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Original Article

Modulation of 8-Oxoguanine DNA Glycosylase 1 (OGG1) Alleviated Anemia Severity and Excessive Cytokines Release during *Plasmodium berghei* Malaria in Mice

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Received 19 Jun 2024
Accepted 14 Aug 2024

Keywords:
Malaria;
Plasmodium berghei;
Anaemia;
Cytokines

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Abstract

Background: The interplay of OGG1, 8-Oxoguanine, and oxidative stress triggers the exaggerated release of cytokines during malaria, which worsens the outcome of the disease. We aimed to investigate the involvement of OGG1 in malaria and assess the effect of modulating its activity on the cytokine environment and anemia during *P. berghei* malaria in mice.

Methods: *Plasmodium berghei ANKA* infection in ICR mice was used as a malaria model. OGG1 concentration and oxidative stress levels in *P. berghei*-infected mice and their control counterparts were assessed during malaria using enzyme-linked immunosorbent assay. OGG1 activity in malaria mice was modulated using treatment with TH5487 and O8-OGG1 inhibitors. The effects of modulating OGG1 activity using OGG1 inhibitors on cytokine release and anemia during *P. berghei* malaria infection were assessed by cytometric bead array and measurement of total normal red blood cell count respectively.

Results: The plasma OGG1 level was significantly upregulated and positively correlated with parasitemia during *P. berghei* malaria in mice. Modulation of OGG1 ameliorated malaria severity by improving the total normal RBC count in TH5487 and O8-treated mice. Modulation of OGG1 with TH5487 caused significant reductions in serum levels of TNF-α, IFN-γ, IL-6, and IL-10. Similarly, OGG1 modulation activity using an O8-OGG1 inhibitor caused a significant reduction in serum levels of TNF-α, IL-2, IL-6, and IL-10.

Conclusion: The findings indicate the involvement of OGG1 in the *P. berghei* malaria infection. OGG1 inhibition by TH5487 and O8-OGG1 inhibitors suppressed excessive cytokine release, and this may represent a novel therapeutic strategy for ameliorating the severity of malaria infection.

Introduction

Malaria is an important parasitic disease with a significant impact on global health due to its high morbidity and mortality (1). The host's immune system interacts with *Plasmodium* species during malaria to influence several responses (2). Severe malaria infection has a poor prognosis due to prolonged immune system hyperactivation caused by parasite invasion and multiplication (3). Malaria is associated with dysregulated cytokine production (4).The equilibrium between the pro-inflammatory and anti-inflammatory responses is maintained by cytokines such as transforming growth factor-β (TGF-β) and IL-10. The interruption of this equilibrium can lead to increased malaria severity and mortality (5). Elevated free radical levels caused by the destruction of hemoglobin in *Plasmodium-infected* RBC damage not only the membrane of the infected RBC but also the membrane of the uninfected RBC, which can lead to malaria anemia (6,7). Severe anemia in young children and pregnant women is associated with high mortality (6,8).

Oxidative stress is an important part of malaria pathophysiology (9). Oxidative stress can lead to oxidative DNA damage during malaria. Guanine is the most vulnerable nucleobase of nucleic acid DNA to oxidation, giving rise to the production of 8-oxo-7,8- dihydroxyguanine (8-OxoG), the most common DNA adduct caused by oxidative stress (10). The OGG1 is a base excision repair enzyme responsible for removing the oxidized base through base excision repair (BER) pathway (11). Previous studies suggested that OGG1-initiated DNA BER resulted in the formation of OGG1/8-OxoG complex which activated small GTPases. These small GTPases in turn generate cell activation signals leading to the increased expression of pro-inflammatory cytokines causing severe inflammatory response (12,13). Downregulation of inflammatory response was observed in the absence of functional OGG1 in several previous studies, which suggests that OGG1/8-OxoG activities can affect disease pathology and modulation of their activity could be a novel therapeutic strategy in certain diseases particularly those associated with inflammation (14-16).

However, the role and involvement of OGG1 in malaria infection is yet to be studied. Hence, we aimed to determine the effect of modulating OGG1 activity on anemia and cytokine environment during *P. berghei* malaria in mice.

Materials and Methods

Research experimental animals and ethical note

Juvenile male ICR mice, aged 4-5 weeks and weighing between 17 and 20 g were purchased from a local supplier and housed at the animal house of the Faculty of Medicine and Health Sciences, University Putra Malaysia. The mice were allowed to acclimatise for 2 weeks prior to experiment. The mice were divided into four groups (n=8) as follows: Group I (Control mice), Group II (Malaria mice day 1), Group III (Malaria mice day 3) and Group IV (Malaria mice day 5).

The Institutional Animal Care and Use Committee at University Putra Malaysia revised and approved the protocols for this study with an ethical approval number (UPM/IACUC/AUP-R001/2022).

Animal infection and sample collection

P. berghei ANKA strain was obtained from the Anatomy laboratory of University Putra Malaysia. Mice were designated as *P. berghei*-infected groups and control group, based on previously reported methods (17). The mice in the *P. berghei*-infected groups received 200 µL of the diluted blood containing $1x10^7$ parasitized RBC via intraperitoneal injection, while the mice in the control group received 0.2 ml

of similarly diluted uninfected blood from a healthy donor mouse. Whole blood was collected from the mice through cardiac puncture on days 1, 3, and 5 after inoculation, under general anesthesia of ketamine (100 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injections. Plasma separation was carried out at 1000 x g for 15 minutes for the determination of plasma OGG1 levels on day1, day 3 and day 5 respectively.

Measurement of parasitemia

Thin blood films from tail venesection were prepared daily for each mouse to monitor parasitemia. The thin blood smears were examined using an Olympus CX31 light microscope with oil immersion at a total magnification of 1000x after being stained with Leishman stain. Specifically, the crenelation method was used to count five different microscopic fields on each slide, each field containing about 200 cells (18). Parasitemia was determined by counting the number of parasitized red blood cells, which have been infected by the parasites. Five fields, each containing approximately 200 cells, were counted. The parasitemia was then calculated as the percentage of parasitized red blood cells [i.e. Parasitemia (%) = (Parasitized red blood cells / Total red blood cells count) × 100] (17).

Determination of OGG1 level during malaria infection

The concentration of OGG1 was quantified using ELISA. The plasma samples were analyzed in duplicate using quantikine Mouse ogg1 ELISA kit (ABclonal® Technology, USA) according to the manufacturer's instructions. Plates were read using a micro plate reader (VersaMax Molecular Devices®, China) at a wavelength of 450 nm.

Drugs preparation

The TH5487 (catalogue no. 6749) and O8-OGG1 (catalogue no. 6236) OGG1 inhibitors were purchased from Tocris Bioscience, USA. They were reconstituted according to the manufacturer's instructions. The drugs were diluted with a vehicle prepared by mixing 90ml of sterile phosphate buffer solution (PBS), 5ml of 5% glycerol, and 5ml of 5% Tween 80 (19). The dose used for each of the TH5487 and O8-OGG1 inhibitor was 30 mg/kg by intraperitoneal injection (19). The prepared drug solutions were stored at -80℃ in freezer before use.

Modulation of OGG1 activity

OGG1 modulation was carried out from the 1st to the 4th day following *P. berghei* inoculation as shown in Table 1.

Table 1: Modulation of OGG1 activity during malaria infection

Group	*Description*
Group I (control)	Uninfected mice that received sterile vehicle (0.2 ml) daily by intraperitoneal injection
Group II (vehicle-treated)	Malaria-infected mice that received sterile vehicle (0.2 ml) daily by intraperitoneal injection
Group III (TH5487-treated)	malaria-infected mice that received TH5487 (30 mg/kg) daily by intraperitoneal injection
Group IV (O8-treated)	Malaria-infected mice that received an O8-OGG1 inhibitor (30 mg/kg) daily by intraperitoneal injection

Measurement of parasitemia and total red blood cell count were carried out daily until the fifth day. Cardiac puncture was performed for every mouse to withdraw about 400–500 µl of blood on day 5 from which serum sample was obtainable. A cytometric bead array procedure for the simultane-

ous detection of IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ and TNF-α was carried out on serum samples collected from *P. berghei* infected and control mice on the 5th day after infection and OGG1 modulation. A commercially available mouse Th1/Th2/Th17 cytometric bead array kit (BD Biosciences, San Jose, CA, USA) was used for the experiment. The staining procedure was carried out in accordance with the instruction manual (BD CBA Mouse Th1/Th2/Th17 cytokine kit, catalogue no. 560485). Sample acquisition was achieved using BD FAC Suite software application embedded in the machine (BD FACS Lyric ™ Flow Cytometer, San Jose). The final cytokines concentrations were determined using FlowJo™ software version 10, USA.

Statistical analysis

GraphPad Prism software (Prism 9, GraphPad Software, Inc., USA) was used for data analysis. Differences in the mean parasitemia and OGG1 between the malaria and control groups were compared using unpaired student's *t*-test. Similarly, the association between OGG1 and parasitemia was determined by Pearson correlation coefficient. Moreover, the differences in the mean total number of normal RBC and cytokine concentrations between the various treatment groups compared to malaria-untreated groups were determined using the one-way analysis of variance with a post hoc test using Tukey's honestly significant difference (HSD) test. A probability value of $P<0.05$ was considered statistically significant. Results were presented as mean ± SEM.

Results

Effect of P. berghei malaria infection on plasma OGG1 levels

The OGG1 levels in control and malaria mice following *P. berghei* inoculation during the early (day 1), mid (day 3), and late (day 5) phases of malaria infection were assessed. No significant difference in the mean OGG1 concentration between malarial (105.8 ± 30.49 pg/mL) and control mice (50.35 ± 12.26 pg/mL) on day 1. However, the means OGG1 concentration were observed to be significantly different between malarial (236.4 ± 40.1 pg/mL) and control mice (50.35 ± 12.26 pg/mL) on day 3 ($P<0.01$), as well as day 5 between the malarial (149.7 ± 34.59 pg/mL) and control (50.35 ± 12.26 pg/mL) mice ($P<0.05$) (Fig.1).

OGG1 was positively correlated with parasitaemia during P. berghei malaria in mice

A significant positive correlation between levels of parasitemia and OGG1 concentration was observed ($r = 0.7048$; $P<0.001$). Therefore, the increased in percentage parasitemia is associated with increasing levels of OGG1 during *P. berghei* malaria infection in mice (Fig. 2).

Effect of modulating OGG1 activity on Total normal RBC count

The mean percentage total normal RBC count differed significantly between the untreated *P. berghei*-infected mice receiving vehicle (39.7 ± 1.18%) and their counterpart receiving TH5487 treatment [57.2 ± 3.81%), *P<0.001*] as well as O8-treatment [(70.30 ± 3.62%), *P<0.001*]) (Fig. 3).

Total number of normal RBC was used as an index of anemia during *P. berghei* malaria in mice. The total percentage of normal red blood cell (RBC) count in control mice ranged from 97.4-98.6% and remained stable. However, a profound reduction in total RBC count was observed in malaria-untreated mice during the late phase of the infection, with about a 60% decrease in total RBC count. However, in TH5487-treated mice, about 43% reduction in total RBC count was recorded, while about 30% reduction in the total number of RBC was recorded in O8-treated mice (Fig. 3).

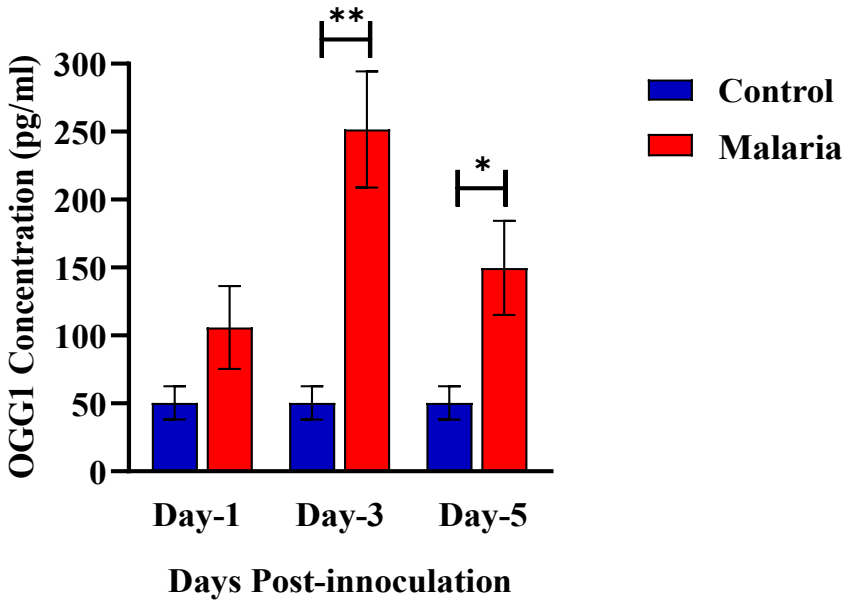

Fig. 1: OGG1 concentrations of *P. berghei* infected and control mice on day 1, day 3 and day 5 following *P. berghei* inoculation. Data were analyzed using Unpaired t-test (two-sided) at $P<0.05$. The results were presented as mean ± SEM with n= 8, *$P<0.05$, **$P<0.01$

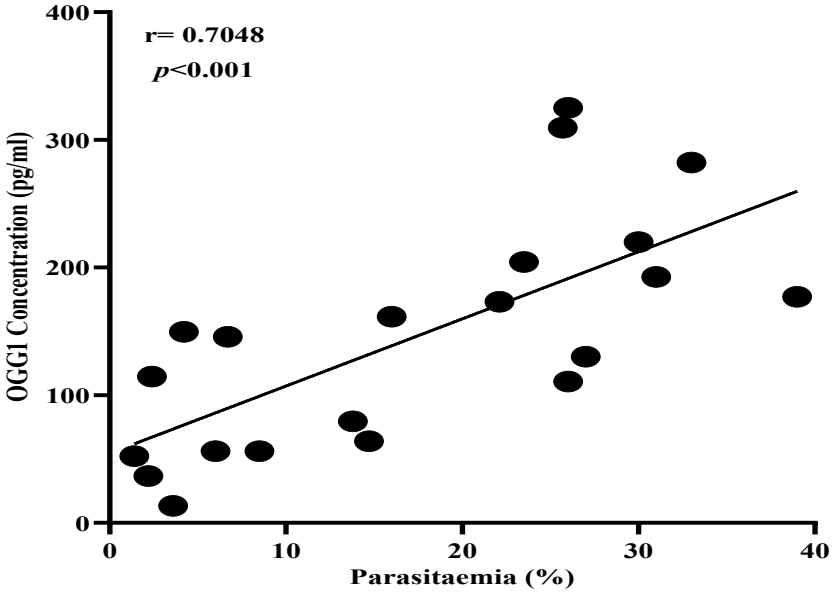

Fig. 2: Pearson correlation between the percentage parasitemia and OGG1 concentration. An increase in percentage parasitemia is associated with a significant increase in levels of OGG1 during *P. berghei* infection (r = 0.7048; ***$P<0.001$), n=8. The data represent pooled results from the experiments

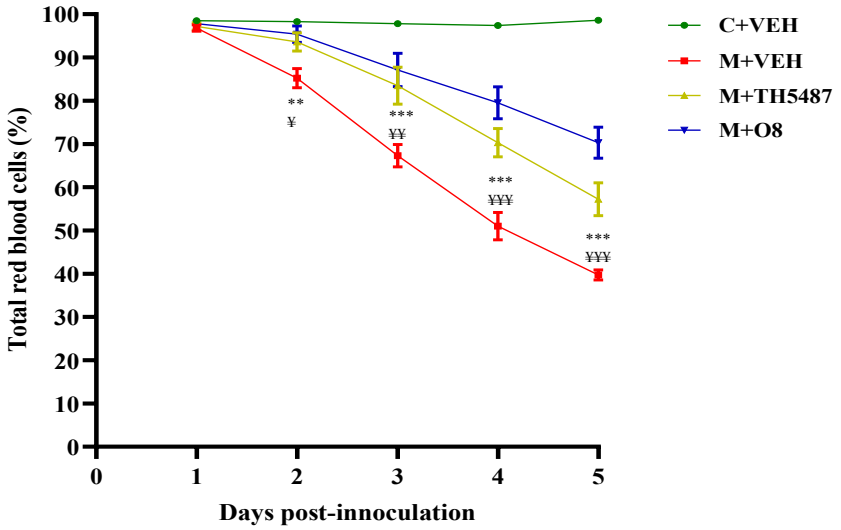

Fig. 3: Comparative mean percentage total RBC count curves for the control mice and *P. berghei*- infected mice that received vehicle (VEH), TH5487 and O8 treatments. Data were analyzed using Unpaired Student's *t*-test at $P<0.05$ between malaria untreated mice and each of the treatment groups. Results were presented as Mean ± SEM (n= 8). The (*) denotes a comparison between the vehicle (VEH) and O8-treated mice and (¥) denotes a comparison between the vehicle (VEH) and TH5487-treated mice. C + VEH= Control + Vehicle; M + VEH= Malaria + Vehicle; M +TH5487= Malaria + TH5487; M + O8= Malaria + O8

Effect of modulating OGG1 activity on cytokine release during P. berghei malaria in mice

There was a significant difference in the mean serum TNF- α concentrations between the untreated *P. berghei*-infected mice receiving vehicle (3235 ± 444 pg/ml) and their counterpart receiving TH5487 treatment [(1903 ± 424 pg/ml), $P< 0.05$] as well as O8-treatment [(1950 ± 171 pg/ml), $P< 0.05$] (Fig. 4). Similarly, the difference in the mean serum IFN-γ concentrations differed significantly between the untreated *P. berghei*-infected mice receiving vehicle (2508 ± 438 pg/ml) and their counterpart receiving TH5487 treatment [(1155 ± 182 pg/ml), $P< 0.05$]. However, no significant difference in serum IFN-γ concentration observed in O8-treated mice [(1876 ± 471 pg/ml), $P> 0.05$] (Fig. 5). Moreover, the mean serum IL-2 concentrations differed significantly between the *P. berghei* malaria-infected mice [(137 ± 10.8 pg/ml)] and O8-treated mice [(94.2 ± 5.18 pg/ml), $P< 0.05$]. Whereas, no significant difference in serum IL-2 concentrations between the *P. berghei* malaria-infected mice [(137 ± 10.8 pg/ml)] and TH5487-treated mice [(141 ± 8.67pg/ml), $P> 0.05$] (Fig. 6).

The mean serum IL-10 concentrations differed significantly between the untreated *P. berghei*-infected mice receiving vehicle (2293 ± 242 pg/ml) and their counterpart receiving TH5487 treatment [(1464 ± 160 pg/ml), $P< 0.01$] as well as O8-treatment [(1334 ± 103 pg/ml), $P< 0.01$] (Fig. 7). There were significant differences in the mean serum IL-6 concentrations between the untreated *P. berghei*-infected mice receiving vehicle (593 ± 122 pg/ml) and the mice receiving TH5487 treatment [(301 ± 23.3 pg/ml), $P< 0.05$] as well as O8-treatment [(224 ± 30.9 pg/ml), $P<0.01$] (Fig. 8). Even though there were some differences in the mean serum IL-4 concentrations (Fig. 9) and IL-17A concentrations (Fig.10) between all the untreated and treated *P. berghei* malaria-infected mice groups following OGG1modulation activity, these differences could not achieve significant levels.

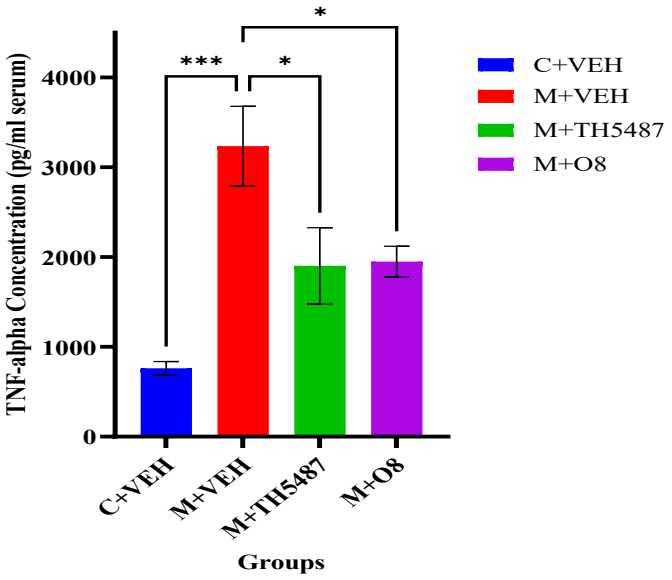

Fig. 4: Effect of modulating OGG1 activity on serum TNF-α concentration on day 5 post *P. berghei* inoculation. C + VEH= Control + Vehicle; M + VEH= Malaria + Vehicle; M +TH5487= Malaria + TH5487; M + O8= Malaria + O8. Data are mean ± SEM, (n=6), *P< 0.05, ***P< 0.001

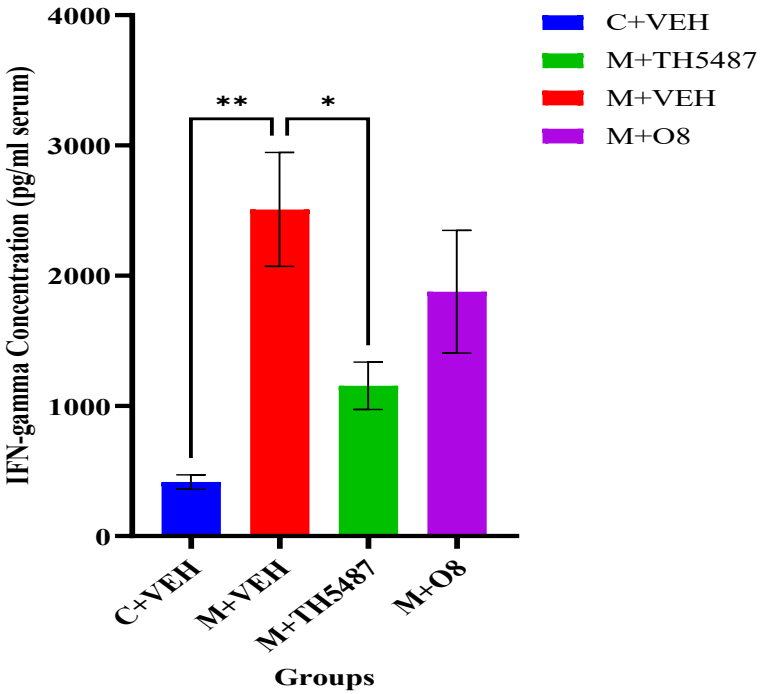

Fig. 5: Effect of modulating OGG1 activity on serum IFN-γ concentration on day 5 post *P. berghei* inoculation. C + VEH= Control + Vehicle; M + VEH= Malaria + Vehicle; M +TH5487= Malaria + TH5487; M + O8= Malaria + O8. Data are mean ± SEM, (n=6), *P< 0.05, **P< 0.01

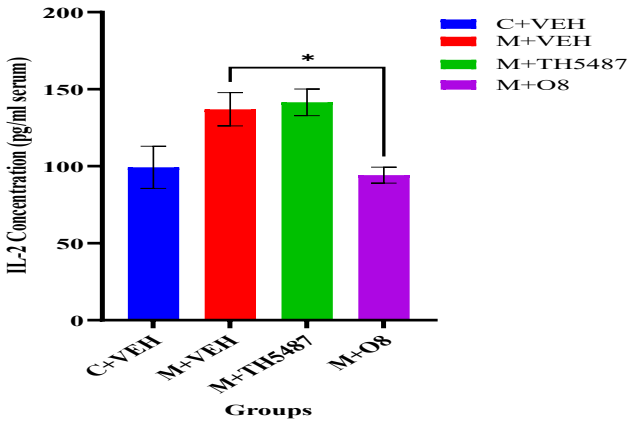

Fig. 6: Effect of modulating OGG1 activity on serum IL-2 concentration on day 5 post *P. berghei* inoculation. C + VEH= Control + Vehicle; M + VEH= Malaria + Vehicle; M +TH5487= Malaria + TH5487; M + O8= Malaria + O8. Data are mean ± SEM, (n=6), *P< 0.05

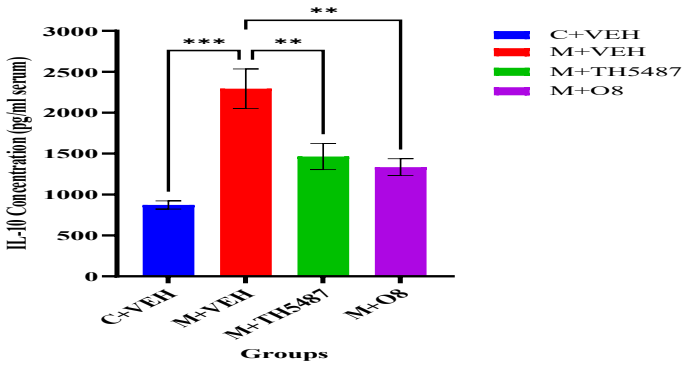

Fig. 7: Effect of modulating OGG1 activity on serum IL-10 concentration on day 5 post *P. berghei* inoculation. C + VEH= Control + Vehicle; M + VEH= Malaria + Vehicle; M +TH5487= Malaria + TH5487; M + O8= Malaria + O8. Data are mean ± SEM, (n=6), **P< 0.01, ***P< 0.001

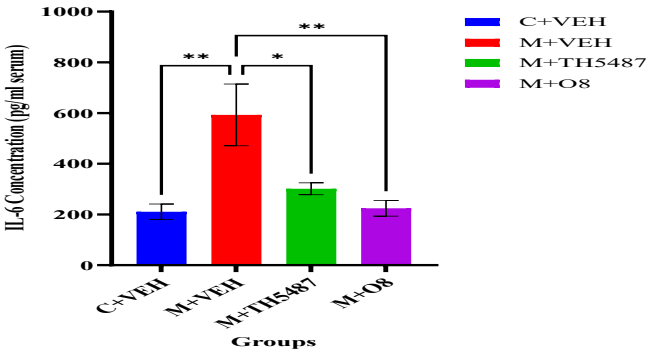

Fig. 8: Effect of modulating OGG1 activity on serum IL-6 concentration on day 5 post *P. berghei* inoculation. C + VEH= Control + Vehicle; M + VEH= Malaria + Vehicle; M +TH5487= Malaria + TH5487; M + O8= Malaria + O8. Data are mean ± SEM, (n=6), *P< 0.51, **P< 0.01

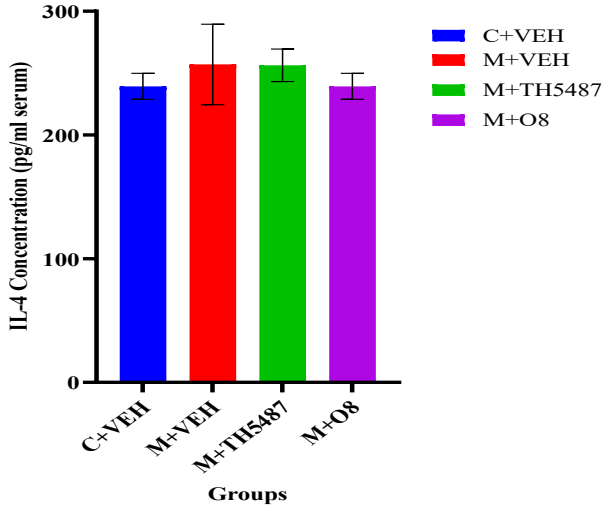

Fig. 9: Effect of modulating OGG1 activity on serum IL-4 concentration on day 5 post *P. berghei* inoculation. C + VEH= Control + Vehicle; M + VEH= Malaria + Vehicle; M +TH5487= Malaria + TH5487; M + O8= Malaria + O8. Data are mean ± SEM, (n=6)

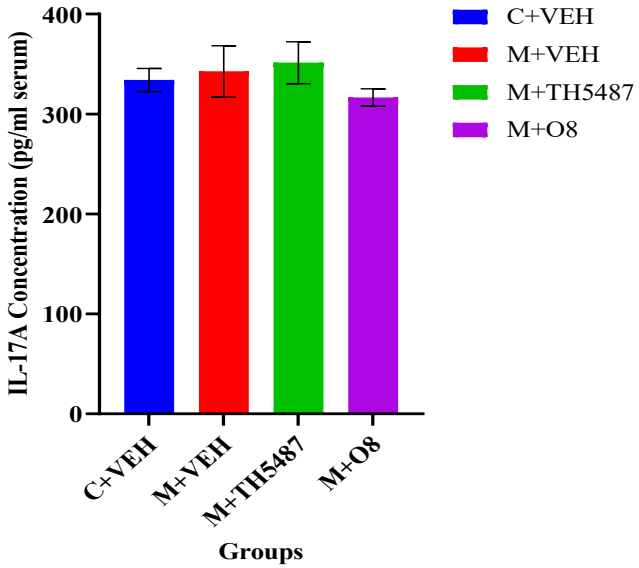

Fig. 10: Effect of modulating OGG1 activity on serum IL-17A concentration on day 5 post *P. berghei* inoculation. C + VEH= Control + Vehicle; M + VEH= Malaria + Vehicle; M +TH5487= Malaria + TH5487; M + O8= Malaria + O8. Data are mean ± SEM, (n=6)

Discussion

In this study, we utilized a *P. berghei* malaria infection model to explore the role of OGG1 and how its modulation can affect cytokine release and anemia during malaria infection. Our findings revealed that *P. berghei* malaria infection elevated OGG1 levels substantially. The elevated levels of OGG1 were observed especially during the mid and late phases of

the infection, suggesting a link between OGG1 activity and malaria infection. OGG1 concentration increases during malaria due to increased oxidative stress, a vital component of malarial pathogenesis (10). The increased in percentage parasitemia caused increased levels of OGG1. Hence, OGG1 significantly correlates with malaria infection. Although studies have not yet associated OGG1 with malaria at present, however, some studies revealed an association of OGG1 with other disease conditions. Huang and colleagues investigated the enzymatic activity of OGG1. They discovered that *K. pneumoniae* infection dramatically boosted OGG1levels and its activity, indicating a strong link between OGG1 and *K. pneumoniae* infection (20). The pathophysiology of acute pancreatitis has been linked to OGG1 activity in a mouse model. In acute pancreatitis, oxidative stress promotes DNA oxidation, accumulating 8-OxoG in the pancreas. Moreover, the 8-OxoG build-up in the DNA might promotes NF-ϰB binding to its complementary sequence in acute pancreatitis (21).

Modulation of OGG1 activity ameliorated malaria severity by improving the total normal RBC count in TH5487 and O8-treated mice. Total red blood cell count and parasitemia were used as indicators for determining severe malarial anemia in children under antimalaria therapy (22). Low total red blood cell count and increased parasitemia were used to monitor severe malarial anemia in children (23). The reduction in the number of RBCs cannot be fully explained by the rupture of the highly infected RBCs and its clearance by splenic macrophages alone (24,25,26,27). It is also believed that the severe anemia that occurs in the later stages of malaria is likely due to the pro-inflammatory cytokines' suppression of erythropoiesis (28,29). Cytokines such as interleukin 1 (IL 1 β) and tumor necrosis factor (TNF-α) are pyrogenic, and their release is cyclic during malaria paroxysms every 48 hours in *P. vivax* and *P. falciparum* infections. Fever can help the host's defence mechanism by delaying the growth of pathogenic microbes with precise temperature requirements (30,31). A rise in parasitemia, which in turn causes anemia is largely responsible for the negative clinical outcome associated with *falciparum* malaria. The high parasitemia and low hemoglobin levels necessitate immediate intervention to avoid severe anemia in malaria patients (32).

Decreased serum levels of TNF-α, IFN-γ, IL-10 and IL-6 were observed in *P. berghei* infected mice treated with TH5487. Similarly, significant decrease in serum levels of TNF-α, IL-2, IL-10 and IL-6 was observed in *P. berghei* infected mice treated with O8 OGG1 inhibitor. The suppression of OGG1 activity using TH5487 and O8 OGG1 inhibitors to downregulate the immunoinflammatory response during malaria infection is perhaps due to the interference with the enzyme's glycosylase activity and inhibition of the OGG1/8-OxoG binding and NF-ϰB activation pathway. Despite the unavailability of reported research regarding OGG1 with malaria, however, studies involving other disease conditions demonstrated that suppression of OGG1 activity using TH5487 caused decreased levels of IL-10, IL-6 and TNF-α in acute pancreatitis (33). Similarly, TH5487 reduced levels of TNF-α and IL-6 during lung inflammation (13). Previous study has shown that the binding of OGG1 to its substrate (8-OxoG) was inhibited by TH5487 leading to decreased levels of TNF-α and IL-6 in the mouse lung. Even though, O8 inhibited OGG1 activity however, the binding of OGG1 to its substrate was not sufficiently prevented by the O8 and therefore could not significantly decrease inflammation like TH5487 (21). Another study reported that O8 OGG1inhibitor caused a modest decrease in the binding of OGG1 to its substrate (34). The insignificant decrease in serum IL-4 in this study may be attributable to the study's focus on primary infection with *P. berghei* because IL-4 is a Th2 cytokine that has been linked to malarial immunity against reinfection

(35). The non-significant decrease in serum IL-4 and IL-17A may also be attributed to insufficient effects of the OGG1 inhibitors to decrease their levels significantly during malaria infection.

Conclusion

The concentration of OGG1 in *P. berghei* malaria-infected mice was significantly elevated and positively correlated with increasing levels of parasitemia, indicating the involvement of OGG1 in malaria pathophysiology. Moreover, modulating OGG1 activity using TH5487 and O8-OGG1 inhibitors alleviated severe anemia and excessive cytokines release during *P. berghei* malaria in mice. These findings suggest that therapeutic strategies targeting OGG1 activity as an adjuvant to existing antimalaria drugs may confer benefits in reducing the severity of malaria infection.

Acknowledgements

The Fundamental Research Grant Scheme supported this work (FRGS), funded by the Malaysia Ministry of Education (Grant no: FRGS/1/2021/SKK0/UPM/02/1).

Conflict of interest

Authors declare no conflicts of interest.

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