COMPARATIVE STUDY OF ANTI-INFLAMMATORY ACTIVITY OF ROSUVASTATIN WITH THAT OF ASPIRIN IN ALBINO RATS

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ABSTRACT

BACKGROUND

Statins are HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitors well known for their hypolipidemic action. More recently, there has been an increased interest in pleiotropic effects of statins like anti-inflammatory action that occurs independent of their lipid lowering effect. Statins exert anti-inflammatory action by preventing the isoprenylation of Rho proteins, a family of small G proteins and subsequent disruption of their functions. Hence, the present study was planned to compare the anti-inflammatory effect of rosuvastatin with that of aspirin on acute inflammation, so that it could be utilised in acute inflammatory conditions as an adjuvant or as a monotherapy avoiding adverse effects of commonly used anti-inflammatory agents like NSAIDS (nonsteroidal anti-inflammatory drugs).

The aim of the study is to compare anti-inflammatory effect of rosuvastatin with that of aspirin on carrageenan-induced inflammation in albino rats.

MATERIALS AND METHODS

18 adult albino rats weighing between 100-150 g of either sex were divided into 3 groups. Control, Standard and test receiving oral normal saline (2 mL/kg), aspirin (100 mg/kg) and rosuvastatin (5 mg/kg) drug solutions, respectively. Acute inflammation model of carrageenan-induced paw oedema was used as tool. An hour after the administration of the drugs to each group, paw oedema was induced with intradermal injection of 0.1 mL of carrageenan (1%) into the plantar surface of the right hind paw of each rat. Volume of inflamed paw was determined using a plethysmometer immediately and also at 30, 60, 120 and 180 minutes after injection. Finally, mean paw volumes at different time intervals were calculated and percentage inhibition of paw oedema with standard and test drugs were determined.

RESULTS

Data was analysed using by Analysis of Variance (ANOVA). Results showed that rosuvastatin has statistically significant ($P<0.05$) anti-inflammatory action reflected by percentage inhibition of paw oedema at different time intervals when compared to control. The antiinflammatory action of rosuvastatin was found intermediate to that of standard (aspirin) and control (normal saline).

CONCLUSION

From this study, it is evident that statins does have anti-inflammatory action. This study results also indicate that statins anti-inflammatory effect is more in late phase of acute inflammation probably due to inhibition of new synthesis of proinflammatory agents. NSAIDS and statins, both of them act via different mechanisms and their actions could be additive. Hence, rosuvastatin could be a promising adjuvant to existing anti-inflammatory drugs in acute inflammatory condition particularly with hyperlipidaemias.

KEYWORDS

Inflammation, Statins, Aspirin, Carrageenan, Paw Oedema, Plethysmometer.

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BACKGROUND

Inflammation is a response triggered by irritation, injury or infection of living tissue, characterised by pain, redness, swelling and sometimes loss of function. Inflammation is broadly classified as acute inflammation and chronic inflammation. Acute inflammation is immediate response to an injurious agent. It is rapid in onset and is of short duration, involves vasodilatation due to action of several mediators like histamine and nitric oxide (No). It is followed by increased permeability of the microvasculature

causing oedema. Tissue macrophages, mast cells and endothelial cells secrete several cytokines, which in turn leads to expression of proteins like selectins and their ligands, which are involved in leucocyte migration at target site. These leucocytes are activated to eliminate the offending agent. Chronic inflammation is inflammation of prolonged duration characterised by presence of lymphocytes and macrophages, proliferation of blood vessels, fibrosis and tissue destruction. Sometimes, inflammation can cause injury and disease when it is inappropriately directed against self-tissues. For instance, hypersensitivity reactions, rheumatoid arthritis, atherosclerosis and it also play a role in type 2 diabetes, Alzheimer disease and cancer.[1]

NSAIDS (nonsteroidal anti-inflammatory drugs) control inflammation by inhibiting synthesis of inflammatory mediators by acting on cyclooxygenase (COX). Other class of drugs, mainly steroidal anti-inflammatory drugs inhibits phospholipase A_2. However, inhibiting COX, there is a possibility of arachidonic acid diversion towards lipoxygenase pathway that could increase the production of inflammatory leukotrienes. NSAIDS and corticosteroids are also associated with many adverse effects.[2] Recently, because of coronary thrombosis in patients taking COX-2 inhibitors some prominent members of this class were withdrawn from the market.[3]

Statins are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors commonly used hypolipidemic drugs. Statins have been supported by both experimental and clinical studies to possess various pleiotropic effects like anti-inflammatory, immunomodulatory, plaque stability and endothelial dysfunction improvement.[3] The level of Inducible Nitric Oxide Synthase (iNOS) protein in cells can be increased over 1000 fold by inflammatory stimuli, which in turn causes increased synthesis of NO and finally vasodilatation. Statins markedly reduced such (iNOS) protein level as assessed by immunohistochemistry. Statins augment endothelial type NO synthase (eNOS) activity by inhibiting small GTPase protein Rho activity.[4,5,6] Rosuvastatin could be a promising adjuvant anti-inflammatory drug with lesser side effects than other anti-inflammatory drugs since it acts via different mechanism. Hence, present study is carefully planned to compare anti-inflammatory activity of rosuvastatin with that of aspirin in albino rats.

MATERIALS AND METHODS

Adult albino rats weighing between 100-150 g of either sex were used for experiment. 18 rats were produced from the central animal house. They were housed in cages in standard laboratory conditions with natural light and dark cycle and at room temperature. The protocol was approved by Institutional Animal Ethics Committee (IAEC) as per guidelines of CPCSEA.

Anti-inflammatory activity of aspirin and rosuvastatin is evaluated in acute inflammatory model of carrageenan-induced paw oedema in albino rats. Animals were divided into the following three groups containing 6 animals in each group. Groups - Control, standard and test receiving oral normal saline, aspirin and rosuvastatin drug solutions, respectively. After an hour of drug administration to each group, paw oedema was induced by an intradermal injection of 0.1 mL of carrageenan (1%) into the plantar surface of the right hind paw of rats. Acute phase of inflammatory reaction, i.e. oedema volume of right hind paw was determined using a plethysmometer immediately and also at 30, 60, 120 and 180 minutes after Carrageenan subplantar injection and all values were tabulated. After the experiment, animals were kept under observation for 24 hrs. period and then transferred back to animal house.

Carrageenan-Induced Paw Oedema Model

Carrageenan is an irritant or inflammogen or a phlogistic agent. Chemically, it is a sulfated polysaccharide from seaweeds. The experimental tissue injury caused by this irritant initiate inflammation is biphasic in nature. The first phase is attributed to the release of histamine, 5-HT and kinins, while the second phase is related to the release of prostaglandins. The well recognised method of Winter et al, 1962 is followed. A 1% w/v suspension of carrageenan prepared freshly is normal saline and injected into subplantar region of right hind paw (usually, 0.1 mL in rats and 0.025-0.05 mL in mice). In control group animals, only vehicle is injected. Test drug usually administered orally or intraperitoneally according to body weight immediately half an hour or one hour before carrageenan challenge. To each rodent, a mark is made at their ankle joint. In drug treated and untreated groups, paw volume is measured up to the ankle joint before and after 3 hours after carrageenan challenge using a plethysmograph filled with mercury. The sophisticated electronic devices are being used nowadays to record the paw volume of rodents. Oedema is found out and % reduction in oedema is calculated.[7,8]

After calculating the mean paw volumes at different time intervals, percentage inhibition of paw oedema by standard and test drug was calculated using the following formula-

$$\% \text{ inhibition} = ((Vc - Vt) \times 100)/Vc.$$

Where, Vc =mean of increased paw volumes in control group, Vt = mean of increased paw volumes in test group, (Vs = mean of increased paw volumes in standard group used in place of 'Vt' to calculate % inhibition by standard drug).

Preparation of Solution of Drugs

1. Aspirin- Standard solution of aspirin is prepared by dissolving the tablet (Asicom 75 mg Comed) in 5 mL of distilled water. The solution has a concentration of 15 mg/mL.
2. Rosuvastatin- Standard solution of rosuvastatin was obtained by dissolving tablet form (rosuvas 5 mg Ranbaxy) in 5 mL of distilled water. The solution has concentration of 1 mg/mL.
3. Normal saline- Normal saline acted as control and was administered as 2 mL/kg.

4. Carrageenan- Carrageenan 1% in 0.9 normal saline was freshly prepared and was used to induce inflammation into the rats paw.

Doses of Drugs-

1. C = Control group receiving normal saline (2 mL/kg).
2. S = Standard group receiving aspirin (100 mg/kg).
3. T = Test group receiving rosuvastatin (5 mg/kg).

Observations- Increase in paw volumes after subplantar carrageenan injection at different time intervals was obtained using mercury plethysmometer in each group and tabulated. Data was then used to calculate mean increase in paw volumes, percentage inhibition of paw oedema in different experimental groups and were tabulated.

Statistical Analysis

Comparison between different groups (aspirin and rosuvastatin) was done by One-Way Analysis of Variance (ANOVA). P value less than 0.05 was considered statistically significant.

Group	N	Mean ± SEM (Standard Error of Mean) Paw Volumes in µmL				
		Initial	At 30 min	At 60 min	At 120 min	At 180 min
C	6	156.2±1.92	163.5 ± 1.78	178.0 ± 1.18*	185.8 ± 1.44*	190.2 ± 1.61
S	6	153.7 ± 2.29	159.3 ± 2.37	166.2 ± 1.85*	170.4 ± 1.78*	174.3 ± 2.0*
T	6	154.3 ± 1.89	160.2 ± 1.62	171.2 ± 1.57*	177.8 ± 1.74*	182.8 ± 1.66*

Table 1. Paw Oedema Volume in Different Groups at Different Time Intervals

In the above table, C = Control group receiving normal saline (2 mL/kg), S = Standard group receiving aspirin (100 mg/kg), T = Test group receiving rosuvastatin (5 mg/kg), N = number of animals in a group. Statistical significant test for comparison was done by ANOVA, *P<0.05 is considered as significant.

Group	N	Percentage Inhibition				
		30 mins.	60 mins.	120 mins.	180 mins.	
S	6	23.4	47.7*	45.8*	42.4*	
T	6	19.1	29.6*	25.1*	21.0*	

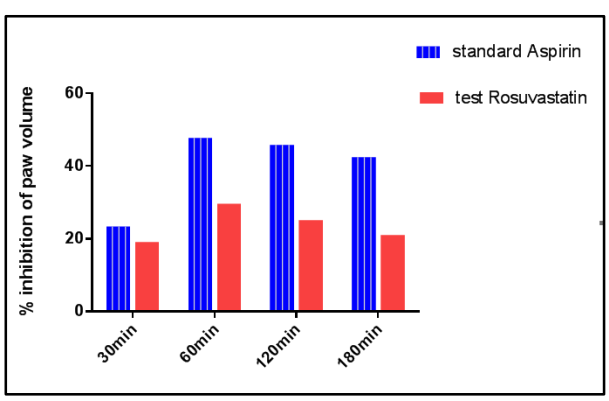
Table 2. % Inhibition of Paw Volume in Different Groups at Different Time Intervals When Compared to Control (C)

S= Standard group, T= Test group, N= Number of animals in a group; *P <0.05 is considered as significant.

Percentage inhibition of paw oedema by standard and test drugs was calculated using the following formula-

% inhibition by test drug = ((Vc - Vt) × 100)/Vc,

Where, Vc =mean of increased volumes in control group, Vt = mean of increased volumes in test group at respective time intervals; (Vs = mean of increased volumes in standard group used in place of 'Vt' to calculate % inhibition by standard drug).



% Inhibition of Paw Volume in Different Groups at Corresponding Time Intervals

RESULTS

Table 1 data shows mean paw volumes of different groups. It is seen from the table that mean basal (zero minute volumes) paw volumes was comparable in all the groups (control- normal saline, standard- aspirin and test-rosuvastatin). There was no significant difference in mean paw volumes of different groups at zero minutes.

From the same table, at 60 minutes, 120 minutes and 180 minutes, the mean paw volume in all the drug-treated groups was statistically significantly lower when compared to control group (P <0.05). In initial 30 minutes, there is no statistically significant difference in the mean paw volumes.

Table 2 percentage inhibition of acute inflammation was greater in aspirin group when compared to rosuvastatin at all-time intervals and the same results are expressed in bar in graph 1.

DISCUSSION

Inflammation is a complex reaction in tissues that consists mainly of the vascular and cellular events that triggered different inflammatory stimuli. Anti-inflammatory drugs works on different aspects of the inflammatory cascade including the synthesis and action of mediators. NSAIDs provide symptomatic relief in acute and chronic inflammation, but do not improve the course of chronic conditions such as rheumatoid arthritis.[9] Statins have been widely used in the treatment of dyslipidaemia since long. More recently, there has been an increased interest in the pleiotropic effects like antiinflammatory, immunomodulatory, plaque stability and endothelial dysfunction improvement. These effects are unrelated to their lipid-lowering action.[10,11,12,13]

In the model of acute inflammation, both simvastatin and atorvastatin showed anti-inflammatory activity comparable to aspirin. Simvastatin therapy significantly

decreased high sensitivity C-reactive protein in patients of metabolic syndrome compared with placebo and resulted in a significant reduction in plasma and lipopolysaccharide-activated monocytic release of interleukin 6 (IL-6) and TNF. Hence, it appears that statins can effectively suppress both acute as well as chronic inflammation by inhibiting the release of various mediators of inflammation.[14,15]

Present study has revealed that statins (rosuvastatin) do have anti-inflammatory activity evident from significantly low mean paw volume, comparatively less increase in paw volume and good percentage inhibition of paw volume. This experimental study suggests that rosuvastatin has anti-inflammatory effect, which is more than control, but less than that of aspirin. The anti-inflammatory activity of rosuvastatin on late phase of acute inflammation induced by carrageenan can be attributed to inhibition of iNOS, inhibition of recruitment leucocytes and interference in process of chemotaxis. Exact mechanism needs to be evaluated. There are evidences from both experimental and clinical studies that statins do have anti-inflammatory activity. Hence, there is a possibility of using statin along with NSAIDS and other anti-inflammatory drugs, so that there is reduction in doses of other anti-inflammatory drugs, there by reduction in the incidence of adverse effects of the later.

With the knowledge and background research information about anti-inflammatory action of statins, proposed mechanism behind different pleiotropic effects of statins, there is a lot of scope for utilising them for benefit of human in different diseases. Few studies evaluating statins use in different human diseases like cardiovascular diseases, type 2 diabetes, Alzheimer's disease, rheumatic diseases and more are already in progress. Our study has revealed that like other statins, rosuvastatin also has anti-inflammatory effect. Therefore, in future, it requires animal studies and large clinical trials to evaluate statins beneficial effects as an adjuvant to existing anti-inflammatory drugs or as monotherapy in the treatment of both acute and chronic inflammatory conditions preferably when associated with hyperlipidaemias.

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