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Article

Effect of Nebulised BromAc® on Mucus Plug in a Mechanically Ventilated Ex-Vivo Ovine Lung Model for Obstructive Respiratory Tract

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Abstract: Mucus plugging of the respiratory tract occurs in airway diseases, including asthma, chronic obstructive pulmonary disease and cystic fibrosis. It can cause blockage of airways, leading to breathlessness and lung failure. Here, we demonstrate the effect of BromAc® in dissolving mucus plugs in an novel ex-vivo ovine obstructive lung model using a ventilatory setup. Mucus simulant was filled into the trachea of freshly slaughtered ovine lungs and ventilated via an endotracheal tube (ETT) using Continuous Mandatory Ventilation. Predetermined single or repeated doses of Bromelain, Acetylcysteine (Ac), BromAc® and saline control were administered via an Aerogen® vibrating nebuliser and ventilated for 30 or 60 minutes. Ventilatory recording of resistance, compliance, tidal volume was conducted and rheology pre and post treatment were measured. A significant decline in airway resistance ($p<0.0001$) compared to saline control was observed when treated with Bromelain, Ac and BromAc®, with the latter showing a stronger mucolytic effect than single agents. The decline in resistance was also effective in shorter timepoint ($p<0.05$) at lower doses of the drugs. Changes in compliance, peak pressure and tidal volume was not observed post-administration of the drugs. Rheology measurements revealed that BromAc®™ significantly reduced the viscosity of the mucin at the end of 30-minute and 60-minute time points ($p<0.001$) compared to the saline control. BromAc® showed complete dissolution of the respiratory mucus simulant and improved ventilatory airflow parameters in the ex-vivo ovine model.

Keywords: mucus plugs; bromelain; acetylcysteine; ventilatory resistance; mucus dissolution; muco-obstructive disease; ovine lung model; cystic fibrosis; viscosity

1. Introduction

Respiratory diseases are third among the top ten diseases worldwide (World Health Organization, 2019); approximately 7.7 million people die from respiratory diseases each year [1]. Patients with severe acute and chronic respiratory inflammatory diseases, including acute respiratory distress syndrome (ARDS), asthma and cystic fibrosis, are known to secrete excessive inflammatory cytokines and mucus material. [2,3]. As a result, many hospitalised patients with respiratory diseases suffer from severe dyspnoea and hypoxaemia. In addition, about 80% of the patients with inflamed lungs secrete thick jelly-like mucilaginous substances that are difficult to clear, leading to respiratory obstruction, lung collapse and distress to patients [4,5]. Although these patients are often intubated to manage hypoxia, the blockage of their airways by thick secretions presents a barrier [6,7] to efficient gas exchange, resulting in respiratory failure, barotrauma in many, and potential mortalities [8,9). In pathological airway conditions, an increase in mucus production occurs primarily due to

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goblet cell hyperplasia or through an increase in their inherent secretory capacity, usually as a response to harmful molecules/chemicals such as cigarette smoking, air pollution or through severe infectious conditions such as in bronchiectasis [10–12] and cystic fibrosis. Over time, the increase in mucus hypersecretion severely obstructs the airflow in these patients, speeding up the decline in lung function. In addition, the variable lung inflammatory responses to infections and foreign substances further compromise the cilia's mucociliary clearance efficiency and affect the mucus's biophysical properties [13]. A reduced mucociliary clearance enhances the susceptibility to chronic airway infections, especially opportunistic airway pathogens such as Pseudomonas aeruginosa (PsA), Haemophilus influenzae, and Staphylococcus aureus. Mucus accumulation in the trachea and impaired mucus clearance are also observed in the intubated patient, particularly the neurologically compromised. Chest physiotherapy is variably effective, and suctioning is a common practice to remove the accumulated mucus; however, limited by access to the proximal airway, repeat bronchoscopy can be logically challenging [14]. The composition of sputum in samples from asthmatics, chronic obstructive pulmonary diseases, cystic fibrosis, COVID-19, and other respiratory disease patients share similarities containing double-stranded RNA, mucins (mainly MUC1, MUC5AC and MUC5B) [15], cell debris and lipids. Bronchoscopy in mucus-plugging is frequently required during mechanical ventilation. Recently, Mitja et al. 2022 [16] reported prominent findings of abundant thick secretion in COVID-19 patients, which was challenging to suction in 91% of patients, and muco-hematic plugs required the use of saline and mucolytic agents in 32% of patients. Further, another study described the abundant presence of mucin, especially MUC5B, in the distal airway regions of COVID-19 patients [16], beyond the reach of bronchoscopy, making the intervention challenging. Therefore, targeted administration of a potent mucolytic and anti-inflammatory agent may have comprehensive patient management benefits. To this end, the mucolytic agent BromAc® (a combination of selective stem bromelain components and Acetylcysteine), is an effective mucin digestive enzyme solubiliser of thick mucus. We previously demonstrated the effect of BromAc® in mucinous tumours [17], oncology [18], COVID-19 [19], and cystic fibrosis sputum [20]. Here, we investigated the efficacy of BromAc® using sputum-plugged ovine lungs as a model of mucus-obstructive airways. We simulated an obstructive lung model using artificial mucin and primarily investigated the mucolytic effect of BromAc® in a ventilated ex vivo ovine lung. The study also compares the efficacy of BromAc® versus Bromelain or Acetylcysteine alone treatments at several time points and concentrations. Further, data on resistance, compliance, peak pressure, tidal volume, and viscosity was collected.

2. Materials and Methods

2.1. Drugs

Bromelain (PPP-20-811) was manufactured by Mucpharm Pty Ltd. 200mg/ml Acetylcysteine (AC) Lot#90410 was clinical grade and purchased from Link Pharma (Australia). All other reagents used were purchased from Sigma Aldrich, Sydney, Australia.

2.2. Preparation of Drugs (Bromelain, AC and BromAc®)

Separate doses of BromAc®, containing 250µg/ml of Bromelain with 20mg/ml Acetylcysteine (BromAc® High) or 125 ug/ml Bromelain with 10mg/ml and 20mg/ml Acetylcysteine (BromAc® Low1 and 2) prepared in 0.9% Sodium Chloride (saline) and pH adjusted to 7.0. Bromelain (125 and 250 µg/ml) was also prepared in Sodium Chloride 0.9% and adjusted to pH 7.0, whilst 10 and 20 mg/ml (1.0 & 2.0%) of Acetylcysteine were prepared by 20% dilution of clinical solution with 0.9% Sodium Chloride and pH adjusted to 7.0. The drugs were prepared fresh daily, stored at 4ºC and equilibrated to ambient room temperature (23ºC) just before use.

2.3. Preparation of Mucus Simulant

Soft mucin extracted from Pseudomyxoma peritonei (PMP) during surgical tumour resection was used to prepare the mucus simulant. They primarily contain mucin, including MUC1 and

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MUC5AC, cellular debris, and double-stranded RNA (dsRNA) from degrading cells, lipids, and other cellular materials [19]. The soft mucin was mixed with phosphate buffer saline (PBS) to obtain a consistency of sticky sputum purulent as in cystic fibrosis patients [21]. Briefly, to 250 ml of PMP mucin, approximately 50 ml of PBS was added and sheared rapidly and continuously several times using a 25 ml serological pipette and then vortexed until an even flow rate was obtained. Determination of the viscosity of the fluid was done using a rotational digital viscometer (Drawell NDJ-55) and was observed to be approximately 20,000 mph.s for all experiments [22].

2.4. Ventilator Settings and Measurements

A Drager Evita XL ventilator was used in the circuit with a size 9.0 endotracheal tube, Aerogen ProX mesh nebuliser, and Fisher & Paykel 850 humidifier connected to medical oxygen and air. Sheep lungs via the trachea were connected to the end of the endotracheal tube. The lungs were placed in a water bath at 37°C. The ventilator was set to continuous mechanical ventilation (CMV) with the following parameters Tidal Volume (VT) – 0.400L; Time Inspiratory – 2.0; Frequency (f) – 9.0 bpm, Pmax – 35 cmH2O; Positive End Expiratory Pressure (PEEP) – 0 cmH20. The Humidifier (Fisher & Paykel 950) was set in Invasive mode; Temperature: 37°C.

2.5. Mucus Simulant Administration and Nebulisation of Drugs in Ovine Lung

Western Sydney Meat Works kindly donated freshly slaughtered ovine cardiorespiratory system, including trachea, lungs and heart. They were stored at 4ºC for immediate use, i.e., within 24-48 hours. After a stable ventilatory period, 10 ml of mucus simulant was administered through the endotracheal tube and into the trachea to simulate mucus obstruction. After 1 minute of ventilation, nebulisation of 5 or 10ml of saline (control), Bromelain, Ac, or BromAc® commenced.

Ventilator recordings for resistance, compliance, peak pressure, and tidal volume were recorded, controlling for peak pressure and tidal volume. Two experiments were completed, one for 30 minutes at intervals of 0, 5, 10, 15, and 30 minutes with a single nebulisation and the other for 60 minutes at intervals of 0, 15, 30, 45, and 60 minutes with double nebulisation. The time point intervals of the long-duration experiment were based on the nebulisation time, which took 30 minutes. Three independent studies (new lung) for each experiment were performed (Figure 1).

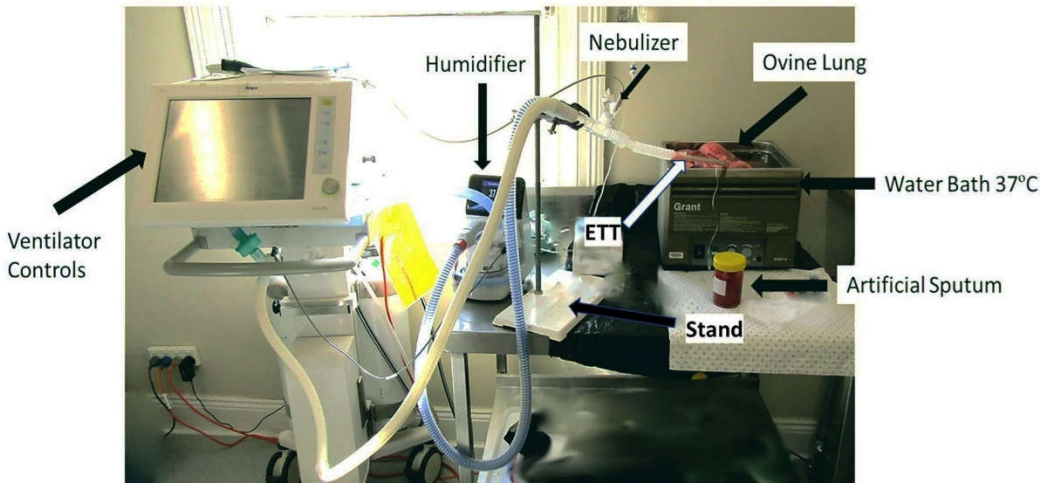

Figure 1. outlines the equipment and the experiment setup used for treating the plugged mucin in the endotracheal tube connected to the ovine lungs. The mucin was inserted into the Endotracheal tube (ETT) and the drug administered through the nebuliser chamber.

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2.6. Rheology Measurements

The viscosity of pre-treated (control) and treated mucus was measured using a digital viscometer (Drawell NDJ-55) at the study start and end points, i.e., 0 and 30 or 60-time points post-treatment with Bromelain, Ac and BromAc®, following the standard measurement protocols described in the instrument manual.

2.7. Statistical Analysis

All analyses are represented as mean and 95% CI. A Two-way ANOVA was performed to analyse the variance across the ventilator parameters, with the comparison between saline control and drug treatment groups carried out using Dunnett's multiple comparison test. The analysis was done using GraphPad Prism V9.4.1. A p-value<0.05 was considered significant.

3. Results

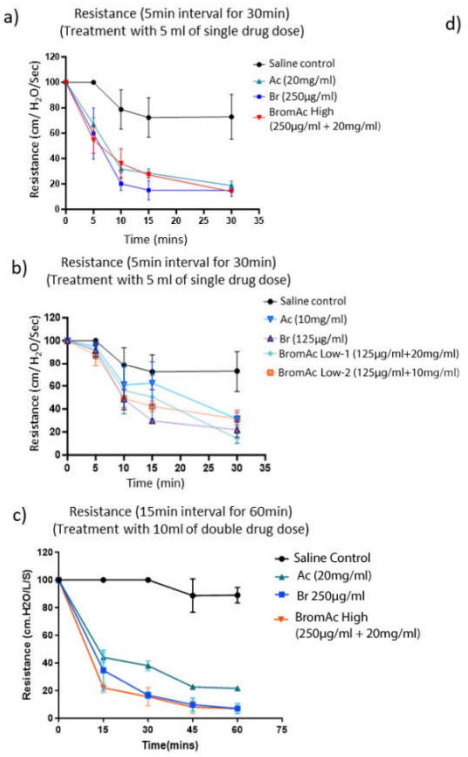
3.1. Effect of Bromelain, Ac and BromAc on Ventilatory Resistance

A significant (p<0.001) decline in resistance was observed with BromAc® High five minutes post-nebulisation compared to saline control. Significance was maintained when compared to single agent Bromelain and Acetylcysteine across the time points (p<0.05).

When the lower concentration of the agents was administered, ventilation for 30 minutes after nebulisation was required before observing a significant decline in resistance (Bromelain alone (p<0.05) and BromAc® Low (p<0.01)).

Over the 60-minute experiment (Figure 2c), airway resistance recovered to 82% with BromAc® High compared to the control, with a similar reading from high-dose Bromelain alone treatment at 60 minutes. More clinically relevant, by the 20-minute mark, BromAc® High had decreased resistance compared to all agents alone, indicating it was the most effective in this experiment.

Figure 2



d) Significance Chart

Time Point (Minutes)	Comparison (with Saline)	Mean Diff (95% CI of Diff)	% Change	P Value Summary
5	BR 250µg/ml	40.3 (5.167 to 75.43)	59.7	*
	AC 20mg/ml	33.17 (-1.966 to 68.30)	66.83	ns
	BromAc(250µg/ml+ 20mg/ml)	45.53 (10.40 to 80.67)	54.47	**
10	BR 250µg/ml	58.57 (23.43 to 93.70)	25.58	***
	AC 20mg/ml	46.87 (11.73 to 82.00)	40.44	**
	BromAc(250µg/ml+ 20mg/ml)	42.57 (7.434 to 77.70)	45.91	*
15	BR 250µg/ml	57.23 (22.10 to 92.37)	20.80	***
	AC 20mg/ml	43.83 (8.701 to 78.97)	39.34	*
	BromAc(250µg/ml+ 20mg/ml)	45.17 (10.03 to 80.30)	37.50	**
30	BR 250µg/ml	57.93(22.80 to 93.07)	20.46	***
	AC 20mg/ml	54.17 (19.03 to 89.30)	25.64	**
	BromAc(250µg/ml+ 20mg/ml)	58.9 (23.77 to 94.03)	19.13	***

Time Point (Minutes)	Comparison (with Saline)	Mean Diff (95% CI of Diff)	% Change	P Value Summary
30	BR125 µg/ml	51 (7.812 to 94.19)	29.97	*
	NAC 10mg/ml	41.43 (-1.754 to 84.62)	43.11	ns
	BromAc (125ug/ml + 20mg/ml)	58.53 (15.35 to 101.7)	19.63	**
	BromAc 125ug/ml +10mg/ml)	41.33 (-1.854 to 84.52)	43.25	ns

Time Point (Minutes)	Comparison (with Saline)	Mean Diff (95% CI of Diff)	% Change	P Value Summary
15	BR 250ug/ml	65.33(14.31 to 116.4)	34.67	*
	AC 20mg/ml	56 (43.37 to 68.63)	44	**
	BromAc(250µg/ml+ 20mg/ml)	78 (65.87 to 90.13)	22	**
30	BR 250ug/ml	83.33 (74.52 to 92.15)	16.67	***
	AC 20mg/ml	62 (49.87 to 74.13)	38	**
	BromAc(250µg/ml+ 20mg/ml)	84.47 (61.49 to 107.4)	15.53	**
45	BR 250ug/ml	78.9 (43.56 to 114.2)	11.01	**
	AC 20mg/ml	66 (24.88 to 107.1)	25.57	*
	BromAc(250µg/ml+ 20mg/ml)	80.67 (45.03 to 116.3)	9.02	**
60	BR 250ug/ml	82 (66.97 to 97.03)	7.87	***
	NAC 20mg/ml	67.33 (49.78 to 84.89)	24.35	**
	BR 250ug/ml & NAC 20mg/ml	82 (66.97 to 97.03)	7.87	***

Figure 2. Representative graphs illustrate the decline in airway resistance over time when treated with BromAc, Bromelain and Ac, at a) higher and b) lower dose for 30 treatments (5minutes intervals), and c) shows higher dose for sixty minutes (18- and 12-minute time intervals). Mean differences per

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cent change and p-values between the treatment groups and saline control are presented in the d) significance chart. No significant change was observed for saline control and the drug treatments at the 0-time point across in the experiments (data not shown). Also, significant changes were only observed at the lower drug concentrations (b) at the 30-minute timepoint. *p<0.05, **p<0.01,***p<0.001 and ****p<0.0001.

3.2. Effect of Bromelain, Ac and BromAc on Ventilatory Compliance, Tidal Volume and Peak Pressure

Limited by the model used in this experiment, we did not observe a significant change in compliance values in lower and higher drug concentrations for the 30- (data not shown) and 60-minute timepoint experiments (Figure 3a).

No tidal volume or peak pressure change was observed between the 60-minute time point and zero across treatment groups compared to the saline control (Figure 3b,c).

Figure 3

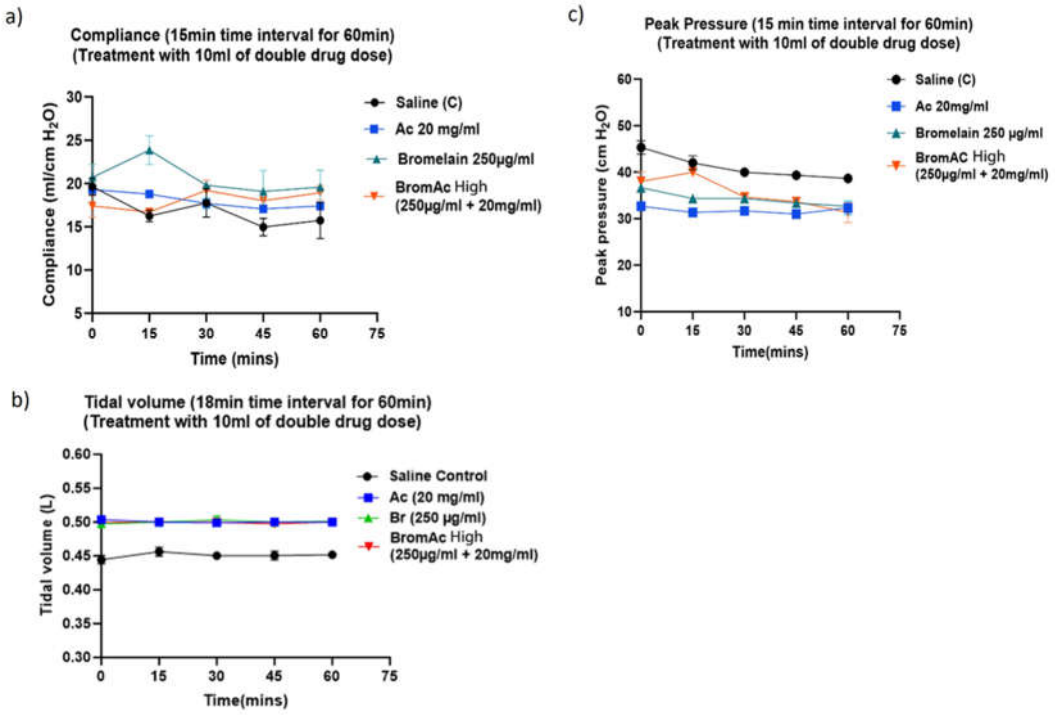

Figure 3. Representative graphs demonstrate the ventilation parameters a) Compliance, b) Tidal volume, and c) Peak pressure over time when treated with higher doses of BromAc, Bromelain and Ac for sixty minutes (15-minute intervals).

3.3. Effect of Bromelain, Acetylcysteine and BromAc®™ on Mucus Viscosity Using Rheology

We observed a significant decline in the viscosity of mucus irrespective of concentration or time point. The greatest potency was observed with BromAc® High, which significantly reduced viscosity measurement (1281.4m.pas) compared to the saline control (12,708.5 mpa.s) ($p<0.0001$) by the end of 30 minutes (Figure 4a).

Similarly, at the 60-minute time point, high dose BromAc® had dissolved the mucin entirely with the viscosity down to 0% ($p<0.0001$) vs saline control (Figure 4c). BromAc®™ Low also decreased viscosity by 30 minutes (Figure 4b).

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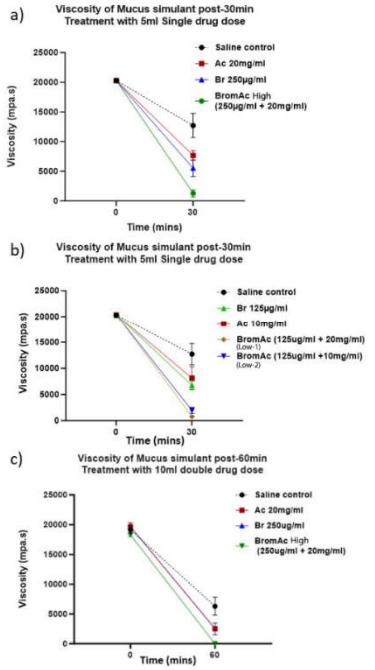
Figure 4



d) Significance Charts

Time Point (Minutes)	Comparison (With Saline)	Mean Diff (95% CI of Diff)	% Change	P Value Summary
0	BR 250ug/ml	0 (-3474 to 3474)	100	ns
	AC 20mg/ml	0 (-3474 to 3474)	100	ns
	BromAc (250µg/ml+20mg/ml)	9 (-3465 to 3483)	99.96	ns
30	BR 250ug/ml	5041 (1567 to 8515)	60.33	**
	AC 20mg/ml	7191 (3716 to 10665)	43.42	***
	BromAc (250µg/ml+20mg/ml)	11427 (7953 to 14901)	10.08	****

Time Point (Minutes)	Comparison (With Saline)	Mean Diff (95% CI of Diff)	% Change	P Value Summary
0	BR 250ug/ml	0 (-3722 to 3722)	100	ns
	AC 20mg/ml	8.333 (-3714 to 3730)	99.96	ns
	BromAc (125µg/ml+ 20mg/ml)	9 (-3713 to 3731)	99.96	ns
	BR+NAC (125ug/ml +10mg/ml)	8.333 (-3714 to 3730)	99.96	ns
30	BR 250ug/ml	5983 (2261 to 9705)	52.92	**
	AC 20mg/ml	4575 (853.3 to 8298)	63.99	*
	BromAc (125µg/ml+ 20mg/ml)	11955 (8232 to 15677)	5.93	****
	BR+NAC (125ug/ml +10mg/ml)	10767 (7045 to 14489)	15.28	****

Time Point (Minutes)	Comparison (With Saline)	Mean Diff (95% CI of Diff)	% Change	P Value Summary
0	BR 250ug/ml	-527.3 (-3388 to 2333)	102.75	ns
	AC 20mg/ml	-528 (-3389 to 2333)	102.76	ns
	BromAc (250µg/ml+ 20mg/ml)	838 (-2023 to 3699)	95.62	ns
60	BR 250ug/ml	3760 (899.3 to 6620)	40.33	**
	AC 20mg/ml	3795 (934.4 to 6655)	39.77	**
	BromAc (250µg/ml+ 20mg/ml)	6301 (3440 to 9161)	0.00	****

Figure 4. Representative graphs show a decline in viscosity when treated with BromAc, Bromelain and Ac, at a) a higher and b) lower dose for 30 minutes and c) higher dose for sixty minutes. Mean differences, per cent change and p-values between the treatment groups and saline control are presented in d) significance chart. *p<0.05, **p<0.01,***p<0.001 and ****p<0.0001.

3.4. Viscosity and Ventilatory Parameters and Relation in the Treatment Group and Control

We observed a direct relationship between the decline in resistance and viscosity, with the best outcomes seen in BromAc® compared to single agents or control (Figure 5a–d). In addition, the reduced viscosity of the mucin improved tidal volume irrespective of the treatment condition (Figure 5d).

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Figure 5

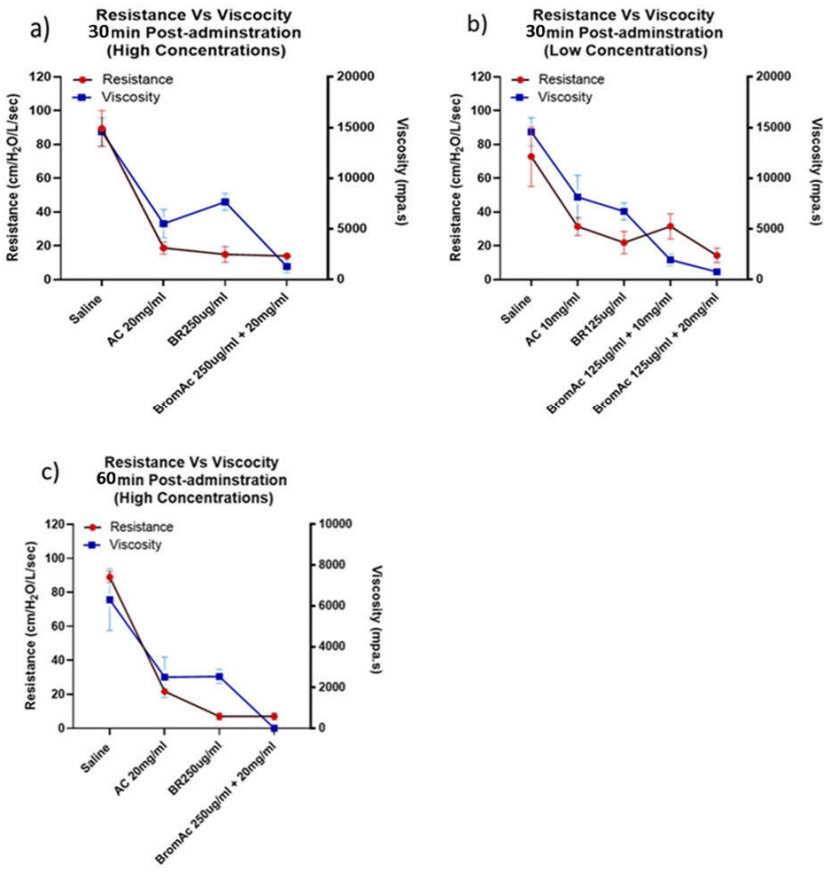

Figure 5. Graphical analysis shows the association between viscosity and resistance post-treatment of Bromelain, Ac, BromAc and saline control at higher doses after a) 30 and c) 60 minutes and b) at a lower dose for 30 minutes**.**

4. Discussion

In the present study, we simulated a respiratory distress model by plugging the ovine lung's airway with artificial mucus material (simulant) via an endotracheal tube connected to sheep lungs. We delivered saline, Bromelain, Acetylcysteine and BromAc® using a standard mechanical ventilatory circuit.

Bromelain, an extract from the stem or fruit of the pineapple plant (ananas comosus), consists of many enzymes such as cysteine proteases, peroxidases, phosphatases, and cellulases [23] and has an array of therapeutic properties, including the ability to solubilise mucinous materials [24,25]. Its mucolytic property has been ascribed to the hydrolysis of peptide and glycosidic linkages in polymeric mucin [26]. Acetylcysteine is a sulfhydryl group donor, potentiating the selected enzymes in bromelain, a reducing agent (antioxidant) and is used as a mucolytic in respiratory diseases since it disintegrates the disulphide bonds interlinking the mucin polymer [27]. BromAc® selectively combines these two agents and has shown great success as a mucolytic for treating the rare tumour of the appendix, pseudomyxoma peritonei (PMP), in phase 2 evaluation [17]. A novel synergistic finding was the combination of cysteine-protease, which breaks peptide bonds, prolines, and disulphide bond breakers.

In our 30- and 60-minute time point studies using BromAc® we identified a significant decline in resistance. Interestingly, the effect was seen within the first five minutes post-nebulisation, with resistance declining by 50%, and by 60 minutes, the resistance was close to a clear airway. Further, the reduction in resistance was directly associated with reduced viscosity of the mucus, which was seen to be the best in BromAc®™ compared to single agents. In muco-obstructive lung diseases such

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as asthma, COPD and cystic fibrosis, or the prolonged ventilated patient, increased accumulation of mucus in the airway is problematic [28]. Mucus in these patients is thicker, tenacious, and not quickly cleared by the normal ciliary and lung. It also harbours opportunistic pathogens, leading to lifetime chronic inflammation, biofilms and frequent exacerbation. Thick mucus causes non-contractile airway narrowing. All these phenomena contribute to reduced lung airflow [29]. Removing these compact mucus plugs is critical in improving the overall condition of these patients. In addition, an intervention that targets biofilms and reduces inflammation is clinically required.

With rheology, there was a significant decline in viscosity of BromAc®mucin, which was indicated by a sharp drop even at the shorter time points; however, BromAc®™, Bromelain and Acetylcysteine all showed almost similar viscometry readings at the 60-minute endpoints. Prolonged exposure to the agents at 60 minutes may have contributed to the similar effect seen between all agents. In the clinical setting, absorption and clearance of the drug are expected to be rapid (ref), making the shorter time points more clinically significant. The drop in viscosity may correlate directly with reduced airway resistance and tidal volume [30]. Saline also showed a drop in viscosity compared to baseline, which could suggest the possible action of monovalent sodium ion [27] or hydration [28] in reducing mucin viscosity.

In the current respiratory model of obstructed or restricted airways by mucinous plugs, the sheep lung model does not fully represent the human lungs because of the absence of the chest wall diaphragm and perfusion. We used post-mortem ovine lungs that could have undergone morphological changes, affecting their elastic property. All ventilator parameters, particularly compliance, are therefore impacted. We attempted to control this by placing the lungs in a water bath. There is a clear difference in the modulation of some parameters, such as airway resistance and viscosity, indicating the clinically feasible solubilisation capability of BromAc®™. Additional research into the effect of therapeutic solutions at shorter intervals post-administration may provide clinically significant outcomes for the obstructed airway.

The passage of air and oxygenation is hampered by thick mucinous secretions in response to airway disease and inflammatory response, providing a highly effective barrier and environment for pathogens and may contribute to alveoli collapse [6,7]. Patients on mechanical ventilation may suffer from lung damage due to increasing pressures required to keep the alveoli open and maintain oxygenation [31,32]. Clearance of a mucinous barrier may provide more efficient gas exchange limit pressure [4] and nosocomial infection. Further, multiorgan failure following respiratory failure is a known complication in ARDS and COVID-19 patients [3,33]. Many patients who succumb to asthma have mucinous casts blocking the airway in addition to construction. Mucus plugs have a long-term effect on airflow in ambulant patients, associated with a decline in lung function.

A clinical phase 1 has been completed with nebulised BromAc® at both concentrations utilised in this ex-vivo study, with results supporting expansion to clinical evaluation in patients with much-obstructive respiratory diseases.

5. Conclusion

We demonstrated ex vivo that BromAc® is a highly effective agent with the potential to dissolve mucus plugging associated with chronic and acute airway diseases. BromAc®™, possibly through its mucolytic properties, also showed improved ventilatory parameters. Clinical evaluation of BromAc® aims to establish the utility in managing muco-obstructive lung diseases in a hospital setting.

Author Contributions: "Conceptualization, DM and SV; methodology, NV, MSE and KP; formal analysis, MSE, NV and KP.; investigation, NV and MSE; resources, DM and SV.; data curation, JA and AM; writing—original draft preparation, NV, MSE and KP; writing—review and editing DM, RM, SV and JA; supervision, DM and SV; funding acquisition, DM. All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest: David Morris and Sarah Valle are shareholders of Mucpharm Pty Ltd. NV, MSE, KP, JA, and AM are employed by Mucpharm Pty Ltd.".

References

1. Schluger NW, Koppaka R. Lung Disease in a Global Context. A Call for Public Health Action. Annals of the American Thoracic Society. 2014;11(3):407-16.
2. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodriguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev. 2020;54:62-75.
3. Mokhtari T, Hassani F, Ghaffari N, Ebrahimi B, Yarahmadi A, Hassanzadeh G. COVID-19 and multiorgan failure: A narrative review on potential mechanisms. J Mol Histol. 2020;51(6):613-28.
4. Wang Y, Zhang M, Yu Y, Han T, Zhou J, Bi L. Sputum characteristics and airway clearance methods in patients with severe COVID-19. Medicine. 2020;99(46):e23257.
5. Kumar SS, Binu A, Devan AR, Nath LR. Mucus targeting as a plausible approach to improve lung function in COVID-19 patients. Med Hypotheses. 2021;156.
6. Hansen CB, Sandholdt H, Moller MEE, Perez-Alos L, Pedersen L, Israelsen SB, et al. Prediction of Respiratory Failure and Mortality in COVID-19 Patients Using Long Pentraxin PTX3. J Innate Immun. 2022.
7. Khan MA, Khan ZA, Charles M, Pratap P, Naeem A, Siddiqui Z, et al. Cytokine Storm and Mucus Hypersecretion in COVID-19: Review of Mechanisms. J Inflamm Res. 2021;14:175-89.
8. Ottestad W, Sovik S. COVID-19 patients with respiratory failure: what can we learn from aviation medicine? Brit J Anaesth. 2020;125(3):E280-E1.
9. Manckoundia P, Franon E. Is Persistent Thick Copious Mucus a Long-Term Symptom of COVID-19? Eur J Case Rep Intern Med. 2020;7(12):002145.
10. Fahy JV, Dickey BF. Airway mucus function and dysfunction. N Engl J Med. 2010;363(23):2233-47.
11. Keir HR, Chalmers JD. Pathophysiology of Bronchiectasis. Semin Respir Crit Care Med. 2021;42(4):499-512.
12. Muñoz G, de Gracia J, Buxó M, Alvarez A, Vendrell M. Long-term benefits of airway clearance in bronchiectasis: a randomised placebo-controlled trial. European Respiratory Journal. 2018;51(1):1701926.
13. Aegerter H, Lambrecht BN. The Pathology of Asthma: What Is Obstructing Our View? Annual Review of Pathology: Mechanisms of Disease. 2023;18(1):null.
14. Trawoger R, Kolobow T, Cereda M, Giacomini M, Usuki J, Horiba K, et al. Clearance of Mucus from Endotracheal Tubes during Intratracheal Pulmonary Ventilation Anesthesiology. 1997;86(6):1367-74.
15. Lu W, Liu X, Wang T, Liu F, Zhu A, Lin Y, et al. Elevated MUC1 and MUC5AC mucin protein levels in airway mucus of critical ill COVID-19 patients. J Med Virol. 2021;93(2):582-4.
16. Serra Mitjà P, Centeno C, Garcia-Olivé I, Antuori A, Casadellà M, Tazi R, et al. Bronchoscopy in Critically Ill COVID-19 Patients: Findings, Microbiological Profile, and Coinfection. Journal of Bronchology & Interventional Pulmonology. 2022;29(3).
17. Valle SJ, Akhter J, Mekkawy AH, Lodh S, Pillai K, Badar S, et al. A novel treatment of bromelain and Acetylcysteine (BromAc®) in patients with peritoneal mucinous tumours: A phase I first in man study. Eur J Surg Oncol. 2021;47(1):115-22.
18. Pillai K, Akhter J, Chua TC, Morris DL. A formulation for in situ lysis of mucin secreted in pseudomyxoma peritonei. Int J Cancer. 2014;134(2):478-86.
19. Coelho dos Reis JGA, Ferreira GM, Lourenço AA, Ribeiro ÁL, da Mata CPdSM, de Melo Oliveira P, et al. Ex-vivo mucolytic and anti-inflammatory activity of BromAc® in tracheal aspirates from COVID-19. Biomedicine & Pharmacotherapy. 2022;148:112753.
20. Pillai K, Mekkawy AH, Akhter J, Valle SJ, Morris DL. Effect of nebulised BromAc®®on rheology of artificial sputum: relevance to muco-obstructive respiratory diseases including COVID-19. bioRxiv. 2021:2021.12.28.474344.
21. Serisier DJ, Carroll MP, Shute JK, Young SA. Macrorheology of cystic fibrosis, chronic obstructive pulmonary disease & normal sputum. Respiratory Research. 2009;10(1):63.
22. Vasconcellos CA, Allen PG, Wohl ME, Drazen JM, Janmey PA, Stossel TP. Reduction in Viscosity of Cystic Fibrosis Sputum in Vitro by Gelsolin. Science. 1994;263(5149):969-71.
23. Taussig SJ, Batkin S. Bromelain, the enzyme complex of pineapple (Ananas comosus) and its clinical application. An update. J Ethnopharmacol. 1988;22(2):191-203.
24. Maurer HR. Bromelain: biochemistry, pharmacology and medical use. Cell Mol Life Sci. 2001;58(9):1234-45.
25. Pillai K, Akhter J, Morris DL. Assessment of a novel mucolytic solution for dissolving mucus in pseudomyxoma peritonei: an ex vivo and in vitro study. Pleura Peritoneum. 2017;2(2):111-7.

 doi:10.20944/preprints202406.1231.v1

26. Akhter J, Pillai K, Chua TC, Alzarin N, Morris DL. Efficacy of a novel mucolytic agent on pseudomyxoma peritonei mucin, with potential for treatment through peritoneal catheters. Am J Cancer Res. 2014;4(5):495-507.
27. Guerini M, Condrò G, Friuli V, Maggi L, Perugini P. N-acetylcysteine (NAC) and Its Role in Clinical Practice Management of Cystic Fibrosis (CF): A Review. Pharmaceuticals (Basel). 2022;15(2).
28. Sheehan JK, Richardson PS, Fung DC, Howard M, Thornton DJ. Analysis of respiratory mucus glycoproteins in asthma: a detailed study from a patient who died in status asthmaticus. American Journal of Respiratory Cell and Molecular Biology. 1995;13(6):748-56.
29. Georas SN. All plugged up — noninvasive mucus score to assess airway dysfunction in asthma. The Journal of Clinical Investigation. 2018;128(3):906-9.
30. Sinha P, Flower O, Soni N. Deadspace ventilation: a waste of breath! Intensive Care Med. 2011;37(5):735-46.
31. Chiumello D, Carlesso E, Brioni M, Cressoni M. Airway driving pressure and lung stress in ARDS patients. Critical Care. 2016;20.
32. Beitler JR, Malhotra A, Thompson BT. Ventilator-induced Lung Injury. Clin Chest Med. 2016;37(4):633-+.
33. Østergaard L. SARS CoV-2 related microvascular damage and symptoms during and after COVID-19: Consequences of capillary transit-time changes, tissue hypoxia and inflammation. Physiological Reports. 2021;9(3):e14726.