

EFFECT OF ROSUVASTATIN AS AN ANTI-INFLAMMATORY AGENT IN ALBINO RATSHARISH KUMAR V S^{1*}, DWAJANI S¹, DHARMVEER GURJAR², UMAKANT PATIL³, VINODKUMAR C.S.⁴

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ABSTRACT

The present study is planned to evaluate anti-inflammatory effect of rosuvastatin. Statins have been shown to exert 'pleiotropic effects' independent of their cholesterol lowering actions that include anti-inflammatory properties. Our finding concealed that rosuvastatin exercises acute anti-inflammatory effect (carrageenan-induced rat paw edema method) and chronic anti-inflammatory effect (Cotton pellet induced granuloma method). Animals received rosuvastatin orally prior to induction of inflammation by injection of carrageenan into rat paw in the acute model and in chronic model; animals were treated for ten days after the implantation of cotton pellets. The Oral administration of rosuvastatin reduced inflammation in both acute as well as chronic inflammatory models. There was a significant reduction in inflammation in both the models when compared to control group (without treatment). The present findings show that rosuvastatin has anti-inflammatory effect in both acute and chronic inflammation.

Key words: Rosuvastatin, anti-inflammatory, rat paw edema, and cotton pellet granuloma.

INTRODUCTION

Statins come under the category of HMG CoA reductase inhibitors which competitively inhibit the activity of HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Inhibition of this enzyme results in a transient, modest decrease in cellular cholesterol concentration. The decrease in cholesterol concentration activates a cellular signaling cascade culminating in the activation of sterol regulatory element binding protein (SREBP), a transcription factor that up-regulates expression of the gene encoding the LDL receptor. Increased LDL receptor expression causes increased uptake of plasma LDL, and consequently decreases plasma LDL-cholesterol concentration. Approximately 70% of LDL receptors are expressed by hepatocytes, with the remainder expressed by a variety of cell types in the body^{1,2}. Statins lower cholesterol levels in people at risk of cardiovascular disease because of hypercholesterolaemia³. As atherosclerosis is considered to be an inflammatory disease, Bickel demonstrated that patients receiving statin therapy had lower levels of all measured inflammatory markers^{4,5}. Despite many studies attributes positive effects of statins, to date, there have been very few or no studies on rosuvastatin as the anti-inflammatory agent in acute and chronic rat model

Research question

- To study the anti-inflammatory activity of Rosuvastatin.
- To compare its anti-inflammatory activity (Acute & Chronic) with Aspirin

MATERIALS AND METHODS**Ethical clearance**

The study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research, and the protocol was approved by the Institutional Animal ethical Committee, S. S. Institute of Medical Sciences and Research Centre, Davangere, Karnataka

Maintenance of rats

Healthy Albino rats (150-250gm), 12 weeks old of either sex, bred locally in animal house of S.S.I.M.S & R.C, Davangere, are used for the study. They are housed under controlled conditions of temp 23±2°C & 10-14 hrs light & dark cycles respectively. The animals are housed individually in polypropylene cages containing sterile paddy husk as bedding and are maintained on normal diet & water ad libitum.

Groups**The experiments are conducted in two methods**

- Acute anti-inflammatory activity - (Paw edema method)
- Chronic anti-inflammatory activity - (Cotton pellet granuloma method)

Acute anti-inflammatory activity - (Paw edema method)

The animals are randomly allocated into three groups of six animals each.

Group 1: Receives Normal saline 0.5 ml orally (Control).

Group 2: Receives Aspirin 300mg/kg body weight orally (treated with known anti-inflammatory drug).

Group 3: Receives Rosuvastatin 20mg/kg body weight orally (test)

Carrageenan induces paw edema method

The aim of this experiment is to evaluate the anti-inflammatory property of rosuvastatin. Group 1 was given normal saline orally, Group 2 was given aspirin orally and for Group 3 rosuvastatin was given orally. After 30 mins of administration, 0.1% carrageenan was injected subcutaneously on plantar side of the hind paw in all the groups. The paw volume of rats was measured by plethysmometer, before and after injection of 0.1% carrageenan at different time intervals (0.5, 1, 2, 3, 4, 5, 6 h). Change in the paw volume, in milliliter (ml), was recorded at above time intervals with reference to the initial volume before administration of the inflammatory agent⁶. Edema volume and mean percentage of inhibition was calculated.

Chronic anti-inflammatory activity - (Cotton pellet granuloma method)

Group 1: Receives Normal saline 0.5 ml orally (Control).

Group 2: Receives Aspirin 300mg/kg body weight orally (treated with known anti-inflammatory drug).

Group 3: Receives Rosuvastatin 20mg/kg body weight orally (test).

The back of the each animal was shaved and prepared after washing with spirit. The line was marked in the dorsal interscapular region to the lumbar region. Rats are anaesthetized with ether. An incision was made in the marked region. Subcutaneous tunnels were formed on both sides and 20 ± 1 mg sterile cotton pellets were inserted. Later incisions were sutured by sterile catgut. Animals were treated for 10 days. On the 11th day animals were anesthetized with ether

and 2 ml of blood was collected for estimation of ALP from retro orbital route and later the animals were sacrificed by cervical dislocation⁷.

Pellets were separated from extraneous tissue and dried at 60 C until constant weight. The net dry weight, i.e., after subtracting the initial weight of the cotton pellet, was determined. The average weight of the pellet of the control group as well as of the test groups was calculated. The percent change of the granuloma weight with respect to the control group was determined and taken as a measure of granuloma tissue formation⁸. The weight of the cotton pellet was calculated.

Table 1: Effect of rosuvastatin on carrageenan induced paw oedema in rats.

Groups	Parameters	Mean oedema volume at different intervals						P*Value, sig	Significant pairs**
		0 min	30 min	1 hr	2 hrs	3 hrs	6 hrs		
Control	Mean	2.2	2.5	3.1	3.7	4.3	5.7	<0.001 HS	1&2, 1&3, 1&4, 1&5, 1&6
	SD	0.1	0.1	0.1	0.1	0.2	0.2		
Standard	Mean	2.1	2.3	2.4	2.6	2.9	3.6	<0.001 HS	1&2, 1&3, 1&4, 1&5, 1&6
	SD	0.1	0.1	0.1	0.2	0.1	0.2		
Test	Mean	2.2	2.3	2.4	2.6	3.1	3.8	<0.001 HS	1&2, 1&3, 1&4, 1&5, 1&6
	SD	0.1	0.1	0.1	0.1	0.1	0.1		
P ^s value		0.74NS	0.007 S	<0.001 HS	<0.001 HS	<0.001 HS	<0.001 HS		
Significant Pairs ^{ss}		-	1&2, 1&3	1&2, 1&3	1&2, 1&3	1&2, 1&3	1&2, 1&3		

- * Repeated measures ANOVA test
- ** Bonferroni t test
- \$ One way ANOVA test

\$\$ Tukey's post hoc test

The mean total oedema responses were reduced significantly by 20 mg/kg of rosuvastatin. The strongest inhibitory effect on the total oedema response which was comparable to that of aspirin was seen (table 2). These observations strongly suggest that oral treatment by statins has anti-inflammatory activities. The footpad swelling represents an acute inflammatory response characterized by the influx of PMN leukocytes. Rosuvastatin blocks the influx of PMN leukocytes into the paw 3 hours after carrageenan injection. Where else, in control group did not affect the ability of leukocytes to migrate at the inflammation site.

Table 2 : Percentage of inhibition of paw oedema in rosuvastatin treated rats

Interval	% of Inhibition					
	Controls		Standard		Test	
	Mean	SD	Mean	SD	Mean	SD
30	-14.6	4.0	-8.4	4.0	-6.6	2.5
60	-39.9	5.1	-14.1	5.1	-14.2	3.6
2	-69.0	7.2	-23.3	5.2	-22.7	4.5
4	-95.3	17.1	-37.2	5.7	-45.5	6.8
6	-159.6	20.4	-68.8	9.2	-79.5	10.1

Effect of rosuvastatin on granuloma formation in cotton pellet-induced granuloma in rats

Granuloma formation was significantly reduced by treatment with rosuvastatin at 20 mg/kg, p.o. ($p < 0.01$) and by aspirin ($p < 0.01$) when compared with control group (Table 3). The difference in the initial weight and weight after 11th day is shown in the table 3. The difference in the cotton pellets weight in standard and rosuvastatin treated rats was statically significant. Where else the differences

were statically significant between control group, standard group and rosuvastatin treated group with control group (table 3).

RESULTS

Effects of rosuvastatin on carrageenan induced rat paw oedema. The animals in the control group showed significant increase in paw oedema volume from first to sixth hour, while aspirin treatment showed inhibition of paw oedema from first hour to sixth hour with percentage of inhibition of $-37 \pm 5.7\%$ during 3rd hour and $-68.8 \pm 9.2\%$ during 6th hour. Treatment with rosuvastatin at a dose of 20 mg/kg ($p < 0.001$) showed significant inhibition of paw oedema at second hour (-22.7 ± 4.5) compared to standard (-23.3 ± 5.2) and control (-69.0 ± 7.2).

Table 3: Effect of rosuvastatin on cotton pellet-induced granuloma in rats.

Groups	Weight of cotton pellets					
	Initial (t1)		After (t2)		Difference (t2-t1)	
	Mean	SD	Mean	SD	Men	SD
Control	0.020	0.000	2.155	0.171	2.134	0.171
Standard	0.020	0.000	0.556	0.038	0.536	0.038
Test	0.020	0.000	0.631	0.033	0.611	0.033

Effect of rosuvastatin on serum ALP in cotton pellet-induced granuloma in rats.

Serum level of ALP was found to increase in the control group but was significantly decreased by rosuvastatin at 20 mg/kg, p.o. (test group) and this difference was statically significant. In aspirin treated rats (standard group), ALP was within the normal range.

DISCUSSION

HMG-CoA reductase inhibitors have been shown to decrease the risk of cerebrovascular and cardiovascular events in patients with coronary artery disease and hypercholesterolaemia⁴. These beneficial effects have usually been attributed to the lipid-lowering properties of the statins. However, recent studies have shown that HMG-CoA reductase inhibitors also offer protection in acute inflammatory processes. Hence in the present study we evaluated the anti inflammatory property of rosuvastatin in carrageenan induced inflammation in acute and chronic rat model.

Carrageenan is known for its classic biphasic effect, first phase mediated by release of histamine and serotonin during the first hour and release of kinins up to 2.5 h, while the second phase is mediated by release of prostaglandins from 2.5 to 6 h^{9, 10, 11}. In the present

study, rosuvastatin showed inhibition of second phase of carrageenan-induced rat paw oedema, suggesting the inhibition of prostaglandins release. In the acute models, statistically difference in the volume of paw oedema in control group, aspirin treated group and rosuvastatin treated group at 3rd hour (table 1 and table 2) and also the infiltration of PMN leucocytes were very few in rosuvastatin treated rats. Taken together, these data indicate that rosuvastatin is able to significantly attenuate the inflammatory process, thus preventing leukocyte infiltration, which might provokes subsequent tissue injury.

During chronic inflammatory conditions, there is elevation of ALP and LDH¹². In the cotton pellet-induced granuloma model, increased concentration of serum enzyme ALP was found to be reduced by treatment with rosuvastatin in rats. Marked decrease in serum enzyme levels of ALP indicates that there was reduction in necrosis of cells, which may be mediated by inhibiting the release of inflammatory mediators like cytokines. Which are responsible for tissue destruction and cell necrosis. Thus, overall results suggest that rosuvastatin showed efficacy in the chronic model of inflammation probably by inhibiting proliferation of macrophages, neutrophils, fibroblasts which lead to reduction in granuloma, antioxidant effect which also contributes in reduction of inflammation^{13,14}. Rosuvastatin treated rats did not show any significant ulceration which is an advantage over the common non steroidal anti-inflammatory drugs.

To conclude, in this study we showed that oral administration of rosuvastatin apart from having lipid lowering activity, it has got anti-inflammatory and antileukocyte accumulation activities in carrageenan induced rat paw oedema model.

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