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Platelet Kinetics and Other Hematological Profiles in Experimental *Plasmodium falciparum* Infection: A Comparative Study between *Saimiri* and *Aotus* Monkeys

Ibulaimu Kakoma[1], Mark A. James[2], Herbert E. Whiteley[1], Frederico Montelegre[3], Margaret Buese[1], Carol J. Fafjar-Whestone[1], Greg W. Clabaugh[1], and Byeong Kirl Baek[4]

University of Illinois[1], College of Veterinary Medicine, 2001 S. Lincoln, Urbana, IL 61801, Tulane University[2], Medical Center, New Orleans, LA, Ponce Medical School[3], Ponce, Puerto Rico, USA.

College of Veterinary Medicine[4], Chonbuk National University, Chonju 560-756, Korea

Abstract: Levels of platelets and other hematological values were monitored in 21 *Saimiri* and 12 *Aotus* monkeys over a period of three weeks post-infection with monkey-adapted Indochina CDC-1 strain of *Plasmodium falciparum*. In both *Saimiri sciureus boliviensis* and *Aotus nancymai* karyotype-1 monkeys the severest thrombocytopenia was observed at 14 days post-infection coinciding with peak parasitemia, neutropenia, lymphocytosis, and anemia associated with severe hemoglobinemia and elevated fibrinogen degeneration products(FDP's). MCH and MCV profiles in *Aotus* monkeys decreased with ascending parasitemia. In contrast, these parameters in *Saimiri* were characterized by a significant compensatory increase correlating with parasitemia. In general, thrombocytopenia was one of the earliest clinical manifestations of the infection with the platelets returning to normal levels shortly after peak parasitemia at 14 days. Platelet kinetics had a strong correlation with hematologic and parasitologic values in the *Aotus* model. No consistent associations were observed between platelet kinetics and other parameters in the *Saimiri* model. These data indicate that the *Aotus* model for malaria is more predictable than the *Saimiri*. Further, platelet turnover rates and recovery provide a useful prognostic parameter during malaria infection. The results are discussed in relation to the value of the two species of monkeys as models for the pathogenesis of human malaria.

Key words: *Plasmodium falciparum*, *Saimiri sciureus boliviensis*, *Aotus nancymai*, parasitemia, hematological values, thrombocytopenia

INTRODUCTION

In human malaria, thrombocytopenia is frequently observed(Beale *et al.*, 1972; Hortsman *et al.*, 1981; Kelton *et al.*, 1983). The mechanism of this clinical manifestation is poorly understood and often partly attributed to autoimmunity (Skudowitz *et al.*, 1973; Sorensen *et al.*, 1984), associated with reduced platelet half life, leading to changes in the platelet surface structures or disseminated intravascular coagula-

tion (DIC) (Vreeken and Cremer-Goote, 1978; Srichaikul *et al.*, 1975; Essien and Ebhota, 1981). It has also been suggested that occasional direct infection of platelets by malaria parasites may play a role in this process (Fajardo, 1973, 1974 & 1979; Arundhati *et al.*, 1984). These observations have been primarily in human clinical malaria, and some of the mechanisms such as the role of DIC remain controversial (Vreeken *et al.*, 1978). In addition to thrombocytopenia, malaria induces numerous hematologic and hematopoietic alterations (Collins and Campbell, 1983; James *et al.*, 1985; Warrell, 1987). In previous studies thrombocytopenia has been reported in *Saimiri* monkeys (James *et al.*, 1985).

The present experimental study was undertaken to compare changes in platelet levels in *Saimiri* and *Aotus* monkeys experimentally infected with the monkey-adapted *P. falciparum* strain, the most important causative agent of severe human malaria. The thrombokinetics were related to hematologic and parasitologic parameters.

MATERIALS AND METHODS

Experimental animals: Healthy adult male *Aotus* and *Saimiri* monkeys obtained from the USAID-Battelle non-human primate colony (Richland, Washington) were used. The criteria for inclusion into the study were negativity in the parasitological and serological test for malaria and freedom from major gastrointestinal parasites. The experimental animals were housed in environmentally controlled rooms with a temperature of 21 to 29°C and 70% humidity; fed on monkey Chow (Ralston Purina Co., St. Louis, MO), supplemented with fresh fruits, essential minerals, and vitamins; and allowed *ad libitum* access to drinking water.

Experimental infection: Each animal was inoculated IV with freshly infected monkey blood containing 10^7 virulent monkey-adapted CDC-1 Indochina-1 organisms. Prior to infection, the experimental animals were sampled and analyzed for hematological and biochemical profiles as previously reported (Kakoma *et al.*, 1985), and briefly described below.

All experimental animals were handled humanely according to USAID/NIH/USDA guidelines. Any animal with persistent anorexia, parasitemia equal to or greater than 10%, or a hematocrit dropping below 50% of the pre-infection (baseline) value was withdrawn from the experiment and treated chemotherapeutically, including blood transfusions when indicated.

Collection of hematological and pathology samples: Blood samples of 200 μl were collected by femoral venipuncture on a weekly basis. The following parameters were analyzed: neutrophils (Neut %), monocytes (Mono %), lymphocytes (Lymph %), platelet (PTLT $\times 10^3$/mm^3), erythrocytes (RBC $\times 10^6$/mm^3), hemoglobin (HB g/dl), mean corpuscular volume (MCV μm^3), and mean hemoglobin concentration (MHC). Basic blood biochemical profiles were also monitored. Fibrinogen degeneration products (FDP's) were determined using the Dade method (Dade, American Hospital Supply, del Caribe, Aguada, Puerto Rico, USA). General observations on appetite, overall health, demeanor, *etc.* were noted daily and body weights were recorded every two weeks.

Experimental design and statistical analysis: Experimental animals were sampled, tested, and analyzed by descriptive statistics, frequency distribution, and one-way variance were performed on an IBM personal computer using Abstat (Anderson and Bell, Cannon City) as previously described (Kakoma *et al.*, 1985 & 1987). The level of significance was established at 0.05% level.

RESULTS

Table 1 summarizes data on descriptive statistics of the two models. Our data show that there are some innate differences in baseline values between the two animal models used. As observed, statistically significant differences were encountered in the following parameters: *Aotus* had higher levels of platelets (Fig. 1),

Table 1. Descriptive statistics of hematologic values (Mean±S.D.) obtained in the two animal models, including standard deviation (±) from the mean

Variables	Days After Infection					
	Saimiri[1]			*Aotus*[2]		
	0	7	14	0	7	14
PLAT	498.8(±82.8)	354.4(±85.6)	130.8(±134.9)*	586.5(±79.3)	274.0(±63.1)	25.3(±9.1)
WBC	8.2(± 2.3)	8.3(± 1.6)	9.6(± 3.6)	8.8(± 1.0)	5.5(± .8)	8.0(±1.4)
RBC	7.3(± .4)	7.5(± .3)	4.8(± 1.2)	6.5(± .2)	6.3(± .2)	4.2(±0.3)
HGB	14.4(± .7)	14.8(± .5)	9.5(± 2.2)	16.7(± .6)	16.1(± .8)	10.2(±.09)
HCT	44.6(± 2.8)	45.7(± 1.9)	29.9(± 6.8)	51.3(± 1.8)	49.5(± 2.5)	31.8(±2.6)
MCV	60.7(± 1.6)	60.4(± 1.7)	62.3(± 3.1)	78.3(± 2.1)	78.2(± 2.6)	75.4(±2.5)
MCH	19.7(± .58)	19.5(± .5)	19.8(± 0.6)	25.5(± .8)	25.5(± .8)	24.2(±0.8)
NEUT	38.8(±14.8)	55.7(±13.3)	27.8(± 19.0)	28.8(± 7.7)	40.0(± 7.7)	15.5(±3.8)
LYMP	47.2(± 9.0)	21.5(±12.2)	61.2(± 11.4)	61.1(± 6.9)	46.0(± 7.1)	73.8(±7.0)
PARA	Negative	.5(± .5)	6.0(± 5.8)	Negative	.6(± .2)	2.1(±1.1)

1 : n=21, 2 : n=12

HGB(Fig. 2), MCV(Fig. 3), and LYMPH (Fig. 4). In contrast, *Saimiri* monkeys have significantly higher baseline values of RBC than *Aotus* monkeys(Fig. 5). Seven days post-infection, there was a significant difference between the species in WBC, RBC, HGB, HCT, MCV, MCH, neutrophils, and LYMPH. The *Aotus* model showed higher levels in MCV, LYMPH, and HGB. Fourteen days after infection, there was a significant difference in only platelets, MCV, and MCH. The *Saimiri* model had higher levels of RBC only. In the correlation analysis, it was demonstrated in the *Aotus* model

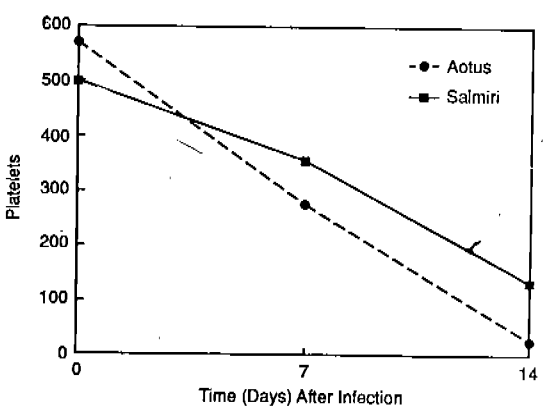

Fig. 1. Relative kinetics of platelets in *Aotus* and *Saimiri* monkeys between Day 0 and 14 post-inoculation.

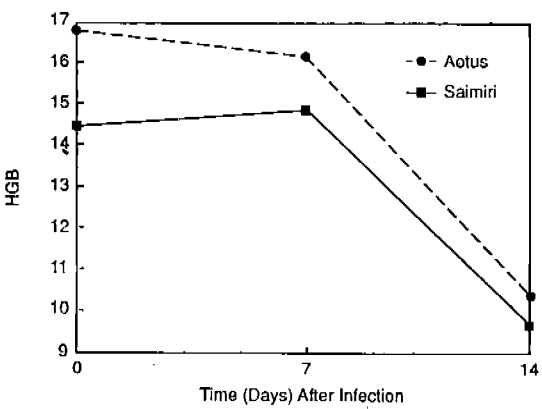

Fig. 2. Hemoglobin levels in *Aotus* and *Saimiri* during the course of *P. falciparum* infection.

that platelet levels had a reverse relationship with MCV prior to infection. At seven days post-infection, there was a strong correlation between platelets, lymphocytes, and parasitemia. Fourteen days after infection, there was a negative correlation with neutrophils and lymphocytes. In the *Saimiri* model, platelets did not show a consistently significant correlation at any point post-infection. Fibrinogen degeneration products were significanted in *Aotus* monkeys versus *Saimiri* and coincided with high parasitemia(Fig. 6), anemia, and thrombocytopenia.

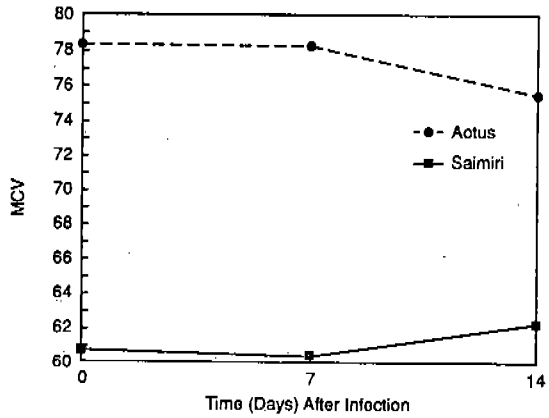

Fig. 3. MCV trends in infected *Saimiri* and *Aotus* monkeys during the course of experimental infection.

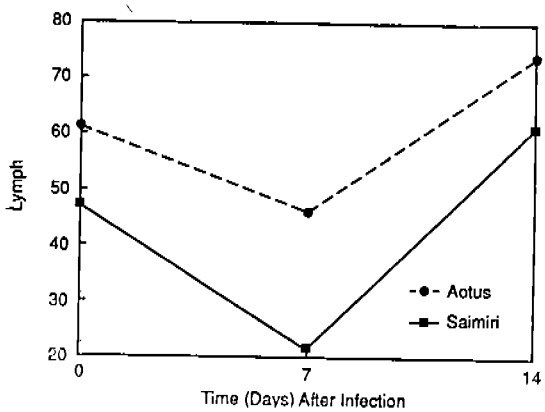

Fig. 4. Levels of lymphocyte counts in *Saimiri* and *Aotus* monkeys during the course of experimental malaria infection.

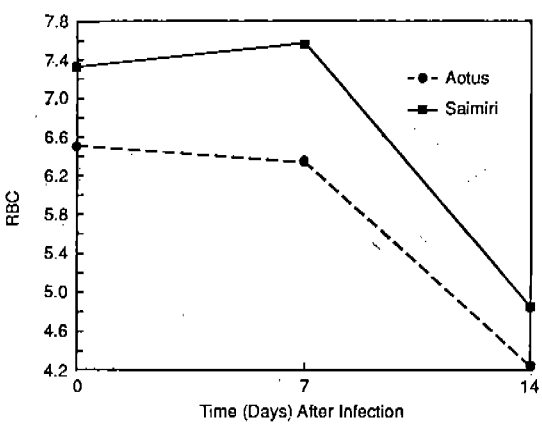

Fig. 5. Red blood cell count profile of *Saimiri* and *Aotus* monkeys between Day 0 and day 14 post-inoculation.

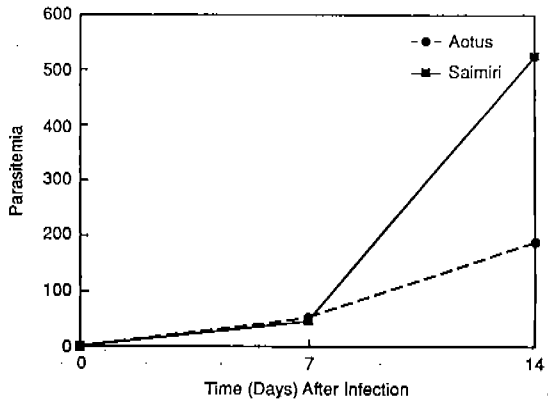

Fig. 6. Profiles of parasitemia in *Aotus* and *Saimiri* monkeys.

Table 2. Correlation between platelet levels, FDP's, and other hematological parameters in the two experimental models. The 1-tail test was used.

	Saimiri[1]			*Aotus*[2]		
	Time-Days					
Variable	0	7[a]	14[b]	0	7[c]	14[d]
PLAT	1.0	1.0	1.0	1.0	1.0	1.0
WBC	−.54	−.31	.29	−.24	.40	.41
RBC	.01	−.34	.57	.64	.30	.70
HGB	.14	−.17	.61	.05	−.17	.62
HCT	−.01	−.33	.64	.24	−.10	.55
MCV	.01	.07	.01	−.67*	−.21	−.27
MCH	.50	.30	.04	−.76*	−.33	−.03
NEUT	.53	−.28	−.28	.02	.37	.91*
LYMPH	.17	−.19	.41	1.7	−.84*	−.93*
PARA		−.48	−.37		−.86*	−.39

*=Indicates strong correlation
1=Critical value (1-tail, 0.05)=+ or −.67
2=Critical value (1-tail, 0.05)=+ or −.73
n=6 for *Aotus* and 12 for *Saimiri*
a=45% of the animals were FDP positive
b=86% of the animals were FDP positive
c=79% of the animals were FDP positive
d=96.7% of the animals were FDP positive
FDP positivity was significantly ($p<0.05$) greater in *Aotus* than in Squirrel monkeys.

DISCUSSION

Complicated falciparum malaria is a multi-systemic disease with major hematologic disturbances (James *et al.*, 1985; Warrell, 1987). Many of the clinical characteristics of human malaria

can be replicated in the non-human primate models especially the *Aotus* monkeys(Collins *et al.*, 1983 & 1985). However, *Saimiri* monkeys have been used by many investigators(Dubois *et al.*, 1984; Hau *et al.*, 1984; Perrin *et al.*, 1984) as an alternate model. Our data strongly suggest that *Aotus* monkeys are more consistent than *Saimiri* for thrombokinetic and other hematologic studies, in that platelet numbers correlate well with many other parameters such as: parasitemia, MCV, MCH, LYMPH, and NEUT. In the case of the *Saimiri* model, many of the parameters were found to be erratic including unpredictable parasitemia trends(Fig. 6). The latter observations are consistent with our previous data(James *et al.*, 1985) and those of others(Dubois *et al.*, 1984; Collins *et al.*, 1988). In general, most parameters in the *Aotus* change drammatically during the severe phase of the disease in a predictable pattern, unlike the erratic fashion seen in *Saimiri* species.

We have demonstrated that the platelet profile during severe *P. falciparum* infection in the *Aotus* monkey is remarkably similar to the patterns observed in the human disease (Beale *et al.*, 1972; Hortsmann *et al.*, 1981; Kelton *et al.*, 1983), including evidence of fibrinogen degeneration products(Srichaikul *et al.*, 1975). The *Aotus* monkey appears to be a reliable model for the study of the various mechanisms of the pathogenesis of falciparum malaria in man. The data also indicate that platelet kinetics may be a useful prognostic indicator for the clinical course of malaria. In our experiments, the increase in parasitemia almost coincided with drop in platelet count and levels of hemoglobin. In human *P. falciparum* malaria, these parameters are very reliable indicators of disease severity and prognosis(Beale *et al.*, 1972). Accordingly, the *Aotus* monkey is a better model than *Saimiri* in that it more closely mimics the malaria syndrome in man.

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REFERENCES

Arundhati, P., Kelly, N.I. and Fajardo, L.F. (1984) Enhanced parasitization of platelets by *Plasmodium berghei yoelii*. *Trans. Roy. Soc. Trop. Med. Hyg.*, **78**:451-455.

Beale, P.J., Cormack, J.D. and Oldrey, T.B.N. (1972) Thrombocytopenia in malaria with immunoglobulin (IgM) changes. *Br. Med. J.*, **1**:345-349.

Collins, W.E. and Campbell, C.C. (1983) Studies on the Indochina I/CDC strain of *Plasmodium falciparum* in Colombiana and Bolivian *Aotus* monkeys and different amphophilines. *J. Parasit.*, **69**:186-190.

Collins, N.E., Skinner, J.C., Pappaioanou, M., Broderson, R.N., Shi-Fong Ma, Filipski, V., Stanfill, P.S. and Rogers, L. (1988) Infection of Peruvian *Aotus nancymai* monkeys with different strains of *Plasmodium falciparum*, *P. vivax* and *P. malariae*. *J. Parasit.*, **74**:392-398.

Dubois, P., Dedet, J.P., Fandeurt, Roussihon, C., Jendoubi, M., Pauillac, S., Mercereau-Puijalon, O. and Pereira da Silva, L. (1984) Protective immunization of the squirrel monkey against asexual blood stages of *Plasmodium falciparum* by use of parasite protein fractions. *Proc. Natl. Acad. Sci. U.S.A.*, **81**:229-232.

Essien, E.M. and Ebhota, M.J. (1981) Platelet hypersensitivity in acute malaria (*Plasmodium falciparum*/infection) in man. *Thromb. Haemostasis*, **46**: 547-549.

Fajardo, L.F. (1973) Malaria parasites in mammalian platelets. *Nature*, **243**:298-299.

Fajardo, L.F. (1979) The role of platelets in infections. *Arch. Pathol. Lab. Med.*, **103**:131-134.

Fajardo, L.F. and Tallent, C. (1974) Malaria parasite within human platelets. *JAMA*, **229**:1205-1207.

Hau, R., Hyde, J.E., Goman, M., Simmons, J.A., Hope, M. and Mackay, J. (1984) Major surface antigen gene of human malaria parasite cloned and expressed in bacteria. *Nature*, **311**:379-382.

Hortsmann, R.D., Dietrich, M., Bienzle, V. and Rasche, H. (1981) Malaria-induced thrombocytopenia. *Blut*, **42**:157-164.

James, M.A., Kakoma, I., Ristic, M. and Cagnard, M. (1985) Induction of protective immunity to *Plasmodium falciparum* in *Saimiri sciureus* monkeys with partially purified exoantigens. *Infect. Immun.*, **49**:476-480.

Kakoma, I., James, M.A. and Jackson, W. (1985) Normal haematologic profiles of male *Saimiri sciureus* squirrel monkeys. *Folia Primatologica*, **44**:102-107.

Kakoma, I. and James, M.A. (1987) Correlative clinical biochemistry and hematologic profiles of laboratory-bred squirrel monkeys (*Saimiri sciureus*). *J. Med. Primatol.*, **16**:261-266.

Kelton, J.G., Keystone, J., Moore, J., Denomme, G., Tozman, E., Glynn, M., Neaone, P.B., Gauldie, J. and Jensen, J. (1983) Immune-mediated thrombocytopenia of malaria. *J. Clin. Invest.*, **11**:832-836.

Perrin, L.H., Loche, M., Dedet, J.P., Roussilhon, C. and Fandeur, T. (1984) Immunization against *Plasmodium falciparum* asexual blood stages using soluble antigens. *Clin. Exp. Immunol.*, **56**:67-72.

Skudowitz, R.B., Katz, J., Lurie, A., Levin, J. and Metz, J. (1973) Mechanisms of thrombocytopenia in malignant tertian malaria. *Brit. Med. J.*, **2**:515-518.

Sorensen, P.G., Mickley, H. and Schmidt, K.G. (1984) Malaria-induced thrombocytopenia. *Vox. Sang.*, **47**:68-72.

Srichaikul, T., Puwasatien, P., Karnjanajetanea, J. and Bokisch, V.A. (1975) Complement changes and disseminated intravascular coagulation in *Plasmodium falciparum* malaria. *Lancet*, **1**:770-772.

Vreeken, J. and Cremer-Goote, T.M. (1978) Haemostatic defect in non-immune patients with falciparum malaria; no evidence of diffuse intravascular coagulation. *Br. Med. J.*, **2**:533-535.

Warrell, D.A. (1987) Pathophysiology of severe falciparum malaria in man. *Parasitology*, **94**:553-576.

=국문초록=

Plasmodium falciparum 감염 실험에 있어서의 혈소판과 혈액치의 변화 —*Saimiri*과 *Aotus* 원숭이의 비교 시험—

University of Illinois[1], *College of Veterinary Medicine, Urbana. IL, 61801,*
Tulane University[2], *Medical Center, New Orleans, LA,*
Ponce Medical School[3], *Ponce, Puerto Rico, USA.*
전북대학교 수의과대학[4]
Kakoma, I.[1], James, M.A.[2], Whiteley, H.E.[1], Montelegre, F.[3],
Buese, M.[1], Fafjar-Whetstone, C.J.[1], Clabaugh, G.W.[1], 백병걸[4]

Saimiri 원숭이 21마리와 *Aotus* 원숭이 12마리에게 *Plasmodium falciparum* Indochina CDC-1충주(원숭이 순응 충주)를 접종시킨 후 3주간 혈소판과 혈액치를 경시적으로 관찰하였던 바, 이들 원숭이(*Saimiri sciureus boliviensis*와 *Aotus nancymai* karyotype-1)는 접종 14일 후에 최고의 기생률, 호중구감소증, 임파구증가증 그리고 심한 혈색소혈증과 섬유소원의 퇴행성 산물(FDP's)치의 증가를 수반한 심한 혈소판 감소성 빈혈증이 관찰되었다. *Aotus*에 있어서는 평균혈색소량(MCH)과 평균혈구용적(MCV)치의 감소와 기생률의 증가를 가져왔으나, *Saimiri*에 있어서는 기생률이 상승함에 따라서 이들 혈액치는 유의적 차이로 상승하는 결과를 가져왔다. 일반적으로 이들 두 원숭이에 있어서 혈소판의 감소 증세와 최고 기생률을 14일 째 나타낸 후 곧 정상 혈액치로 회복되는 소견이 관찰된다. 특히 혈소판의 변화에 있어서 *Aotus* 원숭이는 다른 혈액치의 변화 그리고 기생률과 깊은 상관관계를 갖고 있었으나, *Saimiri*에 있어서는 상관관계를 유지하지 못하였다. 결론적으로 말라리아 연구시 *Aotus* 원숭이가 *Saimiri* 원숭이 보다 우수한 품종임과 말라리아 진단과정에 있어서 혈소판의 회복률과 재생은 말라리아의 감염시기 판정에 유용한 자료로서 활용될 수 있음을 관찰하였으며, 말라리아 병원성 판정에 대한 실험에 있어서 이들 두 종류 원숭이의 혈액치 변화 특이성을 보고하는 바이다.

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