

# **SERRAPEPTASE CLINICAL ABSTRACTS:**

## **1.) Formulation optimization of double emulsification method for preparation of enzyme-loaded Eudragit S100 microspheres.**

**[Rawat M, Saraf S Reader.](#)**

Institute of Pharmacy, Pt Ravishankar Shukla University, Raipur, India.

The present study aimed to develop an oral sustained release microparticulate system for acid labile enzyme-Serratiopeptidase. A 3(2) full factorial experiment was designed to study the effects of the external aqueous phase volume and stabilizer (Tween(R) 80) concentration on the entrapment and size of Eudragit S100 microspheres prepared by a modified double emulsion solvent evaporation technique. The results of analysis of variance tests for both effects indicated that the test is significant. The effect of external aqueous phase volume was found to be higher on the entrapment efficiency of microspheres ( $SSY(1) = 1362.63$ ;  $SSY(2) = 250.13$ ), whereas Tween(R) 80 produced a significant effect on size of microspheres ( $SSY(1) = 944.01$ ;  $SSY(2) = 737.26$ ). Scanning electron microscopy of microspheres demonstrated smooth surface spherical particles. The effect of formulation variables on the integrity of enzyme was confirmed by in vitro proteolytic activity. Microspheres having maximum drug encapsulation ( $81.32 \pm 3.97$ ) released 4-5% enzyme at pH 1.2 in 2 h. The release of enzyme from microspheres followed Higuchi kinetics ( $R(2) = 0.987$ ). In phosphate buffer, microspheres showed an initial burst release of  $25.65 \pm 2.35\%$  in 1 h with an additional  $62.96 \pm 4.09\%$  release in the next 5 h. Thus, formulation optimization represents an economical approach for successful preparation of Eudragit S100 microspheres involving fewest numbers of experiments.

## **2.) Effect of the proteolytic enzyme serrapeptase in patients with chronic airway disease.**

**[Nakamura S, Hashimoto Y, Mikami M, Yamanaka E, Soma T, Hino M, Azuma A, Kudoh S.](#)**

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**OBJECTIVES:** The proteolytic enzyme serrapeptase (SER) is widely used in clinical practice in Japan. We investigated the effect of SER on sputum properties and symptoms in patients with chronic airway diseases. **METHODS:** This study was an open-labelled trial with a non-treatment control group. Patients were randomly assigned to oral treatment with ( $n = 15$ ) and without ( $n = 14$ ) SER 30 mg/day for 4 weeks. Patients collected sputum samples for about 4 h in the morning on the day the trial began and 4 weeks later. We measured the amount of sputum by weighing. Part of each sputum

sample was weighed and then completely dried and reweighed. The percentage solid component, viscosity and elasticity of the sputum were measured. Mucociliary transportability index was measured using ciliated bovine trachea ex vivo. Sputum smears were also prepared to count sputum neutrophils. Patients' symptoms were assessed by a questionnaire that used a visual analogue scale. RESULTS: After 4 weeks of SER treatment, sputum weight in the morning, percentage solid component, viscosity and elasticity of sputum, sputum neutrophil count, frequency of coughing and frequency of expectoration significantly decreased. The mean mucociliary transportability index increased from 13.3 +/- 1.8 to 24.4 +/- 2.5 (P = 0.0103). CONCLUSIONS: SER may exert a beneficial effect on mucus clearance by reducing neutrophil numbers and altering the viscoelasticity of sputum in patients with chronic airway diseases.

**3.) [Clinical study of the efficacy of and tolerance to seaprose S in inflammatory venous disease. Controlled study versus serratio-peptidase]**

[Article in Italian]

**[Bracale G, Selvetella L.](#)**

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This study was designed to compare the efficacy and safety of seaprose S and serratio-peptidase in the treatment of venous inflammatory disease. Forty patients entered the study (11 males, 29 females), mean age 54.3 years (range 30-77), mean weight 74.8 kg (range 51-96), with superficial thrombophlebitis. The trial was conducted following a controlled, between patients, randomized experimental design. Seaprose S was administered as 30 mg tablets at a daily dosage of 90 mg (one tab t.i.d.), and serratio-peptidase as 5 mg tablets, at a dose of 30 mg per day (two tabs t.i.d.), both orally, for 14 days. Twenty patients received seaprose S and 20 serratio-peptidase. The findings indicate that seaprose S was more effective and better tolerated than serratio-peptidase. Although the group of patients assigned to seaprose S had considerably more severe initial symptoms, by the end of treatment spontaneous pain was reduced 68.7% from the baseline mean score (from 3.2 to 1.0), as compared with a 63.3% reduction in the serratio-peptidase group (from 3.0 to 1.1). Pain on pressure was reduced 61.1% with seaprose S (from 3.6 to 1.4), compared to 57.6% with the reference treatment (from 3.3 to 1.4). Edema was reduced respectively 75% (from 1.6 to 0.4) and 56.2% (from 1.6 to 0.7); erythema diminished 72.4% (from 2.9 to 0.8) and 58.3% (from 2.4 to 1.0); nighttime cramps were 61.1% less (from 1.8 to 0.7) compared with 52.9% (from 1.7 to 0.8); hemorrhagic suffusion was 53.3% less (from 1.5 to 0.7) compared with 41.7% (from 1.2 to 0.7); cutaneous dystrophy was reduced by 11.1% (from 1.8 to 1.6) and 7.7% (from 1.3 to 1.2). At the end of the treatment with seaprose S efficacy was assessed as good or excellent in 85% of the cases, compared with 65% for serratio-peptidase. Seaprose S caused no adverse reactions. During serratio-peptidase treatment one patient reported diarrhea, requiring temporary dosage reduction and specific treatment. It can thus be confirmed that seaprose S was effective and well tolerated in patients with inflammatory venous diseases.

**4.) Evaluation of Serratia peptidase in acute or chronic inflammation of otorhinolaryngology pathology: a multicentre, double-blind, randomized trial versus placebo.**

[Mazzone A, Catalani M, Costanzo M, Drusian A, Mandoli A, Russo S, Guarini E, Vesperini G.](#)

Institute of Clinical Otorhinolaryngology, University of Naples, Italy.

The efficacy and tolerability of Serratia peptidase were evaluated in a multicentre, double-blind, placebo-controlled study of 193 subjects suffering from acute or chronic ear, nose or throat disorders. Treatment lasted 7-8 days, with the drug or placebo being administered at a rate of two tablets three times a day. After 3-4 days' treatment, significant symptom regression was observed in peptidase-treated patients. There was also a significant reduction in symptoms after 7-8 days for patients in both treatment groups but the response was more marked in those patients receiving the active drug. Statistical comparison between the two groups confirmed the greater efficacy and rapid action of the peptidase against all the symptoms examined at both stages. Tolerance was found to be very good and similar for both groups. It is concluded that Serratia peptidase has anti-inflammatory, anti-oedemic and fibrinolytic activity and acts rapidly on localized inflammation.

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**6.) Effects of orally administered drugs on dynamic viscoelasticity of human nasal mucus.**

[Majima Y, Hirata K, Takeuchi K, Hattori M, Sakakura Y.](#)

Department of Otorhinolaryngology, Mie University School of Medicine, Tsu, Japan. The effects of orally administered drugs on rheologic properties of nasal mucus were investigated in adult chronic sinusitis patients. The elastic modulus  $G'$  and the dynamic viscosity  $\eta'$  of nasal mucus were determined by an oscillating sphere magnetic rheometer. Both  $G'$  and  $\eta'$  values of the mucus before drug administration

were much higher than optimal viscoelasticity for mucociliary transport. Norfloxacin, an antibacterial agent, reduced the  $G'$  but not the  $\eta'$  of nasal mucus.

Serratiopeptidase, a proteolytic enzyme, reduced  $\eta'$  but did not reduce  $G'$ . S-carboxymethylcysteine, a blocked thiol derivative of cysteine, did not change either  $G'$  or  $\eta'$ . L-cysteine ethyl ester hydrochloride, a sulfhydryl type of agent, reduced both  $G'$  and  $\eta'$ . The results indicate that some of the orally administered mucokinetic agents can improve the abnormal rheologic properties of nasal mucus in chronic sinusitis.

**7.) The effect of an orally administered proteolytic enzyme on the elasticity and viscosity of nasal mucus.**

[Majima Y](#), [Inagaki M](#), [Hirata K](#), [Takeuchi K](#), [Morishita A](#), [Sakakura Y](#).

Department of Otorhinolaryngology, Mie University School of Medicine, Japan.

We have evaluated the effect of serratiopeptidase (SER), a proteolytic enzyme, on the elasticity and viscosity of the nasal mucus in adult patients with chronic sinusitis. SER was administered in a dose of 30 mg/day orally for 4 weeks. Nasal mucus was collected from the nasal cavities of each patient before (week 0) and 4 weeks after the start of the medication (week 4). The storage modulus ( $G'$ ) and the dynamic viscosity ( $\eta'$ ) of each specimen of nasal mucus were determined by an oscillating sphere magnetic rheometer at frequencies of 0.5, 1, 5, 10 and 20 Hz at a constant temperature of 25 degrees C. The dynamic viscosity ( $\eta'$ ) of the mucus at week 4 was significantly lower than that at week 0 (at frequencies of 5, 10 and 20 Hz). No significant differences were observed in the storage modulus ( $G'$ ) between the mucus at week 0 and week 4. SER reduced the viscosity but not the elasticity of the nasal mucus. These findings are discussed in relation to mucociliary clearance.

**8.) The treatment of breast engorgement with Serrapeptase (Danzen): a randomised double-blind controlled trial.**

[Kee WH](#), [Tan SL](#), [Lee V](#), [Salmon YM](#).

We evaluated an anti-inflammatory enzyme drug Danzen (Serrapeptase: Takeda Chemical Industries, Ltd.) on 70 patients complaining of breast engorgement. These patients were randomly divided into 2 groups, a treatment group and a placebo group. A single observer, unaware of the group the patients were in, assessed the severity of each of the symptoms and signs of breast engorgement before treatment was commenced, and daily for 3 days, during which therapy was administered. Danzen was noted to be superior to placebo for improvement of breast pain, breast swelling and induration and while 85.7% of the patients receiving Danzen had "Moderate to Marked" improvement, only 60.0% of the patients receiving placebo had a similar degree of improvement. "Marked" improvement was found in 22.9% of the treatment group and 2.9% of the placebo group. These differences were statistically significant (P less than 0.05). No adverse reactions were reported with the use of Danzen. Danzen is a safe and effective method for the treatment of breast engorgement.

**9.) [Reduction of postoperative swelling. Objective measurement of swelling of the upper ankle joint in treatment with serrapeptase-- a prospective study]**

[Article in German]

[Esch PM](#), [Gerngross H](#), [Fabian A](#). Using a quantitative standardized procedure, the swelling of the ankle produced by supination trauma was measured. In the 66 patients with fresh rupture of the lateral ligament treated surgically at our Department between December 1986 and April 1987, a prospective study of the effect of serrapeptase (Aniflazym) on post-operative swelling and pain was carried out in 3 randomized groups of patients. In the group receiving the test substance, the swelling had decreased by 50% on the third post-operative day, while in the other two control groups (elevation of the leg, bed rest, with and without the application of ice) no reduction in swelling had occurred at that time. The difference is statistically significant ( $p = 0.013$ ). Decreasing pain correlated for the most part with the reduction in swelling. Thus, the patients receiving the test substance more rapidly became pain-free than did the control groups. On the basis of these results, serrapeptase would appear to be an effective preparation for the post-operative reduction of swelling, in comparison with the classical conservative measures, for example, the application of ice.

**10.) [Augmentation by serrapeptase of tissue permeation by cefotiam]**

[Koyama A](#), [Mori J](#), [Tokuda H](#), [Waku M](#), [Anno H](#), [Katayama T](#), [Murakami K](#), [Komatsu H](#), [Hirata M](#), [Arai T](#), et al.

Cefotiam (CTM) is a new cephalosporin with a broad spectrum of activity against both Gram-positive and Gram-negative microorganisms. Cephalosporins are widely used for prophylaxis of infections in patients undergoing thoracotomy. Augmentation by serrapeptase on tissue permeation of CTM was examined in 35 thoracotomy patients with lung cancer. The subjects were divided into two groups according to the method of the administration of CTM. Group I consisted of 17 subjects, each of whom received a single dose of 2 g of CTM alone by an instillation for 30 minutes. Group II consisted of 18 subjects, each of whom received a combination of CTM and serrapeptase; serrapeptase was given 2 tablets (10 mg) each time for three times/day until the day before surgery, and then CTM was administered by the same procedure. The following results were obtained: Individual difference was observed for the permeation of CTM into tissues. Pathologic differences also affected the permeation. Nevertheless, the CTM levels in pulmonary tissues reached about a half of those in the blood in both the single dose group and the combination group, hence sufficient concentrations exceeding MIC<sub>80</sub> for main microorganisms that caused infections in the lung were obtained. The concentrations of CTM in inflammatory tissues have showed lower levels than those of normal tissues in both CTM single dose and the combination groups. Decrease of blood flow volume may have contributed to the reduction in levels of CTM in the inflammatory tissues. The ratio of the concentration of the drug in pulmonary tissues to that in the blood was 29.1 +/- 2.5% in the single dose group, and 44.2 +/- 6.0% in the combination group, the latter showing quite a significant increase ( $P$  less than 0.05). Combined administrations of CTM and serrapeptase deserves more trials in the case when surgical treatments of the lung are

performed. An antiinflammatory effect of serrapeptase in the respiratory system is expected, and in addition, the combined use of CTM and serrapeptase should stimulate permeation of the antibiotic into tissues.

**11.) A multi-centre, double-blind study of serrapeptase versus placebo in post-antrotomy buccal swelling.**

[Tachibana M](#), [Mizukoshi O](#), [Harada Y](#), [Kawamoto K](#), [Nakai Y](#).

A multi-centre, double-blind, placebo-controlled trial was carried out to investigate the clinical efficacy of the anti-inflammatory enzyme serrapeptase in a total of 174 patients who underwent Caldwell-Luc antrotomy for chronic empyema. Eighty-eight patients received 10 mg serrapeptase 3 times on the day before operation, once on the night of the operation and 3 times daily for 5 days after operation; the other 86 received placebo. Changes in buccal swelling after operation were observed as a parameter of the response to treatment. The degree of swelling in the serrapeptase-treated patients was significantly less than that in the placebo-treated patients at every point of observation after operation up to the 5th day ( $p$  less than 0.01 to  $p$  less than 0.05). Maximal swelling throughout all the post-operative points of observation was also significantly smaller in size in the serrapeptase-treated group than in the placebo-treated group. No side-effects were reported.

**12.) [Experimental studies on distribution of cefotiam, a new beta-lactam antibiotic, in the lung and trachea of rabbits. II. Combined effects with serratiopeptidase]**

[Ishihara Y](#), [Kitamura S](#), [Takaku F](#).

Plasma levels and distribution in pulmonary and bronchial tissues of CTM following injection into the jugular vein were investigated in rabbits with experimental pleuritis or pneumonitis as well as in normal rabbits. The experiments also included the assessment of the effect of concomitant administration of serratiopeptidase (TSP). The pneumonitis + TSP group, pleuritis group and pleuritis + TSP group showed a tendency to delayed dissipation of CTM from the plasma, as compared with controls. The CTM concentrations in tissues from the apical region of upper lobe (L1), lateral region of middle lobe (L2) and diaphragmatic region of lower lobe (L3) 30 minutes after injection did not differ significantly between the control and the TSP group, pleuritis group or pleuritis + TSP group. In the pneumonitis group, the tissue CTM concentrations at all 3 sites (L1, L2, L3) were lower than those in the control group. They were increased by the concomitant administration of TSP, with statistical significance of increase in regions L2 and L3. Thirty minutes after the injection of CTM, the pneumonitis group and pneumonitis + TSP group displayed essentially comparable CTM levels in pleural fluid, whereas the CTM concentrations in the pleural fluid were prone to be increased in the pleuritis + TSP group as comparing with the pleuritis group. CTM levels in the tissues of trachea (B0), right and left main bronchi (B1) and lobar bronchi (B2) 30 minutes after the injection did not show any significant difference between control and TSP-treated normal groups. CTM concentrations tended to be increased, yet not significantly, in all these regions in the rabbits with pleuritis administered TSP, compared to those without

**13.) Effect of expectorants on relaxation behavior of sputum viscoelasticity in vivo.**

[Shimura S](#), [Okubo T](#), [Maeda S](#), [Aoki T](#), [Tomioka M](#), [Shindo Y](#), [Takishima T](#), [Umeya K](#).

We studied the effects of expectorants (mucolytic agents) in vivo on the relaxation behavior of sputum viscoelasticity. Seven female and thirty-three male patients (56.8 +/- 19.3 yrs, range: 21 to 82 years old) with a chronic pulmonary disease except bronchial asthma were studied. They were randomly put into the control group or a group which would be given oral treatments with an expectorant for a week after a one week washout period. The groups were as follows: Group I (n = 8), control; Group II (n = 7), Bromhexine hydrochloride 24 mg per day; Group III (n = 10), Ambroxol 90 mg per day; Group IV (n = 9) alpha - Chymotrypsin buccle 100 ch.u. per day; Group V (n = 6), Serratiopeptidase 30 mg per day. In Groups IV & V, frequency dependence of sputum viscoelasticity at the range of  $\omega = 10(-3)$  to  $10(0)$  rad.sec<sup>-1</sup> were clearly changed after the treatments, and the magnitude of the relaxation and its main relaxation time were significantly increased. On the other hand, in Groups I, II & III, no significant changes of the frequency dependences were observed. These findings suggest that proteolytic enzymes administered orally work on the molecular structure of sputum, and break down their linkages between subunits of the structure.

**14.) A new method for evaluating mucolytic expectorant activity and its application. II. Application to two proteolytic enzymes, serratiopeptidase and seaprose.**

[Kasé Y](#), [Seo H](#), [Oyama Y](#), [Sakata M](#), [Tomoda K](#), [Takahama K](#), [Hitoshi T](#), [Okano Y](#), [Miyata T](#).

Using our new method described in a preceding paper, in vivo effects of two proteolytic enzymes such as serratiopeptidase (SER) and seaprose (SAP) on sputa collected from bronchitis rabbits were examined. SER (20 mg/kg) and SAP (30 mg/kg) significantly reduced the viscosity of sputum (P less than 0.05) at the 1-3-h periods and the 4-6-h periods, respectively, after intraduodenal administration. 50 mg/kg of SER also significantly decreased not only viscosity (P less than 0.001) but also amount of freeze-dried substance (P less than 0.05) of sputum at the 1-3-h periods, but SAP did not affect the amount of dried substance. Both enzymes significantly increased the volume of sputum, probably as the result of liquefaction. Thus, mucolytic expectorant activity of both enzymes can be demonstrated first by the reduction in viscosity and next of the increase in volume of sputa. However, the decrease in amount of freeze-dried substance is not always in accord with the reduction viscosity.

**15.) [Studies on the distributions of antibiotics in the oral tissues: Experimental staphylococcal infection in rats, and effect of serratiopeptidase on the distributions of antibiotics (author's transl)]**

**[Aratani H](#), [Tateishi H](#), [Negita S](#).**

A focal infection was prepared by inoculation of staphylococci into rat gingiva. Then, concentrations in oral tissues (gingiva, tongue and masseter), serum and liver of the infected rats which were given ciclacillin, ampicillin, cephalixin and minocycline in a dose of 100 mg/kg p.o. were investigated and effects of serratiopeptidase (20 mg/kg) on these concentrations were studied. 2) Concentrations of ciclacillin in the oral tissues were approximately 10% of a serum level. A gingival concentration was elevated 8.5-fold by pretreatment with serratiopeptidase. A concentration in infected gingiva was 2.5-fold of that of another side of gingiva. 3) Concentrations of ampicillin in the oral tissues were approximately 15% of a serum level. A gingival concentration was elevated 5.7-fold by pretreatment with serratiopeptidase. A concentration in infected gingiva was 2.2-fold of that of another side of gingiva. 4) Concentrations of cephalixin in the oral tissues were approximately 3 to 5-fold of a serum level except that in masseter. A gingival concentration was slightly elevated (1.1-fold) by pretreatment with serratiopeptidase. A concentration in infected gingiva was 1.7-fold of that of another side of gingiva. 5) Concentrations of minocycline in the oral tissues were 1.3 to 7.2-fold of a serum level. A gingival concentration was elevated 2.2-fold by pretreatment with serratiopeptidase. A concentration in infected gingiva was 3.1-fold of that of another side of gingiva. 6) Gingival concentrations of antibiotics were higher than those of tongue and masseter and serratiopeptidase elevated gingival concentrations.

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