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Short Communication

ANTI-INFLAMMATORY ACTIVITY OF HMG CO A REDUCTASE INHIBITORS: A COMPARATIVE STUDY

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ABSTRACT

Objective: The purpose of the present study was to reveal the potential pleotropic anti-inflammatory activity of two hmg-co Reductase inhibitors, Rosuvastatin and atorvastatin in comparison with standard drug diclofenac.

Methods: The method used for screening anti-inflammatry activity was *In vitro* human red blood cell membrane stabilization method.

Results: The results of our study revealed that atorvastatin and rosuvastatin showed effective *in vitro*(500mcg/ml) anti-inflammatory activity when compared with the standard drug diclofenac.

Conclusion: The rosuvastatin was more potent anti-inflamatory drug when compared with atorvastatin.

Keywords: Atorvastatin, Rosuvastatin, Membrane stabilizing, Anti-inflammatory.

The process of inflammation includes activation of the number of enzymes, extravasation of inflammatory cells, release of mediators of inflammation, proliferation, tissue breakdown and repair [1]. It occurs in three different phases. The first phase of inflammation is characterized by an increase in vascular permeability resulted in fluid exudation from the blood into the interstitium. The infiltration of leucocytes, monocytes and macrophages from the blood into the inflamed tissues constitute the second phase of inflammation and the third one comprises of chronic proliferative phase leading to granuloma formation. The screening methods for the evaluation of potential anti-inflammatory agents are aimed to counteract these phases [2, 3]. At present the pharmaceutical research is concentrated to explore the potential pleotropic effects of clinically using safer drugs, as the discovery of a new agent with new therapeutic action is not only a time consuming complex procedure but also create greater financial burden to pharmaceutical companies.

Statins are drugs which are in long term clinical use with adequate safety for the treatment of hyperlipidaemia, which act by inhibiting the enzyme HMG-Co reductase enzyme, a rate limiting enzyme involved in cholesterol biosynthesis [4]. Several clinical research trial reports suggest that the observed clinical benefit of statin therapy is much greater than that expected, which may be due to its potential anti-inflammatory activity. Inflammatory processes have an important role in the pathogenesis of the number of diseases like atherosclerosis and statins exhibit anti-inflammatory activity and reduce these events than expected [5, 6]. Numerous studies suggested that statins antagonize one of the major phagocytic cell, macrophage function, as the macrophages secreting matrix metalloproteinase capable of degrading the extracellular matrix and thus leads to atheromatous plaque to rupture [7, 8].

Our aim was to investigate whether statins exhibit any anti-inflammatory activity independent of its cholesterol lowering effect; so that these agents may be useful in other disease conditions where inflammation is the root cause for disease amplifiation. Two statins atorvastatin(commonly used) and rosuvastatin (potent) were selected and HRBC membrane stabilization method was used for screening *in vitro* anti-inflammatory activity (Gandhisan etal. 1991) [9]. Since HRBC membrane is similar to lysosomal membrane, this method is an important *in vitro* screening method for anti-inflammatory activity as the enzyme released from lysosomes have pivotal role in acute and chronic inflammation and also most of the currently available anti-inflammatory drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [10, 11].

Atorvastatin, rosuvastatin, and diclofenac reference samples were obtained from Ranbaxy pharmaceuticals, Pvt, Ltd, Punjab and are identified by taking IR spectra and which was comparable with reference standard spectra. The selected statins were dissolved in DiMethylSulphoxide(DMSO). 5mg of the drug was disolved in 10 ml* of the solvent so as to get a final concentration of 500mcg/ml and standard drug diclofenac was dissolved in 10 ml* of distilled water to get a final concentration of 50mcg/ml. In order to conduct the anti-inflammatory activity, blood was collected from healthy human volunteer who had not taken any anti-inflammatory drugs for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2%dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline and a 10 %(v/v) suspension was made with isosaline. Prepared the assay mixture by adding 1 ml* of drug solution (Diclofenac 50mcg/ml, statins 500mcg/ml), 1 ml of 0.15M phosphate buffer of pH 7.4, 2 ml of hyposaline and 0.5 ml* of HRBC suspension. 1 ml* of distilled water was used in the control instead of drug solution. All the assay mixtures were incubated at 37^0C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water as 100%[1, 3, 10, 11].

Percentage of haemolysis = [OD of test/ OD of control]100

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula;

Percentage protection = [100-Percentage of haemolysis].

The control represent 100% lyses

All the data are expressed as mean ± S. E. M. Statistical significance was determined by students 't' test; P value less than 0.05 was regarded as significant. The *in vitro* anti-inflammatory activity of rosuvastatin(500mcg/ml) and atorvastatin(500mcg/ml) was found to be 62.8% and 47.4% respectively when compared with 74.8% of protection offered by standard drug diclofenac (50mcg/ml). Data shown in fig: 1.

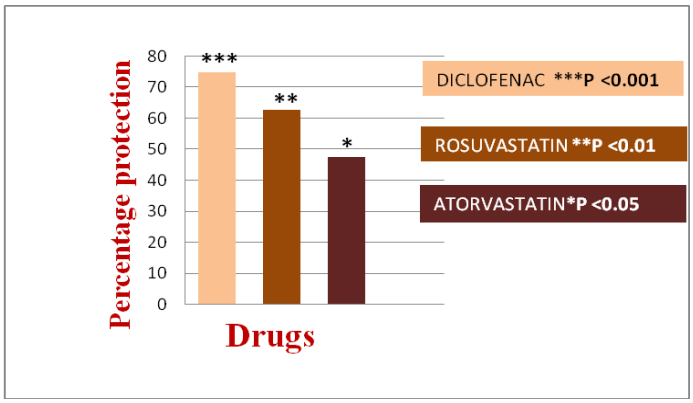

Fig. 1: Graphical representation of antiinflammatry activity of statins

Stabilization of human red blood cells membrane is a commonly used method for screening *in vitro* anti-inflammatory activity. RBC membrane is considered to be physiologicaly similar to lysosomal membrane, as both the membrenes are composed of same types of lipids and proteins. So it is assumed that stabilization of RBC membrane by an agent indirectly implies that the compound can stabilize the lysosomal membrane and exhibit anti-inflammatory activity; as many of the clinically available anti-inflammatory drugs act by stabilizing the lysosomal membrane. Stabilization of lysosomal membrane helps to prevent the release of inflammatory mediators such as bactericidal enzymes and proteases and thus limits the inflammatory process[14].

Non-steroidal anti-inflammatory drugs(NSAIDs) like diclofenac act by inhibiting the lysosomal enzymes cyclooxygenase, an enzyme which is responsible for conversion of membrane phospholipid, arachidonic acid into prostaglandins. Some of the NSAIDs are known to exhibit membrane stabilization due to osmotic loss of intracellular electrolyte and fluid components[15].

The selected statins, atorvastatin and rosuvastatin at 500mcg/ml concentration exhibit significant membrane stabilization effects towards the hypotonicity induced lyses of erythrocyte membrane, which was comparable with standard drug diclofenac. Maximum *in vitro* anti-inflammatory activity was obtained with rosuvastatin (P<0.001) at 500mcg/ml concentration. Further *in vivo* studies using suitable animal models of inflammation will be needed to confirm the role of statins in inflammations.

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