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# The Role of Calcium and Glucose on the Increasing of Parasitemia Value and Hemolysis into Plasmodium falciparum-infected Erythrocyte

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***Abstract***–Erythrocyte infected by *Plasmodium* experiences various changes of shape and function. The permeability increases upon various dissolved material including amino acid, glucose, vitamin, nucleotide, purine, anion/cation and organic/inorganic and also others simple dissolved materials such as sorbitol, choline and chloride-ion. The increasing of permeability is very needed by *Plasmodium* to provide nutrients for internal growth. The objectives of this research were to determine the increasing of parasitemia value and hemolysis on erythrocyte infected by *Plasmodium falciparum.* The medium culture used for growing *Plasmodium falciparum* was RPMI 1640 that produced parasitemia 15%, inoculation was conducted to produce sub-culture that produced parasitemia 20% and divided into calcium, glucose and control (CM 10%) treatment with 3 times replications. Observation was conducted from the first day to sixth day after treatment. Parasitemia and hemolysis parameters as growth indicators were observed. Difference among treatments groups were analyzed using ANOVA and followed by Duncan *Multiple Range Test* (DMRT α=0.05). The result showed that the application of calcium + glucose produced the highest number of parasitemia (11.87±4.71) (means ±SD) and hemolysis (0.278±0.012) compared with others applications i.e. calcium, glucose and control medium culture (10% CM). This application produced significant difference ($p<0.05$). It was concluded that calcium and glucose had important to increase parasitemia and hemolysis of *Plasmodium falciparum*-infected erythrocyte.

***Key words***—*parasitemia, hemolysis, calcium, glucose*

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## I. INTRODUCTION

Malaria is still a health problem in the world, widely spread and endemic especially in tropics and sub-tropics area. Malaria has spread to over 106 countries and infected 41% of the world population, increased from 99 countries in 2010. Data shows that every year about 350-500 million cases of malaria was found and caused 1.5–2.7 million deaths. This would impact on the quality of human resources that can cause various social problems, economic and even affect the national security (World Health Organization, 2011).

Erythrocyte infected with plasmodium experiences shape and functions changes. Permeability of erythrocyte increases to various dissolved materials including amino acid, glucose, vitamin, nucleoside, purine, anion/cation and organic/inorganic, as well as some other simple dissolved materials such as sorbitol, choline and chloride ion. Increased permeability is needed by *Plasmodium* to provide nutritional needs for growth in erythrocyte. The permeability of cell membranes of infected erythrocyte may increase along with the increased role of the transporter or parasite activating channel that exist in the erythrocyte membrane, or an increased in membrane fluidity in which parasites affect lipid or protein that causes structural changes of cell membranes (An and Mohandas, 2010; Haldar and Mohandas, 2007).

The growth process of malaria parasite in the red blood cells plays an important role for the development of next parasytes which then will affect the severity level along with the increasing number of parasytes in the red blood cells. The metabolism of infected cell increase after parasite enter erythrocytes cell. It is indicated by an increased influx of ingredients nutrients such as essential amino acids,

nucleoside, lactic and fatty acids, as well as several kinds of substances that under normal circumstances can not enter the cells. These include cations, small organic ions, amino acids and acids organic. These materials are used by parasite for their development in human body. The development process of malaria parasite can be seen in several stages i.e. an increase in metabolism, movement, growth and proliferation/replication (Doerig *et al*., 2009; Gazarini *et al*., 2007; Harijanto *et al*., 2000).

Rapid growth needs glucose/fructose material and amino acids such as glutamate, aspartic, alanine, leucine, methionine and some vitamins such as calcium pantothenate, PABA, folic acid, and purine and pyrimidine. Therefore, increasing metabolism of infected cell occurs after the parasite enters erythrocyte which characterized by an increased influx nutrient materials such as essential amino acids, nucleosida, lactic and fatty acids, as well as several kinds of substances that under normal circumstances can not enter the cells but now can enter such as hexitol, small organic ions, amino acids and organic acids (Harijanto *et al*., 2010; Gazarini *et al*., 2007). Malaria parasites in erythrocyte require glucose as the main nutrient and produce energy and lactic acid by metabolism via glycolysis. *Plasmodium*-infected erythrocytes may increase the consumption of glucose about 40-100 times greater than normal consumption (Bagnaresi *et al*., 2007; Gazarini and Garcia, 2003; Moreno and Docampo, 2003; Garcia, 1999).

Calcium plays an important role in the process of merozoites invasion and maturation in erythrocytes. Besides the influx of calcium into erythrocyte cell during parasite invasion would lead to an increased of calcium concentration in cytoplasm and as the second messenger in stimulating molecular activity that causes erythrocyte easily infected by parasites (Kirk, 2001; Wasserman *et al*., 1999).

Based on this background, research that can determine the important roles of calcium and glucose on the growth of *Plasmodium falciparum* at asexual phase in human red blood cells (erythrocyte) is needed.

## II. MATERIAL AND METHOD

### Preparing *Plasmodium falciparum* culture medium

Preparation of culture medium was conducted by preparing RPMI-1640 medium, 5% sodium bicarbonate solution, erythrocyte washing medium, human serum and 50% erythrocyte, preparing of *Plasmodium falciparum*-infected blood and removing infected erythrocyte (inoculum) from culture medium.

### Preparing $CaCl_2$ and glucose solution

Using 100 mM $CaCl_2$ solution which had been prepared and 15 μM glucose solution which then given to *Plasmodium falciparum*-infected erythrocyte cell with 20% initial parasitemia.

### Observing hemolysis

Examination of hemoglobin concentration in supernatant solution was carried out by using Microplate reader Birad model 550 instrument with specific wavelength of the hemoglobin i.e. 540 nm. The absorbance unit was cm M.

### Examining *Plasmodium falciparum* parasitemia

As much as 20 μL of erythrocyte cell was taken from each well to make thin blood smears, fixation with methanol and staining with Giemsa 25%. Parasitemia was calculated by using microscope estimation i.e. the percentage of parasites that infected erythrocyte per 1000 erythrocyte which had been observed as well as calculating schizont maturation phase.

### Data analysis

Parasitemia and hemolysis data resulted from each treatment i.e. application of 15 μM calcium, 100 mM glucose and control (CM 10%) were analyzed by using analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) at 95% significance level ($p<0.05$).

## III. RESULT AND DISCUSSION

### A. Parasitemia Profile

Parasitemia was calculated from the estimation under microscope observation by counting the percentage of parasites which infected erythrocyte per 1000 erythrocyte. Based on application of 15 μM calcium solution, 100 mM glucose solution, 15 μM calcium solution+100 mM glucose solution and CM 10% (control) (Table 1), it can be seen that all applications increased parasitemia

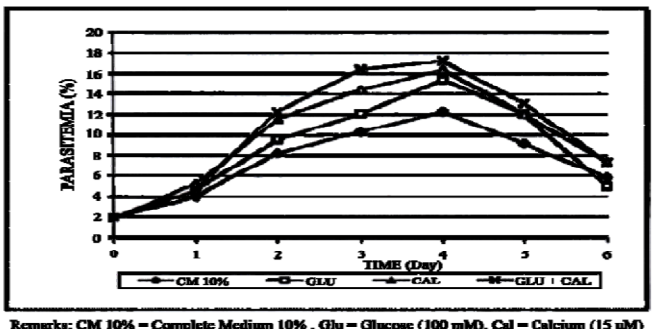
**Table 1**. Means of parasitemia (%) on basic culture medium 10% CM given with 15 μM calcium, 100 mM glucose, 15 μM glucose + 100 mM glucose and 10% CM as control

| Culture medium | Parasitemia (Means±SD) | ANOVA (α<0,05) |
|---|---|---|
| Control (CM 10%) | $8.28^{a}\pm 2.82$ | 0.000 |
| Glucose | $10.97^{b}\pm 4.02$ | |
| Calcium | $11.09^{c}\pm 4.01$ | |
| Calcium + Glucose | $11.87^{d}\pm 4,71$ | |

Remarks: Different small letters at the same row show 5% difference of Duncan Test. 10% CM = 10% Complete Medium

Based on the result, it can be seen that parasitemia on the culture medium sharply increased from the first to the third day and continued to the fourth day. However, the increment on the fourth day was not as sharp as on the first and third day. Parasitemia decreased on the fifth day and sixth day (Fig. 1). The application of calcium + glucose produced the highest increment of parasitemia (11.87±4.71) (means±SD) compared with basic culture medium 10% CM (8.28±2.82), 100 mM glucose (10.97±4.02), calcium (11.09±4.01). Overall, there was significant difference from Anova analysis (α=0.05).

The increasing of parasitemia until the fourth day (Table 1 and Figure 1) showed that calcium and glucose affected the increasing number of erythrocyte infected with *Plasmodium falciparum*. This was consistent with the previous research which studied the value of parasitemia resulted from invasion process and growth or parasite maturation in erythrocyte cell. The results showed that the better and more rapid invasion and maturation process would increase the number of erythrocyte infected with *Plasmodium falciparum* (Haldar and Mohandas, 2007).




Remarks: CM 10% = Complete Medium 10% , Glu = Glucose (100 mM), Cal = Calcium (15 μM)

**Figure** 1. Means of parasitemia (10%) on 10% CM basic culture medium given with 15 μM calcium, 100 mM glucose, 15 μM calcium + 100 mM glucose and 10% CM as control.

Membrane transportation system in *Plasmodium*-infected erythrocyte differs with the membrane transportation system in normal erythrocyte. In infected erythrocyte, the transportation is mediated by new permeable pathway (NPP) which characterized as an anion-selective channels and permeable to various dissolved solutions needed by parasite for the growth. Therefore, more nutrients are taken from extracellular environment. New permeable pathways has three pathways models created by plasmodium to absorb nutrient from the extracellular environment and erythrocyte cytosol namely duct parasitophorous model, window metabolite model and sequential model (Gazarini *et al*., 2007; Kirk, 2001).

Malaria parasite in erythrocyte requires glucose as the main nutrient source by doing metabolism through glycolysis to produce energy and lactic acid. Erythrocyte which infected with *Plasmodium* increase their glucose consumption by about 40-100 times higher than in normal condition. Research using 2-deoxy-D-glucose(2-DOG) as glucose analog showed an increasing glucose concentration in intracellular compartment of *Plasmodium yoelii* parasite which infected mice erythrocyte. This could be due to the mechanism of H1-glucose cotransport which carries glucose passing through parasite cell membrane. This research proves that transporting glucose passing through parasite cell membrane is a passive equilibrative process than concentrative (active) process (Bagnaresi, 2008; Gazarini *et al*., 2007; Goodyer *et al*., 1997).

Calcium plays an important role in the process of invasion of merozoite and maturation process in erythrocyte cell. Besides, the influx of calcium into erythrocyte cell during parasite invasion will lead to an increase of calcium in cytoplasm which roles as a second messenger in stimulating molecular activity that cause erythrocyteeasily infected by parasites (Iwalokun *et al*., 2007; Kirk, 2001).

### B. Hemolysis Profile

Result showed that the application of 15 μM calcium+ 100 mM glucose gave higher absorption value compared with the application of CM 10% (control), 15 μM calcium and 100 mM glucose. Based on the ANOVA statistical analysis (α=0.05), overall, it was known that there was significance difference between medium culture calcium+ glucose (0.278±0.012) and CM 10% as control (0.291±0.003) (Table 2).

**Table** 2. Means of absorption total (cm.M) at basic medium CM 10% and after the application of glucose (100 mM), calcium (15 μM) and calcium + glucose (15μM+100 mM)

| Culture medium | Absorption (Rerata ±SD) | ANOVA(α<0.05) |
|---|---|---|
| Control (CM 10%) | $0.265^{a}$± 0.02 | 0.000 |
| Glucose | $0.264^{a}$± 0.03 | |
| Calcium | $0.278^{b}$± 0.03 | |
| Calcium + Glucose | $0.291^{c}$± 0.03 | |

Remarks: Different small letters at the same row shows difference in Duncan test 5%. CM 10% = 10% Complete Medium

In this research, hemolysis process which caused by the release of merozoite resulted from the maturation process of *Plasmodium falciparum* in erythrocyte cell was assessed and observed from the hemoglobin absorption value. The addition of calcium and glucose produced the highest absorption value compared with other medium cultures. This finding showed similar condition found in parasitemia profile. Principally, the activity of protein phosphorylation of erythrocyte cell membrane changes the cell shape, reduces cell protection ability, changes membrane skeleton settings, changes membrane permeability, ruptures cell membrane to remove merozoite from cell and changes the erythrocyte cell surface against antigen (Tiffert and Staines, 2000).

The role of glucose in increasing maturation leads to the increasing of erythrocyte cell lysis that removes merozoite. The peak expression of PfHT1 mRNA membrane *Plasmodium falciparum* which responsible to the glucose transportation occurred after 8 hours (ring form) parasite invasion in erythrocyte. The second increasing level of PfHT1 mRNA was needed by glucose for cell fission. Variety of the increasing mRNA that encoded PfhT1 mRNA was related with the glucose needs during parasite maturation process in erythrocyte cell. *Plasmodium falciparum* parasite experiences asexual reproduction by producing 16 progeny in each cycle (48 hours) as indicated by different metabolism activity compared with uninfected erythrocyte cell. Energy required by infected erythrocyte cell during metabolism is 20-100 times higher than normal erythrocyte cell (Kato, 2008; Kirk, 2001; Woodrow, 1999).

The increasing level of cytosolic calcium for parasites is important to carry out invasion process, synchronization and gene expression that rupture erythrocyte membrane cell by amatured schizont (Mbengue *et al*., 2012; Gracia, 1999; Wasserman *et al*., 1999). Calcium application increased intracellular calcium concentration by 30% and transglutaminase enzyme in *Plasmodium falciparum*-infected erythrocyte was more dominant at the end stage of schizont phase. This phenomenon was consistent with several studies which concluded that rigidity changes and shape changes of erythrocyte cell at the end phase of parasite maturity was a mechanism to release merozoite resulted from maturation with the lysis of erythrocyte cell. There were no presence of invasion process, synchronization and gene expression that rupture erythrocyte membrane cellby a matured schizont in uninfected erythrocyte cell by *Plasmodium falciparum* (Bolaji *et al*., 2012; Cruz *et al*., 2012; Tiffert and Staines, 2000; Garcia, 1999).

## IV. CONLUSIONS

Calcium and glucose had an important role in determining parasitemia value with the increasing of growth and development of *Plasmodium falciparum* parasite in erythrocyte cell. The role of calcium and glucose was indicated from the increasing of erythrocyte-infected *Plasmodium falciaparum* lysis.

## SUGGESTION

Based on this study, a deeper analysis of the plasmodium parasite growth during intra erythrocyte phase is indispensable. This is because the role of calcium and glucose is not known for certain whether its role in the phase tropozoit or schizon. Further research will be benefited the approach in the management of Plasmodium falciparum became evident.

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