J. Pharm. Pharmacol. 1998, 50: 225–229
Received July 11, 1997
Accepted October 13, 1997

A Study of the Anti-pyretic Effect of Quinine, an Alkaloid Effective Against Cerebral Malaria, on Fever Induced by Bacterial Endotoxin and Yeast in Rats

F. A. SANTOS AND V. S. N. RAO

Department of Physiology and Pharmacology, Health Sciences Center, Federal University of Ceará, Caixa Postal-3157, 60430-270 Fortaleza, CE, Brasil

Abstract

The effect of quinine on fever induced by lipopolysaccharide and brewer's yeast has been investigated in rats.

Oral administration of 50 or 100 mg kg^{-1} quinine, doses which had no effect on normothermic rats, significantly reduced lipopolysaccharide- (50 μg kg^{-1}, i.m.) and yeast- (2 g kg^{-1}) induced fever in rats. Pentoxifylline (100 mg kg^{-1}), a tumour necrosis factor antagonist also attenuated the febrile response induced by lipopolysaccharide, but not that by yeast, in a manner similar to quinine. Piroxicam (5 mg kg^{-1}), a cyclooxygenase inhibitor suppressed both types of fever with a longer duration of action. In addition to its anti-pyretic effect, quinine had a significant anti-inflammatory effect in the carrageenan model of acute inflammation in the hind-paw of rats.

The results indicate the anti-inflammatory and anti-pyretic potential of quinine which might be important in addition to its anti-plasmodial action in the therapy of cerebral malaria.

Quinine is an alkaloid from cinchona bark (Jesuits powder) long-known for its usefulness against cerebral malaria. It still remains the drug of choice in severe chloroquine-resistant *P. falciparum* malaria (Wirima et al 1990) and is administered primarily to patients during the initial period of the illness when fever and other manifestations of the disease are most intense. However, the mechanism whereby it suppresses fever still awaits explanation. Fever, a non-specific manifestation, is in general associated with many pathophysiologic conditions mediated by endogenously produced prostaglandins and cytokines such as interleukin (IL)-1β, IL-6 and TNF-α (Kluger 1991). Nevertheless, it is not clear which of these substances are specifically inhibited by quinine.

Experimental cerebral malaria in mice seems to be mediated by tumour necrosis factor-α (TNF-α) and a close correlation between fever fluctuations and serum levels of TNF-α has been observed clinically (Grau et al 1987; Karunaweera et al 1992). Recent studies have shown the suppressive effect of quinine on TNF-α production in-vitro (Marayuma et al 1994) and in-vivo (Gantner et al 1995) and suggested that attenuation of TNF-α secretion might be the major mechanism of the action of quinine in cerebral malaria.

Exogenous pyrogens such as bacterial endotoxin (lipopolysaccharide) and yeast (brewer's yeast) can induce fever in rats (Kluger 1991). For better characterization of quinine as an anti-pyretic drug we have used these models of pyrexia to evaluate the effects of quinine in comparison with the cyclooxygenase inhibitor piroxicam and the TNF-α antagonist pentoxifylline. Because non-steroidal anti-inflammatory drugs generally have an anti-pyretic effect, we considered it of interest to investigate the effect of quinine in the carrageenan-induced hind-paw oedema model of acute inflammation in rats.

Correspondence: V. S. N. Rao, Departamento de Fisiologia e Farmacologia (CCS), Rua Cel. Nunes de Melo 1127, Caixa Postal-3157, Porangabussu, 60430-270 Fortaleza, CE, Brasil.

Materials and Methods

Animals

Male Wistar rats, 160–180 g, were housed and maintained at 22 ± 2°C with a 12-h light-dark cycle and allowed free access to food (purina chow) and water. Food was withheld for a period of 12–15 h before the rats were used for experimentation.

All experiments were performed between 0800 and 1700 h at 28 ± 1°C, which is considered the thermoneutral zone for rats (Gordon 1990).

Chemicals
Lipopolysaccharide from *E. coli*, brewer's yeast, quinine dihydrochloride and lambda carrageenan were purchased from Sigma (St Louis, MO). The other drugs used were piroxicam (Inflamene, Farmalab, Brasil) and pentoxifylline (Trental, Hoechst, Brasil).

Lipopolysaccharide-induced fever
Lipopolysaccharide was dissolved in sterile pyrogen-free 0·9% saline at 500 μg mL^{-1} and was injected intramuscularly (50 μg kg^{-1}) into the thigh of the rats. The test drugs, quinine (25, 50 and 100 mg kg^{-1}), piroxicam (5 mg kg^{-1}), pentoxifylline (100 mg kg^{-1}), or saline were administered orally (10 mL kg^{-1}) 1 h before injection of lipopolysaccharide. Rectal temperature was measured 1 h before (pre-drug control) and at intervals of 1 h for 5 h, with a digital thermometer (Becton Dickinson, Brasil) inserted 3 cm into the rectum. In preliminary experiments the effects of quinine (25, 50 and 100 mg kg^{-1}) on the rectal temperature of normothermic rats were also investigated.

Brewer's yeast-induced pyrexia
Pyrexia was induced in male Wistar rats by subcutaneous injection of 20% brewer's yeast (10 mL kg^{-1}). The animals were fasted and 18 h later the rectal temperature was recorded by use of a digital thermometer. Animals for which the temperature rise was 1°C above normal were considered fevered. Such animals were allocated to groups of six and were treated orally (10 mL kg^{-1}) with the test drugs, quinine (25, 50 and 100 mg kg^{-1}), pentoxifylline (100 mg kg^{-1}), piroxicam (5 mg kg^{-1}) or saline. The rectal temperature of the rats was recorded at 1-h intervals starting 1 h before drug administration and continuing for 5 h after yeast injection.

Carrageenan-induced rat hind-paw oedema
One hour after oral administration of quinine (25, 50 and 100 mg kg^{-1}), pentoxifylline (100 mg kg^{-1}), piroxicam (5 mg kg^{-1}) or saline (10 mL kg^{-1}) to rats, acute inflammatory oedema was induced by subplantar injection of a suspension of carrageenan (1%, 0·1 mL; Winter et al 1962). The paw volume (mL) was measured with a plethysmometer (Ugo Basile, Milan, Italy) immediately before injection of carrageenan (0 h) and again 3 h later. The difference between the paw volumes at 1 and 3 h was taken as the measure of paw oedema.

Statistical evaluation
All values are expressed as the mean ± the standard error of the mean (s.e.m.) and statistical significance between groups was analysed by one-way analysis of variance then the Student-Newman-Keuls multiple comparison test. P values < 0.05 were considered to be indicative of significance.

Results

Preliminary studies
Preliminary investigations involved recording the temperature changes resulting from administration of various doses of quinine (25, 50 and 100 mg kg^{-1}), piroxicam (5 mg kg^{-1}) and pentoxifylline (100 mg kg^{-1}) in normothermic rats. At the doses employed these drugs had no significant effect on the rectal temperature of normal rats during a 5-h observation period (data not shown).

Lipopolysaccharide-induced fever
Lipopolysaccharide (50 μg kg^{-1}; i.m.) induced a febrile response which reached its peak after 4 h with a increase in rectal temperature of approximately 1·6°C. Quinine at 50 mg but not at 25 mg kg^{-1}, significantly attenuated the febrile response induced by lipopolysaccharide at 3 h and 4 h and quinine at 100 mg kg^{-1} significantly suppressed the effect of lipopolysaccharide on rectal temperature in two distinct periods (Figure 1a). An initial phase fall was observed 1 h after administration. At this time the mean rectal temperature of controls treated with lipopolysaccharide alone was 37·91 ± 0·16°C, whereas for rats treated with quinine (100 mg kg^{-1}) it was 36·59 ± 0·16°C. The second phase fall in rectaltemperature was observed at 3 h and 4 h. Pentoxifylline (100 mg kg^{-1}) also induced a significant anti-febrile effect like that of quinine at 1 h, 3 h and 4 h. However, the anti-pyretic effect of piroxicam (5 mg kg^{-1}) was not evident at 1 h but commenced at 2 h and was found to be more sustained (Figure 1b).

Brewer's yeast-induced pyrexia
The influence of orally administered quinine, piroxicam and pentoxifylline on the rectal temperature of rats is summarized in Figures 2a and 2b. Eighteen hours after subcutaneous injection of brewer's yeast (2 g kg^{-1}), a consistent rise in rectal temperature of approximately 1·6°C was observed in control animals throughout the observation period

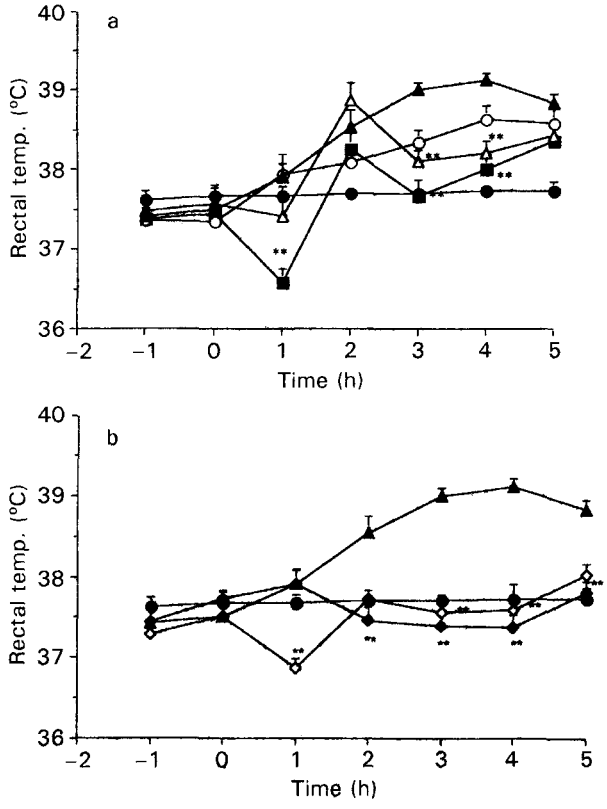

Figure 1. Changes in rectal temperature after oral administration of quinine (a) and pentoxifylline or piroxicam (b) to febrile rats. Fever was induced by intramuscular injection of 50 μg kg^{-1} lipopolysaccharide at 0 h. All drugs were administered 1 h before lipopolysaccharide. ●, normal rats; febrile rats treated with: ▲, normal saline (10 mL kg^{-1}); ○, quinine (25 mg kg^{-1}); △, quinine (50 mg kg^{-1}); ■, quinine (100 mg kg^{-1}); ◇, pentoxifylline (100 mg kg^{-1}); ◆, piroxicam (5 mg kg^{-1}). Values are the means ± s.e.m. of results from six animals. $^{**}P < 0.01$, significantly different from result for saline-treated animals.

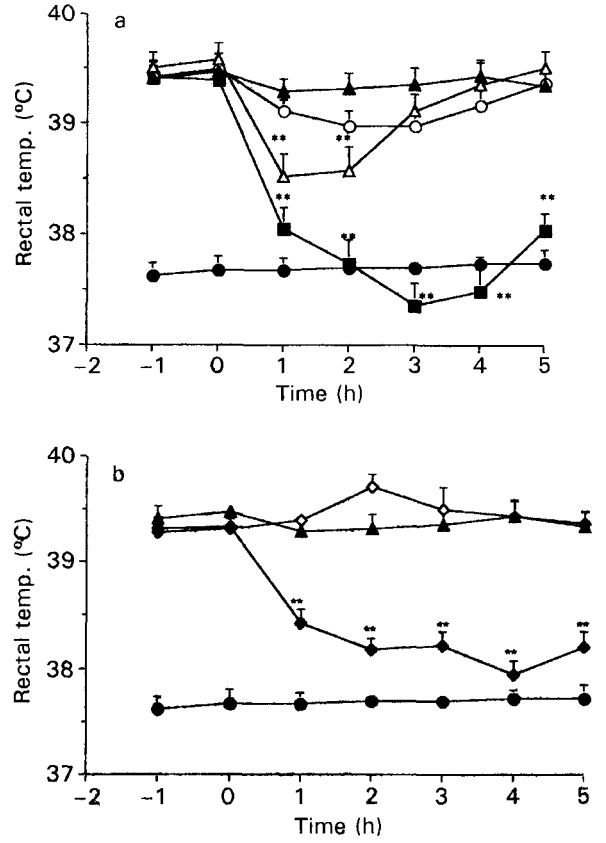

Figure 2. Changes in rectal temperature after oral administration of quinine (a) and pentoxifylline or piroxicam (b) to rats with pyrexia. Pyrexia was induced by subcutaneous injection of 20% brewer's yeast (2 g kg^{-1}) and 18 h later the rats were used for the study. All drugs were administered at 0 h. ●, normal rats; pyrexia rats treated with: ▲, normal saline (10 mL kg^{-1}); ○, quinine (25 mg kg^{-1}); △, quinine (50 mg kg^{-1}); ■, quinine (100 mg kg^{-1}); ◇, pentoxifylline (100 mg kg^{-1}); ◆, piroxicam (5 mg kg^{-1}). Values are the means ± s.e.m. of results from six animals. $^{**}P < 0.01$, significantly different from result for saline-treated animals.

of 5 h. Febrile response did not occur in saline-injected control rats. Administration of 50 mg kg^{-1} quinine, but not 25 mg kg^{-1}, resulted in significant fall in rectal temperature at 1 h and 2 h; injection of 100 mg kg^{-1} induced a more sustained significant anti-pyretic effect throughout the period of study (Figure 2a). The effect of piroxicam (5 mg kg^{-1}) and pentoxifylline (100 mg kg^{-1}) on yeast-induced hyperthermic rats was therefore compared with the effect of 100 mg kg^{-1} quinine. Although pentoxifylline did not modify yeast-induced pyrexia, piroxicam attenuated it in a manner similar to quinine (Figure 2b).

Carrageenan-induced rat hind-paw oedema

Table 1 lists mean paw oedema volumes for control animals and for those treated with the test drugs. In control animals subplantar injection of carrageenan produced a local oedema reaching its maximum intensity 3 h after injection. Pre-treatment of rats with 100 mg kg^{-1} quinine, but not with 25 or 50 mg kg^{-1}, resulted in significant inhibition (69·23%) of paw oedema. Pentoxifylline (100 mg kg^{-1}) did not modify carrageenan oedema and piroxicam significantly inhibited (44·23%) paw oedema.

Discussion

These findings show that quinine exerts both anti-inflammatory and anti-pyretic effects. Clinically, quinine has been the choice drug for severe chloroquine-resistant falciparum malaria because it promotes parasite and fever clearance (Akpede et al 1996). Some suggested mechanisms by which quinine exerts its beneficial effects in cerebral malaria are the inhibition of plasmodial phospholipase A_2 (Zidovetzki et al 1993), prevention of haemoglobin degradation by inhibiting haem polymerase in the intra-erythrocytic trophozoites (Slater 1993; Gabay et al 1994) and suppression of

Table 1. Effects on carrageenan-induced rat paw oedema of single-dose pre-treatment with quinine, piroxicam or pentoxifylline.

Group	Dose (mg kg^{-1})	Paw oedema (mL)	Inhibition (%)
Control	–	0·52 ± 0·04	–
Quinine	25	0·59 ± 0·06	13·46
	50	0·48 ± 0·04	7·69
	100	0·16 ± 0·04*	69·23
Piroxicam	5	0·29 ± 0·02*	44·23
Pentoxifylline	100	0·50 ± 0·05	3·85

Results for paw oedema are means ± s.e.m. (n = 6). *$P < 0·05$, significantly different from the control value.

TNF-α secretion (Gantner et al 1995). Activation of phospholipase A_2 and increased secretion of TNF-α could lead to generation of specific prostaglandins (Dayer et al 1985; Hoeck et al 1993) that participate in inflammatory and febrile reactions (Vane 1976; Strieter et al 1993). Anti-inflammatory drugs that inhibit prostaglandin synthesis are capable of suppressing fever (Flower & Vane 1972). The results of this study show that quinine attenuates the fever induced by both bacterial toxin and yeast. A true anti-pyretic should attenuate the febrile response without affecting the body temperature of healthy afebrile subjects (Kasting 1989). Because quinine at the doses employed in this study did not reduce the rectal temperature of normothermic rats, it could also be termed an anti-pyretic drug.

In lipopolysaccharide-induced fever, quinine at the highest dose (100 mg kg^{-1}) reduced the rectal temperature in two distinct periods. An initial phase hypothermia occurred 1 h after lipopolysaccharide administration and a second phase hypothermia 3 and 4 h after administration. Lipopolysaccharide has been shown to stimulate endogenous production of TNF-α and to induce biphasic fever in guinea-pigs and rabbits (Kluger 1991; Roth & Zeisberger 1995). However, in the current study lipopolysaccharide produced only a monophasic fever. Literature data on the mechanism of lipopolysaccharide-induced biphasic fever are not very clear; it seems to be species-specific and highly influenced by the dose of lipopolysaccharide employed. Nevertheless, pentoxifylline (100 mg kg^{-1}), a class IV phosphodiesterase inhibitor that has previously been shown to inhibit endotoxin-induced TNF-α synthesis (Han et al 1990) produced a magnitude and duration of hypothermia similar to that seen with 100 mg kg^{-1} quinine. The cyclooxygenase inhibitor piroxicam induced an hypothermic response which was not evident at 1 h (first phase) but commenced at 2 h with a much longer duration of action. At present we have no explanation for the early phase hypothermic response of piroxicam and high-dose quinine (100 mg kg^{-1}). The observed anti-pyretic effects of quinine and pentoxifylline might be related to their ability to suppress TNF-α secretion. Piroxicam seems to be devoid of such action. Like quinine, pentoxifylline has been shown to inhibit TNF-α production in animal experiments (LeMay et al 1990; Goldbach et al 1997) and in clinical cases of malaria; in an open randomized controlled therapeutic trial its use as a supportive agent resulted in a trend toward reduced mortality (Di-Perri et al 1995).

Both quinine and piroxicam attenuated brewer's yeast-induced fever with a greater magnitude and duration of action. Lack of effect of pentoxifylline in this model of pyrexia indicates that pentoxifylline possibly blocks endogenously produced TNF-α but not prostaglandins. Finally we were interested to know whether quinine and the phosphodiesterase inhibitor pentoxifylline manifest an anti-inflammatory effect. The results show that quinine and piroxicam, but not pentoxifylline, significantly inhibit the inflammatory oedema response to carrageenan in a manner wherein prostaglandins play a predominant role (Vinegar et al 1987).

In conclusion, these results demonstrate the anti-inflammatory and anti-pyretic effects of quinine probably related to its inhibition of TNF-α and prostaglandins. These actions of quinine and its parasiticidal action might be important to its therapeutic efficacy against chloroquine-resistant cerebral malaria.

Acknowledgements

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) and Financiadora de Estudos e Projetos (FINEP) of Brasil for financial support.

References

Akpede, G. O., Abiodun, P. O., Ambe, J. P. (1996) Aetiological considerations in the febrile unconscious child in the rain forest and arid regions of Nigeria. East. Afr. Med. J. 73: 245–250

Dayer, J. M., Beutler, B., Cerami, A. (1985) Cachectin/tumour necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. J. Exp. Med. 162: 2163–2168

Di-Perri, G., Di-Perri, I. G., Monteiro, G. B., Bonora, S., Henning, C., Cassatella, M., Micciolo, R., Vento, S., Dusi, S., Bassetti, D. (1995) Pentoxifylline as a supportive agent in the treatment of cerebral malaria in children. J. Infect. Dis. 171: 1317–1322

Flower, R. J., Vane, J. R. (1972) Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). Nature 240: 410–411

Gabay, T., Krugliak, M., Shalmiev, G., Ginsberg, H. (1994) Inhibition by antimalarial drugs of hemoglobin denaturation and iron release in acidified red blood cell lysates—a possible mechanism of their anti-malarial effect? Parasitology 108: 371–381

Gantner, F., Uhlig, S., Wendel, A. (1995) Quinine inhibits release of tumour necrosis factor, apoptosis, necrosis and mortality in a murine model of septic liver failure. Eur. J. Pharmacol. 294: 353–355

Goldbach, J. M., Roth, J., Störr, B., Zeisberger, E. (1997) Influence of pentoxifylline on fevers induced by bacterial lipopolysaccharide and tumour necrosis factor-α in guinea-pigs. Eur. J. Pharmacol. 319: 273–278

Gordon, C. J. (1990) Thermal biology of the laboratory rat. Physiol. Behav. 47: 963–991

Grau, G. E., Fajardo, L. F., Piquet, P. F., Allet, B., Lambert, P. H., Vassalli, P. (1987) Tumour necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. Science 237: 1210–1212

Han, J., Thompson, P., Beutler, B. (1990) Dexamethasone and pentoxifylline inhibit endotoxin-induced cachectin/tumour necrosis factor synthesis at separate points in the signaling pathway. J. Exp. Med. 172: 391–394

Hoeck, W. G., Ramesha, C. S., Chang, D. J., Fan, N., Heller, R. A. (1993) Cytoplasmic phospholipase A_2 activity and gene expression are stimulated by tumour necrosis factor: dexamethasone blocks the induced synthesis. Proc. Natl Acad. Sci. USA 90: 4475–4479

Karunaweera, N. D., Grau, G. E., Gamage, P., Carter, R., Mendis, K. N. (1992) Dynamics of fever and serum levels of tumour necrosis factor are closely associated during clinical paroxysms in *Plasmodium vivax* malaria. Proc. Natl Acad. Sci. USA 89: 3200–3203

Kasting, N. W. (1989) Criteria for establishing a physiological role for brain peptides. A case in point: the role of vasopressin in thermoregulation during fever and antipyresis. Brain Res. Rev. 14: 143–153

Kluger, M. J. (1991) Fever: role of pyrogens and cryogens. Physiol. Rev. 71: 93–127

LeMay, L. G., Vander, A. J., Kluger, M. J. (1990) The effects of pentoxifylline on lipopolysaccharide (LPS) fever, plasma interleukin 6 (IL-6) and tumour necrosis factor (TNF) in the rat. Cytokine 2: 300–306

Marayuma, N., Kakuta, Y., Yamauchi, K., Ohkawara, Y., Aizawa, T., Ohuri, T., Nara, M., Oshiro, T., Ohno, I., Tamura, G. (1994) Quinine inhibits production of tumour necrosis factor-alpha from human alveolar macrophages. Am. J. Res. Cell Mol. Biol. 10: 514–520

Roth, J., Zeisberger, E. (1995) Endotoxin tolerance alters thermal response of guinea-pigs to systemic infusions of tumour necrosis factor-α in guinea-pigs. Am. J. Physiol. 268: R514–R519

Slater, A. F. (1993) Chlorquine: mechanism of drug action and resistance in *Plasmodium falciparum*. Pharmacol. Ther. 57: 203–235

Strieten, R. M., Kunkel, S. L., Bone, R. C. (1993) Role of tumor necrosis factor-α in disease states and inflammation. Crit. Care Med. 21: S447–S463

Vane, J. R. (1976) Prostaglandins as mediators of inflammation, Thromboxane Res. 2: 791–801

Vinegar, R., Truax, J. F., Selph, J. L., Johnston, P. R., Venable, A. L., Mckenzie, K. K. (1987) Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed. Proc. 46: 118–126

Winter, C. A., Tisley, E. A., Nuss, G. W. (1962) Carrageenan-induced odema in the hind paw of the rats as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111: 544–554

Wirima, J. J., Khormana, C. O., Macheso, A. F., Heymann, D. L., Campbella, C. C. (1990) In vivo efficacy of quinine treatment for *Plasmodium falciparum* malaria in Malawian children. Ann. Trop. Med. Parasitol. 84: 223–227

Zidovetzki, R., Sherman, I. W., O'Brien, L. (1993) Inhibition of *Plasmodium falciparum* phospholipase A_2 by chloroquine, quinine, and arteether. J. Parasitol. 79: 565–570