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

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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, PGE2 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 E2 (PGE2) 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).

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, PGE2 (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 artemisinin 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 artemisinin (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 artemisinin 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,
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 PGE₂ 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 PGE₂ 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...
mediator that has been widely implicated both in establish-
ment 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 PGE2 production.
Prostaglandins not only bring about vasodilatation by them-
selves but they are also known to potentiate the microvascu-
lar 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 PGE2. 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 decreas-
ing the expression of pro-inflammatory cytokines (Li et
al., 2013). Both TNF-α and PGE2, are chemotactic for pol-
morphonuclear 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 dys-
function and establishment of pro-inflammatory milieu by
modulating various redox sensitive signalling pathways in-
volved 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 neutro-
phils may lead to tissue destruction via the production of re-
active oxygen metabolites, enzymes and cytokines that fur-
ther 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 restor-
ing 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 ex-
perimentally induced inflammation and it has the potential
to be used in ameliorating inflammatory disease conditions.

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FIGURE 4. Effect of artesunate on tissue levels
of MPO in carrageenan induced paw inflam-
mation. 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 di-
clofenac were administered orally once 1h be-
fore inducing inflammation in respective groups.
The results are given as means±SEM. Abbrevi-
ations: 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 treat-
ed group and the carrageenan control group
(ANOVA and LSD post hoc test; p<0.001).


