

The Antipyresis of Chloroquine in Fever Models in Rat

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Abstract

Chloroquine is a 4-aminoquinoline derivative which has been the mainstay of malaria therapy. It is being investigated for its antipyretic effect since it has anti-inflammatory action and suppresses cytokine release and activity. The antipyretic effect of chloroquine was investigated using Lipopolysaccharide and brewer's yeast administered to rat models and compared with pentoxifylline, a TNF α blocker and piroxicam, a prostaglandin (cyclooxygenase) blocker. Chloroquine antipyresis on fever models compared favourably with piroxicam. The effect of pentoxifylline was minimal on brewer's yeast model but significant with LPS mediated pyrexia and comparable with chloroquine. Data suggested that chloroquine had antipyretic effect and therefore raised an important clinical development. Hitherto the inclusion of paracetamol in malarial fever has been a prescription tradition, but clinical finding of paracetamol prolonging of parasite clearance time has raised doubts of its integrated importance in falciparum malaria. Chloroquine alone may be prescribed especially to children or an ancillary treatment should be included but not paracetamol for fever control.

Keywords: Chloroquine, fever models, antipyresis, rat.

Introduction

Chloroquine is a 4-aminoquinoline derivative that has assumed the mainstay of malaria chemotherapy despite occurrences of *Plasmodium falciparum* resistant strains (1, 2). It is usually administered during the early periods of the illness when fever or cachexia is most intense. Fever is a non-specific clinical manifestation associated with various pathophysiological conditions mediated by endogenously produced prostaglandin and cytokines such as tumor necrosis factor (TNF α), interleukin-1 (IL-1) and interleukin-6 (IL-6) (3, 4, 5). These cytokines IL-1, IL-6 and TNF α induce increase in body temperature via direct and indirect actions on the brain and are believed to act as endogenous pyrogens (6, 7). They act at the level of organum vasculosum of the lamina terminalis of the central nervous system inducing the synthesis of prostaglandins which are central mediators in the coordinated response leading to fever. Schizont burst triggers the release of TNF α followed by feverish conditions. Pro-inflammatory enzyme phospholipase-A2 is present during malaria in addition to nitric oxide (NO) generated by TNF α , IL-1 and lymphotoxin. Chloroquine inhibits the production of cytokines and this may be the main mechanism of action in the reduction of fever. Chloroquin

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inhibits IL-1 α and IL-1 β in mixed cell cultures (9) and in lipopolysaccharide (LPS) stimulated monocytes (10). Hydroxy chloroquine also inhibits the production of IL-6 and TNF α (8). Antibodies to TNF α inhibit fever in cerebral malaria (10). Thus if chloroquine attenuates and inhibits these cytokines; IL-1, IL-6 and TNF α and prostaglandin then these functions are important in its mechanisms of action in quenching malaria fever.

The study of antipyresis of chloroquine was undertaken because it has anti-inflammatory effect and so as a non-steroidal anti-inflammatory drug it should have an antipyretic effect. For better characterization of its antipyretic effect the models of pyresis employed were LPS and Brewer's yeast induced fever, carrageenan or formalin inflammatory models with comparative test compounds paracetamol and piroxicam that are cyclooxygenase - I and cyclooxygenase - 2 inhibitors and pentoxifylline a TNF α blocker.

Materials and Methods

Experiment I: Animals

Male albino Wistar rats, 160-180g weight 3 weeks old, bred in the University of Uyo, Animal House, Uyo, Nigeria were used for the study. They were acclimatized for 7 days before the study. The animals had free access to food (chou pellets) and water. To maintain optimal thermo neutrality behaviour; all experiments were performed between 8.00am to 2.00pm with laboratory temperature being $25\pm 1.4^{\circ}\text{C}$ (11). The animals were fasted overnight before the experiments. All animal studies were performed in compliance to guidelines on the use of animals in research of the University of Uyo, Uyo, Nigeria after approval by the institutional animals ethics committee.

Chemicals and Equipment

LPS (Lipopolysaccharide) from *E. coli*, Brewer's yeast and carrageenan (Sigma Mo. USA), formalin (May and Baker, England), Chloroquine sulphate (Nivaquine; May and Baker, Lagos, Nigeria), Pentoxifylline (Trental, Hoescht and Roussel Pharmaceuticals, Kansas City USA), Piroxicam (Feldene, Pfizer Products, USA), Paracetamol (Emzor, Lagos, Nigeria) were used. Other chemicals or reagents were of analytical grade and sourced commercially, rectal clinical digital thermometer (Becton – Dickinson USA) was used. Paw volume measurements were achieved with Ugo Basile Plethysmometer (Camero Verse, Italy).

Test Drugs, Dosages and Temperature Measurements

Chloroquine (10 and 15mg kg⁻¹), piroxicam (5mg kg⁻¹) pentoxifylline (100mg kg⁻¹) paracetamol (15mgkg⁻¹) and normal saline (10ml kg⁻¹) were administered using standard orogastric cannula. Clinical thermometer was inserted 2.3cm into the rat rectum and temperatures were measured in centigrades.

Fever Models

LPS induced fever was achieved according to Pinto *et al* (12). Briefly LPS (50 μg kg⁻¹) was administered intra-muscularly to the animals an hour before the administration of test drugs. Rectal temperatures were taken at hourly intervals for 5h. Only those animals whose rectal temperatures increased 1 $^{\circ}\text{C}$ or above from normal rectal temperature were used for the study.

Brewers' yeast induced fever was achieved by subcutaneous injection of 20% w/v brewer's yeast (10ml kg⁻¹) one hour before test drug administration. Rectal temperatures were measured hourly for a 5h duration. Only animals with a 1 $^{\circ}\text{C}$ and above increase in rectal temperature were used for the study.

Inflammatory Oedema Models

Acute inflammatory oedema was achieved by both carrageenan [(1 % w/v 0.1µl; Winter et al (13)] and formalin [2.5% w/v, 50µl, Murray *et al* (14)] injected subcutaneously to the left hind plantar surface of the animals. The paw volumes were measured with Ugo Basile phethysmometer before and 3h after carrageenan and formalin injections.

Normothermic rats: These were normal rats administered with test drugs (chloroquine 10mg kg⁻¹). Rectal temperatures were measured for a 5h duration after chloroquine administration. This was to find out if the test drugs alter normal rectal temperatures. These rats were not administered LPS or brewer's yeast.

Human Patients

Fever clearance time

22 patients with acute uncomplicated malaria (asexual form parasitaemia less than 4%) aged 24-38 years, weighing 58-70kg with no hepatic or renal dysfunction were chosen for the study. Written informed consent for participation was obtained from all of them. Institutional ethics committee of the hospital where study was carried out approved the study. An episode of malarial fever was defined as temperatures from 37.5°C or higher or 24h fever history. Patients for the study were not administered any antipyretic within 48h of partake in the study. Patients history showed that past administrations of chloroquine had radical cure of the malaria without relapse. The community and the adjoining area, chloroquine sensitive to malaria parasites. Eleven patients were treated with chloroquine in the 2:2:1 dosage ratio, which is 600mg chloroquine base equivalent (Day 1, oral), 600mg chloroquine base equivalent (Day 2, oral) and 300mg chloroquine base equivalent (Day 3, oral).

The other eleven patients were administered orally the same chloroquine dosage regimen of 2:2:1 ratio with an addition, that is paracetamol 500mg x 2 tablets when necessary for over a 90h duration. Body temperatures were measured every 6h using digital clinical thermometer within the 90h duration. Fever clearance time, which is defined as the time from admission (or onset of study) is for ambulatory patients until temperature remains below 37.5°C and thick blood smear remains negative for 24 hours. The parasite clearance time defined as the time for which the thick blood smear remains negative for parasite count for 24 hours was calculated.

Results

Results from the study are shown in Table 1, 2 and 3. Normal rats showed no significant temperature with change chloroquine at 15mg kg⁻¹ and 10mg kg⁻¹ as the average rectal temperature were 37.5±0.1 compared to control. An average of 1°C increase or more above normal rectal temperatures were taken as febrile states. Chloroquine at 10mg kg⁻¹ and 15mg kg⁻¹ reduced significantly the LPS induced pyresis. Pentoxifylline at 100mg kg⁻¹ and piroxicam 5mg kg⁻¹ reduced LPS induced pyresis at significant levels but paracetamol effect was just significant within the 1h, 2h, and 3h intervals. At the 4h and 5h intervals, there was no significant effect on pyresis as rectal temperatures were back to 38°C and above. Pentoxifylline did not reduce pyresis induced by brewer's yeast as rectal temperatures were still above 38°C during the 5h period. Piroxicam effects were significant from the 2h period to the 5h duration. Paracetamol effect was for only 2-hour duration from the 1 to 2h periods as rectal temperatures rose from 2h to 5h.

Table 1. The effects of test drugs or rectal temperature pyrexia induced by LPS at time intervals

Time of measurement (Hours) and rectal corresponding temperatures							
Group	0	1h	2h	3h	4h	5h	
Febrile Rat Normal Saline 10ml kg ⁻¹ (Control)	38.8 ±0.1 ^o C	38.8 ±0.0 ^o C	38.6 ±0.1 ^o C	38.5 ±0.2 ^o C	38.5 ±0.2 ^o C	38.4 ±0.0	
Chloroquine (10ml kg ⁻¹)	38.6 ±0.2 ^o C	37.6* ±0.1 ^o C	37.5* ±0.0 ^o C	37.6* 0.0 ^o C	37.6* 0.1 ^o C	37.6 0.0 ^o C	
Chloroquine (15mg kg ⁻¹)	38.6 ±0.1 ^o C	37.6* ±0.1 ^o C	37.6* ±0.1 ^o C	37.5* ±0.0 ^o C	37.5* ±0.0 ^o C	37.5 ±0.0 ^o C	
Pentoxifylline (100mg/kg)	38.8 ±0.1	37.6* ±0.1	37.5* ±0.0	37.5* ±0.0	37.6* ±0.0	37.6 ±0.1	
Paracetamol (15mg kg ⁻¹)	38.7 ±0.1	37.5* ±0.0	37.6* ±0.1	37.8* ±0.1	38.4 ±0.1	38.0 ±0.00	
Piroxicam (5mg kg ⁻¹)	38.6 ±0.0	37.6* ±0.0	37.5* ±0.0	37.5* ±0.0	37.6* ±0.0	37.5 ±0.1	

Data expressed as mean ±SE (N = 6)

(P* < 0.05) and is statistically significant compared to control group.

On inflammatory oedema models of carrageenan and formalin (Table 3), the percentage reductions of induced oedema, which is an index of anti-inflammatory effect of these agents, are shown in the table. Whereas piroxicam has an elaborate oedema reduction, chloroquine has moderate effects. Pentoxifylline and paracetamol have negligible effects on carrageenan model. Paracetamol effect on formalin was also negligible but pentoxifylline had moderate effect on reduction of inflammatory model of formalin. Pentoxifylline and paracetamol have negligible effect on carrageenan-induced oedema. The effect on formalin-induced oedema was moderate. Chloroquine at 10mg kg⁻¹ was moderate in reducing oedema, however at 15mg kg⁻¹ the effect was significant and showed similar amplitude with piroxicam at 5mg kg⁻¹ for both carrageenan and formalin.

In malaria patients the fever clearance time was 31h for patients who were on chloroquine alone and 25h for patients who took chloroquine and paracetamol. However the parasite clearance time was 14h for patients who took chloroquine alone and 22h for patients who took chloroquine and paracetamol.

Table 2. The effects of test drugs on rectal temperature pyrexia induced by brewer's yeast in rat at intervals

Time of measurement (Hours) and corresponding temperatures						
Group	0	1h	2h	3h	4h	5h
Febrile Rat Control Normal Saline (10ml kg ⁻¹)	39.4 ±0.1 ⁰ C	39.3 ±0.3 ⁰ C	39.2 ±0.3 ⁰ C	39.1 ±0.0	39.1 ±0.1	38.9 ±0.1
Chloroquine (10mg kg ⁻¹)	39.4 ±0.1 ⁰ C	37.6** ±0.1	37.6** ±0.2	37.5* ±0.0	37.6** ±0.1	37.6** ±0.0
Chloroquine (15mg kg ⁻¹)	39.4 ±0.2 ⁰ C	37.5** ±0.1	37.5** ±0.0	37.6** ±0.1	37.6* ±0.1	37.5 0.0
Pentoxifyline (100mg kg ⁻¹)	38.60C ±0.1 ⁰ C	38.8 ±0.0 ⁰ C	39.0 ±0.2 ⁰ C	39.0 ±0.2 ⁰ C	39.0 ±0.0 ⁰ C	38.9 ±0.1 ⁰ C
Piroxicam (5mg kg ⁻¹)	39.2 ±0.00	37.9 ±0.1	37.6** ±0.1	37.6** ±0.0	37.7 ±0.0	36.7 ±0.1
Paracetamol (15mg/kg)	39.3	37.6 ±0.1	37.7 ±0.2	38.1 ±0.0 ⁰ C	38.3 ±0.1 ⁰ C	38.0 0.1 ⁰ C

Data expressed as mean + SE (N = 6)

P** < 0.001, P* < 0.05 is significant in respect of control.

Table 3. The effects of test drugs on carrageenan induced paw oedema and formaline induced paw oedema (in ml)

Treatment	Carrageenan (ml)	Formalin(ml)
Control Normal Saline	1.55±0.03	1.69±0.05
Chloroquine (10mg kg ⁻¹)	1.25±0.04* (55%)	1.30±0.06* (56%)
Chloroquine (15mg kg ⁻¹)	1.20±0.03* (64%)	1.20±0.07** (69%)
Piroxicam (5mg kg ⁻¹)	1.16±0.06** (71%)	1.10±0.03** (86%)
Pentoxifyline (100mg kg ⁻¹)	1.50±0.05 (9%)	1.58±0.08 (6.5%)
Paracetamol (15mg/kg)	1.52±0.02ml (3%)	1.66±0.06 (3%)

Data expressed as mean ± SE (N = 6)

p** < 0.001, p < 0.05 were statistically significant in respect to control groups. (Inhibitory effect) are percentage expression calculated from

$$\frac{(\text{Volume of Paw Oedema (Test drug)} - \text{Volume of Paw Oedema (normal saline)})}{\text{Volume of Paw Oedema (normal Saline)}} \times 100$$

Discussion

Chloroquine attenuates the fever induced by both bacterial toxins LPS and brewers yeast and had no significant effect on normal rat temperature. Potent antipyretics should lower febrile states without affecting normal body temperature. Chloroquine could therefore be classified as a potent antipyretic as it did not affect normal rats but febrile rats.

LPS induces the synthesis of $\text{TNF}\alpha$, which causes fever in laboratory animals (3). The antipyretic effect of chloroquine is not dose related and that of pentoxifylline may be due to the suppression of $\text{TNF}\alpha$ or the inhibition of its synthesis. Prostaglandin synthesis can be activated by $\text{TNF}\alpha$ or phospholipase A_2 , so the inhibition of cyclo-oxygenase by piroxicam may therefore interfere with the cascade of the synthesis of prostaglandin, which induces fever. IL-1 or IL-2 acts on the thermosensory centre by stimulating the secondary signals in elevating febrile states. Cyclooxygenase inhibitors such as piroxicam and chloroquine block prostaglandin synthesis and therefore attenuates fever in LPS treated rats. Paracetamol effect was short because it has no peripheral effect on prostaglandin synthesis. Brewers yeast model induces both $\text{TNF}\alpha$ and prostaglandin synthesis (3). Chloroquine at both doses and piroxicam reduced significantly the pyresis induced by brewers yeast. Chloroquine and piroxicam block $\text{TNF}\alpha$ and prostaglandin, synthesis. Both drugs have long half-lives of elimination (more than 12 hours) hence the sustained effect of these drugs in reducing febrile state for the 5-hours durations. However pentoxifylline only blocks $\text{TNF}\alpha$ release but has no effect on the synthesis of prostaglandin. This may have the poor effect of its administration in Brewer's yeast pyresis model. Paracetamol has no effect on $\text{TNF}\alpha$, and no effect on prostaglandin release from the peripheral stores. It has a short half-life of about 2 hours and its block of cyclo oxygenase - 3 is minimal. These will affect the duration and quality of its antipyresis as monitored. The two inflammatory models carrageenan and formalin showed that chloroquine reduced oedema significantly but not as much as piroxicam. Chloroquine is a slow acting anti-inflammatory agent and this may account for that. Pentoxifylline and paracetamol did not cause any appreciable oedema reduction due to low effect on prostaglandin synthesis.

In malarial patients, the fever clearance time in chloroquine only treated patients and chloroquine plus paracetamol treated patients showed no significant difference. A clinical question arises, if there is any need of inclusion of paracetamol in malarial fever therapy? Moreso as the parasite clearance time was longer in patients who took chloroquine and paracetamol compared with chloroquine only patients. Paracetamol reduces fever but it prolongs parasite clearance time (15) since our study used few patients the result is not conclusive till more patients participate in such studies.

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