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IN VITRO RHEOLOGICAL ASSESSMENT OF MUCOLYTIC ACTIVITY INDUCED BY SEAPROSE

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SUMMARY

Proteolytic enzymes can act on the polymeric structure of the bronchial mucus, shortening the long chain of mucoproteins, DNA and other macromolecules, and thus reducing the viscosity of the mucus facilitating its expectoration. Seaprose (Flaminase, Puropharma) belongs to this class and is a proteinase from *Aspergillus melleus* and it is mainly used in traumatology, orthopaedics, gynaecology and pneumology.

In the present study the *in vitro* activity of increasing concentrations (0.25, 0.5, 1%) of seaprose incubated with bronchial mucus samples (1 ml) was investigated by a rheological technique (transient test) that assesses changes in viscosity and elasticity.

A dose–effect relationship between increasing concentrations of seaprose and the corresponding reductions in bronchial mucus viscosity was found.

There was also a parallel reduction in elasticity after incubation with 0.5%, but an unfortunate distribution of values for 0.25 and 1% concentrations does not allow us to state whether there is a dose–effect relationship for elasticity.

KEY WORDS: seaprose, *in vitro*, rheological assessment, dose–effect curve.

INTRODUCTION

The tridimensional polymeric structure of mucus, due to reciprocal chemical interactions of mucoproteins and DNA, IgA and other macromolecules, also affects it rheological properfies. There are some agents that can interact with the mucus fibrillar architecture, for instance, some chemicals bearing the -SH group can interact with the cysteine-SH group (present in proteins), to break the disulphide bond and reduce the viscosity of mucus and make its mobilization easier [1].

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10–12 min), so authors according to the different techniques used reported a delay up to 2 hours [13–16]; we consider a good result to maintain this delay within 30 min.

The patients were randomly divided in four groups and the samples collected from the first group were used to test the saline effect, while the samples from other groups were used to test the seaprose activity (three different doses).

The mucus had to be mucous, clear and white in appearance. Yellow mucus was discarded.

Viscoelasticity assessment

Viscous and elastic characteristics of the samples were studied by a rheometer (Mod. Viscoelasticimeter by Sefam, Nancy) with a coaxial cylinder (Couette geometry) set up in the rotational mode (transient test) [13]. The inner cylinder is fixed to an axle which is held in position by two torsion wires. The outer cylinder is rotated at a constant rate by a synchronous motor. A transducer, which is fixed to the axle, measures a very low angle of torsion in the wires. After application of a constant shear rate, the apparent viscosity is calculated by measuring the shear stress under steady state conditions (measurement units: Pascal × sec, Pa·s). The elastic modulus is calculated from the stress relaxation curve on the assumption that it may be represented by a simple exponential equation: $G(t) = G_i e^{-t/t_r}$, where t is the time, t_r is the relaxation time and G_i the total modulus at infinity [13] (measurement units:Pascal, Pa). The apparent viscosity is deliberately measured at a single low shear rate ($\gamma = 0.4\ s^{-1}$, chosen to avoid an irreversible breaking of the molecular structure of the mucus. The stainless steel cylinders have diameters of 21.5 mm and 20 mm (gap between the two 0.75 mm) and the inner cylinder is 18.4 mm high. In order to obtain a uniform shear rate and to avoid trapping air bubbles, the bases are conical in shape (angle of the internal cylinder 155.90°, angle of the external cylinder 164.1°), the volume of the sample is 1 ml. The temperature of the external cylinder is maintained at 30°C by water circulating through an annular bath surrounding the cylinder.

To avoid too rapid spreading of the sample between the two cylinders, and to prevent changes in the rheological properties, the descent speed of the internal cylinder is slowed when it makes contact with the mucus.

Linearity was tested against standard oils and is ±1% of the best straight line for a viscosity of 0.5 Pa·s to 60 Pa·s. The block that supports the inner cylinder produces a torsion C of 5.05×10^{-3} N·m·rad^{-1}, an inertia moment of 3.5×10^{-5} kg·m^2 and a natural frequency of 19 Hz. After setting up the sample, the temperature reaches equilibrium within a few minutes under usual measurement conditions.

After the first measurement, taken as the reference, a second measurement was taken under the same experimental conditions. To the same mucus sample, not removed from the cylinder, was added 0.1 ml of a solution of seaprose dissolved in saline. The concentrations of seaprose tested were 0.25, 0.5 and 1% and the incubation was at 37°C for 15 min. After the addition of seaprose only two turns were done to homogenize the distribution of seaprose and then the rheometer was stopped for the period of incubation.

Table I
Values of viscosity and elasticity of mucus samples before and after 15 min incubation with saline and increasing concentrations of seaprose

	Viscosity (Pa·s)			*Elasticity (Pa)*		
Incubation	*Before*	*After*	Δ%	*Before*	*After*	Δ%
Saline						
1	15.65	16.23	+3.70	0.26	0.33	+26.92
2	20.18	20.07	−0.54	0.51	0.75	+47.05
3	46.92	41.85	−10.80	0.79	0.53	−32.91
4	15.67	15.07	−3.83	0.40	0.22	−45.00
5	16.05	13.66	−14.89	0.30	0.23	−23.33
6	22.30	23.67	+5.71	0.52	0.71	+36.53
7	7.69	7.41	−3.64	0.26	0.17	−34.61
8	6.49	5.76	−11.24	0.35	0.40	+14.28
Mean	18.88	17.96	−2.58	0.42	0.41	−1.38
±SD	±12.57	±11.32		±0.17	±0.22	
P		NS			NS	
Seaprose 0.25%						
1	8.39	6.21	−25.98	0.13	0.25	+92.30
2	25.41	15.79	−37.86	0.47	0.21	−55.32
3	18.44	16.58	−10.08	0.26	0.22	−15.38
4	7.35	5.36	−27.07	0.24	0.16	−33.33
5	9.77	7.58	−22.41	0.18	0.11	−38.89
6	12.38	6.67	−46.12	0.22	0.16	−27.27
7	31.10	21.77	−30.00	0.29	0.19	−34.48
8	21.85	13.54	−38.03	0.27	0.21	−22.23
Mean	16.83	11.68	−29.70	0.25	0.18	−16.82
±SD	±8.75	±6.06		±0.10	±0.04	
P		<0.01			NS	
Seaprose 0.5%						
1	25.01	8.33	−64.49	0.42	0.30	−28.57
2	45.22	12.54	−72.27	0.35	0.36	+2.85
3	5.64	2.42	−57.09	0.37	0.16	−56.75
4	21.23	17.67	−16.76	0.40	0.38	−5.00
5	10.39	2.39	−76.99	0.24	0.10	−58.33
6	10.90	5.19	−52.38	0.25	0.16	−36.00
7	8.60	2.47	−71.27	0.40	0.25	−37.50
8	17.44	8.89	−49.02	0.31	0.18	−41.93
Mean	18.05	7.48	−57.53	0.34	0.23	−36.65
±SD	±12.82	±5.53		±0.06	±0.10	
P		<0.01			<0.01	
Seaprose 1%						
1	22.62	2.59	−88.55	0.21	0.30	+42.[illegible]3
2	13.32	6.12	−54.05	0.24	0.16	−33.33
3	9.01	3.07	−65.92	0.22	0.37	+68.18
4	42.15	11.39	−72.97	0.33	0.20	−39.40
5	24.35	1.84	−92.44	0.31	0.14	−54.83
6	12.07	2.42	−79.95	0.12	0.18	+50.00
7	5.59	0.79	−85.86	0.27	0.12	−55.55
8	5.86	0.86	−85.32	0.59	0.45	−23.72
Mean	16.87	3.63	−78.13	0.28	0.24	−5.72
±SD	±12.38	±3.55		±0.13	±0.12	
P		<0.01			NS	