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Review

Serratiopeptidase: A systematic review of the existing evidence

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ABSTRACT

Background: Serratiopeptidase is a proteolytic enzyme prescribed in various specialities like surgery, orthopaedics, otorhinolaryngology, gynaecology and dentistry for its anti-inflammatory, anti-edemic and analgesic effects. Some anecdotal reports suggest it to possess anti-atherosclerotic effects also, due to its fibrinolytic and caseinolytic properties. Despite being widely used there are few published studies regarding its efficacy. Thus, evidence regarding its clinical utility is needed.

Objective: To evaluate the existing evidence regarding efficacy and safety of Serratiopeptidase in clinical practice.

Evidence acquisition: A systematic review of all the published articles of Serratiopeptidase using Cochrane Library, PubMed, MEDLINE, Clinical Trials.gov, Google, Dogpile and a manual search of bibliographies was conducted from 1st May 2011 till 15th July 2012. Further emails were sent to all the leading pharmaceuticals who are manufacturing this enzyme preparation for any additional information. All studies related to Serratiopeptidase which included Randomised controlled trials (RCTs), meta-analysis of RCTs, placebo-controlled, single-blind, double-blind, open label, prospective trials as well as preclinical studies were screened and analysed. The scientific credibility of the studies was graded according to the Scottish Intercollegiate Guidelines Network (SIGN) grading checklist. A total of 24 studies on clinical efficacy of Serratiopeptidase met the inclusion criteria.

Evidence synthesis: Serratiopeptidase search on Cochrane library revealed 16 results among which 9 were Cochrane Central Register of Controlled Trials 2011, issue 4 studies and 7 were Cochrane Central Register of Controlled trials 2012, issue 3 studies. Of these 16 results, 11 were RCTs on efficacy of Serratiopeptidase. PubMed search also revealed 74 results which showed 16 Clinical trials, out of which 9 were RCTs related to Serratiopeptidase. Bandolier online edition (retrieved on 1/5/2011) showed a review 'Serratiopeptidase-finding the evidence' which included 9 RCTs. The evidence supporting the use of Serratiopeptidase as anti-inflammatory and analgesic agent is based on clinical studies which are of poor methodology. Only few RCTs, which are usually placebo control, with a small sample size are there. The dose and duration of treatment was not specified in some studies, and the outcome of the study was not clearly defined in a few. Data on the safety and tolerability of Serratiopeptidase is lacking in these studies.

Limitations: A thorough search of literature was done to include all the relevant studies but we may have unknowingly missed a few of those studies which have not been published or registered with any of these search engines. The clinical evidence obtained have been graded according to the "Scottish Intercollegiate Grading Network" checklist by two separate reviewers and then coordinated together to give the final grading. Any disagreement between the two reviewers was resolved by discussion with the third reviewer. This was done to minimise bias but still the risk of bias cannot be completely ruled out.

Conclusion: Serratiopeptidase is being used in many clinical specialities for its anti-inflammatory, anti-edemic and analgesic effects. It is even being promoted as a health supplement to prevent cardiovascular morbidity. The existing scientific evidence for Serratiopeptidase is insufficient to support its use as an analgesic and health supplement. The data on long-term safety of this enzyme is lacking. Evidence based recommendations on the analgesic, anti-atherosclerotic efficacy, safety and tolerability of Serratiopeptidase are needed.

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1. Introduction

Serratiopeptidase (Serratia E-15 protease also known as serralysin/serratia-protease/serrapeptase) is a proteolytic enzyme that

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has been used for reducing inflammation.^{1–7} Enteric coated oral formulation of this enzyme is being used commonly in various specialities like surgery, orthopaedics, otolaryngology, gynaecology and dentistry for its anti-inflammatory and anti-edemic properties. The use of enzymes like trypsin,⁸ chymotrypsin,⁹ bromelain¹⁰ as anti-inflammatory agents came into practice after it was observed during 1950s in USA that parenteral trypsin could be used to relieve post-surgical inflammation and that due to traumatic injury caused by sports⁹ as well as inflammation due to conditions like rheumatoid arthritis, ulcerative colitis, and atypical viral pneumonia.

This observation was soon followed by the use of Serratiopeptidase² for its anti-inflammatory effects in Japan for the first time. Later during 1960s these parenteral enzyme formulations were replaced by their enteric coated successors to be used orally. During 1980s and 1990s it was proposed by separate research conducted in Europe and Japan that Serratiopeptidase was the most effective agent in reducing inflammation among all other enzyme preparations.^{1,3} Serratiopeptidase hence, has been used for almost 40 years in Japan and Europe for pain and inflammation due to arthritis, trauma, surgery, sinusitis, bronchitis, carpal tunnel syndrome and painful swelling of breasts. Apart from its anti-inflammatory activity, Serratiopeptidase is also said to possess analgesic and anti-atherosclerotic effects. This systematic review is an attempt to critically examine the available evidence which exists as regards the efficacy and safety of Serratiopeptidase.

2. Evidence acquisition

2.1. Search criteria

To identify articles, we systematically searched electronic databases and commercial websites for published and unpublished studies, applied inclusion criteria and performed quality appraisals. The databases searched include the Cochrane Library, PubMed, MEDLINE, the Controlled trials registry Clinical Trials.gov, Google, Dogpile. A manual search from reference lists of relevant articles was conducted and Pharmaceutical companies were contacted through emails for additional information on pharmacokinetics, pharmacodynamics, efficacy and safety of Serratiopeptidase. The

following search terms were used either independently of each other or in combinations: Serratiopeptidase, serrapeptase, serratiopeptidase, proteolytic enzymes, pharmacokinetics, pharmacodynamics, anti-inflammatory agents, danzen, serratiopeptase, serralysin, adverse drug reactions.

2.2. Inclusion criteria

Eligible studies were required to report results on Serratiopeptidase efficacy and safety. All articles available on efficacy were included. All articles were required to be complete and available in english. Studies were selected for inclusion in our systematic review if they met the inclusion criteria: double-blind, single-blind, open label, placebo-controlled, randomised controlled trials, meta-analysis of RCTs, prospective trials or preclinical studies.

A total of 24 studies on clinical efficacy of Serratiopeptidase met the inclusion criteria. Among which 18 were clinical trials (including 14 RCTs, 3 prospective trials and in 1 trial study design not stated) and 6 were preclinical animal studies. As Serratiopeptidase has been in clinical use for inflammation and pain for a number of years and is being promoted these days for its anti-atherosclerotic effects also. But data on its analgesic and anti-atherosclerotic efficacy was found to be lacking. The RCTs were only a few in numbers (14 RCTs in total excluding the duplicated studies, 11 RCTs found on Cochrane Library and 9 on PubMed search). They had a limited population, not more than 150 patients per group in any of the studies and were of short duration. Therefore prospective studies as well as preclinical studies were also included to appraise evidence on efficacy of Serratiopeptidase.

2.3. Quality appraisal

Critical appraisal of studies was conducted using PRISMA¹¹ statement (Fig. 1) while the Scottish Intercollegiate Guidelines Network (SIGN)¹² grading for RCTs was used for original studies by two separate reviewers and then coordinated together to validate the final grading. This was done to facilitate transparent and comprehensive reporting of results. Any disagreement between the two reviewers was resolved by discussion with the third reviewer.

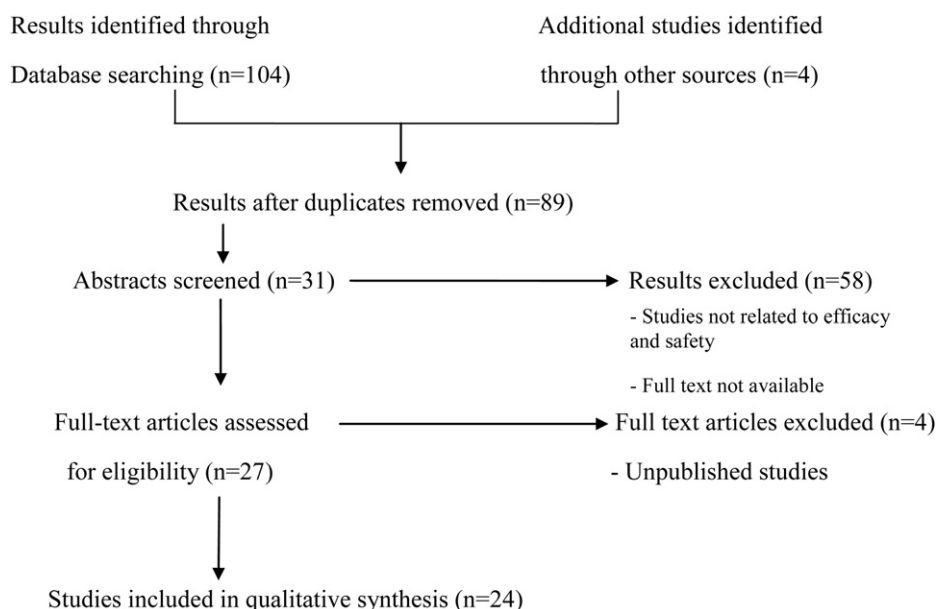


Fig. 1. Preferred reporting items for systematic reviews and meta-analysis (PRISMA)¹¹ flow diagram of studies included in the review of efficacy of serratiopeptidase.

3. Evidence synthesis

Search on Cochrane Library database using keyword ‘Serratiopeptidase’ revealed 16 results among which 8 were RCTs related to efficacy of Serratiopeptidase. A repeated search on the same database using keyword ‘Serrapeptase’ revealed 9 results among which 6 were RCTs. Therefore, a total of 11 RCTs were included in our review excluding those which were duplicated and where full text was not available.

PubMed search using keywords ‘Serratiopeptidase’ or ‘Serrapeptase’ revealed 74 results which showed 16 clinical trials out of which 9 were RCTs related to efficacy of Serratiopeptidase. Thus, a total of 14 RCTs were included in our review. Only those studies were included which were published and where full text was available excluding those with duplicate results. Various published studies have reflected the use of Serratiopeptidase for its anti-inflammatory, anti-edemic and analgesic effects. Some anecdotal studies suggest it to possess anti-atherosclerotic effects also.

There were 6 clinical studies (Table 1) supporting the anti-inflammatory effects of Serratiopeptidase. But among these clinical trials the dose and duration of therapy was not specified in 2 of these studies and were having small sample size, 3 were placebo-controlled trials, while 1 study with active comparator Seaprose S (SAP) had shown Seaprose S to be better than Serratiopeptidase. There were 3 studies in dental patients to see the postoperative anti-inflammatory effects of Serratiopeptidase (Table 1).

In one study by Khateeb et al.,²⁴ Serratiopeptidase + Paracetamol combination was compared to Placebo + Paracetamol in patients after surgical removal of impacted third molars. It was concluded that Serratiopeptidase + paracetamol group showed significant reduction in extent of cheek swelling and pain, but there was no difference in mean maximal interincisal distance between the two groups. Similar study by Chopra et al.²⁵ comparing Serratiopeptidase with ibuprofen/betamethasone/paracetamol/placebo showed serratiopeptidase group to have less mean pain scores and swelling as compared to placebo but the difference was not statistically significant. Moreover, serratiopeptidase was not superior when compared to ibuprofen and betamethasone. The third study by Surachai et al.²⁶ in patients with postoperative swelling after removal of impacted molars did not show any significant difference in degree of facial swelling in the Serratiopeptidase and no medication groups. It was a trial with small sample size and study design not mentioned.

Further 5 studies in different otorhinolaryngology pathologies^{1,16–20} have tried to explore the mucolytic properties of serratiopeptidase. Two were placebo-controlled trials, among which one study¹ showed Serratiopeptidase reduced the symptoms of severity of pain, amount and purulence of secretions but in the second study¹⁶ outcomes were unclear. Another prospective, open label study¹⁹ showed Serratiopeptidase to reduce the viscosity but not the elasticity of secretions in patients of chronic sinusitis. Similarly, one study²⁰ in chronic airway disease patients compared Serratiopeptidase with non-treatment group and concluded it to reduce sputum viscosity, elasticity and neutrophil count. There was only one study¹⁷ in chronic respiratory disease patients with difficulty in expectoration, where Serratiopeptidase was compared with an active comparator Seaprose S and this study showed Seaprose S to be better than Serratiopeptidase in relieving difficulty in expectoration and specific symptoms.

In some studies^{21–23} the role of Serratiopeptidase in increasing the effective concentration of antimicrobials in different tissues was studied. These showed that Serratiopeptidase increased the concentration of antimicrobials in tissues. But among these studies, one was prospective open label trial.²¹ One of the studies²² did not mention the duration of therapy and other was a randomised, open label trial in which Serratiopeptidase + cefotiam was administered

for prophylaxis before surgery, but again duration of treatment was not given.

Bandolier²⁷ online edition (retrieved on 1/5/2011) showed a review “Serratiopeptidase-finding the evidence”. This review concluded that there were no trials of Serratiopeptidase for treatment of back pain, neither there were any trials for its use in heart attack and stroke. Among the 9 RCTs included in this review which were generally said to be of poor methodological quality, five studies were described as double-blind: one was completely un-interpretable, three methodologically weak studies were positive and one trial of apparent high quality was negative.

3.1. Evidence from clinical and preclinical studies

A total of 24 studies on efficacy of Serratiopeptidase were included which met the inclusion criteria. Among these 18 were clinical and 6 were preclinical studies (Tables 1 and 2).

3.2. Clinical evidence

Serratiopeptidase has been used in Surgery (Traumatic and postoperative inflammation,^{4,14} venous inflammatory disease,¹⁵ cystitis, epididymitis³⁴), Orthopaedics (Traumatic swelling after sports injury,⁴ carpal tunnel syndrome,⁷ osteoarticular infection to increase antibiotic concentration at infection site²¹), Otolaryngology (Sinusitis, rhinitis, laryngitis, bronchitis, inadequate expectoration of sputum in bronchial asthma^{1,19,20}), Gynaecology (Engorgement of breasts^{5,35}), Dentistry (Anti-inflammatory and to increase antibiotic concentration at site of infection in periodontitis,³⁶ pericoronitis of wisdom tooth²⁵).

The evidence from clinical studies has been graded according to the checklist given by the Scottish Intercollegiate Guidelines Network (SIGN).¹² The grading showed that the studies supporting the anti-inflammatory and analgesic role of Serratiopeptidase are generally of poor methodology. Firstly the number of studies are only few. Secondly, the studies which are there, either have a small sample size (not more than 150 patients per group in any of the studies) or are of short duration. Moreover, the studies are mostly placebo-controlled trials and lack an active comparator. Also the eligibility criteria and outcome variables are not well defined and finally the statistical methods used for analysing the significance level have not been explained (Table 1).

3.3. Studies in animals

The studies in animals are very few. These have only demonstrated the anti-inflammatory effects of Serratiopeptidase. No specific model has been used in any of these studies to screen for the analgesic and anti-atherosclerotic effects of this enzyme in particular (Table 2). In 3 animal studies^{28–30} Serratiopeptidase was shown to increase the antimicrobial concentration at the site of infection. While in 2 studies^{32,33} using anti-inflammatory animal models it was shown that Serratiopeptidase demonstrated significant anti-inflammatory activity when compared to chymotrypsin, trypsin, aspirin and diclofenac. In another study,³¹ where Serratiopeptidase was compared to active comparator seaprose. Both enzymes showed reduction in viscosity of sputum, but the duration of therapy was not mentioned.

4. Source

Serratiopeptidase is derived from non-pathogenic enterobacteria belonging to genus *Serratia* species E-15. This microorganism was originally isolated during late 1960s from the intestines of silkworm *Bombyxmori* which is its natural habitat.

Table 1
Evidence from clinical and preclinical studies (clinical studies).

Clinical trial	Condition	No. of patients	Study design	Treatment	Control	Duration	Outcome	Sign for grading evidence
<i>Grading of evidence suggesting serratiopeptidase as anti-inflammatory agent</i>								
Esch et al. 1989 ⁴	Postoperative and traumatic swelling (repair of lateral collateral ligament of knee)	66	Randomised	Serratiopeptidase: dose not specified	Application of ice, leg elevation and bed rest	Unclear	Reduction in swelling by 50% on third postoperative day and pain disappeared by 10th day in TSP group	1–
Tsuyama et al. ¹³	Postoperative and traumatic swelling	98/group in two drug groups and 99 in placebo group	Randomised and double blind	Serratiopeptidase: dose not specified	A-4700 tablet and Placebo	Not stated	A-4700 shown to be equally effective to Serratiopeptidase in improving symptoms and reducing swelling	1–
Tachibana et al. 1984 ¹⁴	Postoperative buccal swelling (Caldwell-luc antrotomy)	174 (88 in TSP and 86 in placebo group)	Multicentre, randomised, double blind, placebo-controlled	Serratiopeptidase 10 mg thrice daily (30mg/day)	Placebo	7 days	Buccal swelling reduced significantly in serratiopeptidase group	1+
Bracale et al. 1996 ¹⁵	Superficial thrombophlebitis	40 (20 per group)	Randomised	Serratiopeptidase 10 mg thrice daily (30mg/day)	Seaprose S (SAP) 30 mg thrice daily (90mg/day)	14 days	65% of TSP while 85% cases of SAP showed improvement in specific symptoms like pain, erythema	1+
Kee et al. 1989 ⁵	Breast engorgement	70 (35 per group)	Randomised, double blind	Serratiopeptidase: 10mg thrice daily (30 mg/day)	Placebo	3 days	Moderate to marked improvement in breast pain, swelling and induration seen in TSP group	1+
Panagariya et al. 1999 ⁷	Carpel tunnel syndrome	20 patient	Prospective trial	Serratiopeptidase: 10 mg twice daily with initial short course of nimesulide	–	6 weeks	Clinical improvement seen in 65% patients	2–
–	Back pain, migraine	No randomised controlled trials	–	–	–	–	–	–
<i>Grading of evidence for its use in otorhinolaryngology pathologies</i>								
Mazzone et al. 1990 ¹	Acute or chronic ear, nose or throat disorder	193 (about 96 per group)	Multicentre, randomised, double blind, placebo-controlled	Serratiopeptidase (2 tablets of 5 mg) thrice daily, i.e., 30 mg/day	Placebo (2 tablets thrice daily)	7–8 days	TSP showed good efficacy in reducing symptoms like severity of pain, amount and purulence of secretions, difficulty in swallowing, nasal dysphonia and obstruction and anosmia	1+
Bellussi et al. 1984 ¹⁶	Secretory otitis media	75	Randomised and double blind (for 50 patients)	Serratiopeptidase (0.5 mg/kg/day)	Placebo	10 days	Undefined global judgement Numerous tests but outcomes unclear	1–
Nagaoka et al. 1979 ¹⁷	Chronic respiratory disease with difficult in expectoration	376 patients (125 in Seaprose S, 128 in TSP and 123 in placebo group)	Multicentre, randomised, double-blind, double dummy	Serratiopeptidase (10 mg thrice daily)	Seaprose S (15 mg thrice daily) and placebo	14 days	No significant difference between two groups in showing improvement in expectoration and specific symptoms	1+
Shimura et al. 1983 ¹⁸	Chronic respiratory disease (not bronchial asthma)	40 (<10/group)	Randomised	Serratiopeptidase 30 mg/day	Various mucolytic agents as comparator	7 days	Relaxation of sputum viscoelasticity	1–
Majima et al. 1988 ¹⁹	Chronic sinusitis	–	Prospective, open label	Serratiopeptidase 30 mg/day	–	4weeks	TSP reduced the viscosity of mucus but not its elasticity	2–

Nakamura et al. 2003 ²⁰	Chronic airway disease	29 patient (15 in treatment and 14 in non-treatment group)	Randomized, open label trial with non-treatment control group	Serratiopeptidase 30 mg/day	No treatment	4 weeks	TSP treatment decreased sputum weight, % solid component, viscosity and elasticity, sputum neutrophil count, and other symptoms	1–
<i>Grading of evidence for its role in increasing antimicrobial efficacy</i>								
Okumura et al. 1977 ²¹	Osteoarticular infection	8 patient	Prospective, open label	Serratiopeptidase 30 mg/day + sulbenicillin	Sulbenicillin alone	6 days	TSP increased transfer of sulbenicillin into exudates	2–
Brewer science library 1999 ²²	Perennial rhinitis, chronic rhinitis with sinusitis, chronic bronchitis	93 patient	Randomized, double blind	Serratiopeptidase + Cephalexin	Placebo + Cephalexin	–	TSP group showed improvement in rhinorrhoea, nasal stuffiness, coryza and paranasal sinus shadow	1–
Koyama et al. 1986 ²³	Lung cancer patients undergoing thoracotomy	35 patient (18 in serratio + cefotiam and 17 in cefotiam alone group)	Randomized, open label	Serratiopeptidase (20 mg three times per day) + Cefotiam 2 g	Cefotiam 2 g single dose	Prophylaxis before surgery (days not mentioned)	TSP group had significant higher level of antibiotic in tissues	1–
<i>Grading of evidence for use of serratiopeptidase in dentistry</i>								
Khateeb et al. 2008 ²⁴	Surgical removal of symmetrically impacted mandibular third molars	24	Prospective, intra-individual, randomized, double-blind, Cross-over study	Serratiopeptidase 5 mg + Paracetamol 1000 mg	Placebo + paracetamol 1000 mg	7 days	TSP group showed significant reduction in extent of cheek swelling and pain intensity but no difference in mean maximal interincisal distance between 2 groups.	1+
Chopra D et al. 2009 ²⁵	Postoperative sequel following removal of impacted 3 rd molar	150 patient divided randomly into 5 groups	Randomized, double-blind, placebo-controlled trial	Serratiopeptidase (20 mg thrice daily)	Ibuprofen 600 mg or betamethasone 0.5 mg or paracetamol 1 g or placebo each given thrice daily	7 days	TSP group showed less mean pain scores and swelling as compared to placebo but difference not statistically significant. Also TSP not superior when compared to ibuprofen and betamethasone	1+
Surachai et al. ²⁶	Postoperative swelling after removal of impacted molars	40 (20 per group)	Not stated	Serratiopeptidase (5 mg thrice daily)	No medication	5 days	No significant difference in degree of facial swelling and maximum mouth opening seen in two groups	2–

TSP: serratiopeptidase, and SAP: seaprose.

Table 2
Evidence from clinical and preclinical studies (preclinical studies).

Pre-clinical study	Condition	Animals	Treatment	Control	Duration	Outcome	Conclusion
Mecikoglu et al. 2006 ²⁸	Peri-prosthetic infection	Rats, number not specified	Serratiopeptidase (TSP) + Antibiotic (TSP + A)	Non-treatment (NT) and antibiotic (A) alone groups	2 weeks	Infection persisted in 63.2% in NT, 37.5% in A alone and 5.6% in TSP + A group	TSP effectively eradicated infection and enhanced antibiotic efficacy
Ishihara et al. 1983 ²⁹	Pleuritis and pneumonitis	Rabbits	Serratiopeptidase (TSP) + Cefotiam (C)	Cefotiam alone	30 minutes after injection in jugular vein	TSP increased plasma concentration of cefotiam in both conditions but tissue concentration increased only in pneumonitis group a no change in pleuritis group	TSP ↑ cefotiam efficacy
Aratani et al. 1980 ³⁰	Gingival infection by staphylococci	Rat	Serratiopeptidase (20 mg/kg) + four different antimicro-bials (100 mg/kg per orally)	Antimicrobials alone (Ciclacillin or ampicillin or cephalixin or minocycline)	Not mentioned	TSP ↑ gingival concentration of all four antimicrobial	TSP enhanced the efficacy of antimicrobials
Kase et al. 1982 ³¹	Subacute bronchitis	Rabbit	Serratiopeptidase (20 mg/kg)	Seaprose (SAP) 30 mg/kg	After intra-duodenal administration	TSP and SAP significantly reduced viscosity of sputum ($p < 0.05$) at 1–3 h and 4–6 h, respectively	Both enzymes have mucolytic activity
Jadav et al. 2010 ³²	Paw edema model	Albino rats	Serratiopeptidase (TSP) 10 and 20 mg/kg	Diclofenac 0.5 mg/kg	1, 2, 4 and 8 h after intraplantar formalin injection	TSP at both doses significantly inhibited acute inflammation of paw ($p < 0.0001$) and result comparable with diclofenac	TSP significantly showed anti-inflammatory activity.
Viswanatha Swamy 2008 ³³	Hind paw edema model and cotton pellet induced granuloma	Albino rats	Serratiopeptidase (TSP)	Chymotrypsi (Ch), Trypsin (T) and 2Aspirin (As)	3 h	TSP showed better anti-inflammatory activity as compared to Ch, T, and As	Serratiopeptidase showed significant anti-inflammatory activity

TSP: serratiopeptidase, A: antibiotic, and SAP: seaprose.

The enzyme helps the emerging moth to dissolve its cocoon. Serratiopeptidase is produced by purification from culture of *Serratia E-15* bacteria.³⁷

5. Chemistry

Several strains of bacteria have been isolated from the intestinal canal of silk worms,³⁸ of which five species of *Serratia* have been identified which are all gelatinolytic and produce extracellular protease. These include *S. indica*, *S. marcescens*, *S. plymuthica*, *S. piscatorum*, *S. species E-15*.³⁷ Out of these *Serratia* species strain E-15 produces proteases over three times as much as that produced by *Serratia marcescens*, which is a known protease-producing organism. The *Serratia* protease enzyme has a molecular weight of 45,000–60,000 and is a metalloprotease containing a zinc atom which is important for its proteolytic activity. The amino acid sequence deduced from the nucleotide sequence of the gene encoding for the enzyme reveals that the mature protein of the *Serratia* protease consists of 470 amino acids.³⁹ The enzyme was characterized to be free of any sulphur-containing amino acids like cysteine and methionine. It has been shown that such a type of enzyme protein was found as a protease in *Pseudomonas aeruginosa*⁴⁰ and *Aspergillus oryzae*.⁴¹ The enzyme showed maximal activity at pH 9.0 and at a temperature of 40 °C. The enzyme is completely inactivated by heating at 55 °C for 15 min.³⁷

6. Pharmacokinetics

After oral administration, Serratiopeptidase is absorbed through the intestines and transported directly into the bloodstream.^{42,43} But being a peptide there would be a high propensity of this enzyme to undergo enzymatic degradation in the gastrointestinal tract and low membrane permeability due to the hydrophilic nature of peptides and proteins in general.^{44,45} So these factors could lead to low bioavailability of this enzyme when used therapeutically.

The intestinal absorption of Serratiopeptidase was assessed by measuring its concentration in plasma, lymph and extract of inflammatory tissue of rats by sandwich enzyme immunoassay (EIA) technique.^{42,43} Serratiopeptidase was administered orally to rats and was detected from plasma at ≥ 30 mg/kg dose and in lymph at ≥ 1 mg/kg. Its concentrations in plasma and lymph were dose dependent. It was seen that the peak concentration in plasma and lymph at a dose of 100 mg/kg were 0.87 ± 0.41 and 43 ± 42 ng/ml, respectively, and this peak plasma concentration was achieved 0.25–0.5 h after the dose and disappeared by 6 h. Serratiopeptidase was also detected in carrageenan-induced inflammatory tissue in animals at concentrations higher than that in plasma. It was concluded in the study that Serratiopeptidase is absorbed from the intestine, distributed to the inflammatory site via blood or lymph. Thus, indicating that orally administered Serratiopeptidase is absorbed from intestinal tract and reaches circulation in an enzymatically active form.^{42,43} In rat blood it exists as a complex with plasma protease inhibitor alpha-1 macroglobulin (α_1M) with a molar binding ratio of 1:1 which helps to mask its antigenicity but still retains 20% of its original caseinolytic activity.³ However, pharmacokinetic data including its oral bioavailability in humans is not mentioned anywhere nor is the specific concentration required for its therapeutic action.

7. Pharmacodynamics

Serratiopeptidase is thought to work in three ways.

1. *Anti-inflammatory*: serratiopeptidase reduces swelling by the process of decreasing the amount of fluid in the tissues,

thinning the fluid, and by facilitating the drainage of fluid. In addition, its enzyme activity dissolves dead tissue surrounding the injured area so that healing is accelerated.^{5,6} It may also act by modifying cell-surface adhesion molecules that guide inflammatory cells to their target site of inflammation. These adhesion molecules play an important role in the development of arthritis and other autoimmune diseases.⁶

2. *Analgesic*: it may help alleviate pain by inhibiting the release of pain-inducing amines like bradykinin from inflamed tissues.¹
3. *Fibrinolytic/caseinolytic*: it may be beneficial in atherosclerotic disease as it acts by breaking down fibrin and other dead or damaged tissue without harming living tissue. This could enable the dissolution of blood clots, and atherosclerotic plaques.²²

8. Formulation

Being a protein the major challenge in developing a formulation for this enzyme has been to retain the backbone and folding structure of this enzyme protein during manufacturing, storage and also during digestion and absorption in the stomach and intestines.⁴⁶ To overcome degradation by enzymes in the gastrointestinal tract, Serratiopeptidase is available as an enteric coated tablet. This enteric coating consists of pH sensitive polymers which remains intact in the gastric acidic pH (1.5–3.5) and solubilises in the more favourable alkaline pH (6.5–7.6) of the small intestines.⁴⁷

9. Safety

There are not many published reports of Adverse Drug Reactions (ADRs) to Serratiopeptidase. The only information available is drug companies monographs. The ADRs include allergic skin reactions which could range from dermatitis to extreme cases of Stevens–Johnson syndrome or erythema multiforme, muscle aches and joint pains, gastric disturbances like anorexia, nausea and abdominal upset, cough rarely pneumonitis⁴⁸ and coagulation abnormalities.

10. Dosage

The dose which has been used in clinical studies and mentioned in drug monographs ranges from 10 mg to 60 mg per day. It has to be taken on an empty stomach or at least two hours after eating, and no food should be consumed for about half an hour after taking Serratiopeptidase. The recommended dose of Serratiopeptidase for specific indications, in particular is not mentioned anywhere.

The enzyme activity is measured in units. Serratiopeptidase 10 mg is equal to 20,000 units of enzyme activity.

11. Drug interactions

There are no clinical studies reporting any drug interactions. The only available information is by drug company monographs.

If administered along with Warfarin, Clopidogrel or Aspirin as well as with other natural remedies such as garlic, fish oil and turmeric, there may be increased risk of bleeding or bruising.

12. Discussion

Serratiopeptidase, a proteolytic enzyme, has been used for almost 40 years in Japan and Europe for pain and inflammation. It is usually available as a fixed dose combination with various Non Steroidal Anti-inflammatory Drugs (NSAIDs) like diclofenac, aceclofenac, paracetamol. It is also given along with various

antimicrobials to increase their tissue penetration, e.g. cephalexin, cefotiam, sulbenicillin, etc., by its proteolytic effects.

When combined with NSAIDs it acts as an anti-inflammatory agent. But the efficacy of Serratiopeptidase alone as an anti-inflammatory agent needs to be evaluated in a more elaborate way. In particular the analgesic activity of Serratiopeptidase, which is said to be due to its ability to block the release of biogenic amines, needs to be evaluated in a more comprehensive way by using appropriate analgesic animal screening models. The evidence showing its anti-atherosclerotic effects is only anecdotal. Thus, further extensive clinical studies are needed to prove its worth to be used as a health supplement. There are 2 animal and 8 clinical studies (including 2 studies in dental patients) supporting its use as anti-inflammatory agent but larger, well-designed, controlled trials are warranted to clearly prove its efficacy. Also data on tolerability and long-term safety of this enzyme is lacking. This may be of particular relevance when it is prescribed to patients for anti-atherosclerotic effects.

During the preparation of this review we have done a thorough search of the literature to include all the relevant studies dealing with the efficacy of Serratiopeptidase, but it is possible that some studies may have been missed which have not been published or registered with any of these search engines. Further retrieving all the information from the past is not always possible. Moreover, during grading using SIGN checklist, separate reviewers did the grading and then coordinated together to validate the final grading. Any disagreement between the two reviewers was resolved by discussion with the third reviewer. This was done to minimise bias but still the risk of bias cannot be ruled out completely.

Until there is clear scientific evidence supporting the efficacy of Serratiopeptidase as anti-inflammatory, analgesic and anti-atherosclerotic agent, it is not rationale to prescribe this enzyme on the basis of few studies whose scientific credibility is still not very clear. Thus in future, more well-designed, active comparator controlled trials are needed to clearly define the beneficial effects of this natural enzyme claiming to possess miraculous properties.

Author contribution

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No conflicts of interest with any entity.

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