

EFFECT OF ATORVASTATIN, LOVASTATIN, AND ROSUVASTATIN ON INFLAMMATION IN WISTAR RATS.

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Summary

To evaluate the effects of atorvastatin, lovastatin, and rosuvastatin in acute and subacute model of inflammation and to elicit their interaction with aspirin. Atorvastatin, lovastatin and rosuvastatin in clinical equivalent doses and aspirin in the dose of 54 and 200 mg/kg were administered orally in different groups of Wistar rats weighing 175±25g to study their effect on acute inflammation induced by carrageenan and subacute inflammation induced by subcutaneous implantation of foreign bodies in the axillae/groin. The animals were administered a single dose of various drugs in acute studies and the treatment was repeated once daily for 10 days in subacute studies. On 11th day granulomas were dissected out for noting their dry weight and histopathological studies. For interaction studies the sub anti-inflammatory (SAI) dose of aspirin (54mg/kg) was co-administered with that of atorvastatin (1mg/kg), lovastatin (1mg/kg), and rosuvastatin (1mg/kg) in separate groups. All the three statins individually decreased significantly (P<0.05) pedal edema, granuloma dry weight and granuloma formation. In interaction studies the SAI dose of lovastatin and rosuvastatin produced similar effects when co-administered with sub anti-inflammatory dose of aspirin in both the models of inflammation; while atorvastatin did so only in subacute model. Atorvastatin, lovastatin and rosuvastatin not only showed significant anti-inflammatory activity in both the models of inflammation, but also potentiated anti-inflammatory activity of aspirin in subacute model and atorvastatin failed to do so in acute model.

Keywords: Aspirin, atorvastatin, inflammation, lovastatin, rosuvastatin.

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Introduction

Statins are widely used in clinical practice to treat a variety of dyslipidemia and their clinical efficacy in controlling atherosclerosis, an outcome of prolonged hyperlipidemia is well established. Atherosclerotic endothelial dysfunction is one of the major cause of vascular diseases. Pathogenesis of atherosclerosis comprises a series of highly specific cellular and molecular responses that justify it, as an inflammatory disorder.^[1] Investigations have shown that modified low density lipoproteins(LDL) by injuring the endothelium and underlying smooth muscles to be a major cause for vicious circle of inflammation^[2] that sets in atherosclerosis. Statins not only lower the lipoproteins but, by lowering LDL may break the vicious circle of inflammation. Clinically used statins have been reported to possess other pharmacological actions like ROS scavenging,^[3] P-selectin suppression,^[4] and increased eNOS (endothelial nitric acid synthetase)^[5] suggestive of their anti-inflammatory activity. Similarly, clinical studies have shown atorvastatin to be effective in rheumatoid arthritis^[6] and atorvastatin^[7] as well as lovastatin^[8] in myocardial infarction, both of which are inflammatory conditions. However, certain in-vitro studies have reported lipophilic statins like atorvastatin and lovastatin to be pro-inflammatory.^{[9],[10]} Similarly, some clinical studies indicate neutral effect of atorvastatin in inflammation^[11] and some animal studies have shown similar results with rosuvastatin.^[12] These statins when used along with Non steroidal anti-inflammatory drugs (NSAIDs) like aspirin, could be expected to show additive phenomenon, but such interactions appear to be poorly documented.. In view of the controversial reports regarding anti-inflammatory activity of statins and paucity of information regarding their interaction with NSAIDs like aspirin, the present study was planned to investigate the same in acute and subacute models of inflammation in male Wistar rats.

Materials and methods

ANIMALS:

Adult male healthy Wistar rats weighing 175 ±25 g were obtained from the central animal house, J.N.Medical College Belgaum and were acclimatized to 12:12 h light - dark cycle for 10 days prior to the day of experimentation. They were maintained on standard rat chow pellet (Amrut Brand) and water *ad libitum*. The study was approved by the IAEC constituted as per the guidelines of CPCSEA, New Delhi.

ACUTE INFLAMMATION

Overnight starved with free access to water rats were divided into several groups (n=6 in each) to receive various treatments.. Calculated clinical equivalent doses (mg/kg), 7.2 of atorvastatin, 3.6 of lovastatin, 3.6 of rosuvastatin and 200 of aspirin in 2% gum acacia suspension as vehicle were administered orally in a single dose while, the control group received 0.5ml of 1% gum acacia suspension orally.

Thirty minutes after vehicle, aspirin and lovastatin, two hours after atorvastatin and rosuvastatin administration, 0.05 ml of 1% carrageenan in normal saline was injected into the sub plantar region of the left hind paw, as per the technique of Winter et al.^[13]

A mark was put at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema was measured with the help of plethysmograph by mercury displacement at zero h (immediately after injecting carrageenan) and the procedure was repeated at 0.5,1,3,4 and 5 h.

The percentage inhibition of edema was calculated using formula = $1 - V_t / V_c \times 100$

V_t and V_c were edema volume in drug treated and control groups respectively.

For interaction studies, the sub anti-inflammatory (SAI) dose of statins was determined by administering their doses (lower than therapeutic equivalent dose) in separate groups (n=6 in each) of animals and of all the doses the highest one that failed to suppress carrageenan inflammation significantly was considered SAI dose and was found to be 1mg/kg for all the three statins. The SAI dose of aspirin was taken as established in earlier studies.^[14]

SUBACUTE INFLAMMATION

Rats were divided into several groups of six in each. After clipping the hair in axillae and groin, under light ether anesthesia, two sterile cotton pellets weighing 10 mg each and two sterile grass piths (25X2mm each) were implanted randomly, subcutaneously through a small incision. Wounds were then sutured and animals were then caged individually after recovery from anesthesia. Aseptic precautions were taken throughout the experiment. The rats then received the statins orally in clinical equivalent dose as mentioned in acute studies. The treatment was started on the day of implantation and repeated every 24 hours for 10days. On eleventh day, the rats were sacrificed with an overdose of anesthesia to remove the cotton pellets and grass piths. The pellets, free from extraneous tissue were dried overnight at 60°C to note their dry weight. Net granuloma formation was calculated by subtracting the initial weight of cotton pellet from the weights noted. Mean granuloma dry weight for various groups were calculated and expressed in mg/100g body weight. The grass pith granulomas were preserved in 10% formalin for histopathological studies.

Similarly in the interaction studies, in acute and subacute models of inflammation, SAI dose of aspirin and one of the statins were administered together to separate groups (n=6 in each) in the volume of 10ml/kg orally.

Statistical analysis: Data expressed as mean \pm SEM were analyzed by one-way ANOVA followed by Dunnet's post hoc test and P values ≤ 0.05 was considered significant.

Results

Acute studies

As expected, aspirin significantly ($P<0.05$) reduced paw edema as compared to the controls throughout the observation period with mean values of 0.08 ± 0.04 , 0.28 ± 0.06 , 0.01 ± 0.08 , 0.033 ± 0.02 and 0.02 ± 0.02 at 0.5, 1, 3, 4, and 5 h respectively.

Similarly, all the three statins also showed significant anti-inflammatory activity as compared to vehicle treated controls. The mean paw volume in atorvastatin (7.2mg/kg) treated group were 0.12 ± 0.12 , 0.18 ± 0.04 , 0.23 ± 0.06 , 0.20 ± 0.05 and 0.15 ± 0.05 at 0.5, 1, 3, 4 and 5 h respectively. Likewise the mean values were 0.53 ± 0.11 , 0.38 ± 0.10 and 0.15 ± 0.06 at 3, 4 and 5h respectively in lovastatin (3.6mg/kg) group and 0.33 ± 0.10 , 0.53 ± 0.13 , 0.35 ± 0.16 and 0.20 ± 0.14 at 1, 3, 4 and 5h respectively in rosuvastatin (3.6mg/kg) group.

In interaction studies, the SAI dose of atorvastatin together with that of aspirin failed to show significant anti-inflammatory activity in acute model, while lovastatin(1mg/kg) when co-administered with SAI of aspirin significantly inhibited paw edema with mean values of 0.60 ± 0.09 , 0.40 ± 0.09 and 0.27 ± 0.06 at 3, 4 and 5 h respectively as compared to control . Similarly, rosuvastatin(1mg/kg) when co-administered with SAI dose of aspirin also significantly ($P<0.01$) reduced the paw edema at 1,3,4 and 5 h with mean values of 0.86 ± 0.10 , 0.91 ± 0.09 , 0.68 ± 0.07 and 0.38 ± 0.06 respectively.

Subacute studies

Mean granuloma dry weight (mg % body weight) were 25.05 ± 0.61 , 19.94 ± 0.87 , 22.9 ± 0.55 and 21.44 ± 0.89 in aspirin, atorvastatin, lovastatin, and rosuvastatin groups were significantly ($P<0.01$) lower than control (41.8 ± 0.48). Similarly co-administration of SAI dose of aspirin with that of atorvastatin, lovastatin, and rosuvastatin also produced significant ($P<0.01$) reduction in granuloma dry weight with mean values of 21.33 ± 2.78 , 25.25 ± 2.62 and 20.75 ± 2.87 respectively as compared to the control.

The granulation tissue sections stained with haematoxylin and eosin revealed a marked reduction in thickness of granulation tissue, collagen content and fibroblast number as compared to control in all the treated groups indicating their anti-inflammatory action (Fig 1).

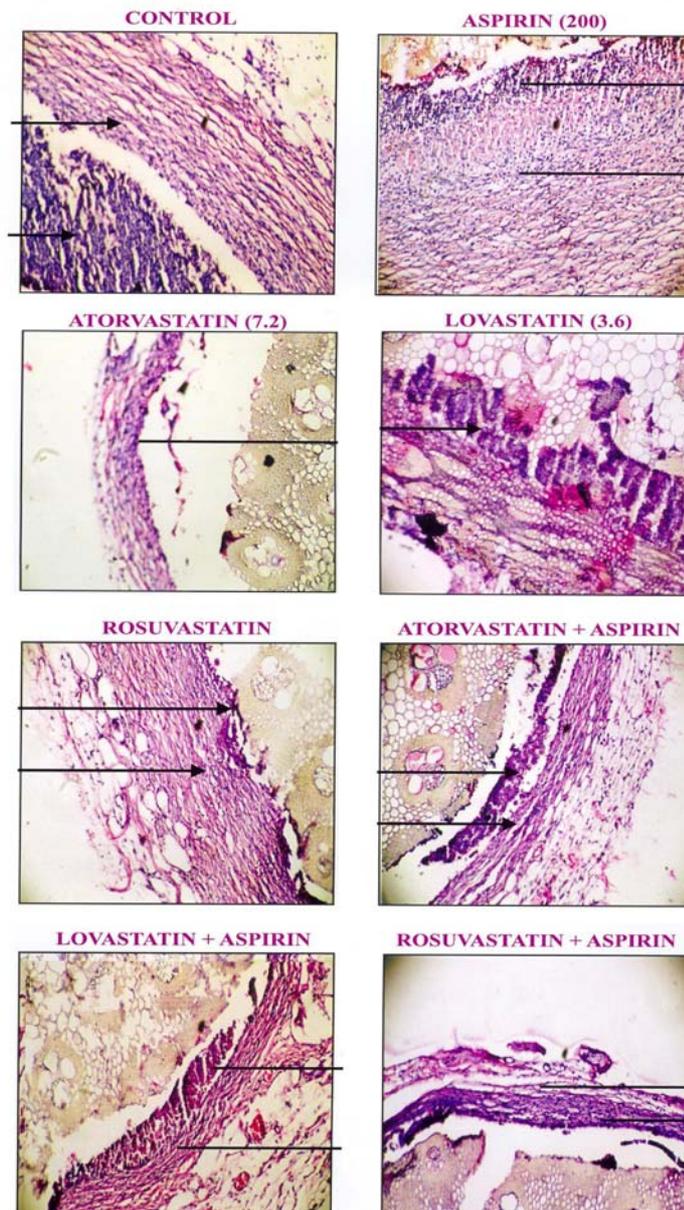
The percentage inhibition of inflammation by various treatments in both the models is shown in Table 1

TABLE 1: EFFECT OF VARIOUS TREATMENTS IN ACUTE AND SUBACUTE INFLAMMATION

S. No	Drugs and dose mg/kg	Percentage inhibition					Granuloma dry weight
		Paw edema					
		0.5 hr	1 hr	3 hr	4 hr	5 hr	
1.	Aspirin (200)	79.15**	45.18*	92.6**	97.47**	98.64**	40.08**
2.	Atorvastatin(7.2)	70.83**	64.6*	82.72**	84.80**	87.7**	52.3**
3.	Lovastatin (3.6)	8.3	-12.2	60.5**	70.9**	87.66**	45.2**
4.	Rosuvastatin (3.6)	33.3	36*	60.5**	73.4**	83.6**	48.71**
5.	Atorvastatin(1) + Aspirin (54)	-12.5	-60	28.4	21.6	20	48.98**
6.	Lovastatin (1) + Aspirin (54)	20.9	-15.4	55.55**	69.63**	78.1**	37.2**
7.	Rosuvastatin (1) + Aspirin (54)	8.3	-66.7**	32.1**	48.12**	68.5**	50.4**

**P<0.01, * P<0.05, as compared to control

FIG1. PHOTOMICROGRAPHS OF GRANULATION TISSUE
(H AND E STAIN 10X)



As compared to control, markedly decreased granulation tissue, collagen content and fibroblast number in drug treated groups.

Discussion

The three statins investigated in the present study include two lipophilic viz. atorvastatin and lovastatin while rosuvastatin is hydrophilic statin. Results of the present study clearly indicate that all the three statins when administered in their clinical equivalent dose showed significant anti-inflammatory activity in acute as well as subacute models of inflammation.

These observations of the present study are in agreement with the earlier experimental reports^[3] wherein, different models of inflammation such as thrombin induced changes in leukocytic function, allergic encephalomyelitis and air pouch models were used. Similarly, statins (atorvastatin,lovastatin) have also been shown to be clinically effective in the treatment of rheumatoid arthritis^[6] and alzheimer's disease.^[15] However, the present findings disagree with some other earlier experimental studies involving atorvastatin and lovastatin wherein they have been reported to be pro-inflammatory. The discrepancy could be explained on the basis of the inflammatory models viz. in-vitro TNF- α (tumour necrosis factor) activated human endothelial cells,^[9] human monocytes^[10] and in-vivo mice leukocytes.^[10] Moreover in contrast to the present finding, rosuvastatin has been reported to possess no anti-inflammatory activity in murine collagen-induced arthritis.^[12] Obviously, the discrepancy could be attributed to the models and species variation in the earlier studies. Similarly, atorvastatin has been reported to be ineffective in suppressing biomarkers of inflammation such as TNF- α , CRP (C-reactive protein) in ischemic cardiomyopathies.^[11] This could be probably due to inadequate delivery of the drug at the desired site due to ischemia.

The sub anti-inflammatory doses of lovastatin and rosuvastatin when co-administered with sub anti-inflammatory dose of aspirin, showed significant anti-inflammatory activity in acute model while, atorvastatin failed to do so. However, all three statins have shown significant synergistic anti-inflammatory activity with aspirin in subacute model which was confirmed by histopathological studies. There are scanty reports involving interaction studies of statins with aspirin. The synergistic interactions between statins and aspirin appear to be pharmacodynamic rather than pharmacokinetic since, atorvastatin has been reported to inhibit expression of cyclooxygenase-2 in rabbit atherosclerotic vascular endothelial cell culture.^[16] However, the pharmacokinetic interaction cannot be ruled out since plasma levels of aspirin/statins have not been monitored in the present study.

The proposed anti-inflammatory activity of statins include suppression of chemotactic proteins,^{[7],[17]} Interleukin-6(IL-6),^[17] P-selectin on platelets,^{[18],[19]} leukocyte transmigration^[17] and to preserve eNOS^[5] an inhibitor of inflammation.

The present findings of synergistic anti-inflammatory activity between aspirin and statins could be extrapolated to clinical situations, patients on statin therapy for dyslipidemia may require smaller doses of aspirin to treat other inflammatory co-morbidities they are suffering from.

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