Clinical and *in vitro* Effect of Dornase Alfa in Mechanically Ventilated Pediatric Non-Cystic Fibrosis Patients with Atelectases

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Key Words

Atelectasis • Mechanical ventilation • Dornase alfa • Children

Abstract

Introduction: At present no evidence-based medical treatment for persistent atelectasis in pediatric non-cystic fibrosis (CF) patients is available. **Method:** To evaluate the use of intratracheally instilled recombinant human deoxyribonuclease (rhDNase) in intubated and ventilated pediatric patients, we performed a single-center observational study on 46 pediatric intensive care patients who had received intratracheal DNase. Patients were classified, according to radiologic findings of atelectasis (group 1) or infiltrates. As controls we examined a historical control group of 17 patients with atelectasis after cardiac surgery, who had been treated with NaCl 0.9% and matched for age and diagnosis with 21 patients from group 1 (subgroup 1a). Radiologic improvement and inflammatory markers in both serum and tracheal aspirates were measured. **Results:** In group 1, 35 patients had 51 atelectases/dystelectases episodes at baseline. 67 % of patients showed radiologic signs of improvement after 24h treatment

with rhDNase. In subgroup 1a, 16 patients had complete resolution of atelectases and minimal change in dystelectases after a treatment of 24 hours rhDNase, compared with the control group of 17 patients, who had 7 atelectases and 10 dystelectases at baseline and an improvement in only 1 out of 17 (6 %) patients after 24h. **Conclusion:** Intratracheal instillation of rhDNase is an effective adjunct to conservative therapy of atelectases in children. Further randomized controlled prospective studies are necessary.

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Introduction

Atelectasis is frequent in children with severe respiratory infections and often requires invasive ventilation. It may even occur during mechanical ventilation [1, 2]. So far no evidence-based treatment for persistent atelectasis in pediatric non-CF patients is available. Common pulmonary complications after surgery are pneumonias and atelectases [3-6]. The incidence of

atelectasis during mechanical ventilation in children was reported as 8 % (Rivera et al.) to 15 % (Thomas et al.) [7, 8]. Several factors contribute to the development of atelectasis: mechanical ventilation changes pro-inflammatory markers by altering respiratory physiology and mucociliary clearance; postoperative restrictive fluid management and diuretic therapy result in thickening and retention of tracheal bronchial secretions. This further contributes to recurrent respiratory infection and decreased lung function [9]. Since there is no evidence-based treatment for persistent atelectasis in ventilated pediatric non-CF patients, there is no “gold standard” for treatment. Current treatment of atelectasis in pediatric pulmonology consists of increasing ventilation parameters (PEEP, PIP and FiO_2), and/or regular physiotherapy, and/or positioning/proning, and/or secretolysis (acetylcysteine, ambroxol), and/or inhaled bronchodilators, and/or antibiotic treatment if infiltrates are suspected or evident and/or intermittent tracheal lavage with saline or secretolytics via endotracheal tube.

The aim of atelectasis treatment is primarily the reduction or complete recruitment of non-ventilated pulmonary segments, secondary as prophylaxis of pulmonary infection (infiltrates, pneumonia). At present, only limited data are available on the elective therapy of atelectasis by intratracheal administration of rhDNase: a literature search on intubated pediatric non cystic fibrosis (CF) patients revealed only case reports of rhDNase therapy and our own recently reported randomized controlled trial of prophylactic use in pediatric cardiac surgery patients [10]. At present, it is not clear whether the reported advantages of therapy with rhDNase are only single case observations or if there is a reproducible clinical benefit.

RhDNase has been used with great success as mucolytic therapy in patients with cystic fibrosis for more than a decade. Several clinical trials [11–13] have demonstrated that a positive effect of rhDNase on rheologic properties of CF patients' sputum results in an improvement of parameters of lung function (FVC, FEV_1) [14]. Furthermore the PEIT-study demonstrated, that rhDNase reduces the rate of pulmonary infections [15, 13]. RhDNase fragments extra-cellular DNA molecules [16–22] in tracheal aspirates and improves flow properties of the mucus in CF-children. In vivo, cooperative patients may expectorate secretion more easily [21, 14]. In sedated and ventilated patients mucus clearance is improved.

A positive effect of rhDNase has also been reported in other diseases with retention of bronchial secretions

[23] like status asthmaticus [24], bronchiectasis [25, 26], atelectasis [8, 24, 27–30], Karthagener-Syndrome [31, 32], chronic bronchitis [33] and bronchopulmonary dysplasia in premature babies [34, 35]. However, there are only limited data on the adjunctive therapy of atelectasis with rhDNase in ventilated children [10, 27, 35–38].

In a previous placebo-controlled study (rhDNase vs. NaCl 0.9 %) on prophylaxis of ventilation-associated complications in intubated children after cardiac surgery, we have demonstrated a significant reduction of the incidence of atelectasis, as well as shortening of ventilation time and length of hospital stay with rhDNase [10]. The results of this controlled trial suggest that rhDNase may also be helpful as interventional therapy of atelectasis during mechanical ventilation.

The present clinical study deals with an off-label use of recombinant human deoxyribonuclease (rhDNase=dornase alfa=Pulmozyme®, Roche AG Grenzach, Germany) for treatment of atelectasis in intubated pediatric non-CF patients. The aim is to provide evidence of a beneficial effect of intratracheally instilled rhDNase in these patients.

Materials and Methods

Patients

This retrospective observational study on mechanically ventilated patients investigates the off-label use of rhDNase as treatment for atelectasis or proven lower airway infection in the pediatric intensive care unit of the Children's University Hospital Tuebingen between 2001 and 2004. All patients with X-ray evidence of atelectasis or infiltrates and who received rhDNase for that reason were included in the study.

Group 1 was defined as patients with atelectasis, a subgroup of which (group 1a) was chosen to match for age and diagnosis. As a control group 17 cardiac surgery patients were matched, who had been treated with NaCl 0.9 % for atelectasis in a RCT [10]. This subgroup 1a did not differ in biometrical parameters from the total group 1.

Study design

Intervention: since rhDNase was administered as a part of patient care and not as a clinical trial, formal approval from an institutional review board or medical ethics committee was not required. RhDNase was instilled into the trachea via the existing endotracheal tube at a dosage of 0.1 mg/kg weight twice daily (max 2×1.25 mg/d) until patients were extubated. The postoperative therapy included mechanical ventilatory support via endotracheal tube, using pressure-controlled ventilation. Blood-gas analysis was done routinely according to the mechanical ventilation within 3 hours of instillation.

The primary endpoint was defined as radiologic improvement in atelectasis or infiltrates. X-rays were interpreted

Group	Patients	Age median (min-max)	All atelectases		Atelectasis before		Atelectasis after 24h		Dystelectasis before		Dystelectasis after 24h	
			before	after 24h	rhDNase	NaCl	rhDNase	NaCl	rhDNase	NaCl	rhDNase	NaCl
1	35	0.48 y (22d -16y)	51	17	35	-	9	-	16	-	22	-
1a	21	0.32 y (15d -1.7y)	25	8	16	-	0	-	9	-	8	-
Controls	17	0.27 y (6d -2.3y)	17	16	-	7	-	6	-	10	-	10

Table 1. Distribution of atelectases events in patient groups.

by two independent radiologists. We have developed the following scoring, since a helpful scoring system for atelectasis is still lacking. Definitions were as follows: a. Atelectasis was defined as subtotal or total density of one or more lobes of the lung on chest X-ray, b. Dystelectasis was defined as subtotal or total reduced ventilation of one or more lobes of the lungs, c. Infiltrate was defined as subtotal or total shadowing on one or more lobes of the lungs.

Secondary endpoints were ventilator parameters (FiO₂, PIP, PEEP, MV/kg), blood gas analysis and C-reactive protein (CrP) in serum, and DNA quantification, white blood cell counts and cytokine expression in tracheal secretions. In this report we will present only results from the first 24 hours of therapeutic intervention.

The primary and the secondary parameters were analysed by student t-test.

Results

Patients

63 patients with episodes of atelectasis or infiltrates were identified, including 56 patients after cardiac surgery, 5 patients with pneumonia, one patient after oesophageal surgery and one patient with meningitis (Table 1). 46 patients were treated with rhDNase and 17 patients, all of whom had undergone cardiac surgery, constituted the control group (median age 0.27 years, min 6 days, max 2.3 years, SD 0.60). These 17 patients were treated with NaCl 0.9 % for atelectasis in a RCT [10].

Of the 46 patients treated with rhDNase, 35 patients (median age 0.48 years, min 22 days, max 16.4 years, SD 4.61) were classified into Group 1 (atelectases), whereas 11 patients (median age 1.74 years, min 27 days, max 14.6 years, SD 4.76) were classified as infiltrates and deleted out of this report. Of the 35 patients in group 1, 21 patients (Group 1a, median age 0.32 years, min 15 days, max 1.65 years, SD 0.36) matched the control group with respect to age and diagnosis. The different groups are described in Table 1.

Fig 1. Radiologic findings of atelectasis and dystelectasis before and 24 hours after rhDNase therapy.

Group	Patients	FiO ₂ (%)		PIP (cmH ₂ O)		PEEP (cmH ₂ O)		MV/kg weight (L)	
		before	after 24h	before	after 24h	before	after 24h	before	after 24h
1a	21	0.53 ± 0.14	0.38 ± 0.1	22 ± 4	20 ± 3	6.3 ± 2.5	5.5 ± 1	0.28 ± 0.2	0.34 ± 0.2
	p-values		0.0001		0.017		0.075		0.13
Controls	17	0.55 ± 0.22	0.47 ± 0.2	21.5 ± 5	22.4 ± 3	4.1 ± 1	4.0 ± 1	0.31 ± 0.2	0.26 ± 0.1
	p-values		0.08		0.28		0.33		0.016

Table 2. Parameters of mechanical ventilation before and after 24 hours. Means ± SD are given.

Radiologic improvement

Atelectases resolved within 24 hours of initiating treatment with rhDNase. In group 1 we found 35 atelectases and 16 dystelectases in 35 patients prior to treatment with rhDNase. 24 hours after treatment a significant improvement in atelectasis (74 %, $p = 0.003$) was found (Figure 1A). In subgroup 1a (21 patients) a significant resolution of all atelectases (100 %) and a small improvement of 11 % in dystelectasis was observed after 24 hours of treatment, $p = 0.0007$ (Figure 1B). In the historical control group, which was treated twice daily with NaCl 0.9 %, we found 7 atelectasis and 10 dystelectasis prior to treatment and resolution of only one atelectasis (6 %, $p = 0.41$) after 24 hours after treatment with rhDNase (Figure 1C).

Mechanical ventilation

All ventilation parameters (positive inspiratory pressure (PIP), positive endexpiratory pressure (PEEP)) improved significantly in the matched group 1a treated with rhDNase, whereas in the control group only a small decrease in FiO₂ was observed (Table 2). Of note, minute volumes (MV) significantly decreased in the control group, whereas an increase was found in the rhDNase group.

Inflammation

Inflammatory markers were assessed in 16 of 21 patients in group 1a before and after 24 hours of rhDNase-therapy and in control patients. There was no significant difference in leukocyte counts in the serum of group 1a compared to the historical control group. On the first and second day of therapy CrP was higher in group 1a (6.4±9 mg/dl and 6.1±8, respectively) compared to the control group (2.3±4 and 5.9±6, respectively), but decreased in both groups equally during the following days. Inflammatory markers in tracheal secretions (Table 3) showed a significant decrease in leukocytes, DNA and IL-8 in patients treated with rhDNase, whereas IL-10 decreased in both groups.

Discussion

Atelectasis treatment should recruit non ventilated pulmonary segments completely or at least partially. A further beneficial side effect may the prophylaxis of pulmonary infection (infiltrates, pneumonia), regardless whether the underlying cause is compression atelectasis, mucus plugs or resorption atelectasis. We were able to recruit almost all atelectases. This suggests mucus plugs to be the most probable cause of atelectasis in our patients.

So far there are only limited data available on elective therapy of atelectasis by intratracheal administration of rhDNase. This retrospective study of off-label use of rhDNase included only mechanically ventilated patients. It was necessary to form a subgroup (1a) of our patients in order to compare them with the available historical control group, taking into account that a comparison between this two groups must be biased per se. The key finding of this retrospective analysis was the difference in atelectases improvement between the two matched groups. We found a resolution of all atelectases (100 %) and a small improvement of 11 % in dystelectasis in the 21 patients in group 1a after 24 hours of treatment with rhDNase, whereas in the historical control group of 17 patients treated with NaCl 0.9 % when suctioning tracheal mucus, we found an improvement in only one atelectasis after 24 hours. As atelectases resolved partially to dystelectasis or totally to normally ventilated areas we distinguished in table 1 the results of rhDNase therapy. As the number of atelectasis decreased after 24 hours of therapy, part of these patients revealed dystelectasis (increased number after 24 hours of therapy).

Under rhDNase therapy respiratory settings improved significantly for FiO₂ ($p = 0.0001$) and PiP ($p = 0.017$) (Table 2). Tracheal inflammation parameters revealed significant decrease in leukocyte counts, DNA content and IL-8 concentration after 24 hours of rhDNase

Group	Patients	Leukocytes (µl)		DNA (mg/ml)		IL-8 (pg/ml)		IL-10 (pg/ml)	
		before	after 24h	before	after 24h	before	after 24h	before	after 24h
1a	16	24964±24570	6561±8887	0.06±0.05	0.03±0.03	47561±46756	28641±47239	64±167	13±15
p-values		0.009		0.042		0.019		0.11	
Controls	10	n.g.	n.g.	0.02±0.03	0.03±0.02	29248±64332	10782±7107	9±6	7±3
p-values				0.26		0.19		0.27	

Table 3. Tracheal inflammatory markers before and after 24 hours. Means ± SD are given.

therapy, whereas in the control group no significant difference could be found. These reduced inflammation parameters suggest that inflammation and consecutive mucus production are the main issues for atelectases and deteriorated respiratory settings. Vice versa rhDNase therapy leads to decreasing inflammation, improving alveolar ventilation by reduction of atelectases and minimizes respirator therapy. Our findings suggest the initiation of a randomized placebo-controlled trial for effective reducing of atelectases in ventilated patients with multiple arms for dosage finding.

Our results support the findings of Hendriks and Erdevé et al. that rhDNase can resolve atelectasis effectively: they found improvement in 17 of 25 patients [9] and in 10 of 12 [37] patients. Our data may differentiate between atelectasis and dystelectasis, because complete resolution of atelectasis was not found after 24 hours in all cases. Therefore this point needs further attention in a subsequent study.

In our study we used a dose of 0.1 mg/kg. However, dosing of rhDNase is still under discussion: Boeuf et al. [27] instilled 0.1 mg rhDNase/kg weight

intratracheally twice daily, while Merkus et al. [38] administered 2.5 mg of rhDNase by nebulization. Because of these different dosing regimens, a dose finding study should be performed.

Conclusion

While there is no evidence-based treatment (for acetylcysteine, physiotherapy and bronchodilators is also still lacking) for persistent atelectasis in pediatric patients, rhDNase seems to be an alternative conservative treatment besides increasing ventilation parameters. Additional therapy with physiotherapy, secretolysis with acetylcysteine or inhaled bronchodilators may enhance improvement when treated with rhDNase. To evaluate this alternative conservative treatment for atelectasis prospectively, a randomized placebo-controlled trial should be initiated. Mechanical ventilation parameters, patient diagnoses and methods for x-ray interpretation should perceive special attention when a RCT is planned.

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