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Effect of artemether on analgesia and inflammation in mice and rats

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Abstract

Artemether, currently one of the first-line antimalarials in Nigeria was evaluated for its ability to reduce pain and inflammation, which are common symptoms of malaria. This study was to ascertain whether artemether has an analgesic / anti-inflammatory effect to complement its anti-parasitic activity against *Plasmodium* species. Acetic-acid-induced writhing test was used to measure peripheral-analgesic activity in mice while the tail-flick and hot-plate methods were used for central-analgesic activity. Fluid-volume-displacement by egg-albumin-induced hind-paw oedema in rats was used to assess for anti-inflammatory effect. Artemether was administered i.p., at three-doses equivalent to therapeutic-dose (1.5 mg/kg) as well as 5 and 10-times higher (7.5 and 15 mg/kg), once (acute) and daily for 7 days (sub-acute). Artemether inhibited peripheral-pain dose-dependently, but this was not significant at 1.5 mg/kg. Statistically significant pain-inhibition ($P<0.05$) greater than that due to dipyrone was provided only at the doses of 7.5 and 15 mg/kg artemether. Analgesic assessment using tail-flick and hot-plate methods did not show any detectable effect even at higher doses, indicating that any pain-relief due to artemether was not centrally-mediated. Artemether reduced the readiness / degree of fluid accumulation after an initial-transitory (30-60 minutes) increase in oedema, following albumin injection. These effects were similar to, but less than those of indomethacin. The data showed that artemether exhibited only mild peripheral analgesic and anti-inflammatory activities which may not be observable at therapeutic-doses and therefore may not, on its own contribute significantly to the relief of pain / inflammation in the treatment of malaria.

Keywords: Artemether; Analgesia; Inflammation; Mice; Rats

Introduction

Artemisinin and its derivatives are now routinely being used clinically as first-line antimalarials both as monotherapy and in combination with other drugs in many countries including Nigeria (Basco and Le-bras, 1993; WHO, 2006). Malaria is often the culprit suspected in any febrile condition that involves pains in the muscles, joints and abdomen as well as gastroenteritis amongst other clinical presentations (White and Pukrittayakamee, 1993; Bouree, 1997). Treatment of malaria is therefore aimed at not only eliminating the offending organism, but also at reducing the body pains and inflammations, which are common symptoms of this disease. Painful stimuli may arise from direct stimulation of sensory pain receptors by various means such as heat, pressure or chemical as well as inflammation (Vogel and

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Vogel, 1997). Inflammation is an injury, irritation or infection of a microvasculature-tissue whereby phagocytic leukocytes and other blood elements infiltrate the inflamed interstitial spaces causing painful swellings (oedema) (Roderick *et al.*, 1980). Most inflammatory mediators are immune mediated chemicals liberated locally in attempt to offset inflammations. The chemotactic factors such as bradykinins and especially prostaglandins amongst other released immune agents (histamine, 5-HT, slow reacting substance of anaphylaxis- SRS-A), are majorly the agent that ultimately results in increased flow and local pooling of blood (oedema), the pathological model of which is often induced in laboratory animals for experimental investigations. Analgesics of the non-steroidal anti-inflammatory drugs (NSAIDs) have aspirin-like properties and some relieve pain by blocking impulse generation at peripheral pain receptors, while others like salicylates or indomethacin merely inhibit a step in the synthetic pathway of cyclooxygenase (prostaglandin-synthetase) to prevent the release of prostaglandins with their attendant oedematous stimulation of pain receptors (Moncada and Vane, 1979). The effectiveness of aspirin-like drugs in inflammation is only in preventing the synthesis and release of prostaglandins that sensitize pain receptors. They are inactive against sharp-stabbing pains in the central nervous system or pains caused by the already released prostaglandins and which can only be blocked with narcotic analgesics like morphine (Vogel and Vogel, 1997). Artemether is a component of artemisinin-combination therapy (ACT) that is also currently the only available parenteral formulation of the artemisinin antimalarials used singly in Nigeria for the treatment of complicated and multi-drug resistant *P. falciparum* (Salako, 1998; Emdex, 2006; WHO, 2006). In the present study, artemether was evaluated for analgesic and anti-inflammatory effects in mice and rats respectively.

Experimental

Animals. Adult Wistar rats and Balb/c mice (male and female) were used throughout this study. The animals were bred locally in the Animal House of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University (ABU) Zaria. Ethical standards were complied with in the course of this investigation in accordance with the Department's Experimental Animals Committee (which approved the study) and which maintained the same standards of care and ethics with the Guiding Principles in the Use of Animals in Toxicology (Society of Toxicology; 1989).

Equipment. Ugo Basile Analgesiometer (7200, Comerio-Varese, Italy); Hot plate (7250, Comerio-Varese, Italy); Ugo Basile Plethysmometer (7150, Comerio Varese, Italy)

Drugs and Chemical-reagents Artemether (Rhone-Poulenc-Rorer, France); Morphine, Indomethacin (Sigma, St Louis Mo., USA); Dipyrone (Shanghai Medicines and Health Product Corporation, China); Glacial acetic acid (general purpose reagent, Johnson Solomon export limited, London); Polyethylene Sorbitan Monooleate (Tween 80; Sigma, USA); Normal saline (0.9% w/v), and Egg albumin (obtained from local source).

Acetic acid-induced writhing-test in mice. The method of Heap *et al.* (1993) was used. Fifteen (15) male mice weighing between 18-25 g were divided into 5 pretreatment groups for 3% v/v Tween 80 (vehicle control), 10 mg/kg dipyrone (standard analgesic), 1.5, 7.5 and 15 mg/kg artemether respectively. Twenty five (25) minutes drug pretreatment and 10 minutes pain writhe counts were used for each mouse. The drugs were administered intraperitoneally (i.p.) per body weight and

0.1 ml of 0.75% glacial acetic acid was also administered *i.p.* into each mouse after 25 minutes drug pretreatment to induce pain. The number of writhing reflex and / or stretching of the abdomen within 10 minutes of pain induction were recorded for each mouse. The mean pain reflex was calculated and the percentage pain inhibition was evaluated as follows:

% Pain inhibition = {(Average writhes in the control - writhes in the treated) x 100} / Writhes in the control

Analgesic effect of acute (tail flick & hotplate) and sub-acute (tail flick) artemether pretreat ment in mice. The Tail Flick Method of Green and Young (1951) and The Hot Plate Method of Woolfe and MacDonald (1944) were used to assess artemether for analgesic effect.

i. Acute tail flick method

Forty two (42) mice weighing between 14-28 g were divided into six (6) groups of seven (7) mice per group matched for weight for the various pretreatments with 3% v/v Tween 80 (control), 1.5 mg/kg (therapeutic dose), 7.5 mg/kg and 15 mg/kg artemether, 25 mg/kg dipyrone (standard peripherally-acting analgesic) and 2 mg/kg morphine (centrally-acting analgesic) respectively. Tail flicking or withdrawal reflex to an applied pressure exerted from an Ugo Basile Analgesiometer was used as an index for pain perception. A stopwatch or clock was pressed on as soon as the pressure was depressed on the mouse-tail and stopped instantaneously at the first visible tail flick or withdrawal. The time at which this effect occurred was then recorded as the pain reaction time for each mouse. The pain reaction times at 0 minute (control) were recorded for all the mice in each group prior to drug pretreatment. The animals were pretreated i.p per body weight and their reaction time to pain was checked periodically at 20, 40, 60, 80, 100, and 120 minutes of drug pretreatments. The mean reaction time for each group of mice was then calculated and compared statistically with the control group.

ii. Acute hot plate method

The hot plate method was also used to assess the central effect of pain perception in mice pretreated with artemether. Sixty (60) mice weighing between 14 -28.5 g were used. The animals were divided into five (5) pretreatment groups of 12 mice per group matched for weight. The plate was heated to 55°C and the pain reflex signs that were used to assess the animals were jumping, withdrawal of paws or licking of paws. A stop clock was started as soon as the animal was dropped on the heated plate and stopped on the first visible sign of any of the above responses. The control reaction times of mice in each group were recorded and the groups were pretreated respectively per body weight with i.p. injections of 3% v/v Tween 80 (control), the three dose levels of artemether (1.5, 7.5, 15 mg/kg) and 2 mg/kg morphine. The animals were tested for pain perception by checking their reaction times on the hot plate at 20, 40, 60, 80, 100 and 120 minutes of drug pretreatment. The mean reaction time for each group of mice was then calculated and compared statistically with the control group.

iii. Sub-acute Tail Flick

For the chronic tail flick experiment, a 7 day pretreatment was used. Twenty four (24) mice matched for weight were divided into four (4) pretreatment groups and treated accordingly in the same manner as above with 3% v/v Tween 80 (control), 1.5, 7.5 and 15 mg/kg artemether respectively for 6 consecutive days. The animals were tested for pain perception at 20, 40, 60, 80, 100, and 120 minutes after the 7^{th} day drug pretreatments. The mean reaction time for each group of mice was then calculated and compared statistically with the control group.

Anti-inflammatory effect of acute and sub-acute artemether pretreatment in rats. The method of albumin-induced hind paw oedema was used to assess artemether for acute and sub-acute inflammation in rats. Ugo Basile

Plethysmometer (Comerio Varese, Italy) apparatus was used to measure the paw volume in the manner described by Alpermann and Magerkurth (1972). Undiluted fresh egg white, 0.1 ml, which induced a short lasting inflammation of about three hours in rats was the irritant used in this experiment as described by Randall and Baruth (1976).

i. Assessment of the Degree of Egg Albumin-Induced Oedema and the Inhibitory Effect of Artemether in Rats

Twenty-four (24) male rats weighing between 167-176.5 g were divided into four (4) groups matched for weight for various pretreatments with 3% v/v Tween 80, albumin alone, 50 mg/kg Indomethacin + albumin and 15 mg/kg artemether + albumin. The control reading of the paw volumes prior to treatments (0 minute reading) was recorded for all the rats in each group. The volume of fluid (ml) displaced when the hind paw of the rat was immersed into the levelled saline fluid in the cuvet of the apparatus was indicated on the pressure transducer. This was used as a measure of the hind paw volumes. The animals were treated i.p. with the various drugs and each treated animal, with the exception of those treated with 3% v/v Tween 80, was injected immediately with 0.1 ml of fresh egg albumin subcutaneously into the plantar side of the left hind paw. The paw volumes were read accordingly as explained above, at the period of albumin administration (0' minute) and at 30, 60, 90 and 120 minutes after albumin administration for each rat in all the groups. The mean fluid volume for each group of rat at the various time intervals was calculated and compared statistically with the control group.

ii. Dose-dependent anti-inflammatory effect of artemether following seven-day pretreatment in rats.

Twenty four (24) adult male Wistar rats weighing between 112-214 g were used for the sub-acute artemether study. Four (4) pretreatment groups of six (6) rats matched for weight were pretreated for 7 days with 3% v/v Tween 80 (control), 1.5, 7.5 and 15 mg/kg artemether respectively. Oedema was then induced in all the rats 20 minutes after the 7th day drug pretreatment and the paw fluid volume was measured at 0, 20, 40, 60, 80, 100 and 120 minutes after albumin injection. The mean fluid volume for each group of rat at the various time intervals was calculated and compared statistically with the control group.

Results

Data from acetic acid-induced writhing-test in mice showed that artemether inhibited peripheral pain dose dependently. Dipyrone provided a percentage pain inhibition of 36.6% while the 1.5 mg/kg therapeutic equivalent of artemether showed 28.1% inhibition which was not statistically significant from the effect of dipyrone ($P > 0.05$, Student's t-test). However, the higher dose levels of 7.5 and 15 mg/kg artemether showed percentage pain inhibition of 62.7% and 85.0% respectively which were statistically significant from that of dipyrone (Table 1).

Acute (tail flick & hot plate) and sub-acute tail flick experiments. The mean reaction times obtained from both the tail flick and hot plate experiments showed no statistically significant or dose dependent effect of artemether at any of the time intervals of pain assessment (Figs 1a and b). However, the morphine-pretreated animals showed statistically significant longer reaction time compared with Tween 80 control at each test period ($P < 0.05$) in both experiments (Fig. 1a and b). Dipyrone provided a statistically significant pain-relief different from that of Tween 80 control only at 20 minutes drug pretreatment (Fig 1a). As with the acute tail flick experiment, there was also no significant difference in the reaction time between the control group and the artemether-pretreated

groups following 7 day pretreatment even though the artemether-pretreated groups generally reacted more readily than the control group (Fig 1c).

Anti-inflammatory Effect of Acute and Sub-acute administration of Artemether in Rats

Results from the acute effect of artemether on rat paw oedema showed variation of 0.34 - 0.36 ml of mean paw fluid volume in the 'non inflamed Tween 80' rats. Therefore normally paw fluid volumes of a rat measured at various time intervals may vary within ± 0.02 ml. The mean paw sizes measured immediately after albumin injection (at 0' minute) were not different from their respective control paw volumes obtained at 0 minute. However, egg albumin induced a progressive increase in paw oedema at 30 - 90 minutes of its injection after which the paw fluid volumes started decreasing. The mean paw fluid volume of the group of rats that received albumin alone varied from 0.34 ml at 0' minute to 0.66 ml at 90 minutes. This variation was statistically different ($P < 0.05$, 1 Way ANOVA) from the non-inflamed Tween 80 control rats at 30, 60, 90 and 120 minutes of oedema evaluation. Indomethacin progressively inhibited fluid accumulation 30 minutes after oedema induction. The peak of oedema in the indomethacin + albumin group of rats was 0.54 ml and this was observed at 30 minutes interval of egg albumin injection; and exactly the same 0.54 ml volume was observed in rats of the albumin alone group at same 30 minutes interval, indicating that the effect of indomethacin still not felt at this period. Indomethacin showed significant inhibitory effect at 90 minutes interval at which the peak oedema of the albumin alone occurred. The peak oedema in the Artemether + albumin group was 0.64 ml and was also observed at 30 minutes of oedema induction. This fluid volume was almost the same as the peak fluid volume of 0.66 ml obtained at 90 minutes interval in rats that received albumin alone. This accumulated fluid in rats pretreated with artemether was significantly more compared with that in the indomethacin + albumin group of rats ($P < 0.05$) and although the accumulated fluid started decreasing after 30 minutes it was still slightly more than that in the albumin alone group at 60 minutes. This observation showed that artemether caused slight, but short lasting inflammatory effect, which enhanced the albumin-induced hind paw oedema. There was no significant difference in the mean paw sizes of rats of various doses of artemether-pretreated groups at all test periods (Fig. 2b) following 7 day pretreatment. Considering the time interval used in this study, the peak oedema in all the three dose levels of artemether-pretreated groups occurred at about 40 minutes interval and the mean paw volume for the various pretreatment groups at 20 and 40 minutes test periods showed that oedema was the same in all the artemether-pretreated groups irrespective of dose and occurred almost in the same degree as that for Tween 80 control.

Table 1: Percentage Pain Inhibition of Acetic acid-induced Writhing-test in Mice

Treatment groups	Tween 80 (control)	Dipyrone (10 mg/kg)	Artemether (mg/kg)		
			15	7.5	1.5
Mean ± SEM	15.3 ± 4.0	9.7 ± 0.3	2.3 ± 1.5	5.7 ± 2.3	11.0 ± 4.5
% Pain inhibition	0	36.6	84.96*	62.71*	28.1

n = 3 rats per group; Pretreatment: i.p. for 25 minutes

* Statistically significantly different from dipyrone compared with the control (Student t-test)

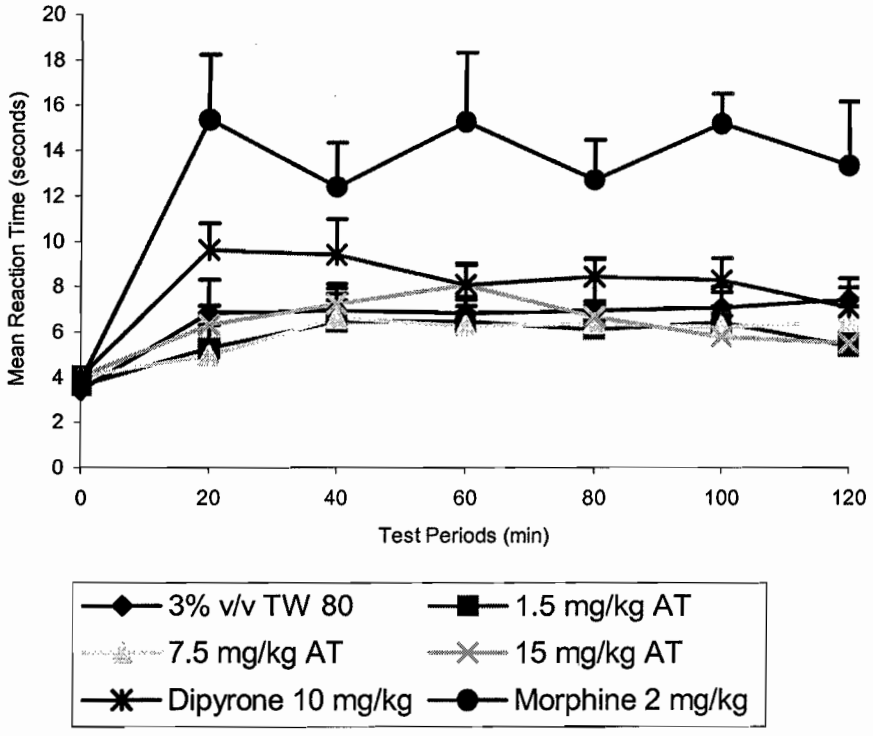

Fig 1. Effect of Artemether Pretreatment on the Reaction Time of Mice.
a. Acute Tail Flick: * significant at all test periods; *[1] significant at 20 minutes

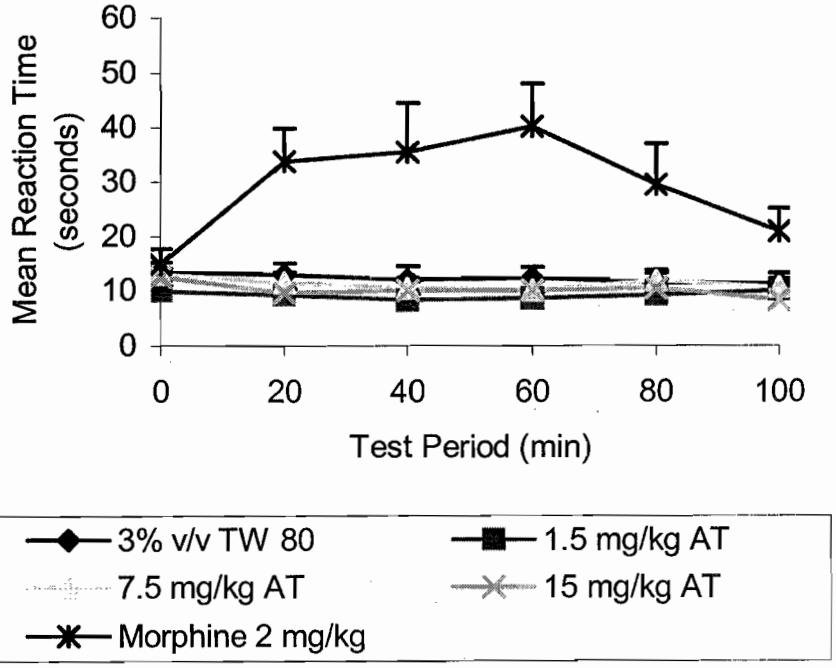

Fig 1. Effect of Artemether Pretreatment on the Reaction Time of Mice.
b. Acute Hot Plate: * significant at all test periods;

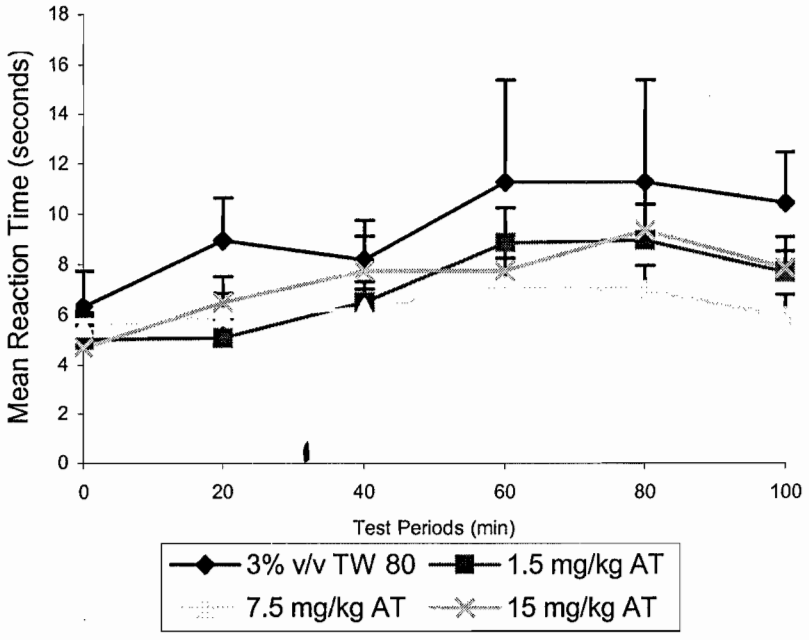

Fig 1. Effect of Artemether Pretreatment on the Reaction Time of Mice.

c. Sub-acute Tail Flick: No significant difference between treatments ($P<0.05$, ANOVA; Student's t-test) AT = Artemether; TW = Tween 80

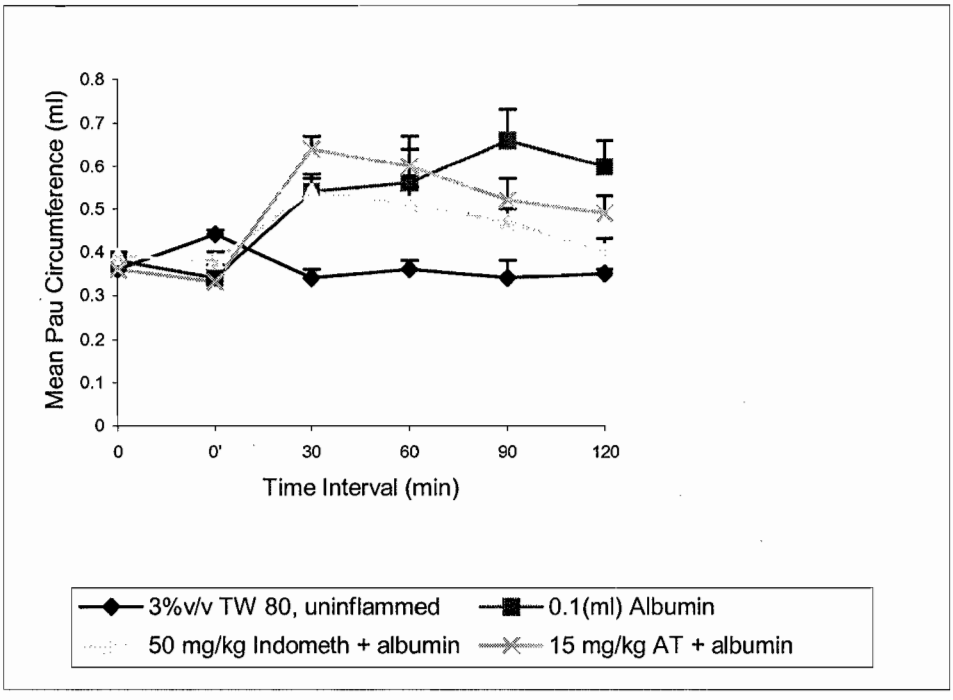

Fig. 2 Degree of egg albumin-induced oedema and the anti-inflammatory effect of artemether in rat paws

a Acute pretreatment: * albumin-induced statistically significant oedema compared with Tween 80 control (Student's t-test)

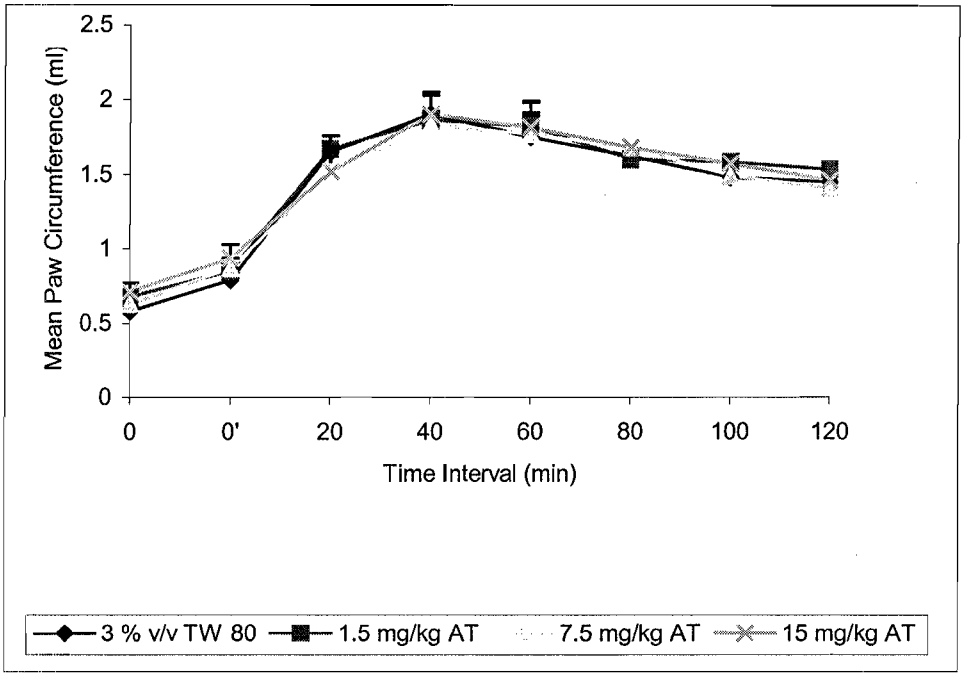

Fig. 2 Degree of egg albumin-induced oedema and the anti-inflammatory effect of artemether in rat paws
bs Dose-effect of 7 day artemether pretreatment on albumin-induced oedema No statistically significant difference between treatments (P> 0.05, ANOVA)

Discussion

Analgesic Effect of Acute and Sub-acute Artemether pretreatment in Mice. This study revealed that artemether inhibited peripheral pain dose dependently and that although pain inhibition was not significant at the normal dose level (1.5 mg/kg), statistically significant pain inhibition different from that of dipyrone occurred at higher dose levels of artemether. This acute study seemed to confirm the Loss of pain response reported with high dose levels (12.5-50 mg/kg) of artemether following 8 - 28 days of administration in both rats and dogs (Brewer *et al.*, 1994). From the tail flick and hot plate experiments used to test for central analgesic activity, artemether seemed to posses no central effect of pain inhibition irrespective of treatment duration (acute or sub-acute). The slight faster response noted with artemether pretreated rats than with Tween 80 control group seemed to be related to the slight sharp localized transient pain at the site of injection and may be the momentary side effect due to injection (Barradel and Futon, 1995). Such peripheral pains are usually assessed using the acetic acid-induced writhing test because they are not easily detected by these tests and this probably explains why only slight protective effect of dipyrone was observed in the acute tail flick experiment. The statistically significant analgesic effect seen with morphine is typical of narcotics, which are strong analgesics used for severe or deep pains, while dipyrone and acetylsalicylates are mild analgesics used to relieve mild peripheral pain and these are usually recommended with most antimalarials to relieve painful side effects like that seen with artemether. Since artemether is not likely to be used at higher dose levels (7.5 and 15 mg/kg) at which it inhibited peripheral pain, it may therefore be used concurrently with mild analgesic agents of the salicylic types to prevent pains at the injection site. The

mechanism whereby artemether provided its peripheral pain inhibition is not known, but it is possible that it may have interfered with the activity of the cyclooxygenase enzyme in the synthesis of prostaglandin or blocked impulse generation at the peripheral pain receptors.

Anti-inflammatory Effect of Acute and Sub-acute Artemether Pretreatment in Rats

Considering the time intervals used in this experiment to assess the degree of egg albumin induced oedema, the peak oedema was obtained 90 minutes after injection of egg albumin after which the paw volume started reducing continuously indicating that egg albumin induces only a short lasting inflammation. The irritants that induce prolonged inflammation or cause the paw oedema to continue increasing for several hours or days are used to study the degree and duration of anti-inflammatory action of drugs (Vogel and Vogel, 1997). Egg albumin induces a short lasting oedema of few hours (> 2 hours, fig 2a) and is therefore suitable for studying the degree of anti-inflammatory actions of drugs. Other irritants used to induce oedema are brewer's yeast, formaldehyde, dextran, sulfated polysaccharides like carrageenan; and paw fluid volume can also be measured by immersion of the paw in mercury (Vogel and Vogel, 1997). In this study, artemether (the test drug) as well as indomethacin (standard anti-inflammatory agent) were not able to prevent accumulation of fluid in the rat paws since oedema was produced in all the pretreated rats. The inhibitory effect of indomethacin indicated by a reduction in fluid accumulation did not occur until about 30 minutes of albumin treatment. This observation could be explained to mean that indomethacin was not maximally absorbed before this time. Artemether (15 mg/kg) also did not show any sign of inhibition on fluid accumulation until about 60 minutes time interval and it also did not reduce the paw volume as much as indomethacin. Thus, artemether as with indomethacin reduced the readiness of progressive fluid accumulation, but showed insignificant anti-inflammatory effect for both the acute and sub-acute pretreatment. However, it is noteworthy that artemether caused a transitory inflammatory effect and enhanced fluid accumulation in rat paws at 30-60 minutes of oedema induction at which the paw fluid volume in the artemether group was more than that in the albumin alone treated rats. This initial increase in fluid volume due to artemether may suggest that a certain degree of inflammation is associated with the use of this drug and this probably could be related to the irregular diffusion from the injection site that is often associated with oily formulations. Since pain is a symptom of inflammation, the sharp transient localized pain that had been reported to be associated with the use of artemether and the slight faster reaction to pain observed in this study might as well be as a result of this initial inflammatory effect and which may or may not be related to the depot effects of the oily formulation of this drug. Tawfik *et al.* (1990) reported that artemisinin, dihydroartemisinin, arteether and artesunate did not also show anti-inflammatory activity when tested on carrageenan-induced oedema.

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