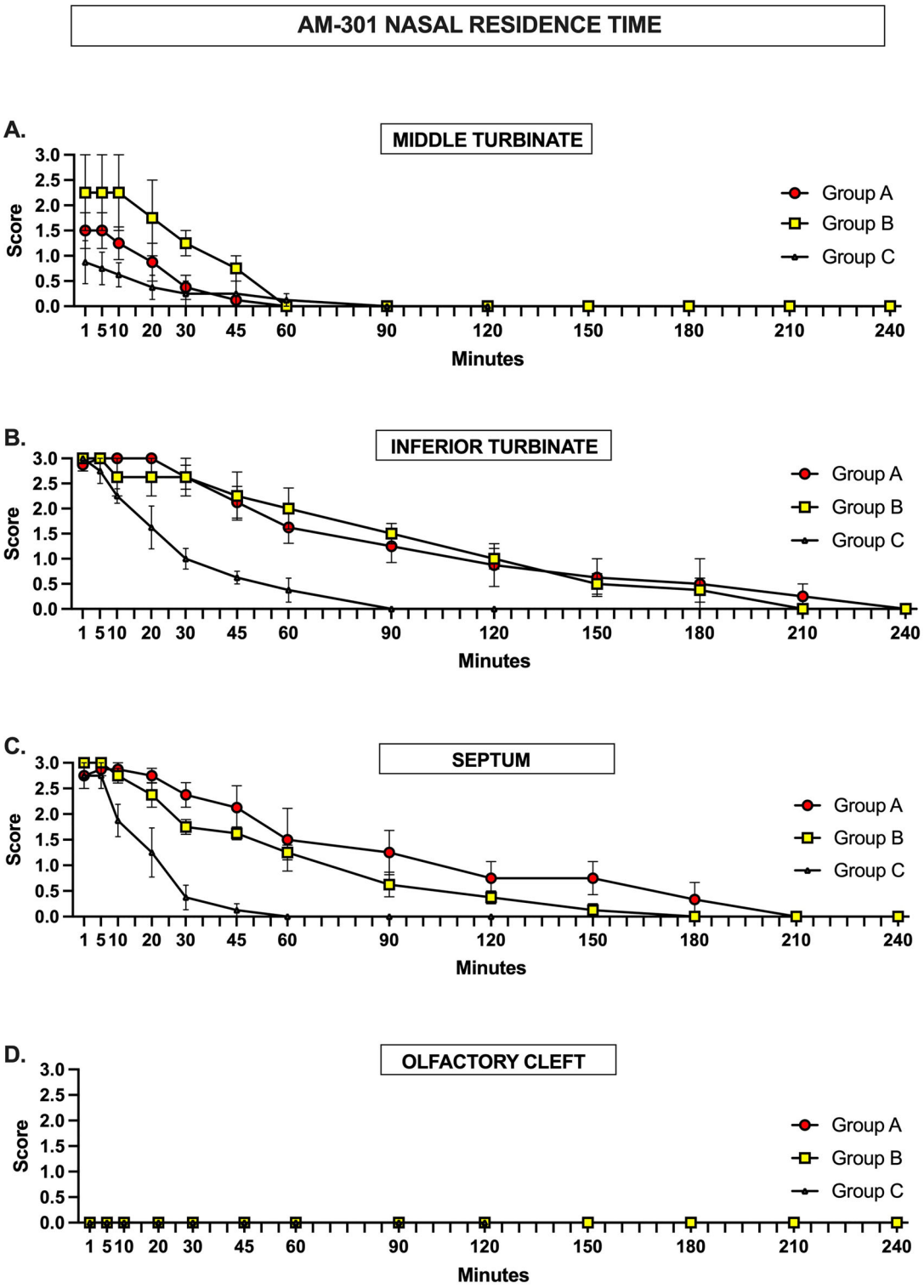


Figure 6. Nasal residence time in different structures of the nose. Results are expressed as mean score and SEM per group ($N = 4$) in (a) inferior turbinate, (b) middle turbinate, (c) septum and (d) olfactory cleft. Nasal residence time was assessed over 240 min. Group A: subjects received 1 spray AM-301/nostril; Group B: subjects received 2 sprays AM-301/nostril; Group C: control group.

oropharynx on endoscopy. Fluorescence appeared in the oropharynx 5 min after administration in all three groups. In the saline control group, fluorescence disappeared after 90–120 min. In the groups receiving AM-301 (Group A and B) fluorescence remained visible for up to 240 min in both Groups A and B. In Figure 8, representative images taken from oropharyngeal endoscopy of one subject each from Group A and Group C show that AM-301 was visible in the oropharynx for up to 240 min (Group A), whereas the saline control could only be detected for 60 min (Group C).

Safety

Both AM-301 and the control-spray were very well tolerated by all subjects and there were no adverse events. There were no

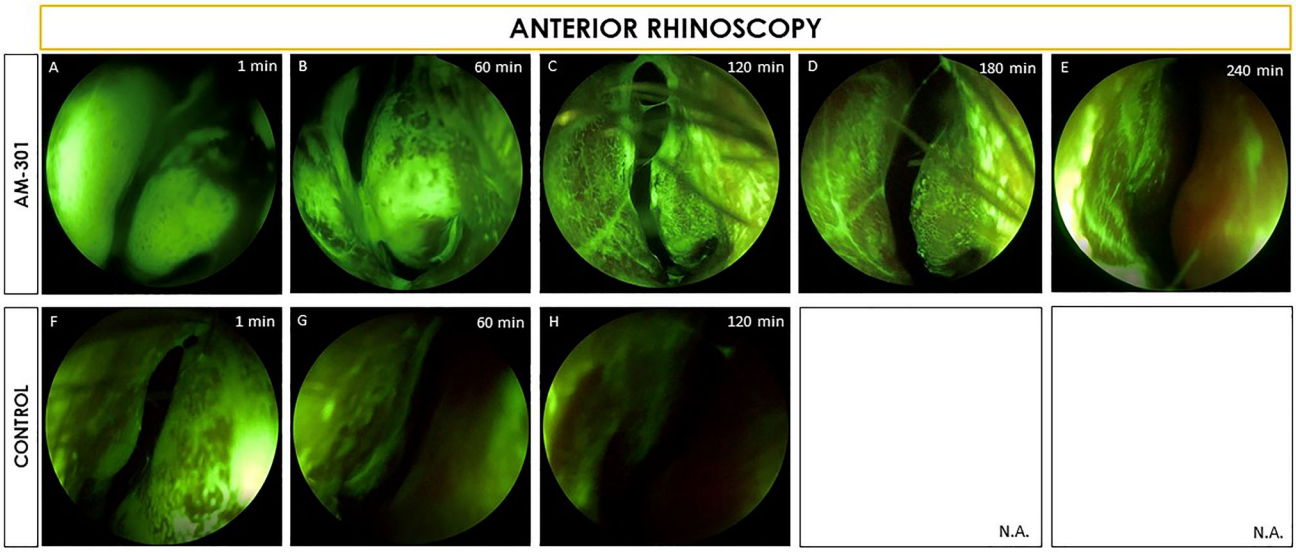


Figure 7. Anterior rhinoscopy. The presence of the formulation in the nose was monitored for 240 min. Panel show representative images taken at different time points (from 0 to 240 min) from videorecording of anterior rhinoscopy of a subject receiving 1 AM-301 spray/nostril (Group A, left nostril) (A–E) and 1 spray/nostril of saline control (Group C, right nostril) (F–H). NA = not applicable (no fluorescence detected).

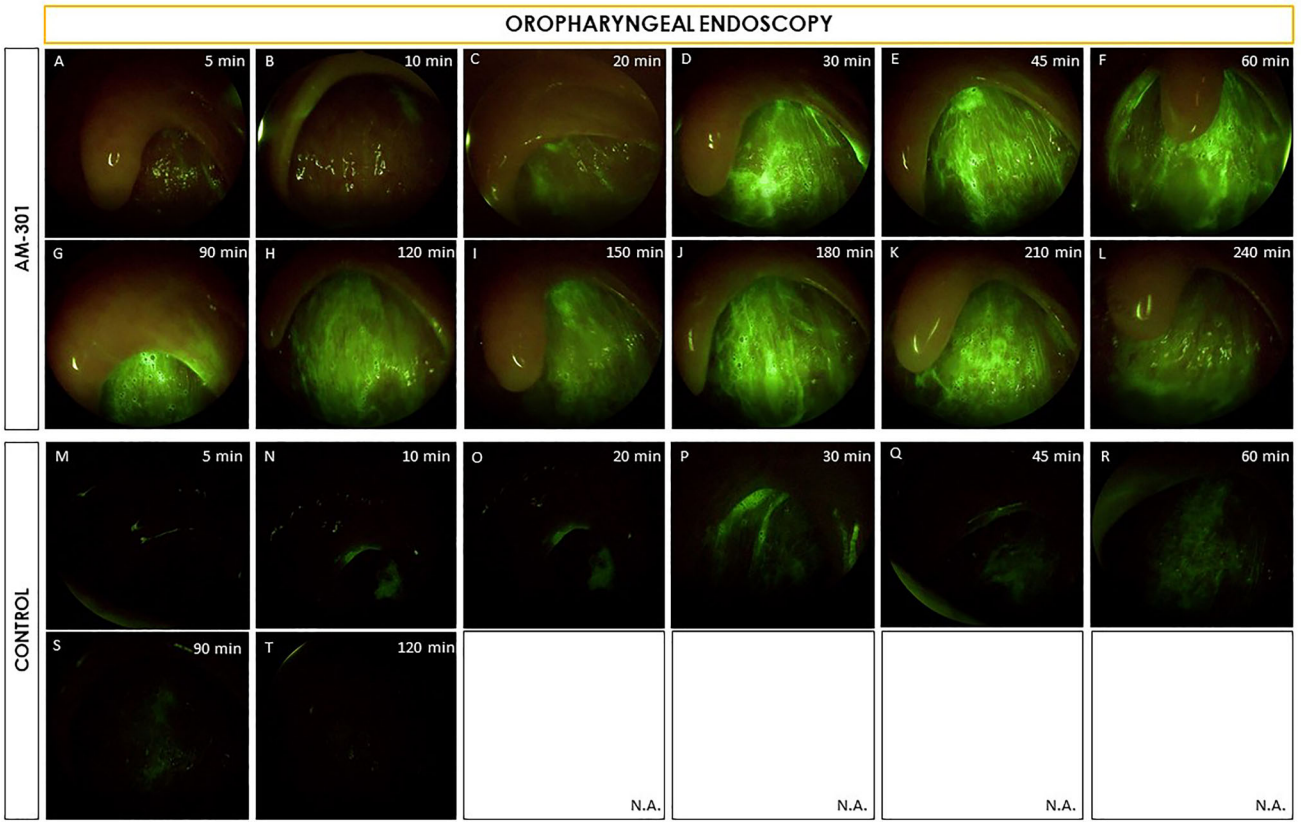


Figure 8. Oropharyngeal endoscopy. The presence of the formulation in the oropharynx was monitored for 240 min. Panel show representative images taken at different time points (from 0 to 240 min) from videorecording of oropharyngeal endoscopy of a subject receiving 1 AM-301 spray/nostril (A–L) and one spray/nostril of saline control (M–T). NA = not applicable (no fluorescence detected).

significant anatomical abnormalities on endoscopic evaluation of the nose and nasopharynx. A very mild dose dependent increase in nasal symptoms was seen on TNSS in both groups that received the AM-301 formulation (Group A and B): two sprays per nostril resulted in a few more symptoms than one spray per nostril. A few symptoms were reported in the saline control group (Group C) (Figure 5). Neither the AM-301 formulation nor the saline solution caused sneezing in any subject. Itching of the nose was reported by two subjects in Group B. Runny nose and nasal obstruction were reported in all study groups. At the end of the study, device tolerability was judged 'very good' by 75% and 'good' by 25% of subjects in Group A. Device tolerability was evaluated 'very good' by 25% and 'good' by 75% of subjects in Group B. All subjects who received the saline solution described the spray as 'very good.' There was little change in vital signs before or after assessment of AM-301: body temperature (36.6 °C ± 0.3 vs.

36.6 °C ± 0.4); systolic (136.6 ± 12 mmHg vs. 130.2 ± 9.4 mmHg) and diastolic blood pressure (78.7 ± 9.9 mmHg vs. 74.3 ± 6.4 mmHg); heart rate fell slightly after the assessment (69.9 ± 10 beats/min vs. 59.0 ± 8.4 beats/min) as subjects relaxed during the 240-min assessment period.

Discussion

The present study was set up to provide a thorough characterization of a new bentonite-based thixotropic gel emulsion (AM-301) in terms of technical characterization (spray characterization, rheological properties) and clinical evaluation of nasal residence time and distribution in different nasal areas and in the throat of healthy volunteers including safety, tolerability. These data are particularly important because results from our previous studies suggest that AM-301 is effective against a broad spectrum of viruses and allergens [15,16] and could be used on a daily basis by a large number of people. The determined nasal residence time is supporting the observed duration of protection in subjects of two other clinical studies with AM-301 (Nehrig et al. [16]; study NCT05122143, not yet published).

The first part of the study was dedicated to the description of the spray characteristics and rheological properties of AM-301. The formulation of AM-301 contains a smectite clay, bentonite, that is intended to trap and inactivate airborne viruses and allergens. It also contains xanthan gum as a thickening polymer that – together with bentonite – contributes to the thixotropic and shear-thinning behavior of AM-301. Both properties are required for the sprayability and efficacy of nasal emulsion sprays. In particular, shear-thinning or the decrease of viscosity at increasing shear rates, is a prerequisite for the successful spraying process of an emulsion-type formulation. Rapid structure regeneration after shear cessation facilitates the return of sufficiently high viscosity that enables AM-301 to form a protective coating inside the nose. Previous reports also support the concept of higher emulsion viscosities in the nasal cavity and prolonged nasal residence time [29].

Spray characteristics of AM-301 were as expected for typical nasal sprays. Addition of fluorescein to AM-301 had no significant impact on the droplet size distribution in terms of deposition within the human nasal cavity. Droplet size distribution is important for characterization of a nasal spray and for safety reasons as droplets smaller than 10 microns could reach the lungs [30,31]. For AM-301 the percentage of droplets below 10 microns was sufficiently low.

Rheological results indicate a fast structure regeneration of AM-301 after mimicking the spraying process by application of a high shear rate (1000 s^{-1}) for 2.5 s. Immediately after cessation of the shear stress, the storage modulus (G′) was again higher than the loss modulus (G″) indicating recovery of the gel-like structure. The absolute recovery of the G′ of AM-301, 240 s after shear stress cessation, ranged from 54.20% to 87.80% and varied between different batches (development batch vs. commercial batch) and temperatures (25 °C vs. 34 °C). The structure regeneration of AM-301 was slightly lower at 34 °C (temperature representative of the nasal cavity), possibly due to the reduction of viscoelastic properties at higher temperatures. However, viscosity was high enough to prevent the formulation running from the nose. This was also indicated by the long nasal residence time of AM-301. A sufficiently high viscosity of the sprayable, shear-thinning emulsion gel in the nose has a higher impact on efficacy than the capacity for complete structure regeneration [29].

In accordance with Kecks et al. [32], 34 °C was chosen for the temperature sweeps. At ambient temperatures during the test setting of around 22 °C, a temperature between 25 °C and 34 °C is expected in the anterior part of the nose when the spray first contacts the mucosa. The anterior parts in the nose, which are effectively reached by a nasal spray, were the site of focus in this study.

In the clinical part of the study, AM-301 was sprayed into the nose of healthy male subjects in order to assess as primary objective its nasal residence time. As secondary objectives, appearance in the oropharynx, safety and tolerability of one or two sprays per nostril as well as nasal symptoms were also evaluated. A group of participants receiving saline solution dyed with fluorescein was added to provide for a baseline residence time of a non-viscous substance (saline solution) and to observe if fluorescein per se (rather than the viscosity of our product) is responsible increasing the residence time of the formulation.

The assessment of the nasal residence time and the observation of the distribution in the nose/oropharynx of a new nasal spray are essential in order to estimate its potential efficacy window. For this purpose, anterior rhinoscopy and oropharyngeal endoscopy were performed to monitor the distribution of fluorescein-labeled AM-301 in the nose (middle turbinate, inferior turbinate, septum and olfactory cleft) and throat of healthy volunteers at time points up to 240 min observation (Figures 6–8). Manual scores from 0 to 3, based on the observation of fluorescein-labeled AM-301, were assigned by the clinician to each structure examined at each time point. This semi-quantitative analysis revealed that AM-301 was mainly distributed on the middle turbinates, septum, and to a large extent, the inferior turbinates. AM-301 did not reach the olfactory cleft. This is consistent with other publications which show that nasal sprays are highly distributed on the inferior and middle turbinates with only slight distribution, if any, to the olfactory region [33,34]. A rich vascular supply allows the inferior turbinates to swell and shrink which helps to regulate airflow, but which is also responsible for the nasal obstruction in allergic reactions and inflammation caused by bacterial or viral infections on the mucosal surfaces. The good and long-lasting coverage by AM-301, especially of the inferior turbinates, by generating a barrier between the incoming allergens and the mucosa for up to 4 h, could explain the reduction in nasal symptoms observed in a grass pollen exposition chamber study [16]. Furthermore, data suggest that one spray of AM-301 per nostril is sufficient to show a wide and sustained distribution in the nasopharynx, that can provide protection from allergens for up to about 210 min. Two sprays of AM-301 per nostril showed to give a similar coverage time of the nasal mucosa and were reported to be safe and well tolerated.

Due to the rheological properties of the formulation, fluorescein-labeled AM-301 was visible on the inferior turbinates and the septum for up to 210–240 min, and on the middle turbinates for up to 60 min, compared with 60 min residence time on these structures seen in the saline-treated control group. The semi-quantitative evaluation of nasal residence time of AM-301 in each specific nasal area seems to underestimate the overall observation of fluorescence in the nose as shown in Figure 7. This discrepancy is because areas of the nose devoid of cilia retain the fluorescein-labeled product for longer due to a reduced mucociliary clearance. These rating differences for specific areas should be taken into account when selecting nasal regions of interest, whereas an overall observation of fluorescence present in the nose (as shown in the panel) includes the additional signal from areas with slower clearance. The clinician rated only specific areas and not the

whole fluorescence in the nose (middle turbinate, inferior turbinate, septum and olfactory cleft).

Importantly, the formulation also reached the oropharynx, remaining in place for up to 240 min compared with 60 min in the control group. This feature is particularly important, since airborne viruses and allergens come into contact with both the nasal mucosa and oropharyngeal cavity [35]. The use of AM-301 to support mucosal functions, in addition to its long nasal residence time, are supported by data from a clinical study performed under conditions of controlled exposure to pollen. Indeed, patients receiving intranasal AM-301 presented a reduction of TNSS lasting for up to 4 h [16].

Vital signs monitored at screening and at the end of the study included body temperature, pulse rate and blood pressure. Importantly, there was little change in vital signs after administration of AM-301, demonstrating no systemic side effects and indicating that the formulation is safe. Moreover, evaluation of the TNSS did not reveal any symptoms specifically elicited by the formulation, since runny nose and nasal obstruction were described by subjects receiving AM-301 and in subjects receiving saline solution. Very mild nose itching was experienced by only 2 subjects who had had two sprays of AM-301 per nostril. Sneezing was not elicited by the spray in any of the subjects. These observations suggest that AM-301 is safe and well tolerated, both after one and two sprays/nostril.

Conclusions

The data presented in this study indicate that AM-301 has a prolonged nasal residence time of up to 3.5 h compared to a saline control. AM-301 is a safe and well-tolerable medical device with features suitable for intranasal administration. Its nasal residence time and distribution encourage its use to protect nasopharyngeal areas. Two nasal spray applications per nostril provide no substantial benefit over a single spray with regards to coverage of the mucosal surface or nasal retention time. Studies of its efficacy against a broad panel of airborne viruses, bacteria and allergens are not focus of this article. This topic will be investigated in the future and results will be presented in separate publications.

Acknowledgments

For scientific editing and formatting of this manuscript we thank Gillian Pritchard, Veronica Francardo and Nicole Grosse.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the medical faculty of the University Tübingen (AM-301-CL-20-02 on 05 May 2021). Because this study involved a class I medical device, it was granted a waiver for formal approval by the German Federal Institute of Drugs and Medical Devices.

Informed consent

Informed consent was obtained from all subjects involved in the study. The consent included the agreement to publish this work.

Author contributions

Conceptualization, rheological part (M.K., M.E.H. and F.F.) and clinical part (S.B., A.K.K. and I.P.H.); methodology, rheological part (M.K. and M.E.H.) and clinical part (S.B and M.M.S.); formal analysis, rheological part (M.K. and M.E.H.) and clinical part (S.B., M.M.S. and I.P.H.); investigation, S.B. and M.M.S.; writing—original draft preparation, M.K., I.P.H., S.B.; writing—review and editing M.K., M.E.H., S.B., M.M.S., F.F., B.M., I.P.H.; supervision A.K.K. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

Funders of this study were involved in conceptualization and manuscript preparation as per author's contribution.

Funding

This research was funded by Altamira Medica (Zug, Switzerland). Altamira Medica and the PI of this study jointly planned and managed the study, while the study was conducted at the Department of Otorhinolaryngology, Head and Neck Surgery of the University Medical Center of the Eberhard-Karls University Tübingen, Germany.

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Data availability statement

Raw data are available from the corresponding author upon request.

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