

Local dornase alfa treatment reduces NETs-induced airway obstruction during severe RSV infection

ABSTRACT

Respiratory syncytial virus (RSV) infection is characterised by airway obstruction with mucus plugs, containing DNA networks in the form of neutrophil extracellular traps (NETs). We investigated the effect of dornase alfa on histopathological NETs-induced airway obstruction and viral load in an age-relevant calf model of severe bovine RSV disease. As compared with the control animals, dornase alfa treatment resulted in a strong reduction of NETs-induced airway obstruction. Viral load in the lower respiratory tract was not different between the two groups. We conclude that NETs form a relevant target for treatment of airway obstruction in severe RSV disease.

INTRODUCTION

Respiratory syncytial virus (RSV) is among the most important respiratory pathogens in children worldwide.¹ Small airway obstruction by dense plugs, composed of mucus and cellular debris from neutrophils and sloughed bronchial epithelial cells, is a key histopathological feature in children with severe RSV-lower respiratory tract disease (LRTD).² Recently, we have implicated neutrophil extracellular traps (NETs) in the development of airway obstruction in severe RSV-LRTD.³

NETs are networks of extracellular DNA covered with antimicrobial peptides and histones, which contribute to the capture and inactivation of bacteria, fungi and viruses, including RSV.⁴ However, there appears a delicate balance between aid and damage to the host as accumulating evidence now suggests that NETs can cause lung injury and may contribute to airway obstruction by trapping mucus into large, viscous plugs.⁴ As such, pharmacological

targeting of NETs, for example, by degradation of the DNA backbone by dornase alfa, may be of therapeutic benefit.

In this study, we hypothesised that local dornase alfa treatment reduces NETs-induced airway obstruction in the airways during experimental severe RSV-LRTD. To test this hypothesis, we used the well-established cognate host-virus model of bovine RSV (bRSV) infection in calves, which is characterised by neutrophilic inflammation with NETs formation and histopathological evidence of (small) airway obstruction, similar to severe RSV-LRTD in children.^{5–7}

METHODS

Twelve 4-week-old, colostrum-deprived dairy calves were infected on day 0 with 3.6 log₁₀ TCID₅₀ of bRSV (Odijk strain, seventh in vivo passage) in a 2 mL volume. Next, the calves (six per group) received either twice daily 5 mL dornase alfa (treatment group, Pulmozyme 1 mg/mL,

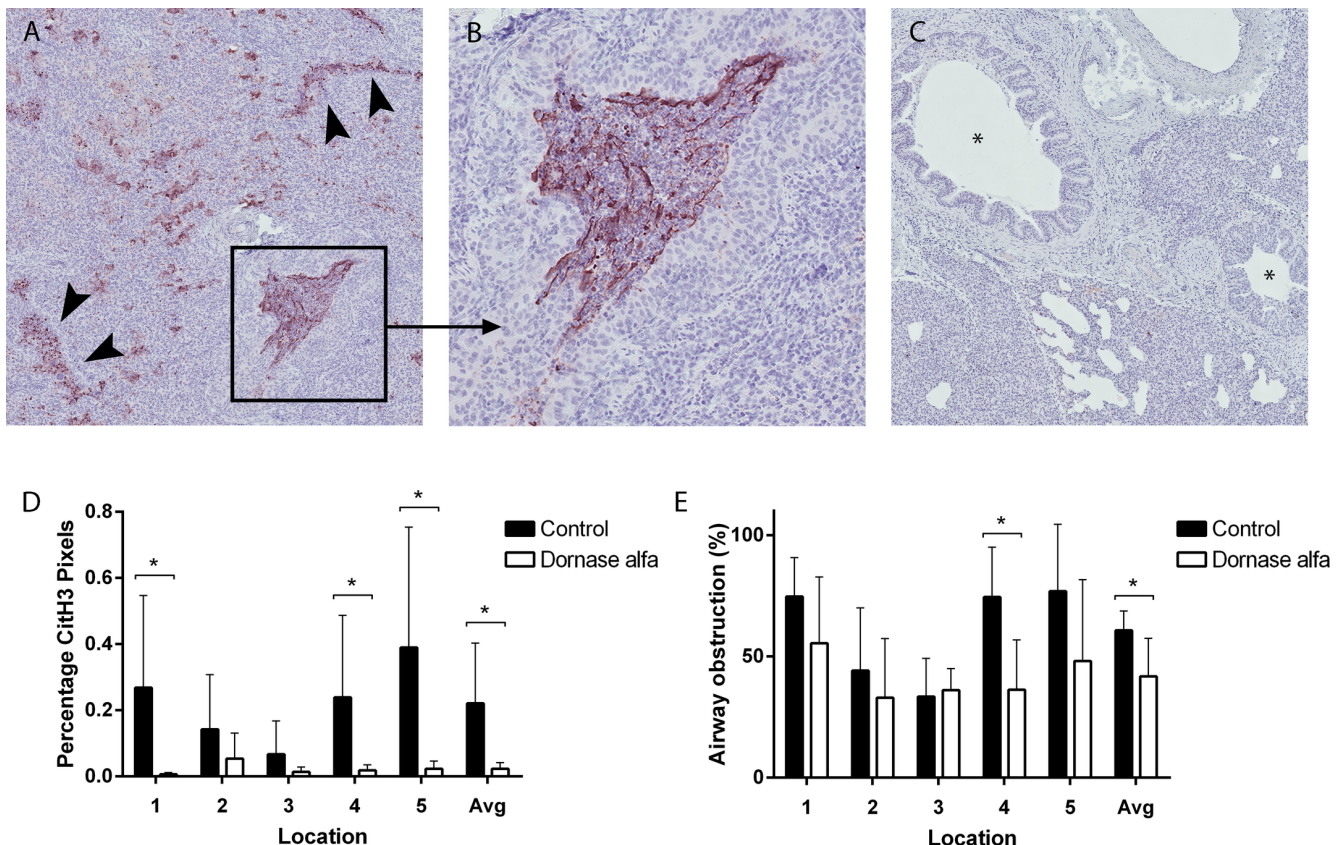


Figure 1 Neutrophil extracellular traps (NETs) degradation by dornase alfa treatment. Immunohistochemistry of lung tissue sections, stained for citrullinated histone H3 (CitrH3) to detect NETs formation. (A) Severe airway obstruction by NETs-rich plugs (black arrowheads and black square) in a saline (control)-treated calf (representative image, magnification 100×), with (B) magnification (450×) of the square. (C) Open airways (asterisks) with absence of NETs-rich obstructing plugs in a dornase alfa-treated calf (representative image, magnification 100×). (D) Percentages of intraluminal airway CitrH3-positive pixels for the five different lung locations (see online supplementary figure 1) in control calves (n=6, black bars) and dornase alfa-treated calves (n=6, white bars), average (Avg) of the five locations: p=0.04. (E) Percentages of partially and completely obstructed airways counted in whole lung tissue sections for the five different locations (see online supplementary figure 2) in control calves (n=6, black bars) compared with dornase alfa-treated calves (n=6, white bars), average (Avg) of the five locations: p=0.03, location 4: p=0.03.

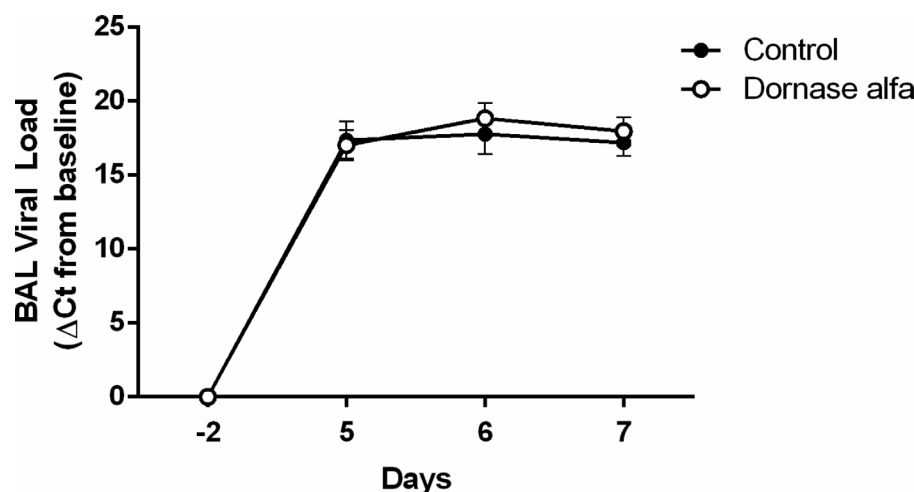


Figure 2 Lung viral load in bronchoalveolar lavage (BAL) from control (black circles) and dornase alfa-treated (white circles) calves during bovine respiratory syncytial virus (bRSV) lower respiratory tract disease, as detected by reverse transcription-PCR. Data are expressed as mean \pm SD of the Δ Ct (total number PCR cycles minus threshold cycle). $n=6$ calves per group, $p=0.1$ between groups.

Hoffmann-La Roche, Basel, Switzerland) or 0.9% NaCl (control group), starting on day 5 after viral inoculation. Further details can be found in the online supplementary methods.

RESULTS

Overall, there was a significant reduction of NETs in the dornase alfa-treated animals compared with the control group (figure 1A–D, $p=0.02$). On average, the percentage of obstructed airways in the dornase alfa group was 31% lower compared with normal saline group ($41.8\% \pm 6.4\%$ vs $60.7 \pm 3.3\%$, respectively, figure 1E, $p=0.03$), with a highest improvement of 51% in the left cranial lung area ($36.3\% \pm 8.4\%$ vs $74.5 \pm 8.4\%$, figure 1E, $p=0.03$). There was no difference in the viral loads between the calves treated with dornase alfa versus normal saline during any of the treatment days (figure 2). There were no differences in the lung inflammatory response as determined by total and differential bronchoalveolar lavage (BAL) cells between the animals treated with dornase alfa or normal saline (see online supplementary results). The calves treated with dornase alfa had a trend towards lower LRTD scores ($p=0.07$) and had reduced extent of hypercapnia on day 7 (51.5 ± 2.8 mm Hg vs 61.7 ± 4.7 mm Hg, $p=0.04$) compared with calves treated with normal saline (see online supplementary results). Interestingly, four out of the six animals in the control group had to be sacrificed prematurely due to reaching their humane end point based on acute deterioration, as opposed to two out of six calves in the dornase alfa group based on

persistent severe disease (see online supplementary results). Further results on lung inflammation and clinical responses can be found in the online supplementary results.

DISCUSSION

Over the last years, NETs are increasingly being implicated in the pathophysiology of multiple respiratory diseases, including cystic fibrosis, COPD, acute lung injury and pulmonary infections.⁴ Specifically, the formation of NETs in the respiratory tract may contribute to airway obstruction, by deposition of large web-like networks with high DNA content, thereby increasing mucus viscosity.^{3,4,8} Previously, we have shown the formation of NETs in bronchoalveolar lavage fluid (BALF) from infants with severe RSV-LRTD, as well as in mucus plugs obstructing the airways of bRSV-infected calves.³ In the present study, we confirmed the hypothesis that NETs are actively involved in airway obstruction during severe bRSV-LRTD. Local dornase alfa treatment strongly reduced the amount of NETs in the airways (figure 1A–D), which was associated with a reduction in airway occlusion on histopathological evaluation (figure 1E). Interestingly, the lysis of NETs led to increased DNA content in BALF, suggesting fragmentation and ‘freeing’ the DNA from disintegrated mucus plugs (see online supplementary results), similar to reports in patients with cystic fibrosis.⁹

On the other hand, disruption of the protective functions of NETs might also be disadvantageous. NETs are able to

capture RSV particles in vitro,^{3,4} and as such, a theoretical concern may be renewed release and enhanced dissemination of immobilised virions on lysis of NETs. However, there was no increased viral dissemination (figure 2), nor evidence of enhanced direct viral-induced lung pathology or inflammation (see online supplementary results) in our in vivo bRSV calf model. These data suggest a relatively limited role of NETs in antiviral defence in the respiratory tract, but future studies must continue to evaluate these potential risks.

In our ‘proof of principle’ study focused on targeting NETs (see online supplementary limitations), we observed improvement of clinical indices, most prominently a relevant reduction in the extent of hypercapnia, on dornase alfa treatment (see online supplementary results). Although these findings need to be interpreted with care because our study was not specifically powered to detect robust clinical effects, this may form the basis of further testing of a potential clinical benefit of dornase alfa and other future interventions in NETs biology (eg, PAD4 inhibitors). The clinical use of nebulised dornase alfa treatment during *mild to moderate* RSV disease in infants has previously been investigated.^{10–12}

While one prospective randomised trial showed a strong reduction in chest X-ray abnormalities, including atelectasis,¹¹ no clinical benefit, and even a statistically non-significant prolonged hospitalisation, of the routine use of nebulised dornase alfa was demonstrated in two subsequent randomised placebo-controlled trials and meta-analysis.^{10,12,13} However, in a case series in mechanically ventilated children with *severe* RSV-LRTD, a population that suffers much more from the typical airway mucus obstruction with atelectasis, dornase alfa nebulisation was of benefit with both clinical and radiological improvement,¹⁴ which is a finding in line with observations in daily clinical practice. Future studies powered for clinical effect must determine if targeting of NETs is of actual clinical benefit during severe RSV-LRTD.

In conclusion, local dornase alfa treatment strongly reduces the amount of NETs in the respiratory tract, leading to less airway obstruction in calves with bRSV-LRTD. These results suggest that NETs contribute to the pathogenesis of airway obstruction during *severe* RSV-LRTD. Targeting NETs formation could prove a promising new treatment to alleviate airway obstruction in severe RSV-LRTD.

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Competing interests None declared.

Ethics approval This study was conducted under legislation of the Dutch Central Authority for Scientific procedures on Animals and after approval of the Body of Animal Welfare of Wageningen University and Research.

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