Effect of Sodium Bicarbonate on Haematological Parameters and Differential Cells in *Plasmodium Berghei*-infected Albino Mice

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ABSTRACT

The current study evaluated the hematological changes in albino mice following infection with *P. berghei* and treatment with sodium bicarbonate; an alkaline substance intended to alkalinize the pH in the parasite environment. Twenty albino mice were randomly divided into five groups of four mice each. Groups 3, 4 and 5 were the test groups and administered 84mg/kg b.w of sodium bicarbonate injection once, twice and thrice respectively. Groups 1 received dH_2O, group 2; only *P. berghei*. Three days later, hematological parameters and differential cells were analyzed. PCV was significantly ($p<0.05$) lower in groups 2(32.00±0.70), 3(34.00±0.70), 4(34.00±0.70), 5(33.00±0.70) compared to control (35.00±0.70). Haemoglobin decreased significantly ($p<0.05$) in group 5 (11.00±0.70) compared to control (11.80±0.70). WBC showed significant ($p<0.05$) increase in the test groups; 2(3600.00±70.71), 3(4600.00±70.71), 4(4800.00±70.71), 5 (4800.00±70.71) compared

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to control (3200.00±70.71). Platelets decreased significantly (p<0.05) in the test groups; 2(90.00±.70), 3(87.00±.70), 4(84.00±.70), 5(86.00±.70) compared to control (92.00±.70). The percentage neutrophils was significantly (p<0.05) higher in group 2(61.00±0.70), significantly (p<0.05) lower in groups 3(58.00±0.70), 4(57.00±0.70), 5(57.00±0.70) compared to control (60.00±0.70). Leucocytes increased significantly (p<0.05) in groups 2(36.00±0.70), 3(38.00±0.70), 4(38.00±0.70), 5(40.0±0.70) compared to control (32.00±0.70). Monocyte was significantly (p<0.05) lower in the test groups; 2(2.00±0.07), 3(2.00±0.07), 4(2.00±0.10) and 5(1.00±0.89) compared to control (4.00±0.07). Eosinophils decreased significantly (p<0.05) in group 2(1.00±0.35), increased significantly (p<0.05) in group 4(3.00±0.70) compared to control (2.00±0.70). Basophils were not detected in neither of the groups. This study revealed that sodium bicarbonate administered to albino mice infected with *P. berghei* caused the elevation of some hematological parameters and differential cells.

1. INTRODUCTION

Many developing countries around the world are often mostly challenged with the scourge of malaria infection. Malaria has become one of the major health problems in developing countries of the world accounting for about 2-3 million deaths yearly. It is a tropical protozoan disease transmitted through female anopheles mosquitoes. It is mainly caused by various species of plasmodium parasite [1]. Four species of intracellular protozoa of the genus Plasmodium cause malaria infection in human beings and they include *P. falciparum, P. vivax, P. ovale, and P. malariae. P. falciparum and P. vivax* cause the most serious forms of the disease [2]. *P. berghei* is the specie that majorly infect rodents such as mice hence its suitability and common use in malaria model experiments. Blood is a tissue that circulates in a virtually closed system of blood vessels. It is composed of red and white blood cells, platelets, suspended liquids known as plasma and serum. Plasma is an extracellular fluid confined within the vascular system. The water and electrolyte composition of plasma is particularly the same as that of intracellular fluid, made up of water, electrolytes, metabolites, nutrients, proteins, enzymes and hormones [3], all playing vital roles towards survival of the cells.

Upon infection, the malaria parasite feeds on the host cell hemoglobin by using cytostome-dependent invaginations. The hemoglobin is transferred into an acidic digestive vacuole (DV) of the parasite and is degraded by proteases providing a source of amino acids and a source of osmolytes [4], thereby generating space and favourable conditions for its growth [5]. The DV pH of the parasite has long been thought to play a major role that favours the degradation of hemoglobin; the protein component of red blood cells responsible for oxygen binding and transportation to cells. Also, the detoxification of heme (a toxic waste product of hemoglobin degradation) and in the event of antimalarial drug action and resistance [6], favour haemoglobin degradation. Several proteolytic enzymes of the parasite such as aspartic and cysteine proteases involved in hemoglobin degradation have pH optima in the range 4.5-5.0 suggesting that the DV maintains an acidic environment [4]. The vacuolar-type proton-pumping ATPase (V-type H^+-ATPase) is thought to be responsible for maintaining an acidic DV [7]. Sodium bicarbonate ($NaHCO_3$) solutions are usually of alkaline pH and are sometimes administered to patients with metabolic acidosis who have both a low plasma HCO_3^- concentration, and a low plasma pH (< 7.2). Since this salt is for the most part completely dissociated in aqueous solution, Na^+, HCO_3^-, and H_2O are effectively added to the extracellular fluid compartment (ECF). Usually, Na^+ molecules are being added without Cl^-, and since HCO_3^- has a tendency to displace Cl^- from the ECF compartment, both effects contribute to increase the "strong ion difference" (SID), thus causing alkalinization of a system [8].

Some of the haematological changes that are associated with malaria infection include anaemia, thrombocytopenia, and disseminated intravascular coagulation [9-12]. Alterations in physicochemical parameters of *P. falciparum* infested blood may vary with levels of malaria endemicity, presence of haemoglobinopathies, nutritional status, demographic factors and the level of malaria immunity [13-14]. Therefore, well informed alterations in blood parameters in malaria infection enable the clinician to establish reliable diagnosis and therapeutic interventions.

Malaria pathogenesis is based mainly on extensive changes in biochemical and haematological parameters [15]. The World health Organization (WHO) criteria acknowledged that some biochemical and haematological features should raise the suspicion of severe malaria [16]. The current study was therefore undertaken to determine the level of Haematological changes following infection of albino mice with *P. berghei* and treating with the alkaline solution; sodium bicarbonate.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental animal models

Healthy albino mice of both sexes weighing between 20-33g each were used for the experiment. The animals were obtained from the zoology Department, University of Jos Nigeria and transported to the Animal House, Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria and acclimatized for seven days before commencement of the experiment, they were fed with standard feed and water *ad libitum*. They were also maintained under standard conditions of humidity, temperature and 12 hours light/darkness cycle. Experiment were conducted in strict compliance with internationally accepted principle for laboratory animal use and care as contained in the Canadian council on animal care guidelines and protocol review [17].

2.1.2 Plasmodium berghei

The plasmodium parasite; *P. berghei* NK 65 was used for the study. It was bought from National Vertinary Research Institute, Vom, Plateau State, Nigeria and kept alive by continuous intra peritoneal passage in mice every four days at the Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria according to [18].

2.1.3 Sodium Bicarbonate

Sodium Bicarbonate injection (Pauco Sodium Bicarbonate; 8.4%w/v) was bought from a pharmaceutical shop in Keffi town, Nasarawa State, Nigeria and stored at between 8-25^{0}C in the laboratory at the Department of Biochemistry and Molecular Biology, Nasarawa State

University, Keffi until commencement of the experiment.

2.2 Methods

2.2.1 Parasite inoculation

The method described by [19] was used for the inoculation of parasite into experimental animals. The inoculums consisted of $5x10^7$ of *P. berghei* parasitized erythrocytes per ml. This was done by first determining the percentage parasitaemia and then the erythrocytes count of the donor mouse. The blood was diluted with phosphate buffer saline in proportions indicated by both determinations. The Albino mice were inoculated intraperitoneally, with 0.2 ml of the already infected blood.

2.2.2 Determination of percentage parasitaemia

To obtain the percentage parasitaemia, thin blood smears were made from the tail of each mouse, fixed with methanol and stained with 10% Giemsa stain. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 100 erythrocytes in random 8 fields of microscope. Parasitaemia was calculated by light microscopy by using the 100× objective lens and the following equation was used

$$\% \text{ Parasitemia} = \frac{\text{Number of Parasitized RBC}}{\text{Total Number of RBC Counted}} \times 100$$

2.2.3 Experimental design

A total of twenty mice were randomly divided into five groups of four mice per group. Groups 1 served as the normal control; not infected with the parasite and not treated with sodium bicarbonate and group 2 served as the positive control; it was infected with *P. berghei* but not treated with sodium bicarbonate while three other groups (3, 4 and 5) were assigned as test groups and administered 84mg/kg b.w of sodium bicarbonate injection once, twice and thrice respectively.

2.2.4 Determination of packed cell volume (PCV)

The packed cell volume was evaluated using the method of Daice and Lewis [20]. Blood sample was collected into a heparinized capillary tube from the tip of the tail of each mouse and sealed

with a plastacin. The tube was then centrifuged using a micro hematocrit centrifuge at 11,000rpm, for 5minutes. PCV was read using the micro hematocrit reader.

$$PCV = \frac{\text{Volume of Erythrocytes in a Given Volume of Blood}}{\text{Total Blood Volume}}$$

2.2.5 Haemoglobin estimation (Hb)

The Hb was determined using the method of [21] Miale (1972). Drapkin's solution is able to hydrolyze the red blood cells leaving haemoglobin to be counted.To a 4ml Drabkins solution in a test tube was added 20µl of well mixed anti-coagulated whole blood. The tube was mixed by inversion and incubated at 25ºC for 5 minutes. Absorbance of the solution was read at 540 nm against a reagent blank. The concentration of haemoglobin was calculated by multiplying the absorbance with a factor of 36.8.

Hb = A_{540} X 36.8

2.2.6 Determination of red blood cell count

This was done using the standard haematological procedure described by [22]. Red blood cells are large and conspicuous when viewed through the microscope, making it easily to be counted in the Neubaeur counting chamber. Well mixed anti-coagulated blood was diluted 1:20 with 10% Na_2CO_3 solution. The mixture was then loaded into an improved Neubauer counting chamber. Appropriate squares were counted and added up to determine the total red cell count.

2.2.7 Determination of total white blood cell (WBC) count

White blood cell count was determined using the method of [23]. Turk's solution (2% glacial acetic acid) hydrolyzes the red blood cells except white blood cells, leaving it to be counted. The blood sample was diluted (1:20) with Turk's solution (2% glacial acetic acid). The diluted sample was loaded into a Neubaer counting chamber with the aid of Pasteur pipette. The total WBC was calculated by counting the required number of squares on the counting chamber under a microscope using the (×1000) magnification.

2.2.8 Determination of platelets count

This was done according to the method described by [24]. Platelets are easily stained by Leishman stain, making them visible enough to be counted. Air-dried thin smears were made from all samples and stained with Leishman stain. These PBS (Platelets blood stains) were examined under light microscope using x100 oil immersion lens. In a monolayer zone of the smear, platelets were counted simultaneously with RBC till 1000 RBC were counted. The number of platelets per 1000 RBC thus obtained was multiplied by 15000.

2.3 Statistical Analysis

The data obtained was analyzed using one-way ANOVA using IBM statistical product and service solution (SPSS) version 23.0 to get the mean values and standard deviations. Further test for level of significance was done using LSD and Duncan tests. The level of significance was set at P < 0.05 for all the data.

3 RESULTS AND DISCUSSION

3.1 Results

Table 1 is the result presentation of the effect of Sodium bicarbonate on the Haematological parameters of *P. berghei*-infected albino mice. PCV was significantly (p<0.05) lower in group 2 (32.00±0.70) and significantly (p<0.05) lower in groups 3 (34.00±0.70), 4 (34.00±0.70) and 5 (33.00±0.70) when compared to the control (35.00±0.70) (group 1). Hb was observed to decrease significantly (p<0.05) in group 5 (11.00±0.70) but no significant decrease (p>0.05) was observed in groups 2 (12.00±0.70), 3 (11.40±0.70) and 4 (11.20±0.70) when compared to the control (11.80±0.70). No significant (p>0.05) decrease was observed for RBC count in all the treatment groups; 2 (8.00±.07), 3 (7.60±.07), 4 (7.52±0.10) and 5 (7.60±0.89) when compared to the control (8.00±.07). WBC count showed significant (p<0.05) increase in all the test groups; 2 (3600.00±70.71), 3 (4600.00±70.71), 4 (4800.00±70.71) and 5 (4800.00±70.71) when compared to the control (3200.00±70.71). Platelets count was observed to decrease significantly (p<0.05) in all the test groups; 2 (90.00±.70), 3 (87.00±.70), 4 (84.00±.70) and 5 (86.00±.70) when compared to the normal control (92.00±.70).

Table 2 shows the result presentation of the effect of Sodium bicarbonate on differential cells of *P. berghei*-infected albino mice. The

Table 1. Effect of Sodium bicarbonate on the Haematological parameters of *P. berghei*-infected albino mice

Treatment Group	Haematological Indices				
	PCV (%)	Hb Conc. (g/dl)	RBCcount (10^{12}/L)	WBC Count (10^6/mm^3)	Platelet Count (10^6/mm^3)
Group 1	35.00±0.70[a]	11.80±0.70[a]	8.00±.07[a]	3200.00±70.71[a]	92.00±.70[a]
Group 2	32.00±0.70[b]	12.00±0.70[a]	8.00±.07[a]	3600.00±70.71[b]	90.00±.70[b]
Group 3	34.00±0.70[c]	11.40±0.70[a]	7.60±.07[a]	4600.00±70.71[c]	87.00±.70[c]
Group 4	34.00±0.70[c]	11.20±0.70[a]	7.52±0.10[a]	4800.00±70.71[d]	84.00±.70[d]
Group 5	33.00±0.70[d]	11.00±0.70[b]	7.60±0.89[a]	4800.00±70.71[d]	86.00±.70[e]

Results are expressed as Mean ± standard deviation, (N=4). Mean values with different letters as superscripts are considered significant at p < 0.05

Table 2. Effect of Sodium bicarbonate on differential cells of *P. berghei*-infected albino mice

Treatment Group	Differential cells				
	NEU (%)	LYM (%)	MONO (10^{12}/L)	EOSINO (10^6/mm^3)	BASO (10^6/mm^3)
Group 1	60.00±0.70[a]	32.00±0.70[a]	4.00±0.07[a]	2.00±0.70[a]	0.00±0.00[a]
Group 2	61.00±0.70[b]	36.00±0.70[b]	2.00±0.07[b]	1.00±0.35[b]	0.00±0.00[a]
Group 3	58.00±0.70[c]	38.00±0.70[c]	2.00±0.07[b]	2.00±0.70[a]	0.00±0.00[a]
Group 4	57.00±0.70[d]	38.00±0.70[c]	2.00±0.10[b]	3.00±0.70[c]	0.00±0.00[a]
Group 5	57.00±0.70[d]	40.0±0.70[d]	1.00±0.89[c]	2.00±0.70[a]	0.00±0.00[a]

Results are expressed as Mean ± standard deviation, (N=4). Mean values with different letters as superscripts are considered significant at p < 0.05

percentage neutrophils was observed to be significantly (p<0.05) higher in group 2 (61.00±0.70) and significantly (p<0.05) lower in groups 3 (58.00±0.70), 4 (57.00±0.70) and 5 (57.00±0.70) when compared to the control (60.00±0.70). Leucocytes increased significantly (p<0.05) in the test groups 2 (36.00±0.70), 3 (38.00±0.70), 4 (38.00±0.70) and 5 (40.0±0.70) when compared to the control (32.00±0.70). Monocyte count was observed to be significantly (p<0.05) lower in all the test groups; 2(2.00±0.07), 3(2.00±0.07), 4 (2.00±0.10) and 5 (1.00±0.89) when compared to the control (4.00±0.07). Eosinophils decreased significantly (p<0.05) in group 2 (1.00±0.35) and increased significantly (p<0.05) in group 4 (3.00±0.70) compared to the control (2.00±0.70) but the increase was non-significant (p>0.05) in groups 3 (2.00±0.70) and 5 (2.00±0.70) compared to the control. Basophyils were not detected in neither of the groups.

3.2 Discussion

Hematological (blood) parameters are crucial markers in diagnosing and determining the actual physiological status of an organism under any kind of physiological state. An organism must have to maintain its blood composition and constituents at a relatively constant and stable physiological conditions for proper functioning, a deviation from which could signal an abnormality in the system. The present study showed that PCV was significantly (p<0.05) lower in group 2, 3, 4 and 5 when compared to the control (group 1). Hemoglobin was observed to decrease significantly (p<0.05) in group 5 but no significant difference (p>0.05) was observed in groups 2, 3 and 4 when compared to the control. Since hemoglobin and hematocrit profiles (such as PCV) relate to the total population of red blood cells in the blood, it could thus imply that sodium bicarbonate may not favour hemoglobin incorporation into red blood cells and a consequent reduction in oxygen binding and transportation [25].

No significant (p>0.05) decrease was observed for red blood cells (RBC) count in all the treatment groups; 2, 3, 4 and 5 when compared to the control. The decrease in erythrocytes and hematocrit counts (PCV) after intraperitoneal passaging of *P. berghei* and intraperitoneal administration of sodium bicarbonate suggests that it may not have stimulated the formation or secretion of erythropoietin in the stem cells of normal mice. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells [26]. Erythropoietin affects the oxygen-carrying

capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and hemoglobin are very important in transferring respiratory gases [27,28]. It may also suggest that sodium bicarbonate could not cause polycythemia. Previous studies have indicated that an increase in the count of erythrocytes and PCV is suggestive of polycythemia and positive erythropoiesis [29-32]. Therefore, sodium bicarbonate cannot be used to restore lost blood during excessive bleeding. The mechanism leading to the increase in erythrocyte count is probably mediated by the anti-oxidant activity [33]. This study however demonstrates that sodium bicarbonate may not possess antioxidant properties as to favour erythrocyte count in albino mice.

WBC count showed significant (p<0.05) increase in all the test groups; 2,3,4 and 5 when compared to the control. The significant increase in white blood cell counts and differential leukocytes in the test animals shows that sodium bicarbonate may have immune boosting and immunostimulatory effects and produced leucocytosis and prevented the leucopenia induced by *P. berghei*. It has been reported that granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins IL -2 IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of white blood cells [34,35]. Since sodium bicarbonate caused increases in white blood cell counts, it is possible that it stimulated the production of these regulatory factors or increased the sensitivity of the committed stem cells, responsible for the production of white blood cells, to these factors.

Platelets count was observed to decrease significantly (p<0.05) in all the test groups; 2, 3, 4 and 5 when compared to the normal control. The significant decrease in platelets after intraperitoneal infection with *P. berghei* and administration of sodium carbonate suggests that sodium bicarbonate may not have stimulated thrombopoietic process in mice infected with *P. berghei*. The significant decrease in platelets count after oral administration of sodium carbonate may indicate that it may not possess the ability to recover platelets.

The percentage neutrophils was observed to be significantly (p<0.05) higher in group 2 and significantly (p<0.05) lower in groups 3, 4 and 5 when compared to the control. Only the circulating neutrophils are accounted for in the WBC count. The half-life of mature neutrophils in circulation is about 7 hours. They irreversibly traverse the vascular endothelium into the tissues, where they die after 1 or 2 days. The main function of neutrophilic granulocytes is phagocytosis of bacteria. This is a complex multistage process that includes engulfment of the organism, incorporation into the cytoplasm, and fusion with a lysosome where enzymes are liberated that will destroy the bacterium while a burst of energy is generated. Their significant (p<0.05) increase in group maybe due to their high mobilization in the presence of *P. berghei*.

Leucocytes increased significantly (p<0.05) in the test groups 2, 3, 4 and 5 when compared to the control. Macrophages and lymphocytes are known collectively as mononuclear leukocytes. Both play important roles in cellular and humoral immunity. These cells are able to exit and re-enter circulation, retaining their function. They may spend time in the tissues or in lymph nodes. The cells of the monocyte-macrophage system have their origin in the bone marrow, deriving from the CFU-GM. They are not stored but are rapidly released into the circulation where they account for 5% of WBC. In tissues, they become macrophages. Significant increase in leucocytes count in this study indicates that the mice immune system was compromised due to infection by *P. berghei* but this was mitigated by the administered sodium bicarbonate.

Monocyte count was observed to be significantly (p<0.05) lower in all the test groups; 2, 3, 4 and 5 when compared to the control. Monocyte-macrophages phagocytose bacteria and particulate material, play a role in the inflammatory reaction, and are important in the immune apparatus where they process antigenic material and "communicate" with T lymphocytes through a cell-cell interaction process. Monocytes are able to secrete interleukin, a substance that potentiates B and T lymphocytes. They participate in fibrinolysis by secreting plasminogen activators.

Eosinophils decreased significantly (p<0.05) in group 2 and increased significantly (p<0.05) in group 4 compared to the control but the increase was non-significant (p>0.05) in groups 3 and 5 compared to the control. Basophils were not detected in neither of the groups. Heavy concentrations of eosinophils are found in the GI tract, lung, and skin. The precise function of these complex cells is not well known. They

possibly play a role in defense against multicellular parasites and in limiting inflammation.

Basophils were not detected in neither of the groups. Basophils constitute about 1 to 2% of circulating leukocytes. Their physiologic role is also not known with precision. In their granules they carry heparin and histamine. IgE can be found bound to their surface in response to specific antigens. Their non-detection in this study implied that there was no antibody response to any antigenic substance.

4. CONCLUSION

The outcome of this studies revealed that sodium bicarbonate administered to albino mice infected with *P. berghei* caused the elevation of WBC, leucocytes, and Eosinophils but decreased neutrophils and basophils count. These are cells usually mobilized during an immune response, hence the suggestion that sodium bicarbonate may possess immunomodulatory effect by posing an unfavorable pH condition to some parasites that prefer to thrive in acidic pH because it is alkaline in nature.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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