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ROLE OF PITAVASTATIN AND LOVASTATIN ON INFLAMMATION USING *IN VIVO* CARRAGEENAN INDUCED PAW EDEMA MODEL

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ABSTRACT

Statins are the medicaments commonly used for the treatment of hyperlipidemia. In addition to these drugs have a negative impact on our immune response. Regular consumption of statins may accelerate inflammatory onset in human physiology. The objective of the present study is to investigate if regular consumption of statins may predict the development of inflammation. The *in vivo* inflammatory activity was evaluated by using carrageenan induced paw edema model in female *albino wistar* rats. The pharmacological response such as paw edema volume and percentage inhibition was quantified by using digital plethysmograph. The result obtained from the aforementioned study showed that there was a significant rise in paw edema volume of both test drugs when compared with carrageenan induced group however, the data of percentage inhibition of paw edema elicited that a remarkable decline of percentage inhibition in treatment groups. The study implied that both test drugs such as Pitavastatin and Lovastatin exhibited edema in animal model due to the release of various proinflammatory mediators. The study claimed that stains use was associated with increased risk for dysregulating immune system.

KEYWORDS: Hyperlipidemia, Carrageenan, Inflammation, Plethysmograph, Inflammation.

INTRODUCTION

Inflammation is defined as the normal physiological response to any kind of unintended stimulus. This stimulus may deregulating the immune response of the host, varying from the

acute transient and highly localized actions to any kind of noxious stimulus. Simple mechanical injury or to the complex persistent response involving the whole organism.[1] The chain of inflammatory reactions are characterised as biochemical, immunological and cellular events which may range in time from reorganisation of the noxious stimulus through the mobilization of natural defence mechanism. Thus inflammation can be defined simply by integrating all these processes, as a complex vascular lymphatic and local tissue reaction elicited in animals by the presence of viable or non-viable irritants.[2]

Statins have been invented for the pharmacotherapy of patients with hyperlipidaemia and hypertension. In addition to their lipid lowering activity, research studies have shown that statins have immunomodulatory properties.[3] It exhibited that because of these aforementioned properties statin use may eventually lead to altered the autoimmune system, In line with this hypothesis, It recently shown in an observational study that statin use was triggered the activation of cyclooxygenase and lipooxygenase pathway.[4]

Statins have elucidate its pharmacological action on autoimmune system by regulating leucocyte-endothelial cell adhesion, reducing nitric oxide production and promote the level of inflammatory cytokines such as tumour necrosis factor (TNF-α), interleukin 1 and interleukin 6. The production of these proinflammatory mediators exhibited biochemical mechanisms of inflammation.[5]

Carrageenan induced paw edema is one of the common method used for the pharmacological screening of drugs, chemicals and phytocompounds for anti-inflammatory activity. The advantages of this method is highly reliable, sensitive and reproducible test for nonsteroidal anti-inflammatory drugs and has long been established as an effective model to understand the invention of anti-inflammatory drugs.[6] This method is effective in oral anti-inflammatory agents; therefore, it has significant pharmacological action for anti-inflammatory agents acting through mediators of acute inflammation.[7] The process of inflammatory reactions are initiated by the induction of carrageenan injection causes an acute and local response. In the early phase, the preliminary mediators such as histamine, serotonin, and bradykinin are involved, whereas prostaglandins and various cytokines such as IL-1β, IL-6, IL-10, and TNF-α are implicated in the second phase.[8]

The scientific studies have been conducted to evaluate the unwanted effect of statins on inflammation in regular consumers. The present study focussed to demonstrate statin use accelerates the risk for the induction of inflammation.

MATERIAL AND METHODS

Carrageenan induced paw edema model.

Animals

Female *albino wistar* rats weighing 150- 200g were collected from the small animals breeding station, Mannuthy, Kerala, India. They were placed in polypropylene cages under standard environmental condition (12hr dark/ 12 hour light cycle: temperature., 25±2˚C, 35-60% humidity, air ventilation) and were supplemented with standard diet (VRK nutritional solutions, Maharashtra, India) and water *ad libitum*. The animals were acclimatized to the environment for 2 weeks prior to experiment use. All the animal experiments were performed after getting the permission from the institutional animal ethical committee (IAEC/M.Pharm/DPS/2018-09).

Preparation of Carrageenan Suspension

1% suspension of carrageenan was prepared by sprinkling 100 mg of carrageenan powder in 10 ml of sodium carboxy methyl cellulose, kept aside and soaked for 1 hour.[9]

Procedure

The rats were divided into 5 groups of 6 animals each. The animals were starved overnight, water given *ad.libitum*, Group I receives vehicle (Sodium CMC), Group II receives 0.1ml of 1% carrageenan in physiological saline into the sub plantar tissue of the left hind paw of each rat. Group III receives standard drug (Diclofenac 10mg/kg). Group IV & V receives Pitavastatin (2 mg/kg) and Lovastatin (10 mg/kg) respectively after thirty minutes, the rats were challenged by subcutaneous injection of 0.1ml of 1% solution of carrageenan into the sub plantar region of the left hind paw except group1. The paw was marked at the level of lateral malleolus just beyond the tibio tarsal joint. The paw volume was measured plethysmographically immediately after the injection at the time intervals of 0, 60,120,180 and 240 minutes.[10, 11]

Percentage inhibition of paw edema was calculated as,

$$\frac{Vc - Vt}{Vc} \times 100$$

Vc is the inflammatory increase in paw volume in a control group of animals.
Vt is the inflammatory increase in drug treated animals.

Statistical analysis

Statistical comparison and significance were analysed by one-way ANOVA followed by Turkeys post hoc multiple comparison test using Graph pad prism 6.00 version. Results were expressed as mean ± SEM (n =6 rat per group). Values of $p< 0.05$, $p< 0.01$ and $p<0.001$ were indicative of statistically significant differences.

RESULT AND DISCUSSION

Effect on paw edema volume

Carrageenan induced group showed an increase in paw volume. It was observed that there was marked increase in the paw volume of both test groups. Paw volume of animals in all groups were recorded at 0, 1st, 2nd, 3rd and 4th hour. In carrageenan induced rat paw edema model of anti-inflammatory activity, the Pitavastatin and Lovastatin showed a significant increase ($p<0.001$) in edema formation from first hour to fourth hour. The highest paw edema formation and least inhibitory effect of Pitavastatin (0.61± 0.00421) and Lovastatin (0.65±0.003416) was found in late phase, that is at third hour when compared with normal control group and standard group. From the data revealed that there was significant ($p<0.001$) increase in paw volume of both test drugs when compared with carrageenan induced group. The study implied that both test drugs showed inflammation in suitable animal model due to the release of proinflammatory mediators.

Percentage inhibition of paw edema

Carrageenan induced group as well as both test drugs exhibited a significant increase in the paw volume in animals. It was observed that there was a significant difference in paw volume at every hour with both test drugs. The percentage inhibition of edema produced at 4th hour for Pitavastatin, Lovastatin and Diclofenac sodium were 8.19, 11.29, 55.55 respectively. It indicated that both test drugs had less anti-inflammatory activity.

Table 1: Effect of Pitavastatin and Lovastatin on paw edema volume in carrageenan induced paw edema in rats.

	RAT PAW VOLUME (ml) (mean ± SEM)				
Groups	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**
Normal control	0.28±0.0021	0.30±0.0008	0.35±0.0021	0.27±0.0034	0.24±0.0042
Carrageenan	0.38±0.0021 ^{c}	0.42±0.00258 ^{ag}	0.52±0.0021 ^{g}	0.53±0.0006^{bg}	0.55±0.0034 ^{bg}
Standard	0.56±0.0034^{c}	0.54±0.0001^{c}	0.50±0.0008 ^{c}	0.45±0.0009 ^{c}	0.43±0.0006 ^{c}
Pitavastatin	0.47±0.0034^{cgr}	0.50±0.0025 ^{crg}	0.60±0.003^{cgr}	0.59±0.004^{cgr}	0.61±0.004^{cgr}
Lovastatin	0.51±0.0074^{cgrz}	0.55±0.0034^{crgz}	0.63±0.0021^{cgz}	0.64±0.0042^{cg}	0.65±0.003^{cgrz}

Values are expressed as mean ± SEM, (n =6). Data was analysed by one way ANOVA followed by multiple comparison Turkey's post hoc test. Test level significance: ^{a}p<0.05 ^{b}p<0.01 ^{c}p<0.001 when compared with normal,^{e}p<0.05 ^{f}p<0.01 ^{g}p<0.001 when compared with standard, ^{p}p<0.05 ^{q}p<0.01 ^{r}p<0.001 when compared with carrageenan induced group,^{x}p<0.01 ^{y}p<0.001 ^{z}p<0.0001 when compared with Pitavastatin treated group.

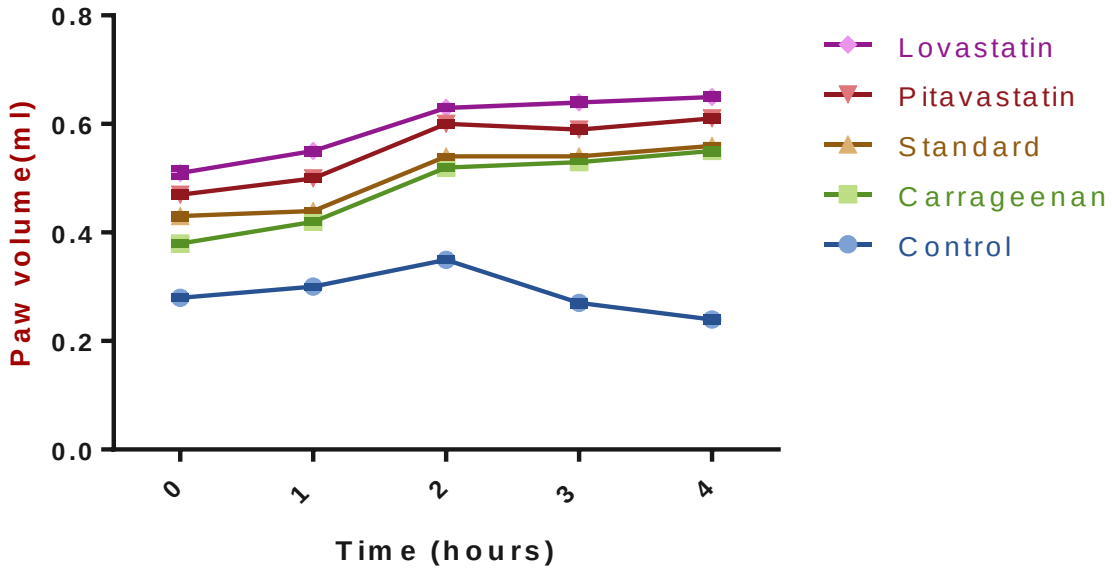

Figure 1: Effect of Pitavastatin and Lovastatin on injected paw volume in carrageenan induced paw edema model.

Table 2: Effect of Pitavastatin and Lovastatin on percentage inhibition paw edema by carrageenan induced paw edema model.

Group	**% INHIBITION PAW EDEMA**			
	1 hr	**2 hr**	**3 hr**	**4 hr**
Standard	31.81	35.18	50	55.55
Pitavastatin	12	10	8.47	8.19
Lovastatin	20	16.92	14.28	11.29

CONCLUSION

The study was aimed at revealing the role of Pitavastatin and Lovastatin on inflammation. The work was designed to determine the evaluation of *in vivo* carrageenan induced paw edema using female *albino wistar* rats exhibited significant increase in paw edema formation due to the release of prostaglandins, histamine, bradykinin etc. The standard drug exhibited drastic increase in percentage inhibition of paw edema However, both test drugs displayed decline in percentage inhibition due to the release of various proinflammatory mediators such as histamine, prostaglandin, Bradykinin. The study clearly explained that the statin consumption resulted the dysfunction of autoimmunity may accelerated the development of edema as an adverse effect.

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