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Anti-inflammatory and antipyretic activities of artesunate in experimental animals

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ABSTRACT

Objective: To evaluate the anti-inflammatory and antipyretic potentials of artesunate in albino mice and Wistar rats respectively.
Methods: For the anti-inflammatory activity, artesunate (5 mg/kg) was administered orally against egg albumin- and xylene-induced inflammation in mice using ibuprofen (50 mg/kg) as standard drug. To assess antipyretic activity, artesunate (5 mg/kg) was administered orally against d-amphetamine- and 2, 4-dinitrophenol-induced pyrexia in rats using ibuprofen (15 mg/kg) as standard drug.
Results: The result showed that artesunate significantly ($P < 0.001$–0.010) reduced inflammation induced by egg albumin and xylene in a time-dependent manner. It also significantly ($P < 0.001$–0.050) and time-dependently reduced pyrexia induced by d-amphetamine and 2, 4-dinitrophenol. These reductions were similar to those produced by the standard drug ibuprofen, and thereby demonstrating that artesunate possesses anti-inflammatory and antipyretic activities.
Conclusions: These results further support the rationale for the use of artesunate in the treatment of malaria, a disease characterized by fever and inflammation and open up possibilities of its usefulness in other inflammatory and feverish diseases.

1. Introduction

Artesunate is a derivative of artemisinin. It is semi-synthetic and used mainly to treat malaria. Malaria is mostly caused by protozoans called *Plasmodium falciparum* and *Plasmodium vivax*. It is still one of the topmost infectious diseases in the world today[1]. The World Health Organization has recommended artemisinin-based combination therapies for the treatment of malaria. Artesunate, as a derivative, is generally well-tolerated and safe, and it has minimal adverse effects[2]. It is a water-soluble artemisinin derivative extracted from the Chinese herb *Artemisia annua*. It is commonly used as an effective and safe anti-malarial drug. It has been recently reported that artesunate has a potential as a new and effective anti-cancer drug[3]. Artemisinin, a naturally occurring endoperoxide, has derivatives with anti-malarial properties and forms the basis of the present global treatment approach for *Plasmodium falciparum* malaria. These derivatives include artesunate, artemether and dihydroartemisinin, which produce more effective reductions in parasitaemia and provide more rapid symptom relief than other anti-malarial drugs. Among these derivatives, artesunate is the most therapeutically useful artemisinin derivative indicated for the treatment of mild to severe malaria infection. Because of its therapeutic efficacy and the need for appropriate dosing, many researchers have directed their attention towards evaluating

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the pharmacokinetics of artesunate and its active metabolite dihydroartemisinin[4]. The artemisinin derivatives have now become pivotal in the treatment of falciparum malaria and artemisinin combination therapies are used in the second and third trimesters of pregnancy[5]. Traditional Chinese medicine, with its 5000 years of practice, occupies a unique position among all traditional medicines. Recently, artemisinin-type sesquiterpene lactones from *Artemisia annua* have been found to be active against malaria, cancer cells, and schistosomiasis. Moreover, artemisinin and its semi-synthetic derivative artesunate are effective against certain viruses such as human cytomegalovirus, herpes simplex virus type 1, Epstein-Barr virus, hepatitis B virus, hepatitis C virus and bovine viral diarrhea virus[6]. There is little or no scientific report on effect of artesunate on inflammation and pyrexia. This study, therefore, aimed at assessing whether artesunate possesses anti-inflammatory and antipyretic properties.

2. Materials and methods

2.1. Drugs

Ibuprofen caplets (Brustan-N by Ranbaxy Pharmaceuticals, Nigeria) and artesunate tablets (Artesunat by Mekophar Chemical Pharmaceutical Joint-Stock Company, Vietnam) were used for the evaluation.

2.2. Experimental animals

Albino mice and Wistar rats with weights ranging 22–32 g and 154–298 g, respectively were obtained and kept in the animal house of Faculty of Pharmacy, University of Uyo. The animals were kept in plastic cages under good light and well ventilated housing condition. They were fed with standard pellet feed (Grand Cereals Ltd., Plateau State, Nigeria) and allowed to access to water except during the experiment. All experimental procedures involving animals were conducted in accordance to Organization for Economic Co-operation and Development guidelines and approved by Animal Ethics Committee, Faculty of Pharmacy, University of Uyo.

2.3. Determination of anti-inflammatory activity of artesunate against egg albumin-induced inflammation

The inflammatory agent used in this model was fresh egg albumin. Albino mice of either sex used were fasted for 24 h before the commencement of the experiment and were not given water during the experiment. The animals were divided into three groups with six animals per group. The linear circumference of the subplantar surface of the right hind paw of each mouse was measured before injecting 0.1 mL of fresh egg albumin to cause inflammation[7,8]. Group 1 animals were used as control and given 5 mL/kg of normal saline intraperitoneally. Groups 2 and 3 animals were administered with 5 mg/kg and 50 mg/kg of artesunate and ibuprofen orally, respectively. Artesunate was the test drug while ibuprofen served as the standard drug. The treatments were administered to all the animals 30 min after induction of inflammation. Thereafter, the linear circumference of the inflamed right paw was measured using a vernier calipers every thirty minutes for 5 h.

2.4. Xylene-induced ear oedema

Albino mice of either sex were used. They were fasted for 24 h before the commencement of the experiment and were not given water during the experiment. Thereafter, they were divided into three groups containing six animals each. Two drops of xylene were applied at the inner surface of the right ear of mice to induce inflammation and allowed to react for 15 min. Group 1 animals were given 5 mL/kg of normal saline intraperitoneally as control. Groups 2 and 3 animals were given 5 mg/kg and 50 mg/kg of artesunate and ibuprofen orally, respectively. All treatments were given to the animals 30 min after inducing inflammation. Thereafter, animals were sacrificed under anesthesia using chloroform and both ears were cut off. The difference between the weights of the left and right ear was recorded as oedema induced by xylene[9,10].

2.5. Evaluation of antipyretic activities

2.5.1. D-amphetamine-induced pyrexia in rats

Adult albino Wistar rats of either sex were fasted for 24 h but water was given *ad libitum* before the commencement of the experiment. Basal rectal temperatures of the animals were recorded with the aid of a digital thermometer. Thereafter, d-amphetamine (5 mg/kg) was given to the animals intraperitoneally. Within 30 min following the administration of d-amphetamine, animals with increased temperature of 1 ˚C were selected and divided into three groups of six animals per group. Group 1 animals were administered with 5 mL/kg of normal saline intraperitoneally as the control. Group 2 animals were given 5 mg/kg of artesunate orally. Group 3 animals received 15 mg/kg of ibuprofen orally which served as the standard drug. Rectal temperatures were obtained at 0.5 h and thereafter recorded hourly for 5 h[10,11].

2.5.2. 2, 4-Dinitrophenol (DNP)-induced pyrexia in rats

Adult albino Wistar rats of either sex were fasted for 24 h but water was given *ad libitum* before the commencement of the experiment. The basal rectal temperatures of all animals were taken and thereafter 10 mg/kg of DNP was administered intraperitoneally.

Animals that developed a rise in temperature by 1 ˚C within 30 min after DNP administration were divided into three groups of six animals per group. Group 1 animals were given normal saline (5 mL/kg) intraperitoneally as control. Group 2 animals received 15 mg/kg of artesunate orally. Group 3 animals received 15 mg/kg of ibuprofen orally which served as the standard drug. Rectal temperatures of all the animals in each group were then obtained and recorded at an hourly interval for 5 h[10,11].

2.6. Statistical analysis

Analysis of data obtained was done using the statistical software GraphPad Instat (version 3.10) and results were expressed as mean ± SEM. One-way ANOVA was used to assess differences between the treated and control groups and followed by Tukey Kramer multiple comparison post-test. Statistical significance was taken at $P \leqslant 0.05$.

3. Results

3.1. Egg albumen-induced inflammation

When artesunate was tested against egg albumin-induced paw oedema in mice, it showed a significant reduction ($P < 0.001$–0.01) in oedema compared to control. This reduction was also time-dependent. The effect was, however, less pronounced than that observed with ibuprofen (Table 1).

3.2. Xylene-induced ear oedema

The result showed that artesunate inhibited xylene-induced ear oedema in mice compared to control. This inhibition was statistically significant ($P < 0.001$). The percentage inhibition of inflammation caused by artesunate was far less than that obtained with ibuprofen (Table 2).

3.3. D-amphetamine-induced pyrexia

In this model, artesunate produced a significant ($P < 0.001$–0.05) and time-dependent reduction in the elevated rectal temperature of rats compared to control. This reduction compared favourably with that produced by ibuprofen (Table 3).

3.4. DNP-induced pyrexia

It was observed that artesunate reduced DNP-induced pyrexia and this reduction was significant ($P < 0.001$–0.01) and time-dependent compared to control. The antipyretic effect was, however, less than that of ibuprofen (Table 4).

Table 1
Anti-inflammatory activity of artesunate against egg albumin-induced oedema.

Groups	Dose (mg/kg)	Oedema (mm)						
		0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	5	2.68 ± 0.03	4.01 ± 0.04	4.03 ± 0.04	4.14 ± 0.09	4.01 ± 0.30	3.45 ± 0.37	3.88 ± 0.03
Artesunate	5	2.77 ± 0.09	3.61 ± 0.07**	3.69 ± 0.07*	3.05 ± 0.05**	3.14 ± 0.03**	3.14 ± 0.06*	2.80 ± 0.08**
Ibuprofen	50	2.67 ± 0.22	3.45 ± 0.05**	3.32 ± 0.07**	3.37 ± 0.06**	3.09 ± 0.01**	2.83 ± 0.05*	2.75 ± 0.02**

Values are represented as mean ± SEM. *: $P < 0.01$, **: $P < 0.001$ compared to control ($n = 6$).

Table 2
Effects of artesunate on xylene-induced inflammation.

Groups	Dose (mg/kg)	Weight of right ear (g)	Weight of left ear (g)	Increase in weight (g)	Inhibition (%)
Control	5	0.067 ± 0.002	0.037 ± 0.002	0.030 ± 0.000	-
Artesunate	5	0.053 ± 0.004	0.036 ± 0.002	0.020 ± 0.000*	33.30%
Ibuprofen	50	0.053 ± 0.002	0.043 ± 0.002	0.010 ± 3.790*	66.67%

Values are represented as mean ± SEM. *: $P < 0.001$ compared to control ($n = 6$).

Table 3
Effect of artesunate on d-amphetamine-induced pyrexia.

Groups	Dose (mg/kg)	Rectal temperature (˚C)						
		BT	0 h	1 h	2 h	3 h	4 h	5 h
Control	5	36.80 ± 0.14	39.50 ± 0.21	39.80 ± 0.11	39.40 ± 0.11	39.00 ± 0.32	39.84 ± 0.37	39.73 ± 0.31
Artesunate	5	36.80 ± 0.21	37.60 ± 0.23	38.48 ± 0.29**	38.45 ± 0.30**	37.00 ± 0.26**	36.87 ± 0.04**	36.77 ± 0.08**
Ibuprofen	15	37.20 ± 0.14	38.40 ± 0.12	38.49 ± 0.13**	38.44 ± 0.15**	38.20 ± 0.31**	37.97 ± 0.31*	37.57 ± 0.26**

Values are represented as mean ± SEM. *: $P < 0.05$, **: $P < 0.001$ compared to control ($n = 6$). BT: Basal temperature.

Table 4
Effect of artesunate on DNP-induced pyrexia.

Groups	Dose (mg/kg)	Rectal temperature (°C)						
		BT	0 h	1 h	2 h	3 h	4 h	5 h
Control	5	36.90 ± 0.16	39.00 ± 0.44	38.80 ± 0.20	39.90 ± 0.14	39.48 ± 0.17	38.85 ± 0.12	39.74 ± 0.11
Artesunate	15	37.00 ± 0.13	39.43 ± 0.14	39.47 ± 0.06*	38.92 ± 0.18**	38.66 ± 0.24**	38.61 ± 0.25**	37.54 ± 0.25**
Ibuprofen	15	37.20 ± 0.08	39.60 ± 0.16	38.73 ± 0.15**	38.38 ± 0.11**	38.20 ± 0.17**	38.09 ± 0.19**	37.97 ± 0.20**

Values are represented as mean ± SEM. *: $P < 0.01$, **: $P < 0.001$ compared to control ($n = 6$). BT: Basal temperature.

4. Discussion

Artesunate is an artemisinin derivative used primarily for the treatment of plasmodium infection. Its mechanism of action is majorly attributed to the presence of endoperoxides. Artemisinin also possesses anti-leishmanial, anti-schistosomal and anti-viral effects[12]. In this research work, the drug artesunate was evaluated for anti-inflammatory and antipyretic activities in mice and rats, respectively using various experimental models.

Inflammation is regarded as a mechanism of innate immunity that is the immediate first line defense to illness or pathogen[13]. It is the body's protective response as it fights to rid itself of both the cause of injury as well as the effect[14]. On the other hand, pyrexia is a clinical sign of inflammation. It functions in retarding the growth of temperature-sensitive microorganism, increasing metabolism of body cells while stimulating the immune reaction and the process of phagocytosis. Both inflammation and pyrexia are seen as cellular responses to infectious agents involving similar mediators. Pyrexia induced by DNP showed a higher and stable temperature compared to that induced by d-amphetamine. Anti-inflammatory, analgesics and antipyretics have similar fundamental mechanism of action, and their difference lies in the spectrum of activity. The corticosteroids are known to be very potent anti-inflammatory drugs but lack antipyretic and analgesic properties. Paracetamol has both analgesic and antipyretic activities with little anti-inflammatory activity, whereas the nonsteroidal anti-inflammatory drugs (NSAIDs) are known to have anti-inflammatory, antipyretic and analgesic effects[15]. NSAIDs are indicated in the treatment of inflammatory disorders, pyrexia and pain. There are various classes of NSAIDs. These include salicylates, propionic acid derivatives, acetic acid derivatives, enolic acid derivatives and selective cyclooxygenase 2 (COX-2) inhibitors. Nonsteroidal anti-inflammatory drugs exhibit their effects through the inhibition of COX enzymes, which results in the blocking of prostaglandins synthesis, thereby hindering the process which mediates inflammation and pyrexia.

The oedema produced by egg albumin is a biphasic response which has an early response phase mediated by histamine, serotonin and kinins, whereas the late response phase is linked to the release of prostaglandins and mediated by bradykinin, polymorphonuclear cells and leukotrienes[15,16]. The result shown in Table 1 revealed that artesunate produced its effect by acting on the early and late phases of inflammation.

Xylene can promote the activity of phospholipase A enzyme[17]. It is therefore useful in evaluating the role of this enzyme in the pathogenesis of inflammation. Substance P is also partially involved in the mediation of xylene-induced ear oedema[18]. Its release from the sensory neurons results in vasodilatation and plasma extravasation, which suggests its involvement in neurogenic inflammation, thus resulting in the ear oedema in mice. Other mediators that are involved in xylene-induced ear oedema are histamine, kinin and fibrinolysin. These mediators can promote vasodilation and increase vascular permeability[19]. The effect of artesunate on this model of inflammation can thus be attributed to its ability to inhibit the activities of these mediators and phospholipase A.

Dinitrophenol acts as a proton ionophore which causes increased level of intracellular calcium, muscle contraction and hyperthermia[20]. The result shown in Table 3 demonstrated that artesunate possesses antipyretic activity. This effect could be due to its ability to decrease the permeability of calcium ion.

Amphetamine is one of the sympathomimetic drugs which triggers increase in release of serotonin that in turn activates the synthesis of prostaglandins from arachidonic acid in the hypothalamus to cause fever[21,22]. Artesunate showed a reduction in the elevated rectal temperature of rats. In the model of amphethamine-induced pyrexia, this effect may be mediated through the inhibition of serotonin release or the same mechanism as ibuprofen.

In general, NSAIDs produce their anti-inflammatory and antipyretic effects through inhibition of prostaglandin synthesis[23]. The antipyretic action is possibly due to a decrease in the brain concentration of prostaglandin E2 especially in the hypothalamus. Artesunate may have inhibited COX-2 expression through its action on COX-3[24] and consequently inhibiting prostaglandin E2 biosynthesis so as to reduce its elevated plasma level. Besides prostaglandin synthesis, there are other mediators underlying the pathogenesis of fever, and inhibition of such mediators may cause antipyresis[25]. Artesunate may also enhance the synthesis of endogenous antipyretics like vasopressin and arginine[26] and may have caused genetic down-regulation of COX-2 expression in certain cell types.

The result of this study has established that artesunate possesses anti-inflammatory and antipyretic potentials. These findings further support the usefulness of the drug in the treatment of malaria that is associated with these symptoms, and open up possibilities of its use in other inflammatory diseases. Further investigation should

be carried out to find out the actual mechanism through which it exhibits these effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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