EFFECT OF PLASMODIUM BERGHEI PARASITAEMIA ON LABORATORY INDUCED GASTRIC ULCER IN MICE

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Abstract

Background: Malaria is an important infection in Africa affecting all age groups although its severity depends on the relative immunity of the individual. Malaria has been found to have effects on the gastric mucosa, which include congestion with capillary stasis, necrosis, ulceration and haemorrhage. This study aimed at the effect of malaria parasitaemia on laboratory induced gastric ulcer in mice.

Methods: Thirty-six mice divided into 6 groups thus: Group 1. no malaria parasite and ulcer (control); Group 2. Parasite without ulcer; Group 3. Parasite and ulcer; Group 4. Ulcer without parasite; Group 5. Parasite, ulcer and chloroquine treatment; and Grop 6. Parasite, ulcer and Artemether treatment. These were used for the study. The mice were parasitized with 20% innoculum of *Plasmodium berghei* and ulcer induced with 70% alcohol and pylorus ligation. Parameters such as degree of parasitization, ulcer diameter, gastric acidity and packed cell volume (PCV) were measured in the various groups and comparison made where necessary. The experimental part of the study took 18 days.

Results: The severity of ulceration in the group with ulcer and parasite (11 ± 1.72 mm); and group with ulcer but no parasite (3.62 ± 1.6mm) was compared and was significant (p = 0.002). The groups with parasite showed gradual reduction in the packed cell volume (PCV) as the parasite load increased while the group without parasite showed increase in PCV over the time of study. The ulcer diameters in the groups treated with chloroquine (4.83 ± 2.48mm) and Artemether (5.00 ± 0.89) was not statistically significant (p = 0.889).

The restoration of PCV was better for the treated group than the untreated group. The pH of the gastric content in the untreated group was lower than the treated groups. The parasite clearance by chloroquine and artemether was significant (p = 0.04) compared to the untreated groups.

Conclusion: Malaria parasitaemia has a significant influence on gastric ulceration, PCV and to some extent gastric secretion. Chloroquine and artemether are sensitive drugs to *Plasmodium berghei.*

Keywords: malaria, parasitaemia, gastric ulcer, chloroquine, artemether.

Introduction

The Greek physician Hippocrates, who lived in the 5th century BC, was the first to describe the characteristic symptoms of a malaria infection as intermittent often relapsing fever accompanied by drenching sweats and followed by shaking chills. Centuries later, the medical almanac of 1888 referred to malaria as one of the great scourges of humanity, wreaking havoc in human civilizations throughout history and claiming more lives than any other infectious disease[1,2].

Malaria is still a major health problem, causing an estimated 300 million cases of illness and killing 1-2 million people mostly infants every year. About 2.2 billion people (41% of the world's population) are now in danger of contracting malaria. It has a high mortality and morbidity especially in children, the non-immune and pregnant women[3,4].

Peptic ulcer is found in about 10% of the population and main causes include *Helicobacter pylori* and non-steroidal anti-inflammatory drugs[5]. Malaria has also been found to have both direct and indirect effects on the gastric mucosa in humans presenting with gastrointestinal symptoms especially in children termed "choleric malaria"[6].

In view of the increasing peptic ulcer disease in our population and the pandemicity of malaria infection, the present study aims at studying the effect of malaria parasitaemia on gastric mucosa, gastric acidity and gastric ulcer; and the effects of treatment on these parameters.

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Methodology

Thirty six mice of 6 groups (average weight 20-25 grams) from the animal house of the College of Medicine, University of Ibadan were used for the study. The mice fed on normal chow meal *ad libitum*.

Each group was kept in a separate cage with food and water and under optimal conditions of temperature 22-25^{0}C and relative humidity of 40-70% with 12 hour day time/night time cycle.

The animals were parasitized with *Plasmodium berghei* collected from the laboratory of Dr. O.G Ademowo of the Institute of Medical Research and Training (IMRAT), College of Medicine, University College Hospital, Ibadan.

The mice were divided into 6 groups:

Group 1 – No malaria parasite, no ulcer (control)
Group 2 – Malaria parasite no ulcer
Group 3 – Malaria parasite and ulcer
Group 4 – No malaria parasite, but ulcer
Group 5– Malaria parasite, ulcer and chloroquine treatment
Group 6 – Malaria parasite, ulcer and Artemether treatment

The mice were parasitized with 20% innoculum of Plasmodium berghei using the intraperitoneal route, ulcer induced with 70% alcohol and pylorus ligation (Bolarinwa et al)[5] and pH measured using a standard pH meter and blood taken for blood film examination from the tail of the mice. The degree of parasitaemia was checked at day 3, day 5 and day 7 along with the PCV. The percentage parasitaemia was estimated from thin blood film. The blood films were made by staining with Leishman stain using standard protocols. The slides were examined on x100 (oil immersion) microscope for percentage parasitaemia.

For the untreated group, ulcer was induced on day 7 with 70% alcohol and pylorus ligation after 24 hours fast[7]. The gastric acidity (pH) and ulcer indices were assessed after 4 hours of pylorus ligation.

For the treated group, the drugs were administered orally using the microintubator. Chloroquine at a dose of 10mg/kg for 2 days; and 5mg/kg for the 3rd day was commenced on day 8. The gastric acidity (pH) and ulcer indices were assessed on day 11 (24 hours after completion of therapy). Also, artemether at a dose of 3.2mg/kg first day; and 1.6mg/kg for the next 4 days was commenced on day 8.The gastric acidity (pH) and ulcer indices were assessed on day 13 (24 hours after completion of therapy). Both were used following standard dose regimen of 3 and 5 days for chloroquine and artemether respectively. The bench-work was concluded after 18 days.

All the mice were fasted for 24 hours before the gastric acidity (pH) and ulcer indices were estimated. The pH of gastric content was measured with an automatic pH meter by insertion into the stomach and the gastric acidity estimated using pH = -log (H^+).

The stomach was harvested and ulcer diameter measured using a tape measure. Gastric samples were kept in formalin for histology. These specimens were examined and the ulcers were scored by measuring the diameter of the ulcers and other morphologic changes were noted. The tissues were put into cassettes and were subsequently transferred to the tissue processor overnight. The processed tissues were embedded in paraffin wax and then cut into 5 microns sections by microtome machine. The sections were mounted on the slides and stained with haematoxylin and eosin and covered by cover-slips; and the slides were reported under the microscope. Physical observations were made over the time of study. Following treatment, the activity of the animal and their feeding rate were better. The data were analysed using SPSS version 16. Continuous variables were compared using the Student t test, while discontinuous variables were compared using the $\chi 2$ test. The level of significance was set at $p \leq 0.05$. Findings were presented in tables and figures.

Results

Six groups of 6 mice each were studied totaling 36 mice. The weights ranged from 20 – 25gm with mean weight 21.5gm.

Parasite Changes With Packed Cell Volume

The animals were inoculated with 20% Plasmodium berghei. There was an initial fall in the percentage parasitaemia and subsequent gradual increase over time. With intervention (drugs) there was a decline in the parasitaemia as seen in table 1.

The packed cell volume in the control group increased gradually over the time of study. The groups with parasite showed a decline in PCV with increasing parasitaemia.

pH and Gastric Acidity

Reduction in pH and increase gastric acidity were seen in parasitized mice. An improvement in these parameters occurred with treatment especially the artemether group 6. (Table 2)

Ulcer Study

Within the 4 hours of ulcer induction with pylorus ligation and 0.1ml of 70% ethanol, the parasitized mice showed significant gastric mucosa ulceration. Also, the animals without ulcers but parasites showed congestion of the gastric mucosa. ($P < 0.05$)

Therapy

With treatment, there was a significant reduction in percentage parasitaemia ($p<0.05$). The pH and gastric acidity showed improvement. The gastric ulceration in the treatment groups were less than the untreated groups. The parasite clearance by chloroquine and artemether was significant ($p < 0.05$). But artemether has a slightly higher clearance although not statistically significant.

Table 1: Average PCV changes with increasing parasitaemia over 7 days

	Pcv %				**Parasitaemia %**		
	Day 0	Day 3	Day 5	Day 7	Day 3	Day 5	Day 7
(Control) Group 1	56	57	57	58			
Group 2	57± 1.75	52±4.59	50±2.28	35±1.94	17.8±2.99	30.7±3.01	40±1.79
Group 3	52±6.47	53±8.73	49±5.90	44.6±6.28	15.2±1.47	31.5±3.012	39.7±2.73
Group 5	56.8±7.81	51.8±5.46	40.5±6.19	41.2±16.98	18±2.45	32.8±6.62	39.6±16.25
Group 6	57±6.19	55.8±3.49	51.7±4.80	47.3±7.00	15.3±2.14	27.7±2.07	36.8±3.92

Table 2: Average pH and acidity of gastric content

	pH	**Acidity mol/L**	**ulcer**
Group 1	5.6±0.31	$2.8x10^{-6}$ ±1.78	-
Group 2	5.3±0.33	$5.0x10^{-6}$ ±4.29	-
Group 3	5.5±0.54	$3.2x10^{-6}$ ±12.28	11±1.79mm
Group 4	6.3±0.15	$5.2x10^{-7}$ ±1.75	3.7±1.633mm
Group 5	5.0±2.06	$1.0x10^{-5}$ ±7.44	4.8±2.483mm
Group 6	6.4±2.90	$4x10^{-7}$ ±12.62	5±0.894mm

Table 3: Differences in the gastric acidity and PCV between the antimalarial treated and untreated groups

	pH	**Acidity (mol/L)**	**PCV (%)**	**Ulcer index**	**Parasitaemia %**
Group 3	5.5±0.54	$3.2x10^{-6}$ ±12.28	44.6±6.28	11±1.79mm	39.7±2.73
Group 5	5.0±2.06	$1.0x10^{-5}$±7.44	47.6±16.98	4.8±2.48mm	21.4±9.13
Group 6	6.4±2.90	$4x10^{-7}$ ±12.62	53.2±7.01	5±0.89mm	16.2±2.90

Table 4. Difference in the average degree of parasitaemia, PCV, gastric acidity and ulcer indices in chloroquine (CQ) and artemether (ART) treated mice.

	Group 5 (CQ)	**Group 6 (ART)**
PCV (%) Pretreatment	41.2±16.98	47.3±7.01
Post Treatment	47.6±19.56	59.2±4.57
Parasitaemia Pretreatment	39.6±16.98	36.8±7.01
Post treatment	21.4±19.56	16.2±4.57
pH	5.0±2.05	6.4±0.23
Acidity (mol/L)	$1.0±10^{-5}$ ±7.42	$4x10^{-7}$ ±2.62
Ulcer index (mm)	4.8±2.4	5±0.89

HISTOLOGY REPORT

The biopsies were processed at the Department of Pathology, University College Hospital, lbadan, Nigeria. Haematoxylin and Eosin stains were used.
Sections of unparasitized mice show normal gastric mucosa glands lined by columnar epithelium with other layers appearing normal. There is congestion of the mucosa in the mice with parasite but no ulcer. (Figure 1)

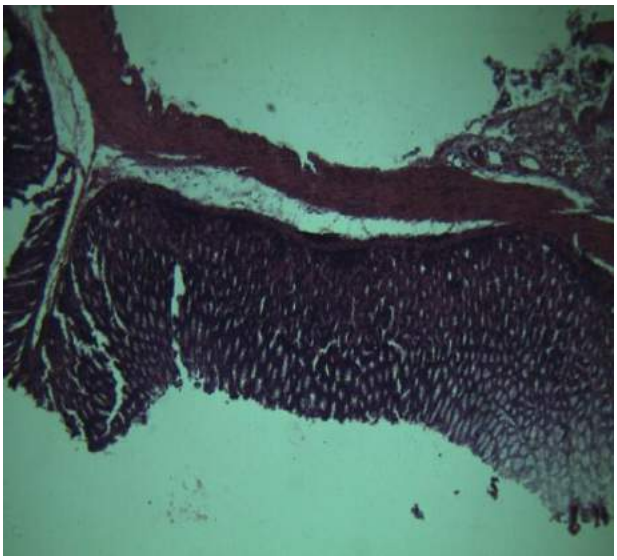

Fig 1. Normal gastric mucosal biopsy with mild congestion (Groups 1 and 2)

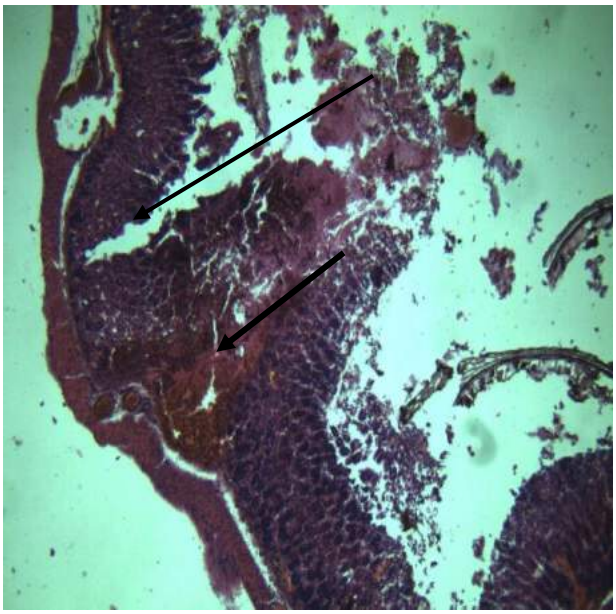

Fig 2. Section of gastric tissue showing severe gastric ulceration, haemorrhage, necrosis and infiltration by acute inflammatory cells consistent with acute severe gastric ulcer (Group 3).

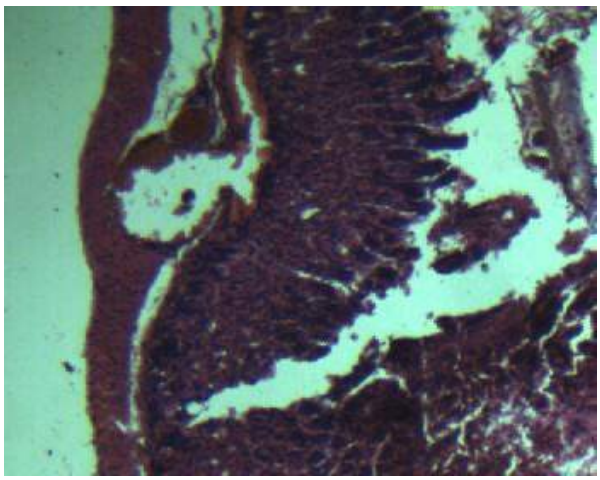

Fig 3: Section shows gastric tissue with ulceration and congestion consistent with gastric ulcer. (Group 4).

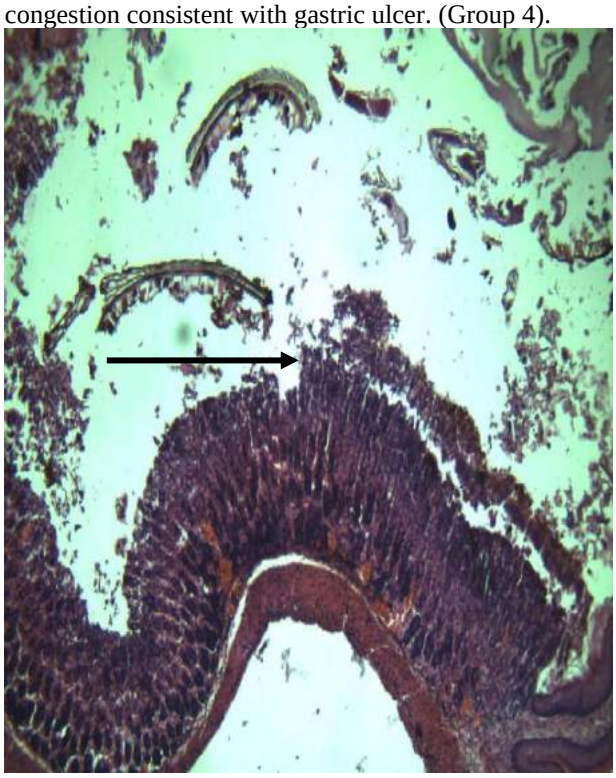

Fig 4: Section of gastric biopsy showing gastric mucosa erosion (Groups 5 and 6)

Section of the group with ulcer and parasite shows ulceration of the gastric mucosa extending beyond the muscularis propria with associated haemorrhage and congestion; and focal area of necrosis and infiltration by acute inflammatory cells consistent with severe gastric ulcer. (Figures 2 and 3).

Sections of the treated groups show erosion of the gastric mucosa with mild infiltration by acute inflammatory cells consistent with gastric erosion. (Figure 4)

Other Observations

The parasitized mice showed reduced activity and anorexia while the un-parasitized ones were more active and were feeding well.

Discussion

The effects of *Plasmodium berghei* parasitaemia on laboratory gastric ulcer in mice was evaluated in 36 mice which were grouped into six.

The decline in PCV with increasing parasitaemia in our study supports the fact that anaemia is a common finding in malaria. This is comparable with the studies in humans by other workers (Francis and Warell 1993 and Menendez 2000)[8,9]. The cause of decline in packed cell volume as a result of malaria is multifactorial, there is lysis of parasitized erythrocytes, immune mediated haemolysis and reduction in the life span of the erythrocytes (Phillips et al 1992, Smith et al 2002)[10,11,12,13]. Kai et al[14] also suggested that anaemia is due to both a great increase in clearance of uninfected cells and a failure of an adequate bone marrow response.

Chloroquine and artemether were sensitive drugs to P berghei parasitaemia as they significantly clear the parasitaemia in the mice studied. Various studies have shown that these drugs are very potent (Nakazawa 2005)[15]. Nakazawa in Japan found that chloroquine is effective in treating P berghei in early and recrudescence phases. Artemether was better in restoring the packed cell volume to nearly pre-morbid state 24 hours after dosage completion than chloroquine. The clearance of parasitaemia by artemether was slightly greater than chloroquine though both showed a considerable clearance to *Plasmodium berghei*. This is comparable with reported observation in Gambia (White el)[16] and that of Myint et al.[17] in 1987 where artemether was reported to have a faster malaria parasite clearance time than quinine.

Parasitaemia was noted to have influence on the gastric mucosa and gastric content.

The parasitized mice showed lower pH and higher gastric acidity compared with the unparasitized counterpart (Jimmy et al., 2014).[18] There was also marked congestion of the gastric mucosa in the parasitized mice.

The ulcers in the parasitized animals were remarkable with moderate to severe congestion of the gastric mucosa.

The congestion noticed in the untreated groups were not present in the treated group and the ulcer diameters were less in the treated group. This showed that with increasing parasitaemia, there is increased likelihood of capillary stasis, haemorrhage and congestion. Despite this, absorption of antimalarial drugs is generally adequate (Francis et al., 1993)[8]. The study by Eweka and Adjene in Benin, Nigeria suggested that antimalarial like artesunate may contribute to the changes found in the stomach in contrast to our finding of decrease gastric mucosa pathology with the use of chloroquine and artemether.[19] Artemether was able to neutralize the acidity of the gastric content better than chloroquine although the mean ulcer diameter for the 2 groups was not remarkable.

The anorexia and reduced activity noticed in the parasitized mice is comparable to the symptoms seen in malaria in humans. After 24 hours of commencement of therapy, the animals feeding and activity improved considerably.

Conclusion

Our study demonstrated that malaria parasitaemia can increase the severity of gastric ulceration and also in the absence of previous history of gastric ulcer; high level of parasitaemia can cause gastric mucosal congestion and erosion. Chloroquine and Artemether showed a remarkable effect on the clearance of parasitaemia and improvement in the packed cell volume. From the study, Artermether show a higher efficacy than chloroquine.

It is recommended that similar studies should be done with other species of Plasmodium on a larger scale and different mode of inducing gastric ulcer should be employed.

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