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Evaluation of anti-Inflammatory activity of hydroxychloroquine and simvastatin combination in experimental animals.

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ABSTRACT

In this study, combination of hydroxychloroquine and simvastatin was studied for its anti-inflammatory activity in rats. Thirty albino Wistar rats of either sex were divided into five groups i.e. control (received saline); hydroxychloroquine alone (received 24 mg/kg of hydroxychloroquine); Simvastatin alone (received 1.3 mg/kg of simvastatin); high dose combination of hydroxychloroquine (24 mg/kg) and simvastatin (1.3 mg/kg); low dose combination of hydroxychloroquine (12 mg/kg) and simvastatin (0.6 mg/kg) of six animal each. The anti-inflammatory activity of the drugs was tested in carrageenan-induced rat-paw oedema. The high dose combination of hydroxychloroquine and simvastatin showed 91 % inhibition and low dose combination of hydroxychloroquine and simvastatin showed 92.3 % inhibition on carrageenan induced rat paw oedema at 9 hour. The results of this study revealed that combination of hydroxychloroquine and simvastatin has significantly higher ($p<0.001$) anti-inflammatory activity than hydroxychloroquine or simvastatin alone.

Keywords: anti-inflammatory activity, hydroxychloroquine, simvastatin, rat paw oedema.

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INTRODUCTION

Inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality"[1]. It is a disorder involving localized increases in the number of leucocytes and a variety of complex mediator molecules. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and alzheimer's disease [2]. It is believed that current drugs available such as opoids and NSAIDs drugs are not useful in all cases of inflammatory disorders, because of their side effects, economy and potency [3].

Antimalarial agents have been used to treat some rheumatic diseases such as lupus erythematosus since the 1800s [4]. Hydroxychloroquine an antimalarial drug has broader anti-inflammatory efficacy. It has been proven to be effective in rheumatoid arthritis (it is classified as disease-modifying anti-rheumatic drug, or DMARD) and in systemic lupus erythematosus. Hydroxychloroquine is an anti-inflammatory drug which target mononuclear/microglial cell activation, APP processing [5].

In the last decade low grade inflammation has been identified as pivotal pathogenic factor in the development of atherosclerosis. Numerous inflammatory parameters, such as cell adhesion molecules, cytokines, chemokines, and acute phase reactants, have been identified as valuable risk markers for the prediction of cardiovascular events.

Statins are currently the best known pharmaceutical intervention for CVD. In addition to their low-density lipoprotein (LDL)-cholesterol-lowering effect, statins exert anti-inflammatory actions by stimulating the expression of peroxisome proliferators-activated receptor. (PPAR)-γ [6]. In this study, combination of hydroxychloroquine and simvastatin was investigated for its anti-inflammatory activity using carrageenan-induced rat paw oedema.

MATERIAL AND METHODS

Chemicals

Hydroxychloroquine (HCQ) and simvastatin (SS) were provided by Ipca Laboratories Ltd., Mumbai, India. All other chemicals used were of IP/AR grade.

Equipment

Plethysmometer (UGO BASILE, Italy) was used for measurement of rat paw edema.

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Experimental Animals

Albino Wistar rats weighing 300-350g of either sex were procured from Central Animal Facility, Birla Institute of Technology and Science, Pilani, India. The animals were housed in 37cm x 23cm x 16cm polypropylene cages with maximum 3 animals per cage and acclimatized for a period of 7 days. Individual animal was identified by a mark on tail with permanent marker and cages were identified with label pasted on cages with relevant information. Animals were housed at a temperature of 24 $\pm$ 2 ^{o} C and relative humidity of 30 to 70 % in an air conditioned area. A 12:12 (light: dark) cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet. The experimental protocols were approved by the Institutional Animal Ethics Committee of the Institute. All animal experiments were carried in accordance with guidelines of CPCSEA.

Experimental design

Rats were divided into five groups consisting of six animals each.

Acute inflammation was produced by the sub-plantar administration of 0.1ml of 1%w/v carrageenan in normal saline (0.9% NaCl) in right hind paw of rats. The Group I (Control) was treated with 1ml of saline, Group II with HCQ (24 mg/kg of HCQ; p. o.) Group III with SS (1.3 mg/kg of SS; p.o.), Group IV was treated with high dose of HCQ (24 mg/kg; p.o.) plus SS (1.3 mg/kg; p. o), Group V was treated with low dose of HCQ (12 mg/kg; p.o) plus SS (0.6 mg/kg; p. o). Rat paw volume was measured before the injection and then at 1,2,4,6 and 9 hr after carrageenan injection using rat paw edema meter (IITC Woodland Hills, USA. Model 520). The animals were treated with the drugs 60 min before the administration of carrageenan. Data obtained were used to calculate % inhibition of inflammation at different time points. Results were statistically treated for determining the level significance.

% inhibition of inflammation was calculated using the formula,

Where % inhibition= 100[Vc-Vt/Vc]

Where 'Vc' represents oedema volume in control and 'Vt' oedema volume in treated with test extracts.

Statistical analyses

Results are expressed as mean $\pm$ SD. The statistical analysis was performed by One Way Analyses of Varience (ANOVA) followed by Dunnet's t-test. The $p<0.05$ was considered as statistically significant.

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RESULTS

Sub-plantar injection of carrageenan in rat resulted in time dependant increase in paw thickness. Carrageenan-induced inflammation was significantly (*P<0.05*) reduced in all phases of the experiment after treatment with HCQ and SS alone and in combination.

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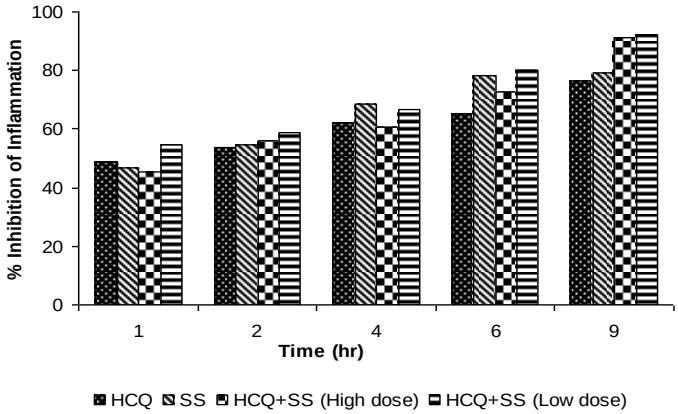

Figure 1: Mean % inhibition of inflammation by hydroxychloroquine, simvastatin, high dose combination of hydroxychloroquine with simvastatin and low dose combination of hydroxychloroquine with simvastatin

The group treated with high dose combination of HCQ and SS (24 mg/kg + 1.3 mg/kg) reported 91% inhibition of rat paw edema. While animals treated with low dose combination (12 mg/kg + 0.6 mg/kg) showed 92.3 % inhibition of rat paw edema. Maximum reduction in rat paw edema was observed at 9h after drug treatment. Moreover the reduction in rat paw edema was significantly more in animals treated with low dose combination of HCQ and SS as compared to their individual components.

DISCUSSION

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation [7]. It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2h after carrageenan injection), chemical mediators such as histamine and serotonin play their role, while in second phase (3-4 h after carrageenan injection) Kinin and prostaglandins are involved [8]. Our results suggest that administration of HCQ and SS alone and in combination inhibited the edema from the first hour and during all phases of inflammation, which is probably the inhibition of different aspects and chemical mediators of inflammation.

Moreover the low dose combination of HCQ and SS has shown activity significantly more (*P<0.001*) at 9 hr than the high dose and individual treatment doses. This shows that the potentiation of anti-inflammatory activity of HCQ by adding SS is dose independent. This also suggests that the action of SS may be reaching the saturation as there was no enhancement when dose of SS was doubled. In group treated with the combination of low dose of HCQ and SS, the activity was found to be more significant, particularly at 6 and 9 hr period (significant level at $p<0.05$ and <0.01 respectively). Probably presence of SS is extending the duration of action of HCQ.

It can be concluded from the results of this study that the combination of hydroxychloroquine and simvastatin has a significant anti-inflammatory activity which will open new avenues in the treatment of inflammation. Further studies involving other models of inflammation and well controlled clinical trials are required in the development of a effective and safe anti-inflammatory alternative.

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