Original Article

Evaluation of anti-inflammatory effect of atorvastatin in rats

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Abstract

Aim: The aim of this study is to evaluate the anti-inflammatory activity of atorvastatin in carrageenan induced rat paw oedema. **Methods and Material:** Anti-inflammatory activity of atorvastatin 3mg/kg and 8mg/kg were assessed in carrageenan induced paw edema in rats (n=6) where they were compared with control and ibuprofen group. **Results:** In carrageenan induced paw edema in rats, both groups of atorvastin showed anti-inflammatory effect ($p<0.01$). Anti-inflammatory activity of atorvastatin 8mg/kg was comparable to ibuprofen. **Conclusions:** The result of this study if substantiated by further experimental and clinical research suggest that atorvastatin may play important role in inflammatory disorders, especially when there is coexisting hyperlipidemia.

Keywords: carrageenan, paw oedema, anti-inflammatory.

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INTRODUCTION

Inflammation, a common clinical condition is said to be a complex protective reaction in the vascularized connective tissue due to variety of exogenous and endogenous stimuli causing cell injury and is characterized by the reaction of blood vessels, leading to accumulation of fluids and leukocytes in the extravascular tissue. This complex phenomenon involves endogenous chemical mediators such as histamine, 5-hydroxytryptamine, various chemotactic factors, bradykinin, leukotrienes and prostaglandins (Kumar V *et al.*, 2004). Pain is a major symptom of inflammatory disease. Many NSAIDs like aspirin, indomethacin and ibuprofen are in clinical use but, all of these are not completely devoid of adverse effects. Hence the search for safer and better anti-inflammatory agents other than NSAIDs continues. Some drugs unrelated to NSAIDs like HMG-CoA reductase inhibitors (statins) like atorvastatin, lovastatin (Schmidt GW, 2002; Tondon V *et al.*, 2005), rosuvastatin (Tondon V *et al.*, 2005) have been reported to posses anti-inflammatory activity in experimental models though they are not routinely used in the treatment of inflammatory disorders. Moreover, clinical studies on rheumatoid arthritis (McCarey DW *et al.*, 2004), ischemic heart disease and alzheimer's disease (Jick H *et al.*, 2003) indicates that statins like atorvastatin, cerivastatin having anti-inflammatory activity. Paradoxically, statins like lovastatin (Schmidt A *et al.*, 2002) and atorvastatin (Kiener PA *et al.*, 2001) have been reported to possess pro-inflammatory activity in *in-vitro* studies while some statins have been reported neutral effect on inflammation (Bleske BE *et al.*, 2006). The statins are a well-known class of cholesterol-lowering drugs that inhibit the enzyme 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Grundy SM, 1988). They are the class of drugs most widely used for the prevention of primary and secondary coronary heart disease (Corsini A *et al.*, 1995). Highly selling and most commonly prescribed drug is atorvastatin (Youssef S *et al.*, 2002). In view of the controversial reports regarding anti-inflammatory activity of statins, the present study was planned to explore anti-inflammatory activity of atorvastatin in male *Wistar* rats

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using their clinical equivalent doses in an acute (carrageenan induced inflammation) model.

MATERIAL AND METHODS

The animal experiments were carried out in accordance with the guidelines set by CPCSEA. The study was approved by the Institutional Animal Ethics Committee. A total 30 male albino *Wistar* rats of age 6-8 weeks and weight 120-150 grams were used in rat model of acute inflammation (carrageenan induced rat paw edema). The animals were from the same stock and were acquired from Haffkine Biopharma Corporation Parel. Six rats were included per group. The rats were accommodated in polypropylene cages with grill on top. Food, water and bedding of clean paddy husk were provided. The rats were allowed one week period of acclimatization in animal laboratory at room temperature. Body weight of the rats was recorded on first day of initiation of study. Animals were fed on standard pellet diet. Water was provided in glass bottles with stainless steel sipper tubes. Food and water were given *ad libitum*. Atorvastatin powder (pure form) and ibuprofen powder (pure form) were obtained from Cipla pharmaceuticals. The suspension of atorvastatin and ibuprofen powders were made in distilled water by using 2% gum acacia. Carrageenan was obtained in powder form from Sigma Co. St. Louis. The rat carrageenan paw model as described by Van Arman *et al*. (1971) was used. The rats received oral saline 2.5ml/kg (group 1), ibuprofen 108mg/kg (group 2), atorvastatin 3mg/kg and atorvastatin 8mg/kg(group 4) for 6 days daily. On third day of the study 1hr after administration of drugs hind paw volumes were measured with the help of plethysmograph. After baseline measurements of paw volume, 0.1ml of 1 %(w/v) solution of carrageenan in saline was injected into plantar region of right hind paw of rats of all groups. The paw volumes were measured at 3 hr and 6 hr. Same procedure repeated on day 6 but on left paw. Parameters analyzed for evaluation of anti-inflammatory effect were

1. Comparison of paw volumes at various time intervals within group and between the groups on day 3 and 6.
2. Percentage inhibition of edema in different groups on day 3 and 6.

Percentage inhibition of oedema calculated as change in paw volume of control group at 6 hr minus change in paw volume of test group at 6 hr divided by change in paw volume of control group at 6 hr multiply by 100.

Statistical Analysis

The difference of rat paw volume at various time intervals within the group was estimated using Repeated Measures ANOVA followed by Dunnett test and between different groups was estimated using One Way ANOVA followed by Tukey multiple comparison test. p value less than 0.05 was considered as significant.

RESULTS

Table 1 shows basal mean paw volume was comparable in all groups. Paw volumes in all groups at 3hour and 6 hour on day 3 and day 6 were statistically significantly increased from baseline value.

Table 1: Comparison of paw volume at Baseline, 3hr and 6 hr on day 3 and 6 in all groups

Study groups	Paw volume (mean ± SEM) in cm On day 3			Paw volume (mean ± SEM) in cm On day 6	
	Baseline	3 hr	6 hr	3 hr	6 hr
Control	5.12± 0.12	7.43 ± 0.13***	9.55 ± 0.41***	7.83 ± 0.19***	9.95± 0.14***
Ibuprofen	5.07± 0.09	5.95± 0.08***	6.30 ± 0.08***	5.75 ± 0.08***	6.32 ± 0.06***
Atorvastatin3mg/kg	5.12 ± 0.06	7.05± 0.09***	8.05 ± 0.06***	6.37 ± 0.14***	7.45 ± 0.25***
Atorvastatin8mg/kg	5.08±0.10	6.2 ± 0.13***	6.57 ± 0.19***	5.88 ± 0.13***	6.35 ± 0.15***

Results are given as mean ± SEM. comparison of paw volume at different time interval with baseline value within group. ***p<0.001 when compared to baseline value

Table 2 clearly shows that paw volume in all three groups at 3 hour and 6 hour on day 3 and day 6 statistically significantly lower when compared to control group.

Table 2: Comparison of paw volume at 6 hr between the groups on day 3 and 6

	Control	Ibuprofen	Atorvastatin 3 mg/kg	Atorvastatin 8mg/kg
Paw volume at 6 hr on day3	9.55 ± 0.41	6.30 ± 0.08**	8.05 ± 0.06**	6.57 ± 0.19**
Paw volume at 6 hr on day 6	9.95± 0.14	6.32 ± 0.06**	7.45 ± 0.25**	6.35 ± 0.15**

Results are given as mean ± SEM..comparison of paw volume between the groups. significance **p<0.01

Table 3: Percentage inhibition of oedema on day 3 and 6

Study groups	Change in paw volume (cm) at 6 hr on day 3 (% inhibition)	Change in paw volume (cm) at 6 hr on day 6 (% inhibition)
Control	4.43 ±0.31	4.83±0.06
Ibuprofen	1.23±0.08 (72.18)	1.25±0.07 (74.14)
Atorvastatin 3mg/kg	2.93±0.02 (33.83)	2.33±0.24 (51.73)

Atorvastatin 8mg/kg	1.48±0.14 (66.54)	1.27±0.07 (73.81)

DISCUSSION

As mentioned earlier, the present study was planned to investigate the influence of atorvastatin on acute inflammation in male. Numbers of studies have been conducted for determining the role of statins in inflammation. These studies have shown conflicting results about role of statins in inflammation. Some studies showed anti-inflammatory activity of statins (Schmidt GW, 2002; Naito Y *et al*., 2006) while some studies showed pro-inflammatory activity (Kiener PA *et al*, 2001, Schmidt A. *et al.*, 2002) or no anti-inflammatory activity (Plamer G *et al.*, 2004). The present study was undertaken to clarify conflicting anti-inflammatory results. We chose atorvastatin because it is the most widely prescribed statin for hypercholesterolemia and presents one of the most favourable safety profiles of the available statins (Youssef *et al.*, 2002). When we compared anti-inflammatory activity of Atorvastatin 3 mg/kg and 8mg/kg groups with control group on day 3 and 6, they showed highly significant difference (table 2 and table 3). Thus atorvastatin showed significant anti-inflammatory activity. Atorvastatin 8 mg/kg group did not show any significant difference with Ibuprofen group (table 2 and table 3). Thus anti-inflammatory activity of Atorvastatin 8 mg/kg group was comparable with Ibuprofen group.. Percentage inhibition of edema of Atorvastatin 8 mg/kg group was comparable with Ibuprofen group on day 3 and day 6. Percentage inhibition of edema of Atorvastatin 3 mg/kg group increased from 33.83% on day 3 to 51.73 % on day 6. Percentage inhibition of edema of Atorvastatin 8 mg/kg group increased from 66.54% on day 3 to 73.81% on day 6 (table 3). Thus anti-inflammatory activity of atorvastatin increased from day 3 to day 6. Similar findings were reported by Barsante *et al.,* 2005 who studied the anti-inflammatory and analgesic effect of atorvastatin in rat model of adjuvant-induced arthritis. In arthritis induced rats, the increase in paw volume was inhibited by the administration of atorvastatin 1-10 mg/kg from days 10 to 15. These results were comparable with the results of our study. Atorvastatin 8 mg/kg showed more inhibition of paw volume than Atorvastatin 3mg/kg. Maximum anti-inflammatory effect of Atorvastatin 8 mg/kg group was seen on day 6. McCarey DW *et al.* (2004) conducted a larger randomized placebo-controlled study investigating atorvastatin as a disease-modifying antirheumatic drug (DMARD) in rheumatoid Arthritis. In this 116-patients study, patients received either 40 mg of atorvastatin per day or placebo in addition to current DMARD therapy; DMARDs were not allowed to be changed during the 6-month study. At the end of 6 months, the group receiving atorvastatin showed statistically significant improvements in the 28-joint Disease Activity Score (DAS28). The mechanism of anti-inflammatory action of atorvastatin cannot be proposed on the basis of the present findings. However, several mechanisms have been proposed in earlier reports. Atorvastatin has been observed to abolish arterial macrophage infiltration and monocyte chemotactic protein (MCP-1) (Bustos C. *et al*., 1998; Ikeda V *et al*., 1999). Atorvastatin is reported to interfere mainly with nuclear factor – kappa B pathway (Schmidt GW, 2002; Ortego M *et al.*, 1993) which is activated by Geranyl geranylated proteins (intermediate metabolite in cholesterol biosynthesis) and plays a pivotal role in transcriptional regulation of cytokines, chemokines, adhesion molecules and acute phase proteins such as CRP. Further evidence for an anti-inflammatory effect of statins is provided by their inhibition of cyclooxygenase-2 (COX-2) expression in a rabbit model of atherosclerosis and in cultured vascular smooth muscle cells stimulated by cytokines (Hernandez-Presa *et al.*, 2002). Statins have been shown to increase both the expression and the activity of eNOS in vitro and in vivo (Endres M *et al*., 1998; Lauf U *et al*., 1998; Kano H *et al*., 1999). NO from endothelial cells is thought to be anti-inflammatory. Some studies reported the pro-inflammatory activity of the statins (atorvastatin, simvastatin, lovastatin etc) was demonstrated in *in vitro* studies using mouse monocytes, human monocytes (Kiener PA *et al*., 2001) and umbilical vein endothelial cells (Schmidt A. *et al*., 2002). The observed pro-inflammatory activity was due to enhanced infiltration of macrophages and neutrophils in the mouse peritoneal cavity, increased production of inflammatory cytokines TNF-α, 1L-1β, MCP-1, 1L-8 (Kiener PA *et al.*, 2001) in mouse monocytic cell culture and super induction of E-selectin, Intercellular adhesion molecule (ICAM-1) in cultured human umbilical vein endothelial cells (Schmidt A. *et al.*, 2002). These studies results were against our study results. The discrepancy could be due to experimental methodology and the difference in the species. It was interesting to note that the anti-inflammatory activity of atorvastatin showed improvement as the study progressed from day 3 to day 6. This perhaps indicates that administration of atorvastatin for a prolonged period as is used for treating hypercholesterolemia may show more pronounced anti-inflammatory effect. As a corollary of the present observations, an atherosclerotic patient with inflammatory co-morbidities may require reduced anti-inflammatory doses of NSAIDs to relieve the

inflammation. However this speculation needs to be confirmed clinically.

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