**In Vitro Effects of rhDNase on Sputum Rheology in Cystic Fibrosis Patients**

Étienne Ghiringhelli,¹ Matthieu Robert de Saint Vincent,¹ Jérémy Patarin,¹ Stéphane Mazur,² Marie Perceval,³ and Isabelle Durieu²

1 Rheonova, Grenoble, France, contact@rheonova.fr; 2 CRML, Hospices Civils de Lyon, Hôpital Femme Mère Enfant, Lyon, France

**Context**

Sputum abnormal viscoelasticity causes cystic fibrosis (CF) patients difficulties to expectorate. To promote clearance, recombinant human deoxyribonuclease I (rhDNase) is often prescribed in its purified form (Dornase alpha) as it is expected to fluidise the mucus by selectively cleaving DNA. The mucolytic efficiency of rhDNase has been assessed,²⁻³ but neither sputum heterogeneity nor sample history were taken into consideration in previous investigations. The present study establishes a unified rheological protocol to validate in vitro the rheological effect of rhDNase on sputum.

1. Shak et al. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum, **PNAS** 87, 9188 (1990)

**Protocol**

**Sample Collection**

CF patients Interrupt rhDNase treatment 48 h Spontaneous expectoration

> 1 mL sample; storage @ 4°C

**Sample Preparation**

Control: saline solution (control) rhDNase, 2 µg/mL - dilution in saline solution

Sputum is composed of highly viscoelastic mucus plugs included in a less viscoelastic matrix. This heterogeneity induces artefacts in rheology measurements.

French patent FR 2570108, filed 26 July 2012

**Rheological Measurements**

Rheology is the study of the flow of matter that exhibits a combination of elastic, viscous and plastic behaviours by combining elasticity and fluid mechanics.

- **In phase contribution:** $G' = \frac{\tau \cos \delta}{\gamma}$
- **Out of phase contribution:** $G'' = \frac{\tau \sin \delta}{\gamma}$
- **tan δ phase tangent** $\tan \delta = \frac{G''}{G'}$

$G'$ and $G''$ a priori depend on frequency and exerted strain.

Reference values are taken in the linear regime (1 Hz, 1%) where this dependency is weak.

**Results**

In vitro addition of rhDNase:

- Significantly reduces the elastic modulus $G'$ by 43% (N = 27, $p = 0.01$).
- Likely reduces the viscous modulus $G''$ by 35% (N = 32, $p = 0.02$).
- Does not significantly affect the phase tangent (N = 23, $p = 0.22$).

**Concluding Remarks**

1. The thinning effect of rhDNase is retrieved by measuring in vitro the rheology of CF patients sputa. Our results are in line with those reported in highly heterogeneous sputum samples,²⁻³ suggesting that rhDNase likely affects both the mucus plugs and the embedding matrix in a similar way.

2. Elastic and viscous moduli are both reduced in comparable proportions. The sputum thins without globally becoming more liquid-like or gel-like.

**Perspectives**

The mechanism by which rhDNase promotes clearance remains unclear with in vitro testing. While we evidence a global thinning effect (reduction in both $G'$ and $G''$), a proper fluidisation of the mucus would imply a significant increase in $\tan \delta$, modifying the gel-like structure. Further investigations would thus be necessary to better understand the mucolytic mechanism of action.