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Antimalarial drug artesunate affords protection against carrageenan induced acute inflammation in rat

Vijay L. KUMAR[1]*, B. GURUPRASAD[1], Priyanka CHAUDHARY[2]

[1] Department of Pharmacology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110 029, India
[2] Department of Plant Molecular Biology, Delhi University South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi -110 021, India.

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ABSTRACT: Artesunate, an antimalarial drug, has been shown to inhibit the release of inflammatory mediators in various disease conditions. The present study was carried out to evaluate the anti-edematogenic effect of artesunate in the rat paw edema model. Inflammation was induced in the hind paw of rat by sub-plantar injection of 0.1 mL of 0.5% carrageenan and the paw volume was measured up to a fixed mark just before the injection and then after 3 h. The difference in two volumes gave a measure of edema formation. At 3h the level of TNF-α, PGE_2 and myeloperoxidase were estimated in the inflamed paw tissue. Treatment of rats with single dose of artesunate at 50 and 150 mg/kg produced a dose-dependent inhibition in paw inflammation where a significant reduction in edema volume and mediator release was observed. Our study shows that by inhibiting the release of inflammatory mediators artesunate affords protection against acute inflammation induced in the rat paw and suggests that it has a potential to be used in the treatment of inflammatory disease conditions.

Introduction

Inflammation, an adaptive response of tissues to noxious stimuli, involves various vascular and cellular events. Acute inflammatory response is characterized by edema formation as a result of an increase in vascular permeability, extravasation of fluid, proteins and infiltration of neutrophils. The establishment and perpetuation of inflammatory process is mediated through orchestrating effects of various mediators among which tumor necrosis factor- α (TNF-α) and prostaglandin E_2 (PGE_2) play an important role (Medzhitov, 2010; Funk 2001). These mediators are also released in various disease conditions to a variable extent and inhibition of their synthesis and release is well known to ameliorate some of these conditions or to alter the responsiveness to drugs (Kulmaytcki and Jamali 2005).

*Address correspondence to: Vijay L. Kumar, kumarvl98@gmail.com
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The aerial parts of the plant *Artemisia annua* have been used in Chinese traditional medicine for the treatment of malaria and several other disease conditions like asthma, bronchitis, sore throat, hemorrhoid and lupus erythematosus (Anamed; WHO, 2006). Artemisinin, an active constituent of this plant, has been shown to possess low water solubility and oral bioavailability. Its semi-synthetic derivative artesunate, on the other hand, is water soluble, exhibits better oral bioavailability and is well known for its efficacy as combination therapy in *Plasmodium falciparum* positive complicated malaria (Graziose *et al.,* 2010; Ferreira *et al.,* 2010). It has been reported to exhibit immunomodulatory and protective effect in murine models of systemic lupus erythematosus and sepsis respectively (Jin *et al.,* 2009; Li *et al.,* 2008; Li *et al.,* 2010). In animal models of various diseases and *in vitro* culture of synoviocytes, it has been shown to inhibit the release of inflammatory mediators like nitric oxide (NO), TNF-α, PGE_2 (Mirshafiey *et al.,* 2006; He *et al.,* 2011; Wang *et al.,* 2011; Li *et al.,* 2013). The present study

was carried out to evaluate the efficacy of artesunate in inhibiting edema formation and mediator release in a well established paw edema model widely used for the screening of anti-inflammatory compounds and to compare it with that of standard anti-inflammatory drug diclofenac (Otterness and Bliven,1985).

Materials and Methods

Experimental animals

The study was carried out on Wistar rats of either sex weighing between 130-150 g which were maintained at ambient temperature and had free access to food and water. The experiments were carried out as per the guidelines of Institutional Animal Ethics Committee following due approval (631/IAEC-11).

Experimental procedure

Rats were divided into five groups (n=6) of which Group I served as normal control (NC). In the remaining four groups, inflammation was induced in the hind paw by sub-plantar injection of 0.1 mL of 0.5% carrageenan (Winter *et al.,* 1962). Rats in Group II served as control and those in Group III and Group IV were treated with artesunate (50 mg/kg, A50 and 150 mg/kg, A150) and in Group V were treated with diclofenac (10 mg/kg, D10). The drugs were given orally 1 h before injecting carrageenan. Paw volume was measured up to a fixed mark on the lateral malleolus before (0 h) and 3 h after injecting carrageenan using a plethysmometer and the edema volume was calculated by taking the difference between the two volumes. At the end of study the rats were sacrificed, the paw tissue was dissected out and kept at -80 °C for biochemical analysis.

Estimation of tissue TNF-α and PGE_2 levels

A tissue homogenate (10%) was prepared in cold phosphate buffered saline (pH 7.4) and centrifuged. The levels of TNF-α and PGE_2 were measured in the supernatant by ELISA using kits (Cayman Chemical Company, USA and Gen-Probe, France respectively) and the levels were expressed as pg/mg tissue.

Estimation of tissue myeloperoxidase (MPO) levels

MPO level was measured in the tissue homogenate (10%) prepared in 50 mM phosphate buffer containing 0.5% hexadecyl trimethyl ammonium bromide (pH 6.0) by the method of Bradley *et al.* (1982) and expressed as µmol/mg tissue.

Statistical analysis

The values are expressed as mean ± SEM and one way ANOVA followed by post-hoc analysis (LSD) was carried out to compare the control group with drug treated groups using SPSS programme version 11.5.

Results

Sub-plantar injection of carrageenan in the hind paw of rat produced an increase in the paw volume at 3 h due to edema formation. Treatment of rats with artesunate produced a dose-dependent inhibition in paw inflammation and the edema volume in A50 and A150 groups was 0.47±0.02 and 0.21±0.01 mL against 0.74±0.02 mL in carrageenan control (36 and 72 % inhibition). The edema volume in D10 group was 0.31±0.02 mL (58 % inhibition) (Fig. 1). The increase in paw volume with carrageenan was associated with a marked increase in the levels of TNF-α to 10.95±0.18 pg/mg tissue against 4.98±0.14 pg/mg tissue in NC group. Like diclofenac,

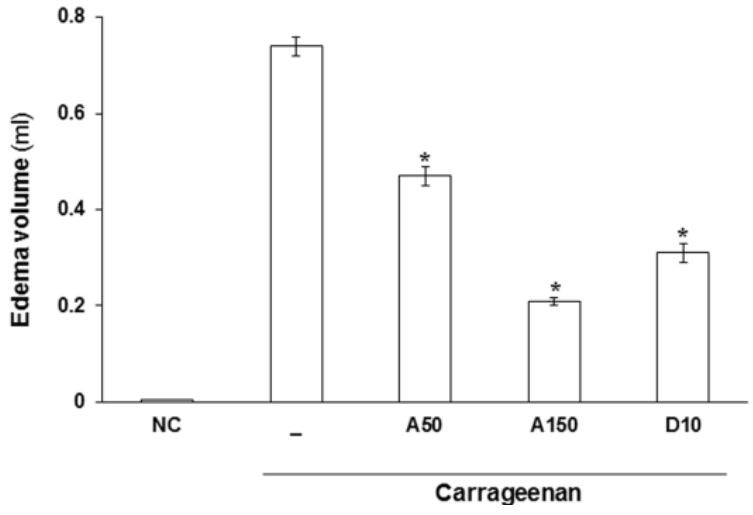

FIGURE 1. Effect of artesunate on edema volume in carrageenan induced paw inflammation in rat. Inflammation was induced by single sub-plantar injection of carrageenan in the hind paw and the edema volume was measured at 3h. The drugs artesunate and diclofenac were administered orally once 1h before inducing inflammation in respective groups. The results are given as mean±SEM. Abbreviations: NC, normal control; -, carrageenan control; A50, artesunate 50 mg/kg; A150, artesunate 150 mg/kg; D10, diclofenac 10 mg/kg. Star indicates the statistically significant difference in the mean values in the drug treated group and the carrageenan control group (ANOVA and LSD post hoc test; $p<0.001$).

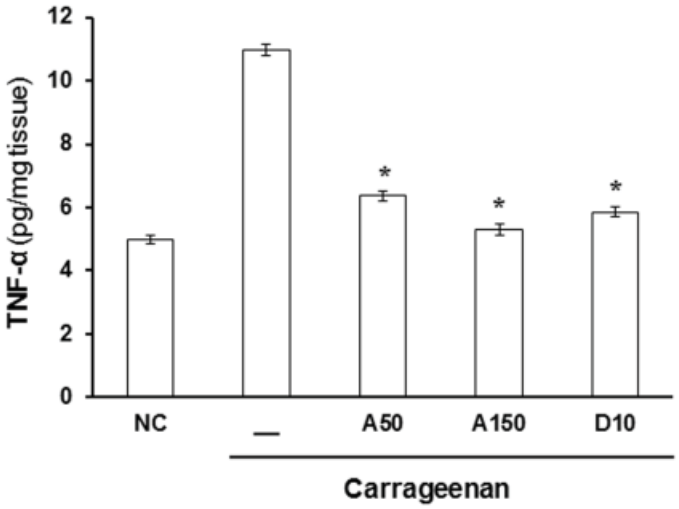

FIGURE 2. Effect of artesunate on tissue levels of TNF-α in carrageenan induced paw inflammation. Inflammation was induced by single sub-plantar injection of carrageenan in the hind paw and the TNF-α level in the paw tissue was measured at 3h. The drugs artesunate and diclofenac were administered orally once 1h before inducing inflammation in respective groups. The results are given as mean±SEM. Abbreviations: NC, normal control;-, carrageenan control; A50, artesunate 50 mg/kg; A150, artesunate 150 mg/kg; D10, diclofenac 10 mg/kg. Star indicates the statistically significant difference in the mean values in the drug treated group and the carrageenan control group (ANOVA and LSD post hoc test; $p<0.001$).

artesunate produced a significant reduction in tissue levels of TNF-α. The levels of TNF-α in A50, A150 and D10 groups were 6.36±0.06, 5.30±0.17 and 5.86±0.17 pg/mg tissue (Fig. 2). In inflamed paw, tissue levels of PGE_2 increased to 76.51±2.18 pg/mg tissue as compared to 55.56±0.79 pg/mg tissue in NC group. Treatment with artesunate brought down the tissue levels of PGE_2 and the effect of artesunate was comparable to that of diclofenac (60.40±0.41 pg/mg tissue in A150 and 60.49±0.22 pg/mg tissue in D10 groups) (Fig. 3). Peak inflammation at 3 h following carrageenan injection in the hind paw of rat was associated with neutrophil infiltration resulting in an increase in the tissue levels of MPO. The levels of MPO in carrageenan control group were 112.46±5.87 µmol/mg tissue against 41.30±2.32 µmol/mg tissue in NC group. Treatment with artesunate produced a dose-dependent reduction in MPO levels and its effect was comparable to that of diclofenac. The levels of MPO in A50, A150 and D10 groups were 53.18±1.72, 34.20±1.90 and 40.28±3.43 µmol/mg tissue (Fig. 4).

Discussion

Artesunate, a semi-synthetic derivative of artemisinin extracted from the plant *Artemisia annua*, is a well known drug for treating malaria and has been shown to inhibit the release of mediators of inflammation in various disease conditions. The present study was carried out to evaluate the efficacy of artesunate in inhibiting experimentally induced edema in the rat paw model. Sub-plantar injection of carrageenan produced an increase in the paw volume at 3 h due to the involvement of vascular and cellular events that are mediated through the release of various pro-inflammatory mediators (Funk, 2001). TNF-α is one such

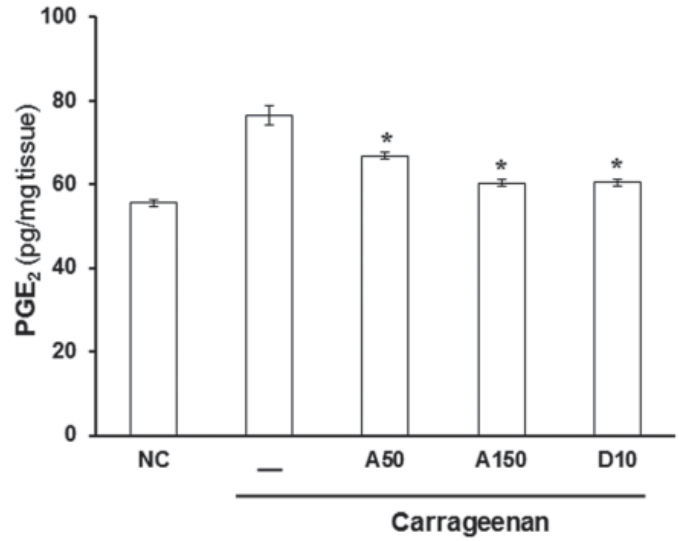

FIGURE 3. Effect of artesunate on tissue levels of PGE_2 in carrageenan induced paw inflammation. Inflammation was induced by single sub-plantar injection of carrageenan in the hind paw and the PGE_2 level in the paw tissue was measured at 3h. The drugs artesunate and diclofenac were administered orally once 1h before inducing inflammation in respective groups. The results are given as mean±SEM. Abbreviations: NC, normal control; -, carrageenan control; A50, artesunate 50 mg/kg; A150, artesunate 150 mg/kg; D10, diclofenac 10 mg/kg. Star indicates the statistically significant difference in the mean values in the drug treated group and the carrageenan control group (ANOVA and LSD post hoc test; $p<0.001$).

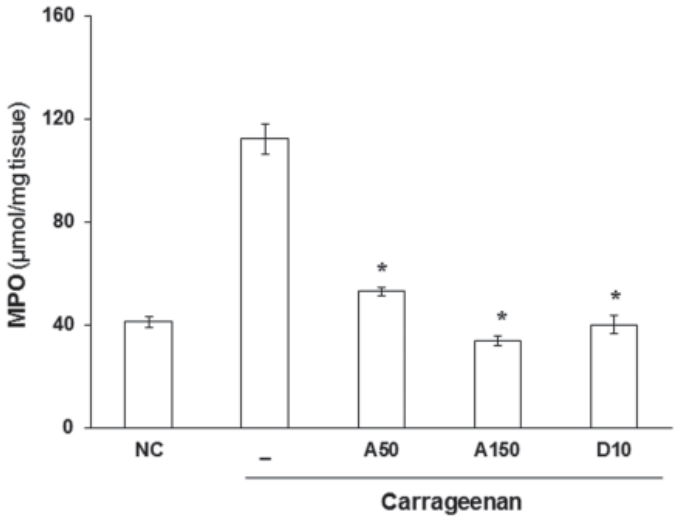

FIGURE 4. Effect of artesunate on tissue levels of MPO in carrageenan induced paw inflammation. Inflammation was induced by single sub-plantar injection of carrageenan in the hind paw and the MPO level in the paw tissue was measured at 3h. The drugs artesunate and diclofenac were administered orally once 1h before inducing inflammation in respective groups. The results are given as mean±SEM. Abbreviations: NC, normal control; -, carrageenan control; A50, artesunate 50 mg/kg; A150, artesunate 150 mg/kg; D10, diclofenac 10 mg/kg. Star indicates the statistically significant difference in the mean values in the drug treated group and the carrageenan control group (ANOVA and LSD post hoc test; $p<0.001$).

mediator that has been widely implicated both in establishment and perpetuation of the inflammatory process and its levels were found to be high in the inflamed paw tissue. Further, the edema formation induced by carrageenan was associated with concomitant increase in PGE_2 production. Prostaglandins not only bring about vasodilatation by themselves but they are also known to potentiate the microvascular effects of other mediators such as histamine, substance P and bradykinin (Williams and Morley, 1973; Williams and Peck, 1977; Ricciotti and FitzGerald, 2011). Treatment with artesunate produced a dose-dependent inhibition of edema formation that was accompanied by a reduction in the levels of TNF-α and PGE_2. Artesunate has earlier been reported to decrease the levels of these mediators in animal models of sepsis and uveitis (Li *et al.,* 2010; Wang *et al.,* 2011). Recently, it has been reported to attenuate inflammatory symptoms and to prevent cartilage and bone destruction by decreasing the expression of pro-inflammatory cytokines (Li *et al.,* 2013). Both TNF-α and PGE_2 are chemotactic for polymorphonuclear cells and the accumulation of these cells at the site of inflammation was substantiated by a marked increase in the levels of MPO, a heme protein that serves as a marker for these cells and contributes to vascular dysfunction and establishment of pro-inflammatory milieu by modulating various redox sensitive signalling pathways involved in an inflammatory response and generating pro-oxidants (Rekdal *et al.,* 1994; Issekutz *et al.,* 1982; Beutler and Cerami, 1986). These cells play an important role in the development and full manifestations of acute inflammation (Wright *et al.,* 2010). An exaggerated recruitment of neutrophils may lead to tissue destruction via the production of reactive oxygen metabolites, enzymes and cytokines that further amplify the immune response (Schreck and Baeuerle, 1994; Perkins, 2007). Treatment with artesunate produced a marked reduction in the levels of MPO in the inflamed paw tissue. The inhibitory effect of artesunate on mediator release at the site of inflammation was comparable to that of diclofenac. Like aqueous extract of aerial parts of the plant *A. annua,* artesunate has earlier been reported to ameliorate oxidative damage by suppressing pro-oxidants and restoring the activities of antioxidants (Ho *et al.,* 2012; Szeto and Benzie, 2006).

Thus, the present study shows that artesunate, a semi-synthetic derivative of artemisinin extracted from *A. annua* inhibits edema formation and mediator release in experimentally induced inflammation and it has the potential to be used in ameliorating inflammatory disease conditions.

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