

# Altered Vascular Reactivity Induced By Malaria Parasites

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## ABSTRACT

**Objective:** In this study, we have examined the possibility that there is altered vascular reactivity due to the direct interaction between parasitized erythrocytes and vascular endothelial cells.

**Method:** Ring preparations of rat aorta were studied using standard in vitro techniques, the rings were mounted in 20 ml organ baths containing PSS under an initial load of 1g, maintained at 37°C at pH 7.4 and isometric contractions were recorded electronically. Rings were allowed 90 minutes to equilibrate before the commencement of the various protocols:

- \* Dose responses to phenylephrine (PE) and other vasoactive agents (high-K<sup>+</sup>)
- \* Acetylcholine (ACh) – induced relaxation in phenylephrine-contracted rings (pre-contraction was induced by EC<sub>70</sub> concentration of phenylephrine)
- \* ACh-induced relaxation in PE-precontracted, endothelium-denuded rings
- \* Also, relaxation responses to acetylcholine was investigated through application of a single (EC<sub>70</sub>) concentration of acetylcholine in rings exposed to blood with varying concentrations and dilutions of parasitized blood and varying durations of exposure.

**Results:** Incubation with parasitized blood resulted in a significant increase in maximum contractile response to phenylephrine in the rat aortic rings ( $p < 0.05$ ) but no effect to the base line. Analysis of the whole dose-response curve (using paired t-test) showed a significant left-ward shift following the addition of parasitized blood ( $p < 0.05$ ), EC<sub>70</sub> (M) values increasing from  $7 \times 10^{-7}$  to  $5 \times 10^{-6}$  M. Following exposure to parasitized blood, the magnitude of ACh-induced relaxation responses reduced significantly from  $73 \pm 3.6$  to  $24.75 \pm 7.25\%$  in rat aortic rings ( $p < 0.05$ ). ACh relaxations were significantly enhanced ( $p < 0.05$ ) at 5-minute exposure; however at longer durations, ACh-relaxations were variable and inconsistent. The lesser the dilution, due to increased volume of parasitized blood, the lesser the relaxation response. Following endothelium removal, there was a marked impairment in endothelium-dependent relaxation responses to ACh in both the control and incubated vessels. Exposure to parasitized blood did not significantly alter contractile responses induced by potassium depolarization.

**Conclusions:** This gives evidence in support of an endothelium-dependent action of malaria parasites as vascular effects of malaria parasites are mediated, at least in part, via endothelium-dependent mechanism(s).

**Keywords:** Malaria, vascular, reactivity, tissue specificity

# Reactividad Vascular Alterada Inducida por los Parásitos de la Malaria

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## RESUMEN

**Objetivo:** En este estudio, hemos examinado la posibilidad de que exista una reactividad vascular alterada debido a la interacción directa entre los eritrocitos parasitados y las células endoteliales vasculares.

**Método:** Se estudiaron preparaciones de anillo de aorta de rata usando técnicas in vitro estándar. Los anillos fueron montados en baños de órgano de 20 ml que contenían solución salina fisiológica (SSF) con una carga inicial de 1g, mantenida a 37°C con un pH de 7.4, y las contracciones isométricas fueron registradas electrónicamente. A los anillos se les dio un tiempo de 90 minutos para permitir que se equilibraran, antes del comienzo de los varios protocolos.

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- \* Respuestas a la dosis de fenilefrina (FE) y otros agentes vasoactivos ( $K^+$  alto)
- \* Relajación inducida mediante acetilcolina (Ac) en los anillos contraídos con fenilefrina (la pre-contracción fue inducida mediante una concentración  $EC_{70}$  de fenilefrina)
- \* Relajación inducida mediante Ac en anillos despojados de endotelio. Pre-contraídos con FE.
- \* También, se investigaron las respuestas de relajación a la acetilcolina a través de la aplicación de una sola concentración ( $EC_{70}$ ) de acetilcolina en anillos expuestos a la sangre con diversas concentraciones y diluciones de sangre parasitada y distintas duraciones de exposición.

**Resultados:** La incubación con sangre parasitada tuvo como resultado un aumento significativo en la respuesta contráctil máxima a la fenilefrina en los anillos aórticos de las ratas ( $p < 0.05$ ) pero ningún efecto a la línea de base. El análisis de toda la curva de respuesta a la dosis (usando la prueba *t* pareada) mostró un desplazamiento significativo hacia la izquierda tras la adición de sangre parasitada ( $p < 0.05$ ),  $EC_{70}$  (M), aumentado los valores de  $7 \times 10^{-7}$  a  $5 \times 10^{-6}$  M. Tras la exposición a la sangre parasitada, la magnitud de las respuestas a la relajación inducida por Ac se redujo significativamente de  $73 \pm 3.6$  a  $24.75 \pm 7.25\%$  en los anillos aórticos de ratas ( $p < 0.05$ ). Las relajaciones por Ac mejoraron significativamente ( $p < 0.05$ ) a los 5 minutos de exposición. Sin embargo, a duraciones más largas, las relajaciones por Ac fueron variables e inconstantes. Mientras menor era la dilución, debido al aumento de volumen de la sangre parasitada, menor era la respuesta de relajación. Una vez retirado el endotelio, se producía un marcado deterioro en las respuestas de relajación dependiente del endotelio, ante el Ac, tanto en los recipientes de control como en los encubados. La exposición a la sangre parasitada no alteró de manera significativa las respuestas contráctiles inducidas por la despolarización del potasio.

**Conclusiones:** Esto provee evidencias en apoyo a una acción dependiente del epitelio, por parte de los parásitos de la malaria, por cuanto los efectos vasculares de los parásitos de la malaria se hallan mediados, al menos en parte, por los mecanismos dependientes del endotelio.

**Palabras claves:** Malaria, vascular, reactividad, especificidad del tejido.

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## INTRODUCTION

Malaria causes more deaths worldwide than any other parasitic disease. *Plasmodium falciparum* is responsible for severe malaria and an estimated 1.5–2.7 million deaths occur from malaria each year (1). Many aspects of the molecular biology, immunology and epidemiology that govern the pathogenesis of this parasite are still unclear and as such, insight into the complexity of malaria pathogenesis is vital for understanding the disease. Malaria has been reported to produce alterations of cardiovascular function (2, 3). Reports in the literature are conflicting: whereas Supanaranond *et al* (4) and Dyer *et al* (5) reported a fall in blood pressure in falciparum malaria, Shida *et al* (2) observed that with a rising parasitaemia, cardiac output increased, despite a reduced heart rate. The mean arterial blood pressure was lower in experimental rats than controls when parasitaemia exceeded 20%, whereas systolic right ventricular pressure was elevated only at the highest levels of parasitaemias, decline in the total peripheral vascular resistance, marked anaemia and an increase in plasma volume have been reported in *P. berghei* infection (2). The reported changes in BP appear to be related to the stages of malaria infection and levels of parasitaemia.

Many processes contribute to the pathogenesis of severe malaria; interactions of parasitized red blood cells (pRBCs) with endothelium are especially important. Severe falciparum malaria is associated with tissue ischaemia related

to cytoadherence of parasitized erythrocytes to microvascular endothelium and reduced levels of NO and its precursor, L-arginine (6). The vascular endothelium plays a central role in the pathogenesis of severe falciparum malaria. Parasitized red cells adhere to constitutive and cytokine-inducible receptors on the microvascular endothelium, resulting in sequestration and vascular obstruction, impaired perfusion and tissue dysoxia in critical organs (7–10). Rosetting is defined by the agglutination of nonparasitized erythrocytes around red cells containing mature forms of the parasite, and probably contributes to the intravascular sequestration of erythrocytes, (11). Cytoadherence of parasitized red blood cells to endothelium and concomitant activation of mononuclear blood cells may be responsible for a local synthesis of cytokines, or even neurotransmitters that remain to be identified (11). This cytoadherence is associated with *in vitro* and histopathological evidence of endothelial inflammation and damage (7, 8, 12, 13). However, there have been no studies examining endothelial function in SM *in vivo*. *In vitro*, NO reduces the expression of cytokine-inducible adhesion molecules on endothelial cells (14) and decreases cytoadherence of parasitized erythrocytes to the microvascular endothelium (15). Impaired *in vivo* endothelial NO production in malaria is likely to exacerbate these processes.

An additional mechanism of reduced NO availability has recently been described in disease states with intravascular haemolysis (16). Erythrocyte rupture results in increase

cell-free haemoglobin and plasma arginase (17, 26) leading to increased NO consumption and plasma L-arginine catabolism, respectively, and an overall reduction in NO bioavailability (6, 16). Because haemolysis is found in malaria, these processes may also contribute to NO deficiency, endothelial dysfunction and pathogenesis in SM. Endothelial function is characterized by the ability of vessels to dilate in response to increased shear stress or chemical agonists and is inversely related to endothelial activation (18). As acute falciparum malaria is associated with impaired circulatory control and the tendency to postural hypotension is worsened significantly by antimalarial treatment with the quinoline antimalarials, quinine and mefloquine (4). Possible functional changes in the responses of vascular smooth muscle to various vasoactive agents, following exposure to malaria parasites, were investigated.

## SUBJECTS AND METHODS

Blood with the human malaria parasite model, *Plasmodium falciparum*, was obtained from patients (University of Benin Teaching Hospital) presenting with high malaria parasitaemia and fever. Parasitaemia was assessed from Giemsa-stained thin blood films. Ethical approval was received before the commencement of the study; all procedures involving experimental animals were performed in compliance with local animal welfare laws, guidelines and policies. The animals used throughout this study (male Wistar rats), were housed, cared for and acclimatized in the animal house (before the experiments). Rats were anaesthetized and killed by decapitation; the thoracic aorta was rapidly dissected out and placed in ice-cold, oxygenated, modified physiological salt solution (PSS). This solution consisted of (in mM) NaCl (119), KCl (4.7),  $\text{CaCl}_2$  (2.5),  $\text{MgSO}_4$  (1.2),  $\text{H}_2\text{O}$ ,  $\text{KH}_2\text{PO}_4$  (1.2),  $\text{NaHCO}_3$  (24.9) and glucose<sup>11</sup> (1.1), pH 7.4. It was then cleaned of loosely adhering fat and connective tissue and cut into 2 mm rings. Each aortic ring was suspended in an organ bath containing 20 ml of well-oxygenated (95%  $\text{O}_2$ +5%  $\text{CO}_2$ ) PSS at 37°C. Rings were allowed 90 minutes to equilibrate before the commencement of the various protocols. Force generation was monitored by means of an isometric transducer (Grass model FT.03 isometric transducer) connected to a Grass multichannel polygraph. The resting tension in the aortic rings was adjusted to 1.0 g which was found to be the optimal tension for inducing a maximal contraction in preliminary experiments. The aortic strips were first contracted by 80 mM  $\text{K}^+$ , these responses being taken as 100%. Contractile responses were each expressed as a percentage of the contraction previously induced by 80 mM KCl. Dose-response tests to various agonists (phenylephrine) were carried out by cumulative addition of the agonists to the bath. High  $\text{K}^+$  contractions were observed by increases in  $\text{K}^+$  concentrations (prepared by equimolar substitution of NaCl with KCl) (19).

## Relaxation Responses

Total acetylcholine were assessed cumulatively in rings pre-contracted with  $10^{-6}$  M ( $\text{EC}_{70}$ ) PE. The magnitude of relaxation was compared with the pre-contraction induced by PE. Ach-induced relaxation was employed in assessing the functional integrity of the vascular endothelial cells. The endothelium was removed by rubbing the intimal surface with a cotton swab, successful removal being functionally confirmed by the absence of a relaxation to  $10^{-4}$  M acetylcholine chloride (ACh) (19).

## Data Presentation and Statistical Analysis

Contractile responses are expressed as either “gram force” or “%” of maximal response. Unless otherwise specified, results shown in the text, tables and figures are presented as means  $\pm$  SEM and comparison of the means effected using student's *t*-tests. A *p*-value  $< 0.05$  was considered as statistically significant. In the experiments carried out in precontracted rat aortic rings, contractile responses to vasoconstrictor agents are expressed as a percentage of the maximal contraction ( $E_{\text{max}} = 100\%$ ) produced by the corresponding vasoconstrictor agent. In these experiments, sigmoidal concentration-response curves for the vasorelaxant effects of acetylcholine were fitted using the programme Origin<sup>TM</sup> 5.0 (Microcal Software Inc., Northampton, USA),  $\text{EC}_{50}$ ,  $\text{EC}_{70}$  values (concentrations producing 50% and 70% maximum responses) were determined graphically. Contractile responses to cumulative concentrations of phenylephrine (in the presence or absence of parasitized blood) are expressed as a percentage of the maximal contraction ( $E_{\text{max}} = 100\%$ ) reached in the control concentration-response curves. These curves were analysed by a sigmoidal curve-fitting analysis programme (Origin<sup>TM</sup> 5.0).

## RESULTS

The dose-response to phenylephrine and its modification by parasitized blood are presented in Fig. 1. Parasitaemia re-

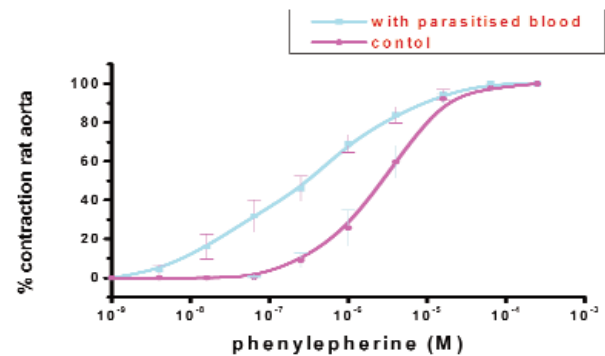


Fig. 1: Influence of parasitaemia on phenylephrine concentration response curves in rings of rat aorta. Responses are expressed as percentage (%) of maximal contractile response induced by phenylephrine. Data are means  $\pm$  SE ( $n = 6$ ).

sulted in a significant ( $p < 0.05$ ) enhancement (left-ward shift) of phenylephrine dose-response curve. The  $EC_{50}$  and  $EC_{70}$  values for phenylephrine contractions in the various ring preparations are calculated from the graph as  $7 \times 10^{-7}$  for the control and  $5 \times 10^{-6}$  M for the tissues exposed to parasitized blood. Incubation with parasitized blood alone did not affect the resting tension in the rings studied, neither did it bring about the relaxation of the phenylephrine precontracted rings when added to the organ baths (not shown).

### Relaxation Responses to Acetylcholine

Endothelium-dependent acetylcholine-induced relaxation responses were examined in  $10^{-7}$  M phenylephrine-precontracted rings. The dose-dependent acetylcholine-induced relaxations in rat aortic rings are expressed as percentage responses. Parasitaemia significantly ( $p < 0.05$ ) attenuated acetylcholine relaxations (Fig. 2), particularly at higher

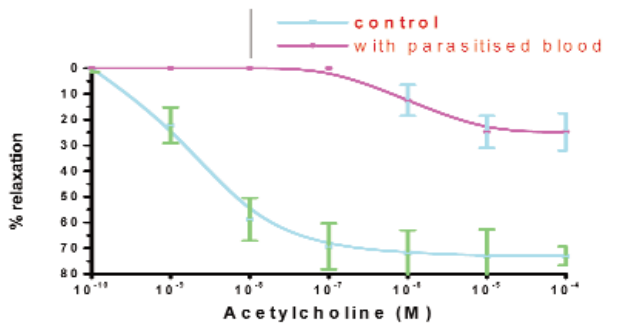


Fig. 2: Acetylcholine concentration response curves in rat aorta expressed as percentage (%) of  $EC_{70}$  phenylephrine. Ach-induced relaxations are significantly attenuated following exposure to parasitized blood. Data are means  $\pm$  SE of tissues ( $n = 6$ ).

concentrations of acetylcholine following exposure to parasitized blood. In separate experiments (not shown), removal of endothelium ( $n = 7$ ) abolished Ach-induced relaxation of phenylephrine contractions.

### Characterization of the Effect of parasitaemia on Ach Relaxation

In order to characterize the effect of parasitaemia on Ach-induced relaxation, the effect of a single application of Ach [ $EC_{70}$ ] (which was calculated from those of the control groups for acetylcholine concentration response curve) on  $10^{-7}$  M phenylephrine – precontraction was examined, using this protocol, rings were exposed to parasitized blood 15 minutes prior to addition of phenylephrine and maintained throughout the remaining part of the protocol. Two variants of this protocol were examined.

#### (a) Effect of increasing volume of parasitized blood

Graded increases in the volume of parasitized blood (while maintaining the duration of exposure at 15 minutes) resulted in significant ( $p < 0.05$ ) attenuation of Ach-induced relaxation responses (Fig. 3).

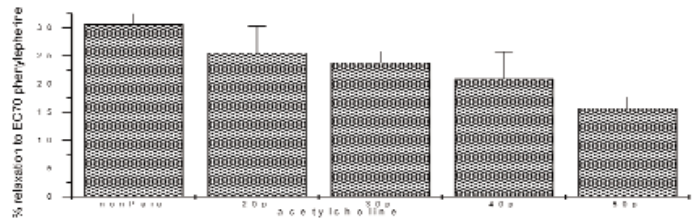


Fig. 3: Acetylcholine relaxation response in the artery expressed as percentage of  $EC_{70}$  phenylephrine contraction in control and following exposure to varying volumes of parasitized blood. Data are means  $\pm$  SE of tissues ( $n = 6$ ).

#### (b) Effect of increasing duration of exposure

In these experiments, Ach relaxations were assessed in arterial rings following varying durations of exposure to parasitized blood, at a particular level of parasitaemia. The results are presented in Fig. 4. Ach-relaxations were

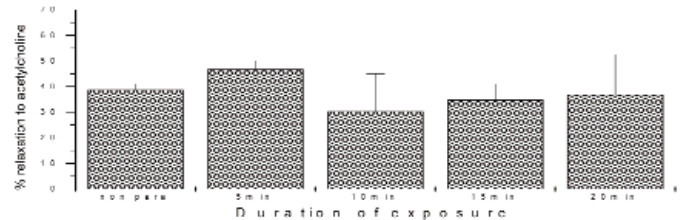


Fig. 4: Effect of duration of exposure to normal and parasitized blood on acetylcholine induced relaxation of phenylephrine contractions in the arteries. Data are means  $\pm$  SE of tissues ( $n = 6$ ).

significantly enhanced ( $p < 0.05$ ) at 5-minute exposure; however at longer durations, Ach-relaxations were variable and inconsistent.

### Effect of Parasitaemia on $Na^+ - K^+$ ATPase Activity

The protocol for assessing membranal  $Na^+ - K^+$  ATPase enzyme activity was done by using  $K^+$  - induced relaxation as an indicator. A biphasic response to potassium ion contraction was observed (Fig. 5). There was an increasing

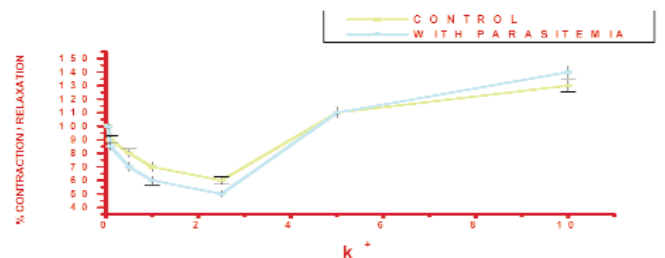


Fig. 5: Potassium-induced relaxation (an indirect indicator of  $Na^+ - K^+$  ATPase activity) in aortic rings from control rats and rats exposed to malaria parasites ( $n = 6$ ).

relaxation to  $K^+$  at lower concentrations, but enhanced contractility at higher concentrations of  $K^+$ . Exposure of the



rings to parasitized blood did not significantly alter the magnitude of  $K^+$  - induced relaxation.

## DISCUSSION

The tone of blood vessels is determined by their responsiveness to contracting agonists. In the present studies, incubation with parasitized blood resulted in a significant increase in maximum contractile response to phenylephrine in the rat aortic rings ( $p < 0.05$ ). Analysis of the whole dose-response curve (using paired t-test) showed a significant leftward shift at final bath concentrations of  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$  and  $10^{-5}$  following the addition of parasitized blood ( $p < 0.05$ ),  $EC_{70}$  (M) values increasing from  $7 \times 10^{-7}$  to  $5 \times 10^{-6}$  M. ( $p < 0.05$ ).

The enhanced contractions with parasitized blood could be due to the nonspecific immune inflammatory response of the host to the malaria parasite, with release of various mediators (11). Cytoadherence associated endothelial inflammation and damage (12, 7, 8, 13) of parasitized red blood cells to endothelium and concomitant activation of mononuclear blood cells may be responsible for a local synthesis of cytokines, or even neurotransmitters that remain to be identified (11), local release of cytokines and nitric oxide [NO] (20–22), histamine, (23) impaired cerebral synthesis of serotonin, dopamine and norepinephrine, and enhanced production of histamine (24). The enhanced production of histamine and NO will lead to vasodilation while the local cytokine production will affect the baseline contractions on the tissues during incubation and in the absence of an agonist, but this was not observed in the present study as contractile effects were only initiated by use of an agonist.

Following exposure to parasitized blood, the magnitude of Ach-induced relaxation responses reduced significantly from  $73 \pm 3.6$  to  $24.75 \pm 7.25\%$  in rat aortic rings ( $p < 0.05$ ), this could occur due to the interactions of the parasites with the vessel walls and blood components associated with endothelial inflammation and damage (12, 7, 8, 13). In the time course, relaxation responses to acetylcholine, observed by varying the time duration for incubation of the parasitized blood with the aortic rings, showed diminished relaxation with increased time for incubation; on analysis this was not found to be significant ( $p < 0.05$ ) beyond five minutes. Ehrich *et al* (25) had reported that the transaminases increased tenfold and the cholinesterase decreased from 943 U/l to 271 U/l when Lewis inbred rats were infected with *Plasmodium berghei* K173. With this we would have expected an increased in relaxation as the time of incubation with parasitized blood increased. However, our result suggested that beyond 5 minutes of exposure, the magnitude of relaxation was independent of the duration of exposure.

Because haemolysis is found in malaria, these processes may also contribute to NO deficiency as erythrocyte rupture results in increased cell-free haemoglobin and plasma arginase (17, 26), leading to increased NO consumption and plasma L-arginine catabolism, respectively, and an overall

reduction in NO bioavailability (16), endothelial dysfunction and pathogenesis in malaria. The results of this study which show decreased relaxation with increased volumes of blood used during incubation may be explained by this increased cell-free haemoglobin and an overall reduction in NO bioavailability. But the long-standing notion that haemoglobin universally scavenges NO and thus that erythrocytes act as a permanent sink for this molecule does not hold true always as it is now known that NO can be released as well as scavenged by haemoglobin. The role played by the endothelium and the function of NO still remains unclear as Maneerat *et al* (27) had reported that there was no difference in nitrate levels and (28) in blood NO in *P. berghei* infected mice. Zhang *et al*, (29) reported that with up-regulated expression of VCAM-1 protein in cerebral and common carotid arteries of rats, there was an impaired endothelium-dependent relaxation, while Yeo *et al* (30) observed that infusion of up to 12 g of intravenous L-arginine hydrochloride which improves endothelial function in adults did not affect the haemodynamic or biochemical status in patients with severe malaria. In our studies, following endothelium removal, there was a marked impairment in endothelium-dependent relaxation responses to ACh in both the control and malaria exposed vessels of all the vessels studied.

Exposure to parasitized blood did not significantly alter contractile responses induced by potassium depolarization. This is further evidence in support of the endothelium-dependent action of malaria parasites – since it is well reported that endothelium-dependent vasorelaxants are ineffective against  $K^+$  contractions (31, 19). We observed that there was no significant difference with parasitized tissues or tissues incubated with parasitized blood and those that were incubated with non-parasitized blood and/or control when the  $Na^+ K^+$  ATPase was investigated. Also, the magnitude of potassium-induced relaxation following a period of  $K^+$ -free exposure (a functional indicator of  $Na^+-K^+$  ATPase activity) was not significantly altered; this suggests that malaria parasitaemia does not alter vascular membranal  $Na^+-K^+$  pump activity.

This study suggests that interaction between malaria parasites and the blood vessel wall (*in vitro*) results in functional changes in the contractile state of vascular smooth muscle possibly through the release of vasoactive agents from both the red cells and vascular endothelium. This may contribute to/or exacerbate the abnormal haemodynamics observed in the capillary microcirculation of numerous vascular beds. Furthermore, the vascular effects of malaria parasites are mediated, at least in part, *via* endothelium-dependent mechanism(s). Further studies to determine the nitric oxide levels following parasitaemia may resolve the conflict between the present *in vivo* and *in vitro* observations.

## REFERENCES

- World Health Organization, Communicable diseases cluster. Severe falciparum malaria. *Trans Soc Trop Med Hyg* 2000; **94**; **Suppl 1**: S1–90.
- Shida KK, Buarong Lewchalermvongse, Lorrin WP, Plasmodium berghei: Malaria infection causes increased cardiac output in rats, *Rattus rattus*. *Exp Parasitol* 1989; **68**: 253–9.
- Anigbogu CN, Adigun SA. Blood pressure, heart rate and autonomic reflexes in plasmodium berghei malaria infection. *NQJ Hosp Med* 1994; **6**: 6–11.
- Supanaranond W, Davis TM, Pukrittayakamee S, Nagachinta B, White NJ. Abnormal circulatory control in falciparum malaria: the effects of antimalarial drugs. *Eur J Clin Pharmacol* 1993; **44**: 325–9.
- Dyer JR, Davis TM, Giele C, Annus T, Garcia-Webb P, Robson J. The pharmacokinetics and pharmacodynamics of quinine in the diabetic and non-diabetic elderly. *Br J Clin Pharmacol* 1994; **38**: 205–12.
- Ugwu AC, Nwokocha CR. Nitric Oxide; the motivator of body systems. *African J Biomed Res* 2001; **4**: 1–5.
- Turner G, Morrison H, Jones M, Davis T, Gatter K, Buley L et al. An immunochemical study of the pathology of fatal malaria: evidence for widespread endothelial activation and a potential role for ICAM-1 in cerebral sequestration. *Am J Pathol* 1994; **145**: 1057–69.
- Turner GD, Ly VC, Nguyen TH, Tran TH, Nguyen HP, Bethell D et al. Systemic endothelial activation occurs in both mild and severe malaria. Correlating dermal microvascular endothelial cell phenotype and soluble cell adhesion molecules with disease severity. *Am J Pathol* 1998; **152**: 1477–87.
- Marchiafava E, Bignami A. On Summer-Autumnal Fever. London: New Sydenham Society 1994.
- Taylor TE, Fu WJ, Carr RA, Whitten RO, Mueller JG, Fosiko NG et al. Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nat Med* 2004; **10**: 143–5.
- Gachot B, Vachon F. Physiopathology of cerebral malaria. *Presse Med* 1995; **24**: 642–6.
- MacPherson GG, Warrell MJ, White NJ, Looareesuwan S, Warrell DA. Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *Am J Pathol* 1985; **119**: 385–401.
- Pongponratn E, Turner GD, Day NP, Phu NA, Simpson K, Stepniewska NT et al. An ultrastructural study of the brain in fatal Plasmodium falciparum malaria. *Am J Trop Med Hyg* 2003; **69**: 345–59.
- De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA Jr, et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995; **96**: 60–8.
- Serirom S, Raharjo WH, Chotivanich K, Loareesuwan S, Kubes P, Ho M. Anti-adhesive effect of nitric oxide on Plasmodium falciparum cytoadherence under flow. *Am J Pathol* 2003; **162**: 1651–60.
- Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *JAMA* 2005; **293**: 1653–62.
- Morris CR, Kato GJ, Poljakovic M, Wang X, Blackwelder WC, Sachdev et al. Dysregulate arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA* 2005; **294**: 81–90.
- Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 2007; **115**: 1285–95.
- Ebeigbe AB, Aloamaka CP. Barium and strontium as calcium substitute for contractile responses in the rat tail artery. *Comp Biochem Physiol* 1985; **82C**: 213–16.
- Clark I, Chaudri G. The balance of useful and harmful effects of TNF with special reference to malaria. *Ann Inst Pasteur Immunol* 1988; **139**: 305–6.
- Clark IA, Rockett KA, Cowden WB. Possible role of nitric oxide in conditions clinically similar to cerebral malaria. *Lancet* 1992; **340**: 894–6.
- Heddi A. Malaria pathogenesis: a jigsaw with an increasing number of pieces. *Int J Parasitol* 2002; **32**: 1587–98.
- Maegraith BG, Onabanjo AO. The effects of histamine in malaria. *Br J Pharmacol* 1970; **39**: 755–64.
- Enwonwu CO, Afolabi BM, Salako LA, Idigbe EO, al-Hassan H, Rabi RA. Hyperphenylalaninaemia in children with falciparum malaria. *QJM* 1999; **92**: 495–503.
- Ehrich JH, Beck EJ, Haberkorn A, Meister G. Causes of death in lethal rat malaria. *Tropenmedizin und Parasitologie* 1984; **35**: 127–30.
- Morris CR, Morris SM Jr, Hagar W, Van Warmerdam J, Claster S, Kepka-Lenhart D et al. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? *Am J Respir Crit Care Med* 2003; **168**: 63–9.
- Maneerat Y, Wilairatana P, Udomsangpetch R, Looareesuwan S. Lack of association between CSF nitrate and sera nitrate in falciparum malaria infection. *Southeast Asian Trop Med Public Health* 2001; **32**: 268–74.
- Nahrevanian H, Dascombe MJ. Nitric oxide and reactive nitrogen intermediates during lethal and nonlethal strains of murine malaria. *Parasite Immunology* 2001; **23**: 491–501.
- Zhang R, Jia G, Bao J, Zhang Y, Bai Y, Lin L et al. Increased vascular cell adhesion molecule-1 was associated with impaired endothelium-dependent relaxation of cerebral and carotid arteries in simulated microgravity rats. *J Physiol Sci* 2008; **58**: 67–73.
- Yeo TW, Lampah DA, Gitawati R, Tjitra E, Kenangalem E, Granger DL et al. Safety profile of L-arginine infusion in moderately severe falciparum malaria. *PLoS One* 2008; **11**: 3–6.
- Ebeigbe AB. Calcium pools for noradrenaline and potassium-induced