ANTI-INFLAMMATORY EFFECT OF THE SERRATIOPEPTIDASE – RATIONALE OR FASHIONABLE: A STUDY IN RAT PAW OEDEMA MODEL INDUCED BY THE CARRAGEENAN

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Abstract: Introduction – Many drugs, including serratiopeptidases, are marketed without proven efficacy in clinical trials. It is protein in nature and claimed to be effective orally.

Methods – 24 albino wistar rats, 6 each in following groups were assigned – (1) Control group (distilled water, orally) (2) Diclofenac (6.75 mg/kg, IP) (3) serratiopeptidase (5.4 mg/kg, orally) (4) Combination of serratiopeptidase (5.4 mg/kg, orally) and diclofenac (2.25 mg/kg, IP). Inflammatory agent, carrageenan (0.1 ml of 1% w/v) was injected subcutaneously in the ether anesthetized rat hind paw, half an hour after the administration of drug. Rat paw volume up to lateral malleolar process was recorded with plethysmometer at various time periods.

Results – Percentage formation and inhibition of oedema in serratiopeptidase or combination groups were not significantly different than control group. Both were significantly less for diclofenac group.

Conclusion – Serratiopeptidase was not effective in this animal model of oedema/inflammation.

Key words: enzyme analgesic anti-inflammatory drugs rational use of drugs

INTRODUCTION

Inflammation is one of the most common suffering of human being and anti-inflammatory drugs are one of the most commonly used one. Probably, most commonly used drugs are Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). However as they are effective, they are associated with side effects as well as cost to the patients. Thus their judicious use is mandatory. There are many drugs which are marketed without clear scientific evidence of efficacy. One such drug is serratiopeptidase.

Serratiopeptidases are obtained from bacteria, *Serratia* species, belonging to enterobactericeae family (1). It is a protein and endopeptidase in nature with molecular weight of 60 daltons (2). Generally, proteins
are digested to constituent amino acids and then amino acids are absorbed, not the parent protein molecule. Still this drug is claimed to be efficacious when given orally.

This drug is not marketed as drug in western world where regulatory bodies are very strict and they issue notices from time to time to drug manufacturers (3-4). They are marketed and promoted in countries like India and other south East Asian countries (5). This drug does not find its mention in US pharmacopeia (6) or British National Pharmacopeia (7) or Indian Pharmacopeia (8); nor in standard textbook of pharmacology like – Goodman and Gilman (9).

Thus we wanted to evaluate the anti-inflammatory effect of this drug in carrageenan induced rat paw oedema model. (10) This is one of the models used for the evaluation of anti-inflammatory effect in animals. The study objectives were to measure the absolute oedema values, to assess the change in rat paw volume at various time points as compared baseline and inhibition of oedema formation as compared to control group. Adverse effects or death among the experimental animals was also noted.

**MATERIALS AND METHODS**

Experiment began after written approval from Institutional Animal Ethics Committee, registered under Animal (Scientific Procedures) Act, 1986. A homogenous group of adult albino wistar rats (14 males and 10 females) were included in the study. Animals were procured from Haffkine Institute, parel, Mumbai. They were housed in animal house. One week time was allowed for their acclimatization. They were given water and food ad libitum. They were kept nil by mouth for overnight before the day of experiment.

The experiment consisted of 24 animals, 6 each in 4 groups:–

1. Control group given distilled water orally
2. Diclofenac sodium IP (6.75 mg/kg, Intra-peritoneally [IP]).
3. Serratiopeptidase group (5.4 mg/kg, orally)
4. Combination of serratiopeptidase (5.4 mg/kg, orally) and diclofenac sodium (subtherapeutic dose 2.25 mg/kg, IP). Sub-therapeutic dose was used to evaluate the additional synergistic effect of serratiopeptidase, if any.

Then, 0.1 ml of 1% (w/v) of freshly prepared carrageenan solution was injected subcutaneously in the anesthetized rat hind paw, half an hour after the administration of drug, in morning time approximately between 9–10 am. Rats were anesthetized using ether soaked cotton rolls placed under bell jar. Then rat paw volume up to lateral malleolar process was recorded at 0 (baseline – immediately after the injection of carrageenan), 1, 2, 3, 4, 5, 6 and 24 hours respectively. After the carrageenan injection rats were allowed to feed ad libitum. The volume of rat hind paw was recorded in millimeters of displacement of mercury in plethysmometer.

**Efficacy parameters**

Absolute oedema scores were recorded.
These recordings were further analyzed by two formulae – percentage oedema and percentage inhibition of oedema.

1. Percentage oedema formation within group (compared to baseline ‘0’ hours) =
   \[
   \frac{\text{Volume at Nth hour (Vn) – Volume at base line (Vo)}}{\text{Volume at baseline (Vo)}} \times 100
   \]

2. Percentage oedema inhibition compared to control between groups at same time point =
   \[
   \frac{\text{Volume of the test group (Vt) – Volume of control at same hour (Vc)}}{\text{Volume of control group (Vc)}} \times 100
   \]

These were recorded as per above time points and are mentioned in observation table.

**Statistical analysis**

The quantitative data was expressed as mean±standard deviation (SD) while qualitative data was expressed as proportion or percentages. The percentage oedema formation within group was analyzed using repeated measures ANOVA followed by Dunnet’s post test. One way ANOVA with Dunnet’s post-hoc test was used for the analysis of percentage edema formation between groups. The statistical analysis was done using graphpad instat software version no. 3.7. The P value less than 0.05 was considered as statistically significant.

**RESULTS**

The oedema scores in all four groups are mentioned in Table I.

Significant rise in paw volume was seen in control, serratiopeptidase and in the combination group at many time point interval (Table I). The rise in oedema volume for diclofenac group was statistically significant only at 3, 4 and 6 hours.

The oedema formation within the groups rose uniformly from 1 hour to 6 hours and

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Control group (mean in mm±SD)</th>
<th>Diclofenac group (mean in mm±SD)</th>
<th>Serratiopeptidase group (mean in mm±SD)</th>
<th>Combination group (mean in mm±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.08±0.58</td>
<td>3.44±0.42</td>
<td>4.07±0.64</td>
<td>4.72±0.41</td>
</tr>
<tr>
<td>1</td>
<td>3.38±0.69</td>
<td>3.76±0.66</td>
<td>4.58±0.55**</td>
<td>5.38±0.48**</td>
</tr>
<tr>
<td>2</td>
<td>3.63±0.89</td>
<td>3.92±0.76</td>
<td>4.98±0.59**</td>
<td>5.80±0.55**</td>
</tr>
<tr>
<td>3</td>
<td>3.80±0.92</td>
<td>4.12±0.98**</td>
<td>5.55±0.80**</td>
<td>6.12±0.54**</td>
</tr>
<tr>
<td>4</td>
<td>3.85±1.06</td>
<td>3.98±0.84*</td>
<td>5.55±0.77**</td>
<td>6.32±0.48**</td>
</tr>
<tr>
<td>5</td>
<td>4.40±0.89**</td>
<td>3.88±0.68</td>
<td>5.78±0.85**</td>
<td>6.55±0.45**</td>
</tr>
<tr>
<td>6</td>
<td>4.55±0.97**</td>
<td>3.96±0.49*</td>
<td>6.05±0.79**</td>
<td>6.70±0.47**</td>
</tr>
<tr>
<td>24</td>
<td>4.28±99**</td>
<td>3.74±0.42</td>
<td>4.67±0.66**</td>
<td>5.50±0.57**</td>
</tr>
<tr>
<td>P value (by repeated measures ANOVA)</td>
<td>0.0004</td>
<td>0.0435</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*The volume measurements represent the measurements of length mercury column displacement in mm in plethysmometer. *P value <0.05, **P value <0.01 and ***P value <0.001 by Dunnet’s post-hoc test.
after that was found to decline at 24th hour. As compared to control group values, the percentage oedema formation was significantly less only in diclofenac group at 4, 5, 6 and at 24 hours. Serratiopeptidase and combination group did not show significant reduction as compared to control group values at all time intervals (Table II and Fig. 1). The percentage inhibition of oedema compared to the control group was found more in the diclofenac group than serratiopeptidase group or combination group at all time points (Table III and Fig. 2).

![Percentage Change in Oedema]

**Fig. 1**: Percentage oedema formation compared to baseline ('0' hr value) for all experimental groups.
The negative sign indicates inhibition of oedema while positive value indicates increase in oedema.

**DISCUSSION**

There are many doubts raised about the efficacy of serratiopeptidases (11). It is marketed as orally effective anti-inflammatory drug despite of its protein nature. There are many enteric coated formulations available which claim that they are not destroyed by gastric acid and get absorbed by intestines, circulate in blood to reach the site of inflammation. This further raise issues like even after enteric coating how it is protected from intestinal digestive enzymes – esp. proteases – those of its own kind. Though there is some evidence that proteins may be absorbed orally, it is applicable to only few proteins in ‘extremely low’ amounts (12-13). This amount may not sufficient to produce efficacy of a ‘protein drug.’ In addition to this there is hardly any evidence present for the oral absorption of serratiopeptidases. Also, why it is not recognized as foreign protein by immune system and not fixed by it remains to be answered. The other pharmacokinetic data of the drug is also not well studied – for e.g. protein binding, metabolism, tissue penetration; half life and volume of distribution are not known. Its status of use in pregnant and lacting females is not known.

Our results demonstrated that there is
lack of efficacy of serratiopeptidases when given alone. Also, in combination with sub-therapeutic dose of diclofenac no additional synergistic effects were observed. We have chosen sub-therapeutic dose of diclofenac in combination group with the expectation to observe the anti-inflammatory properties of serratiopeptidase, if any. However, this study failed to show anti-inflammatory effects of serratiopeptidase alone or in combination. Diclofenac alone in therapeutic doses was effective in reducing oedema. The results obtained were different in another study with formalin injection model for induction of acute as well as chronic oedema. In this study serratiopeptidase group was found to be effective equally as compared to diclofenac (0.5 mg/kg) (14). In another similar animal experiment, it was found that serratiopeptidases were effective when injected into joint during eradication of staphylococcal infection. However it was given locally, not orally. In another experiment for evaluation of distribution of cefotiam in lung and trachea of rabbits combined with serratiopeptidases (injected), it was found that cefotiam concentration was not significantly increased in respiratory track (15).

The evidence of efficacy of serratiopeptidase is based on some animal experiments, personal letters or uncontrolled trials with poor quality. Whatever studies conducted in randomized double blind manner are generally of poor quality with either randomization failure or other methodological flaws in it (16).

Thus firm evidence about efficacy of serratiopeptidase is lacking. Also, ignorance of safety data of such agents may be dangerous. US FDA regularly serves notices to marketing of the drugs which are not approved. It even serves notices to online marketing websites (17). It is requirement of US FDA to do at least one placebo controlled clinical study to get approval for the marketing. However in India such norms are rarely followed. Its lack of mention of this drug in standard textbooks of pharmacology shows that it is not unanimously accepted by medical community.

Serratiopeptidase, alone or in combination with NSAIDs is very costly and prescribed very commonly by physicians and more commonly by surgeons. This adds significantly to cost of treatment. It is claimed to have no side effects (18). When asked about the drug, many physicians were also unaware about the active ingredient in various formulations, its mechanism of action, rationale of combination with NSAIDs and cost of treatment. Pharmaceutical companies make aggressive marketing of such drugs and doctors are pursued to prescribe it. Sadly so, there lies hardly any scientific rational for it.

Below is the comparison of cost of various formulations of serratiopeptidase either alone or with diclofenac combination as compared to diclofenac alone. One can clearly observe the combination or serratiopeptidase monotherapies are costlier. [19] The average cost of plain serratiopeptidase is more than 43 Indian rupees (INR) whereas diclofenac alone costs Rs 8.5. The combination of diclofenac and serratiopeptidase ranges from 31 to 71. This similarly applies to aceclofenac and ibuprofen based combinations. Even combinations with tramadol are also available.
Rational use of medicine is important. Often drugs are prescribed ‘fashionably’, but not rationally. There are extensive marketing campaigns by pharmaceutical companies for these drugs. In countries like India, where affordability of medicines is still very low, one should be very cautious about prescribing such drugs. The readers should also acknowledge the limitations of this study like use of only one animal model or only one inflammatory agent and small number of rats per group. Thus, we encourage further experimental and clinical research in this area.

About 40 years before, Prof. Cochrane, a pioneer in clinical epidemiology, coined a concept of evidence ladder stepped by efficacy (can it work?), effectiveness (does it work?) and cost-effectiveness (is it worth using?). In the era of evidence based medicine, lack of firm efficacy data, while effectiveness and cost-effectiveness is far away, poses an objection on the use of such drugs. There must be further research on these aspects of a drug, apart from simply efficacy. Also, there should be emphasis on the rational use of drugs in the medical curricula.

Conclusion

From this animal experiment, it could be concluded that serratiopeptidase is not effective as an anti-inflammatory agent. Further it adds to cost and may add to side effects as well. Thus it is recommended to conduct human studies and find out its efficacy in humans.

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