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Original Article

Myocardial Dysfunction: A Primary Cause of Death Due To Severe Malaria in A *Plasmodium falciparum*-Infected Humanized Mouse Model

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Abstract

Background: Our study aimed at substantiating the recent claim of myocardial complications in severe malaria by experimentally inducing severe *Plasmodium falciparum* infection in a humanized mouse model employed as human surrogate.

Methods: Twenty five humanized mice were inoculated with standard *in vitro* cultured *P. falciparum* and blood extracts collected from the inner cardiac muscles of infected mice that died were examined for the presence of the infectious cause of death. The therapeutic effect of quinine on 7 mice severely infected with *P. falciparum* was also evaluated.

Results: All the 25 humanized mice inoculated with the *in vitro* cultured *P. falciparum* revealed peripheral parasitemia with a total of 10 deaths recorded. Postmortem examination of the inner cardiac muscles of the dead mice also revealed massive sequestration of mature *P. falciparum* as well as significant infiltration of inflammatory cells such as lymphocytes and monocytes. Post-mortem evaluation of the inner cardiac muscles of the *P. falciparum*-infected mice after quinine therapy showed significant decline in parasite density with no death of mice recorded.

Conclusions: Data obtained from our study significantly corroborated the findings of myocardial dysfunction as the primary cause of death in recent case reports of humans infected with *P. falciparum*.

Introduction

Malaria is the world's leading parasitic disease and affects approximately 40% of the world's population (approximately 2.4 billion people) in more than 100 countries (1). A life threatening illness arising from malaria infection, usually referred to as severe malaria, may occur in 1 – 2 % of infection (2, 3). Of the five clinically relevant *Plasmodium* species (*Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, *P. knowlesi*), *P. falciparum* mainly accounts for severe malaria, an important cause of morbidity and mortality in infected hosts (4). Studies have shown that mortality resulting from severe malaria is higher in children, due to inflammatory responses that are characterized by proliferation of leukocyte and platelets in the vessels of the implicated organs (5, 6), than in adults, with severe malaria that are commonly reported to have only sparse leukocyte and platelet sequestration in the vessels of implicated organs (6).

Severe malaria (SM) can have many manifestations and involve multiple organs. These manifestations include cerebral malaria (CM), severe malaria anemia (SMA), acute respiratory distress syndrome (ARDS), hyperparasitemia, hypoglycemia, metabolic acidosis, jaundice, and renal failure (1). However, myocarditis was reported as a rare manifestation of *falciparum* malaria (7). “These clinical manifestations of severe malaria are thought to occur because of a combination of a high parasite burden and the sequestration of mature *P. falciparum*-infected erythrocytes (IEs) in microvascular beds throughout the body” (8) which leads to microvascular obstruction (3), as well as metabolic disturbances, such as acidosis (9) and release of damaging inflammatory mediators (10, 11), which can combine to cause severe disease and death of the human host. The sequestration of mature *P. falciparum*-infected erythrocytes serves as an immune evasion strategy by the parasite that prevents the removal of parasitized erythrocytes in the spleen, thus facilitating parasite survival (12,

13). “In addition to parasite-induced pathology, a large body of work indicates that host immune responses to parasites also play an important role in CM pathogenesis” (14). A robust proinflammatory response mediated by activated T cells and cytokines, as well as recruitment of activated leukocytes to the implicated organs, have been associated with severe malaria (5, 10). Evidence from mouse models of experimental cerebral malaria (15) as well as from patients with severe malaria (16), has identified tumor necrosis factor (TNF) as an important proinflammatory cytokine associated with malaria pathology.

Amongst the plethora of manifestations due to severe malaria, cerebral malaria (severe *Plasmodial* infestation of the cerebrum) has been the central focus of most studies (17, 18), and has consistently been reported as the most common clinical presentation and cause of death (1). Cerebral malaria (CM) is a serious complication of *P. falciparum* infection. In adult patients with cerebral malaria, previous neuropathological studies have indicated the presence of adhesion and sequestration of *P. falciparum*-infected red blood cells (iRBCs) in brain microvessels, ring hemorrhages (RHs), and Dürck's granulomas as well as axonal injury (19) and blood-brain-barrier (BBB) dysfunction (20). However, the syndrome of cerebral malaria in children is different from that in adults (21).

Recently, only very few case reports have suggested complications in the heart of *P. falciparum*-infected patients as a primary cause of death due to severe malaria (22, 23). These cases revealed myocardial injury due to acute lymphocytic myocarditis with myocardiolysis. To our knowledge, this myocardial dysfunction seen in human patients with severe malaria has not been experimentally demonstrated using mouse models.

Our study utilized an experimental humanized mouse model to substantiate the claim

that myocardial complications may indeed be the primary cause of death due to severe malaria caused by *P. falciparum*, so as to negate previous assertions (1, 7) that cardiac functions were well preserved in severe malarial infections.

Materials and Methods

Development of experimental humanized mouse model

Mice: Balb/c mice weighing 20 – 33 g were used. They were housed in standard mosquito-netted metal cages under standard conditions of light and temperature and were maintained on a standard diet and water ad libitum. They were acclimatized for 14 days and were treated in accordance with guidelines for animal care approved by the Animal Ethics Committee of the Igbinedion University, Okada, Nigeria.

Modification of mice: The Balb/c mice were modified by using pharmacological compounds (4mg aspirin/kg body weight and 4mg anhydrous doxycycline eq./kg body weight) to retard their innate immune responses (Javeed et al. (24); Bellahsene and Forsgren (25); Imade et al. (26)) followed by engraftment of human blood (Imade et al. (26); Moreno et al. (27)).

Infection of experimental humanized mouse model with Plasmodium falciparum

Thirty humanized Balb/c mice were divided into six experimental groups with each group having 5 mice. One of the experimental groups represented the control group, while the remaining experimental groups represented the test group. The 25 humanized mice in the test group were inoculated with clinical isolates of *P. falciparum* that had been previously cultured in vitro (Trager and Jensen (28); Lambros and Vandenberg (29)) to obtain the parasite density (8%) required for inducing in vivo infection, while the 5 humanized mice in the control group were not inoculated with *P.*

falciparum. 0.5ml of the in vitro-cultured *P. falciparum* was intraperitoneally injected into the humanized Balb/c mice to induce in vivo infection. For the mice that died after inoculation with *P. falciparum* culture, postmortem was performed according to previously described standard procedures (30) to collect cardiac blood materials from the inner layers (myocardium and endocardium) of the animal's heart so as to determine the presence of the infectious cause of death. Thick and thin blood films of the extracted cardiac materials were then hematologically stained with 3% Giemsa (BDH, England) according to the method of WHO (31) and examined for *P. falciparum* parasites. For the mice that survived after inoculation with *P. falciparum* cultures, venous blood samples were collected to prepare thick and thin films that were subsequently stained and examined for *P. falciparum* parasites. All films were examined under a digital/analog research microscope (Motic China Group Co., Ltd.). Parasitemia was also calculated according to the method of WHO (31).

Antimalarial chemotherapeutic investigation of Plasmodium falciparum-infected humanized mouse model

Fourteen *P. falciparum*-infected humanized mice that survived the infection phase were equally divided into two experimental groups with each group representing the control and test groups respectively. To evaluate the effect of drugs on the behavior of *P. falciparum*, the *P. falciparum*-infected humanized mice in the test and control groups were orally treated with 73 mg quinine/kg body weight and 4 mg dimethyl sulfoxide/kg body weight three times daily for four days respectively (26, 27). During the course of treatment, postmortem was done on the infected mice that died, according to the procedure of Schlam and Carroll (30), and extracted cardiac blood materials from the inner muscles were hematologically stained and examined for *P. falciparum* parasites, according to the methods of WHO (31). Parasitaemia was also expressed as a percentage of

red blood cells infected, in accordance to the assertion of Moody (32), who stated that a 1% parasitaemia represented 50,000 parasites/ μl of blood. For the mice that survived during the course of treatment, venous blood samples were collected to prepare thick and thin films that were subsequently stained and examined for *P. falciparum* parasites. However, for the infected mice that survived after the four days chemotherapeutic protocols, some were sacrificed and their hearts were removed to obtain cardiac blood materials from their inner muscles, which were also hematologically stained and examined for *P. falciparum* parasites according to the methods of WHO (31) and the assertion of Moody (32). Net mean chemosuppression due to drug administration was calculated according to the formulation of Bassey et al. (33) with slight modifications (26).

Data analysis

Mean values were expressed as mean and standard error of the mean. Chi-square test were used to determine the level of significance, and *P*-value less than 0.05 ($P < 0.05$) were considered significant. The software

SPSS version 16 was employed for the data analysis.

Results

Parasite density obtained from five clinical inocula, their 92 hours synchronized in vitro cultures, as well as their growth factors (degree of parasitemia) are recorded in Table 1. As recorded in Table 1, varying parasite densities were obtained for the five different *P. falciparum*-infected human blood samples (A – E) used as inocula for the in vitro cultures. Parasite densities obtained from the in vitro cultures were markedly different from the values of their inocula after 92 hours of incubating synchronized cultures of the blood samples. Sample A had the highest growth factor (82-fold increase), while the lowest growth factor (62-fold increase) was recorded in sample C. All the synchronized in vitro subcultures were, however, diluted using different dilution factors so as to obtain in vitro cultures with the same parasite biomass (8%), from which equivalent volume (0.5ml) were used as inocula for in vivo infection of humanized mice.

Table 1: Parasite density obtained from in vitro culture of *Plasmodium falciparum*-infected human blood

Human blood samples	Parasite counts						
	Inocula		92 hours Synchronized cultures		Diluted 92 hours Synchronized cultures		Growth factors
	($\times 10^3/\mu\text{l}$)	(%)	($\times 10^3/\mu\text{l}$)	(%)	($\times 10^3/\mu\text{l}$)	(%)	
A	20.22	0.40	1,640	32.8	400	8	82
B	18.15	0.36	1,368	27.36	400	8	76
C	24.36	0.49	1,519	30.38	400	8	62
D	26.73	0.54	1,809	36.18	400	8	67
E	21.58	0.43	1,527	30.53	400	8	71

($\times 10^3/\mu\text{l}$) = $\times 1000$ cells/microliter.

Table 2 shows hematocrit obtained from the peripheral blood of *P. falciparum*-infected humanized mice that survived the infection phase. In the control group containing humanized mice that were not infected with *P. falciparum* (Table 2), mean hematocrit recorded

ranged between $40.20 \pm 0.74\%$ and $40.8 \pm 0.86\%$; while in the test group containing humanized mice that were infected with *P. falciparum*, it ranged between $31.58 \pm 0.60\%$ and $40.48 \pm 0.34\%$. When the values of hematocrit obtained within the control group were compared, no sig-

nificant difference ($P=0.996$) was observed. There was however, a significant difference ($P< 0.001$) in the values of mean hematocrit obtained within the test group when they were compared. When the values of mean hematocrit obtained between the control group and test group were compared, a significant difference ($P= 0.002$) was also observed. During the infection phase, the values of mean hematocrit obtained in the control group significantly increased, while the values of mean hematocrit in the test group were significantly decreased. A total death of 10 infected mice was record-

ed in the test group. The first death was recorded on day 3 of infection. Hematological examination of the dead mice revealed a *P. falciparum* parasite density of $2.54\pm 0.00\%$ in the inner cardiac muscles of the dead mice. Another three deaths were recorded on day 4 of infection with an overall mean *P. falciparum* parasite density estimated at $2.73\pm 0.04\%$. Three deaths were each recorded on day 5 and 6 of infection respectively with overall mean *P. falciparum* parasite density estimated at $2.85\pm 0.05\%$ and $2.80\pm 0.05\%$ respectively.

Table 2: Hematocrit obtained from the humanized mice during the infection phase

Days of infection	Humanized mice that survived infection and their mean haematocrit			
	Number of mice that survived infection		Mean hematocrit (%)	
	Control group	Test group	Control group	Test group
0	5	25	40.20 ± 0.74	40.48 ± 0.34
1	5	25	40.40 ± 0.93	39.08 ± 0.08
2	5	25	40.40 ± 0.93	35.64 ± 0.47
3	5	24	40.40 ± 0.75	34.56 ± 0.52
4	5	21	40.60 ± 0.75	33.72 ± 0.94
5	5	18	40.60 ± 0.68	32.47 ± 0.55
6	5	15	40.80 ± 0.86	31.58 ± 0.60

Figure 1 represents photomicrographs of the cardiac blood materials obtained from the inner cardiac muscles of a humanized mouse that died of malaria infection caused by *P. falciparum*. The photomicrographs revealed a morphological alteration in the blood picture of the infected mouse, as indicated by a significant proportion of lysed erythrocytes in the thin films, as well as a proliferation of lymphocytes, monocytes, and mature trophozoites of *P. falciparum* that were largely at the same stage of growth in both the thick and thin films. Photomicrographs of other infected mice that died had similar patterns with that shown in Fig. 1.

Table 3 represents parasite density and parasite chemosuppression obtained from the peripheral blood of *P. falciparum*-infected mice

treated with quinine and dimethyl sulfoxide (DMSO). In the group of mice treated with quinine, mean parasite counts were estimated at $1.06\pm 0.05\%$ and $0.16\pm 0.01\%$ at day 0 and 4 of therapy respectively; while in the group treated with DMSO, parasite counts were estimated at $1.09\pm 0.03\%$ and $1.13\pm 0.00\%$ at day 0 and 4 of therapy respectively. Unlike the DMSO therapy, where a total of 6 deaths were recorded, no death was recorded in the experimental group of *P. falciparum*-infected humanized mice treated with quinine. Quinine was well tolerated in the *P. falciparum*-infected humanized mouse model, and it exhibited a highly potent effect by significantly clearing ($P< 0.001$) the parasite density of the infected mice, as indicated by net mean chemosuppression of 83.09% at day 4 of therapy.

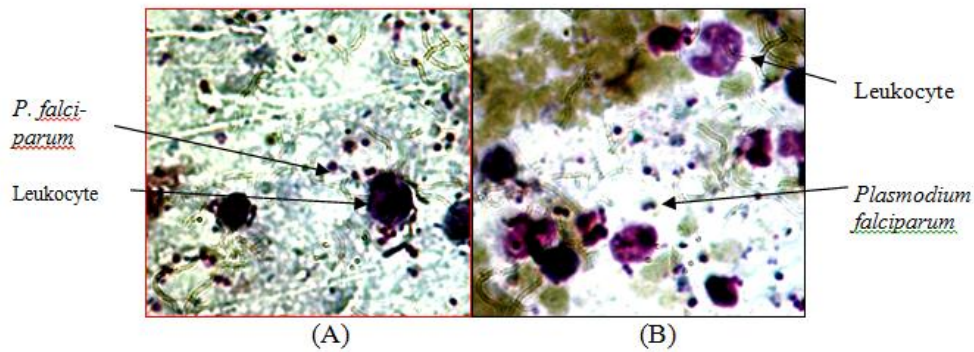


Fig. 1: Photomicrographs of the cardiac blood materials obtained from the inner cardiac muscles of a humanized mouse that died of malaria infection. (A) Thick film of cardiac blood extracts showing massive sequestration of mature *P. falciparum* and infiltration of leukocytes, predominantly lymphocytes and monocytes; (B) Thin film of cardiac blood extract revealing extensive lysis of erythrocytes, massive sequestration of mature *P. falciparum* and extensive infiltration of lymphocytes and monocytes. Giemsa, $\times 1000$ (A) and $\times 1000$ (B); original magnification. (Source: Photomicrographs were obtained from our study)

Table 3: Parasite density and parasite chemosuppression in the peripheral blood of *Plasmodium falciparum*-infected mice treated with quinine and dimethyl sulfoxide

Days of ther.	Infected mice treated with quinine (n = 7)				Infected mice treated with DMSO (n = 7)		
	NTS	($\times 10^3$ cells/ μ l)	(%)	chem. (%)	NTS	($\times 10^3$ cells/ μ l)	(%)
0	7	53.13 \pm 2.52	1.06 \pm 0.05	0.00	7	54.41 \pm 1.27	1.09 \pm 0.03
1	7	40.26 \pm 2.37	0.80 \pm 0.05	28.87	7	58.54 \pm 1.57	1.17 \pm 0.03
2	7	22.98 \pm 1.43	0.46 \pm 0.03	58.60	7	59.68 \pm 2.29	1.19 \pm 0.05
3	7	14.39 \pm 1.10	0.29 \pm 0.02	70.89	3	54.78 \pm 0.49	1.10 \pm 0.01
4	7	7.79 \pm 0.71	0.16 \pm 0.01	83.09	1	56.60 \pm 0.00	1.13 \pm 0.00

Note: Ther. represents therapy, NTS represents number that survived, n = number of mice in each experimental group, Chem. represents chemosuppression, DMSO represents dimethylsulfoxide, Mean parasite counts in the inner cardiac layers of infected mice treated with DMSO that died on day 3 and 4 of therapy were 2.98 \pm 0.05% and 2.99 \pm 0.11% respectively, Values of parasite counts are represented as mean \pm standard error of mean.

Table 4 shows hematocrit obtained from the peripheral blood of *P. falciparum*-infected humanized mice treated with quinine and dimethyl sulfoxide. Values of mean hematocrit recorded during the chemotherapy phase ranged between 29.71 \pm 0.29% and 35.1 \pm 0.51% for *P. falciparum*-infected mice treated with quinine; while it ranged between 25.00 \pm 0.00% and 29.86 \pm 0.34% for *P. falciparum*-infected mice treated with dimethyl sulfoxide. When the values of hematocrit obtained from *P. falciparum*-infected mice treated with quinine from day 0 to 4 of therapy were compared, a significant

difference ($P < 0.001$) was observed. There was also a significant difference ($P < 0.001$) in the values of hematocrit obtained from *P. falciparum*-infected mice treated with dimethyl sulfoxide from day 0 to 4 of infection when they were compared. When the values of mean hematocrit obtained from the *P. falciparum*-infected mice treated with quinine and those of *P. falciparum*-infected mice treated with dimethyl sulfoxide were compared, a significant difference ($P = 0.013$) was also observed. While the values of mean hematocrit obtained from day 0 to 4 of therapy signifi-

cantly increased in the *P. falciparum*-infected mice treated with quinine, the values of mean hematocrit obtained from day 0 to 4 of therapy significantly decreased in the *P. falciparum*-infected mice treated with dimethyl sulfoxide.

At the end of treatment of the infected mice with quinine and DMSO respectively, post-mortem evaluation of the parasitological responses of the mice to antimalarial therapy were also conducted, as shown in the photo-

micrographs displayed in Fig. 2. The photomicrograph of quinine-treated mice (Fig. 2A) showed an absence of knobs on the surface of their erythrocytes with a relatively low parasitemia, unlike the photomicrograph of DMSO-treated mice (Fig. 2B) that showed a proliferation of knobs produced by the sequestered *P. falciparum* over the surface of their host erythrocytes, with a relatively high level of parasitemia.

Table 4: Hematocrit obtained from the *Plasmodium falciparum*-infected mice treated with quinine and dimethyl sulfoxide

Days of infection	Mean haematocrit (%)	
	Infected mice treated with quinine	Infected mice treated with DMSO
0	29.71±0.29	29.86±0.34
1	30.00±0.31	29.29±0.29
2	31.29±0.52	27.00±0.31
3	32.72±0.52	25.83±0.31
4	35.14±0.51	25.00±0.00

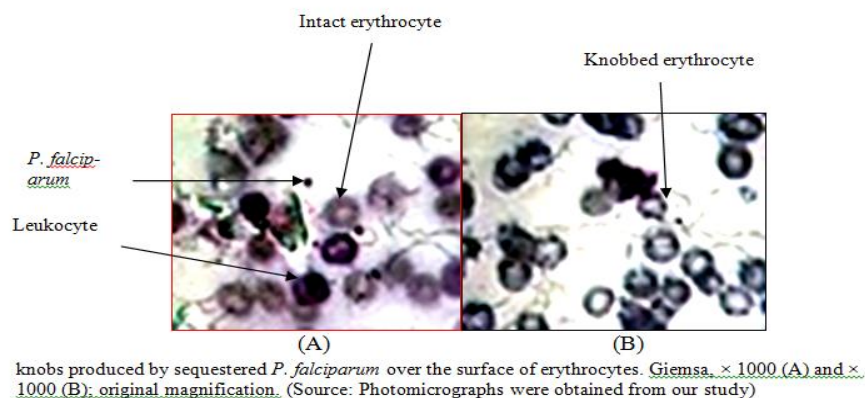


Fig. 2: Photomicrographs of the cardiac blood materials obtained from the inner cardiac muscles of humanized mice that were sacrificed at the end of treatment with quinine and dimethyl sulfoxide. (A) Thin film of cardiac blood extract revealing monocytes and intact erythrocytes with absence of knobs as well as a significantly low *P. falciparum* biomass. (B) Thin film of cardiac blood extracts showing a proliferation of knobs produced by sequestered *P. falciparum* over the surface of erythrocytes. Giemsa, × 1000 (A) and × 1000 (B); original magnification. (Source: Photomicrographs were obtained from our study)

Discussion

Our study substantiates the findings obtained from recent case studies in human patients which revealed that myocardial functions may not be well preserved in severe malaria disease, contrary to previous assertions

(1,7) which indicated that myocardial functions was well preserved even in severe malaria disease. According to previous studies (34-36), myocardial damage may be caused by a plethora of factors that may probably be the result of mechanical (microcirculatory obstruction), metabolic (systemic acidosis and

related tissue hypo-oxygenation), or humoral mechanisms. Besides, cardiotoxicity arising from antimalarial chemotherapy may also rarely induce myocyte apoptosis which could cause damage to the myocardium (37).

To substantiate that myocardial dysfunction may be the primary cause of death due to severe *P. falciparum* infection, our study infected humanized mice (employed as human surrogates) by inoculating *P. falciparum*-infected human blood which had been cultured in vitro (Table 1) into their peritonea, and subsequently employed hematological procedures to examine the inner muscles of the hearts of *P. falciparum*-infected humanized mice that died, for the presence of the infectious cause of death.

All the 25 humanized mice inoculated with the in vitro-cultured *P. falciparum* at a density of 8% revealed the presence of *P. falciparum* parasitemia in their peripheral blood, thus, significantly agreeing with the findings of previous studies (26,38). The presence of peripheral parasitaemia in the humanized mice indicates that some of the parasites may have successfully circumvented the innate defenses mounted by peritoneal phagocytes (39-41). Parasite's survival, in spite of the enormous innate defenses exerted in the peritonea of hosts, may be due to a significant attenuation of inflammatory responses, as a result of repeated intraperitoneal injection of the immunosuppressive drugs (41-43). The high receptivity of the human erythrocyte component of the blood (43,44) that were repeatedly injected into the peritonea of the immunosuppressed mice, which provided a suitable habitat and nutrient for the parasites, may have also accounted for the parasite's survival.

Death of the *P. falciparum*-infected humanized mice was first recorded on day 3 of the infection phase of the experiment. Unlike the parasite density recorded in the peripheral blood before the death of the infected mice, a relatively high parasite biomass (Table 2) were recorded in the blood extracts obtained from the inner muscles of the heart in the dead

mice. This significant difference in parasitemia may explain the massive sequestration of *P. falciparum* from the peripheral veins into deep seated capillaries of organs as a result of severe malaria infection (38,45,46). As was similarly recorded in *P. falciparum*-infected humans that died of acute lymphocytic myocarditis caused by cytokine-mediated low vascular resistance triggered by parasite-derived pyrogens (22,23), excessive level of proinflammatory cytokines arising from the high parasite burden in the infected humanized mice that died may have also resulted in a progressive immune pathology that lead to complication, such as myocarditis, which was characterized by extensive sequestration of matured *P. falciparum* parasites into the deep seated capillaries of the myocardial vessels, massive infiltration of inflammatory cells, such as lymphocytes and monocytes, as well as extensive lysis of erythrocytes (Fig. 1). The uninhibited cytoadherence and resultant sequestration of parasitized and non-parasitized erythrocytes into the deep seated microvasculature may have blocked blood flow, thus, limiting the local oxygen supply, which may have hampered mitochondrial ATP synthesis, ultimately resulting in death of the infected mice (36,47,48). As was also observed in *P. falciparum*-infected humans, the significant decline in the hematocrit of the humanized mice infected with *P. falciparum* (Table 2) may have complementarily accounted for the death of the infected mice. Anemia, which results in the destruction of non-infected human erythrocytes mediated by free-oxygen radicals that damaged the membrane of the human erythrocytes, may have been caused by high parasite burden in the infected mice which induced increased inflammation in the peripheral blood (49).

Our study also demonstrated the usefulness of quinine (a standard antimalarial drug) as a potent therapy for severe malaria caused by *P. falciparum*. In the *P. falciparum*-infected humanized mice treated with quinine, there was significant apoptosis of *P. falciparum* parasites in the blood of peripheral and deep-seated capil-

laries when compared to DMSO therapy, as indicated by a net mean chemosuppression of 83.09 % (Table 3). The programmed death of *P. falciparum* parasites may have been induced by a variety of agents including drugs (50), exposures to bilirubin resulting in reactive-oxygen species increase (51), and binding of platelets to infected erythrocytes (52). Indeed, all the seven infected mice treated with quinine survived the *P. falciparum* infection, unlike in the DMSO therapy where only one infected mouse survived at day 4 of therapy. The results obtained from treatment with quinine in our study further confirmed that cardiotoxicity due to quinine administrations will rarely occur, since quinine is well tolerated when administered at the appropriate therapeutic dose. The significant increase in the hematocrit of quinine-treated mice (Table 4) also demonstrated the efficacy of quinine therapy against severe *P. falciparum* infection. To further demonstrate the chemotherapeutic efficacy of quinine against *P. falciparum* infection, blood materials extracted from the inner muscles of the heart of infected mice treated with quinine and DMSO were examined in our study for the presence of sequestered parasites. Unlike in the quinine-treated mice that had an absence of knobs on the surface of erythrocytes, with a relatively low parasitemia (Fig. 2A); the mice treated with DMSO showed a proliferation of knobs produced by sequestered *P. falciparum* over the surface of the host erythrocytes (Fig. 2B), indicating that parasites may have reached the late trophozoite stage as a result of ineffective antimalarial chemotherapy. As indicated above in this present study, all the results obtained showed significant correlation to those recorded in humans, thus, alluding to the potential immense value of our new humanized mouse model as a valuable research tool for studying other human diseases. Though not extensively demonstrated in our study, and thus, open to further studies, the successful induction of severe malaria disease in our new mouse model which resulted in the proliferation of mature *P. falciparum* par-

asites and activation of cells responsible for adaptive immunity, could provide valuable proteins that could be extracted, purified, and employed as vaccine candidates against *P. falciparum* infection.

Conclusion

Data obtained from our study experimentally demonstrated that myocardial complications due to severe malaria may indeed be the dominant cause of death recorded in *P. falciparum*-infected hosts, thus, significantly corroborating the findings of myocardial dysfunction as the primary cause of death in recent case reports of humans infected with *P. falciparum*. We therefore, support the recommendation that there is a need to reevaluate the current perspectives on the pathophysiology of myocardial dysfunction in the course of severe malaria disease.

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