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EFFECTS OF SEAPROSE ON SPUTUM BIOCHEMICAL COMPONENTS IN CHRONIC BRONCHITIC PATIENTS: A DOUBLE-BLIND STUDY VS PLACEBO

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Summary: *Seaprose is a semialkaline proteinase endowed with proteolytic effect and antiinflammatory activity tested in different clinical trials. There is clinical evidence that seaprose reduces sputum viscoelastic properties in chronic hypersecretory bronchitis. The present study evaluated (in a double-blind design vs. placebo) the activity of seaprose on bronchial inflammation, mucus glycoprotein secretion and bronchial humoral defence mechanism in chronic bronchitic patients clinically stable (10 per group). Markers of bronchial inflammation (albumin, albumin/total protein ratio) and bronchial infection (DNA), of mucus glycoproteins (fucose and N-acetylneuraminic acid) and of humoral defence mechanism (secretory-IgA) were tested in sputum. We found that ten-day treatment with seaprose (90 mg/day) reduced sputum albumin during the observation period, the difference being statistically significant at the 18th day. The sputum albumin/total protein ratio also decreased by 50% at the end of the study. In the same group, sputum DNA, secretory-IgA, fucose and N-acetylneuraminic acid remained unchanged after treatment. The placebo group did not show any significant changes in the sputum marker substances. This study provides experimental evidence for the antiinflammatory activity of seaprose on bronchial mucosa in chronic bronchitic patients studied in a stable phase of their disease. Furthermore the drug does not seem to affect mucus glycoprotein secretion or secretory-IgA production.*

Introduction

Acute and chronic hypersecretory lung diseases are characterized by production of mucus from the tracheobronchial tree above normal in amount and often abnormal in chemical components and physical properties (1). The mucus hypersecretion impairs ventilatory function and mucociliary transport, thus favouring bacterial colonization. The therapeutical strategy to be pursued in hypersecretory lung diseases is to reduce sputum production by the antibiotic and the antiinflammatory treatment, and to decrease the pathological viscoelastic properties of the musus by a mucoactive drug. Among the different pharmacological strategies to correct tracheobronchial hypersecretion, proteolytic enzymes and proteases have been proposed.

Seaprose, also called onoprose, is a semialkaline

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reagent assay (14), fucose by Gibbon's method (15), N-acetylneuraminic acid (NANA) by thiobarbituric acid assay (16), and macromolecular dry weight (MDW) after lyophilization of the sputum. Secretory-immunoglobulin-A (s-IgA) was assayed by ELISA. Micro-well plates were coated with 200 µl of goat anti-human secretory component diluted 3:1000 in 0.1 M $NaHCO_3$ coating buffer pH 9.6, overnight. The wells were washed and blocked with 0.5% bovine serum albumin in 0.15 M phosphate buffered saline at pH 7.4 (PBS) containing 0.01% Tween 20 (PBS-T). Standards of s-IgA or samples diluted in PBS (100 µl) were incubated for 2 h; then the wells were washed and 100 µl of rabbit anti-human alpha chain-peroxidase conjugate, diluted 1:1000 in PBS-T, was added to each well and incubated for 2 h. After being washed, the plates were developed with a peroxidase substrate and read at 492 nm. The assay range was between 5 and 200 ng/ml

Statistical evaluation

Data were evaluated by Student's t test for paired data, or by ANOVA, to assess any statistical difference ($p < 0.05$) either intra-treatment or between the two treatments.

Results

On admission into the study, the two groups (seaprose and placebo) proved to differ from each other in sputum concentration of DNA ($p < 0.01$), albumin ($p < 0.02$), fucose ($p < 0.05$), DMW ($p < 0.05$) and albumin/total protein ratio ($p < 0.01$), although there was no difference in NANA, total protein, s-IgA concentration and in lung function tests (Tables I and II). This artifact was due to the standard randomization followed in the study protocol, which caused an "unfortunate allocation" in the placebo group originating the differences in

Table I *Mean value (± S D) of sputum marker substances on admission (T-0) and after 10 (T-10), 14 (T-14) and 18 days (T-18) of observation in chronic bronchitics receiving placebo treatment*

	T-0	T-10	T-14	T-18
DNA mg/dl	1225	1222	1090	1065
	(392)	(441)	(519)	(612)
Albumin mg/dl	85.9	70 2	77.6	78 4
	(42 4)	(66 7)	(63.6)	(61.8)
s-IgA mg/dl	309	338	334	324
	(109)	(88)	(73)	(76)
Fucose mg/dl	152.8	141.8	145.3	147.6
	(29.5)	(50.8)	(39 4)	(46 0)
NANA mg/dl	21 2	17 2	15.9	15.3
	(21 5)	(19.2)	(17.9)	(18 9)
Total prot. mg/dl	2415	2368	2264	2261
	(547)	(900)	(895)	(934)
DWM mg/ml	54 0	49 1	50.3	50 6
	(10 5)	(21 0)	(18 1)	(18.6)
Alb/Total prot × 100	3 6	2.9	3 3	3 5
	(1.8)	(1.3)	(1 3)	(1.5)

Table II *Mean value (± S D) of sputum marker substances on admission (T-0) and after 10 (T-10), 14 (T-14) and 18 days (T-18) of observation in chronic bronchitics receiving seaprose treatment*

	T-0	T-10	T-14	T-18
DNA mg/dl	588	550	491	552
	(431)	(438)	(384)	(382)
Albumin mg/dl	41 1	31.5	28.0	20 1*
	(20.0)	(12.2)	(13.1)	(9.5)
s-IgA mg/dl	351	354	347	348
	(112)	(92)	(80)	(126)
Fucose mg/dl	135 5	124 7	131.3	131 8
	(49 7)	(54 1)	(72.2)	(57.0)
NANA mg/dl	10 9	12.3	8.1	10.7
	(8 0)	(6 3)	(5 0)	(3 5)
Total prot mg/dl	1928	1926	1909	1894
	(602)	(686)	(830)	(816)
DWM mg/ml	42 0	41 2	40 4	40 6
	(11 1)	(18.89)	(24 3)	(15.1)
Alb/Total prot × 100	2 0	1 5	1.6	1 1
	(1 2)	(0 6)	(0.6)	(0 4)

* Albumin: T-0 vs T-18: $p < 0.05$

The reduction of sputum albumin concentration after seaprose treatment was not due to spontaneous decrease of local infection, since the DNA concentration remained stable during the study period. Our results show that seaprose treatment had an antiinflammatory effect in stable chronic bronchitis which remained for 8 days after the withdrawal of treatment.

Our results represent clinical experimental evidence of the antiinflammatory activity of seaprose in pneumology, confirming previous reports in traumatology, gynaecology and surgery (2–5). In a previous report (6) we have described, in the same group of patients receiving seaprose, a significant reduction in sputum viscosity and elasticity. These rheological changes have been ascribed to the proteolytic activity of seaprose which could break the inter- and intra-molecular bonds of the mucus glycoprotein network. We hypothesize that the reduction in sputum albumin concentration in patients receiving seaprose could slightly contribute to changes shown in the rheological properties of bronchial secretion. In fact, *in-vitro* and *in-vivo* studies have shown that changes in sputum viscoelastic properties are positively related to the concentration of macromolecular components such as mucin, protein and lipid (22). Glycoprotein-protein interactions significantly contribute to the sputum rheological properties: *in-vitro* investigations revealed that the addition of albumin to human isolated mucus glycoproteins induced a significant increase in viscoelastic properties directly proportional to albumin concentration (23).

Conclusions

Our study has shown that ten days' treatment with seaprose (90 mg/day) decreased significantly the sputum albumin concentration, marker of bronchial inflammation; this pharmacological effect remained significant eight days after the end of the treatment. We conclude that seaprose evidenced an antiinflammatory effect on bronchial mucosa of chronic bronchitic patients, suggesting that the drug may be an effective tool with potentiality in clinical conditions characterized by acute and chronic mucus hypersecretion.

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