

# Specificity of Vascular Reactivity and Altered Response in Experimental Malaria

CR Nwokocha<sup>1</sup>, IO Ajayi<sup>2</sup>, DU Owu<sup>1</sup>, AB Ebeigbe<sup>2</sup>

## ABSTRACT

**Objective:** Adherence of erythrocytes infected with *Plasmodium falciparum* (*P. falciparum*) to microvascular endothelial cells (sequestration) is considered to play an important role in parasite virulence and pathogenesis. In this study, we have examined the possibility that there is altered vascular reactivity due to the direct interaction between the parasitized erythrocytes and vascular endothelial cells and that it could be tissue specific.

**Method:** Ring preparations of blood vessels from the rabbit carotid and rat aorta were studied using standard organ bath techniques. Dose response curves for phenylephrine (PE) and acetylcholine (Ach)-induced relaxation were constructed in rings pre-contracted with PE.

**Results:** Incubation of rat aortic rings with parasitized blood resulted in a significant ( $p < 0.05$ ) increase in maximum contractile response to phenylephrine in the rat aortic rings but there was no effect on the rabbit carotid artery. The dose-response curve showed a significant ( $p < 0.05$ ) left-ward shift following the addition of parasitized blood. Parasitized blood had no effect on baseline in both tissues.

Following exposure to parasitized blood, the magnitude of Ach-induced relaxation responses reduced significantly ( $p < 0.05$ ) in rat aortic rings and ( $p < 0.05$ ) in rabbit carotid rings; relaxations to acetylcholine was more pronounced in the aortic compared to the carotid rings.

**Conclusions:** Malaria altered vascular reactivity through an endothelium-dependent mechanism. The regulation of vascular tone by various vasoactive agents following exposure to malaria parasites might be altered in a vessel-specific manner. This may contribute to or exacerbate the abnormal haemodynamics observed in the microcirculation of numerous vascular beds in malaria.

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

## Especificidad de la Reactividad Vascular y Respuesta Alterada en la Malaria Experimental

CR Nwokocha<sup>1</sup>, IO Ajayi<sup>2</sup>, DU Owu<sup>1</sup>, AB Ebeigbe<sup>2</sup>

## RESUMEN

**Objetivo:** Se considera que la adhesión de eritrocitos infectados con *Plasmodium falciparum* (*P. falciparum*) a las células endoteliales microvasculares (secuestro) juega un papel importante en la virulencia parasitaria y la patogénesis. Este estudio examina la posibilidad de que se produzca reactividad vascular alterada debido a la interacción directa entre los eritrocitos parasitados y las células endoteliales vasculares, y que la misma se deba al tejido específico.

**Método:** Preparaciones de anillos de vasos sanguíneos de carótida de conejo y aorta de rata, se estudiaron usando técnicas de baño de órgano normales. Las curvas dosis-respuesta de fenilefrina (PE) y relajación inducida por acetilcolina (Ach) fueron construidas en anillos pre-contraídos con PE.

**Resultados:** La incubación de anillos aórticos de rata con sangre parasitada trajo un aumento significativo ( $p < 0.05$ ) de la respuesta contráctil máxima a la fenilefrina en los anillos aórticos de rata, pero no hubo efecto sobre la arteria carótida de conejo. La curva dosis-respuesta mostró un cambio significativo ( $p < 0.05$ ) hacia la izquierda luego de que se añadiera sangre parasitada. La sangre parasitada no tuvo efecto en ninguno de los dos tejidos con respecto a los valores iniciales (línea de

From: <sup>1</sup>Department of Basic Medical Sciences, The University of the West Indies, Kingston 7, Jamaica and <sup>2</sup>Department of Physiology, University of Benin, Benin City, Nigeria.

Correspondence: Dr CR Nwokocha, Department of Basic Medical Sciences, The University of the West Indies, Kingston 7, Jamaica. E-mail: chukwuemeka.nwokocha@uwimona.edu.jm

base). *Luego de ser expuesta a la sangre parasitada, la magnitud de las respuestas de relajación inducida por acetilcolina, se redujo significativamente ( $p < 0.05$ ) en los anillos aórticos de rata, y ( $p < 0.05$ ) en los anillos de carótida de conejo. Las relajaciones con respecto a la acetilcolina fueron más pronunciadas en los anillos aórticos en comparación con los anillos de carótida.*

**Conclusiones:** *La malaria alteró la reactividad vascular a través de un mecanismo dependiente del endotelio. La regulación del tono vascular por los varios agentes vasoactivos tras la exposición a los parásitos de la malaria, podría ser alterada de manera vaso-específica. Esto puede contribuir o exacerbar la hemodinámica anormal observada en la microcirculación de numerosos lechos vasculares en la malaria.*

**Palabras claves:** malaria, reactividad vascular, especificidad vascular

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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 patho-genesis of this parasite are still unclear as such insight into the complexity of malaria pathogenesis is vital for understanding the disease.

Adherence of erythrocytes infected with *Plasmodium falciparum* to microvascular endothelial cells (sequestration) is considered to play an important role in parasite virulence and pathogenesis (2). Adhesion of IEs to host endothelial receptors is mediated by members of a large diverse protein family called *P falciparum* erythrocyte membrane protein 1 (PfEMP1) (2–4). These interactions leads to increased expression of proinflammatory cytokines, such as gamma interferon IFN- $\gamma$  (5, 6), tumor necrosis factor alpha TNF (7–9), lymphotoxin alpha (LT $\alpha$ ) (10) and interleukin-10 (IL-10) (11) which have important regulatory roles in local vascular proliferation (12–14) and reactivity, through induction of inducible nitric oxide synthase (iNOS) in vascular endothelium (15) and the upregulation of cell adhesion molecules such as ICAM-I on the surface of endothelial cells (16).

The interactions of the infected erythrocytes and vascular endothelium could also cause the release of neurotransmitters (17, 18), impaired cerebral synthesis of serotonin, dopamine and norepinephrine, histamine (19, 20), endothelial cell activation (21), leading to local metabolic derangements (22, 21). Other factors contributing to the pathogenesis of malaria include erythrocyte rupture which results in increased cell-free haemoglobin and plasma arginase (23), leading to increased nitric oxide (NO) consumption and plasma L-arginine catabolism, respectively, and an overall reduction in NO bioavailability (17, 24–26).

Many workers have reported some levels of tissue/organ specificity with regards to the spleen, liver, kidney and brain (2, 15, 27, 28) and even receptor specificity (29) as it concerns sequestration of infected erythrocytes. The binding of infected erythrocytes and activation of the vascular

endothelial cells through the various receptors may thus be vessel dependent or specific.

The aim of the study was to investigate possible functional changes in the response of vascular smooth muscle from different tissues to various vasoactive agents, following exposure to malaria parasites. This becomes important as arterial disorders do not show uniform manifestations through-out the body, but rather are specific to certain vascular systems in individual patients.

## SUBJECTS AND METHODS

### *Animals and experimental design*

Animals were obtained and kept at the Animal house of the Faculty of Medical Sciences for this study. During the entire treatment period, animals were kept at constant room temperature with 12 h of light/dark cycles, and were fed with standard rat food and water *ad libitum*. The animals include male rats weighting 150–180 g and rabbits weighting 1–1.5 kg. The animals were treated according to the faculty ethics and animal regulations Act.

### *Vascular tissue studies*

The thoracic aorta from rats and carotid arteries from rabbits were used for this study, they were rapidly dissected out and placed in ice-cold, oxygenated, modified physiological salt solution (PSS). This solution consisted of (in mM) 119 NaCl, 4.7 KCl, 2.5 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>·H<sub>2</sub>O, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 24.9 NaHCO<sub>3</sub> and 11.1 glucose, pH 7.4. It was then cleaned of loosely adhering fat and connective tissue and cut into ~2mm rings. Each ring was suspended in an organ bath containing 20 ml of well-oxygenated (95% O<sub>2</sub> – 5% CO<sub>2</sub>) 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 (Model 79D, Grass, Quincy, MA, USA). The resting tension in the aortic rings was adjusted to 1.0 g while that of the carotid was adjusted to 2 g, which was found to be the optimal tension for inducing a maximal contraction in preliminary experiments. The rings were first contracted by 80 mM K<sup>+</sup> and this response was

taken as 100%. Contractile responses were each expressed as a percentage of the contraction previously induced by 80 mM KCl. Dose-response to phenylephrine was carried out by cumulative addition of the agonist to the bath in the presence or absence of parasitized blood.

#### Relaxation responses

The relaxation responses to acetylcholine were assessed cumulatively in rings pre-contracted with  $10^{-6}$ M ( $EC_{70}$ ) PE in the presence or absence of parasitized blood. The magnitude of relaxation was compared with the pre-contraction induced by PE (30).

#### Statistical analysis

Results are presented as means  $\pm$  SEM and comparison of the means was done using student's *t*-tests. A *p* value  $< 0.05$  was considered as statistically significant. Contractile responses are expressed as percentage (%) of maximal response to 80 mM KCl. The concentration-response curves for acetylcholine were constructed using a computer software Origin™ 5.0 (Microcal Software Inc, Northampton, USA) and  $EC_{50}$ ,  $EC_{70}$  values (concentrations producing 50% and 70% max. responses) were determined graphically.

## RESULTS

#### Contractile responses to phenylephrine in rat aorta and the effect of parasitaemia

The dose-response curves for phenylephrine are presented in Fig. 1. Parasitaemia resulted in a significant ( $p < 0.05$ )

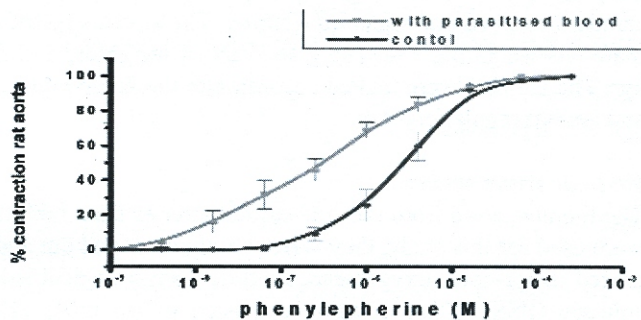


Fig. 1: Influence of parasitaemia on phenylephrine concentration response curves in rings of rat aorta. Data are means  $\pm$  SE ( $n = 6$ ).

enhancement (left-ward shift) of phenylephrine dose-response curve. The  $EC_{50}$  and  $EC_{70}$  values for phenylephrine contractions in the various ring preparations were  $7 \times 10^{-7}$  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 pre-contracted rings when added to the organ baths (not shown).

#### Contractile responses to phenylephrine in rabbit carotid and the effect of parasitaemia

The dose-response curves for phenylephrine are presented in Fig. 2. Parasitaemia resulted in no change of phenylephrine

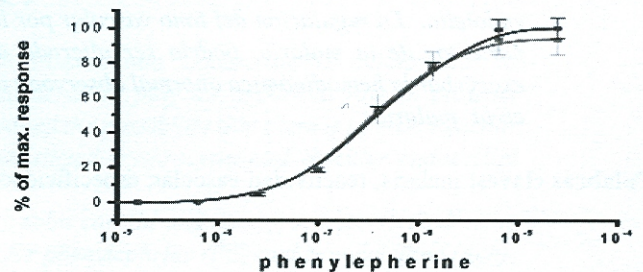


Fig. 2: Phenylephrine concentration response curves in carotid rings in control (●) and following exposure to parasitized blood (■). Data are means  $\pm$  SE ( $n = 6$ ).

dose-response curve. The  $EC_{50}$  and  $EC_{70}$  values for phenylephrine contractions in the various ring preparations remained the same for the control and 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 pre-contracted rings when added to the organ baths.

#### Relaxation responses to acetylcholine in rat aorta and the effect of parasitaemia

Endothelium-dependent acetylcholine-induced relaxation responses were examined in  $10^{-7}$  M phenylephrine-precontracted rings. Parasitaemia significantly ( $p < 0.05$ ) attenuated acetylcholine-induced relaxations particularly at higher concentrations of acetylcholine following exposure to parasitized blood (Fig. 3).

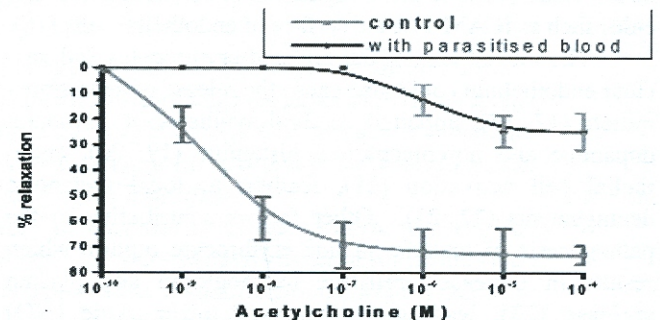


Fig. 3: Ach-induced relaxations of aorta following exposure to parasitized blood. Data are means  $\pm$  SE of tissues ( $n = 6$ ).

#### Relaxation responses to acetylcholine in rabbit carotid and the effect of parasitaemia

Endothelium-dependent acetylcholine-induced relaxation responses were examined in  $10^{-7}$  M phenylephrine-precon-

tracted rings. Parasitaemia significantly ( $p < 0.05$ ) attenuated acetylcholine-induced relaxations particularly at higher concentrations of acetylcholine following exposure to parasitized blood (Fig. 4).

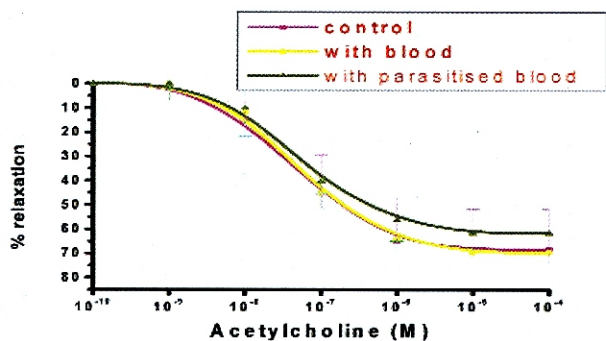


Fig. 4: Ach-induced relaxations of rabbit carotid rings following exposure to parasitized blood. Data are means  $\pm$  SE of tissues ( $n = 6$ ).

#### 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. 5. Ach relaxations were signifi-

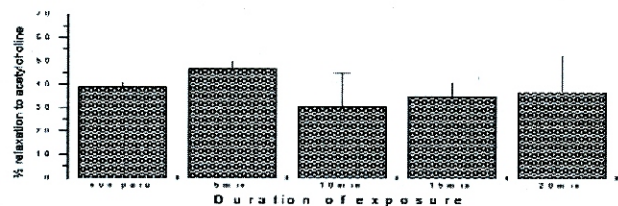


Fig. 5: Effect of duration of exposure to normal and parasitized blood on acetylcholine ( $EC_{70}$ ) induced relaxation of phenylephrine ( $EC_{70}$ ) contractions in the arteries. Data are means  $\pm$  SE. ( $n = 6$ ).

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

## 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 but not in rings of the rabbit carotid. Analysis of the whole dose-response curve showed a significant leftward shift of the curve following the addition of parasitized blood. There was increased sensitivity to phenylephrine in aortic rings exposed to parasitized blood, but parasitaemia did not affect the baseline values. We also observed a significant reduction in the magnitude of Ach-induced

relaxation following exposure to parasitized blood in both the carotid and aortic rings.

Shida *et al* (31) had earlier reported decline in the total peripheral vascular resistance, but our observations were of an enhanced vascular reactivity *in vitro*. The enhanced contractions with parasitized blood could be due to the non-specific immune inflammatory response to the malaria parasite, with release of various mediators into the blood stream, local synthesis of cytokines (8, 9, 20), or even neurotransmitters (17, 18), associated endothelial inflammation and damage (32, 33), nitric oxide availability (26, 34, 35), endothelial cell activation leading to loss of vascular integrity (21). All these can contribute to the amplification of the vasoconstrictor response, suggesting that interaction between malaria parasites and the blood vessel wall (*in vitro*) results in functional changes in the contractile state of vascular smooth muscle (36).

Acetylcholine did not relax ring preparations denuded of endothelium; this was the same for denuded rings incubated with malaria blood. Malaria is reported to cause an increased expression of proinflammatory cytokines (6–9, 22), we anticipated these substances may increase relaxation as they are vasodilators, but that was not the case. It is possible that this could be due to the haemoglobin released through haemolysis (23), a decreased NO availability (26), or even the presence of cholinesterase (37).

Such alteration in endothelial function may be responsible for modulating the regulation of vascular smooth muscle tone (38). Our observations on the effects of time/duration of exposure of malaria parasitaemia on vascular tissues shows that these effects are most experienced within a short duration of time, as such endothelial regeneration and functional changes associated with it are not expected to mimic those seen with major vascular diseases like intimal and adventitia thickening following repeated endothelial damage (38).

However, there are reports documenting the differences in arteries undergoing vascular remodelling and their responses to various stimuli (38, 39). Many workers have reported organ specificity with regards to sequestration of infected erythrocytes and subsequent organ damage (2, 27, 28) and even receptor specificity (29). Our findings suggest some arterial specificity of vascular reactivity following exposure and endothelial injuries in the vessels studied (38). This may explain the non-uniform arterial disorders in individuals presenting with malaria, especially cerebral malaria.

It is, however, unclear as to how this specificity contributes to the different response to vasoactive agents, the up regulation of receptors (29, 40, 41) or even expression of adhesive molecules. All these may contribute to the vascular tissue specificity which may play major roles in disease outcomes (41). Further work is needed to realize the reactivity of other vascular tissues across organs and species to know if endothelial adhesion across these vessels shows

any tissue specificity. An understanding of the expression and distribution of NOS isoforms and adhesion factors in endothelial cells may contribute to better understanding of the structural and physiological changes in malaria induced alteration to vascular reactivity; this could help in the development of targeted interventions in malaria.

This study reports that interaction between malaria parasites and the blood vessel wall (*in vitro*) results in functional changes in the contractile state of vascular smooth muscle which could be time dependent. The regulation of vascular tone by various vasoactive agents following exposure to malaria parasites might be altered in a vessel specific manner. Our observation of enhanced response to contractile agents and reduced relaxation response to acetylcholine displayed some vessel specificity which may be due to species difference, as such caution should be employed in the application of these results.

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