ALTERATIONS IN CAPILLARY PERMEABILITY IN RHESUS MONKEYS WITH *PLASMODIUM KNOWLESI* INFECTION

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INTRODUCTION

The pathophysiological changes in the host in response to malaria infection are very complex. It has been postulated that in malaria, changes in the capillary permeability occur causing increased leakage of plasma proteins from the circulation into the extravascular compartments. This could be one mechanism for the lesions where parasitized erythrocytes are clumped in animal brain capillaries (Kontos *et al.*, 1980). A blood-brain barrier breakdown has been demonstrated in rhesus monkeys with acute *P. knowlesi* (Migasena and Maegraith, 1967). Increased capillary permeability as a result of increased surface pore area available for diffusion and increased capillary permeability coefficient has been demonstrated in patients with *P. falciparum* and rhesus monkeys infected with *P. knowlesi* or *P. coatneyi* (Areekul *et al*, 1984, 1985a, b, c). In order to ascertain these alterations, all these parameters were therefore studied in rhesus monkeys throughout the whole course of *P. knowlesi* infection.

MATERIALS AND METHODS

The studies were performed on 3 healthy rhesus monkeys (*Macaca mulatta*). They were anesthesized with intravenous injection of Sodium pentobarbital (10-15 mg/kg). About 5 microcuries of ^{125}I-human serum albumin (^{125}I-HSA) were injected intravenously into each monkey. Blood samples were taken at 10, 15, 20, 30, 40, 50 and 60 minutes for determination of radioactivity and plasma albumin concentration. Each monkey was then infected with *P. knowlesi* by intravenous injection of 2.0 to 4.0 ml of whole blood containing 0.5-2.0 million trophozoites from a donor monkey. The studies were repeated at various intervals during the whole course of the infection.

Radioactivity of the plasma samples were transformed into the logarithmic values and the regression equation for the time-concentration curves was compiled. The transcapillary escape rate (TER) was derived from the slope of the regression equation. Plasma volume was calculated from the radioactivity at time zero by retropolation of the time-concentration curve and from the injected volume of ^{125}I-HSA measured by weighing the syringes before and after injection. The clearance (Cl) and outflux of albumin were calculated by multiplying the plasma volume and the intravascular albumin mass (IVM) by TER respectively. The area of the capillary membrane available for free diffusion and the membrane thickness ($A_s/\Delta X$) and the specific permeability coefficient (P_s) were determined by the following formula described earlier (Areekul *et al.*, 1984, 1985a, b, c).

$$\frac{A_s}{\Delta X} = \frac{Cl}{D} \quad \text{..... (1)}$$

$$P_s = \frac{Cl}{A_m} \quad \text{..... (2)}$$

where D, the diffusion coefficient of albumin, is 0.085×10^{-5} cm^2/second (Schultz and Heresmans, 1966); A_m, the total membrane surface area in 100 g muscle, is 5000 cm^2 (Landis and Pappenheimer, 1963).

Table 1

Showing the plasma volume, intravascular mass of albumin (IVM), transcapillary escape rate (TER), clearance (Cl), outflux, A_s/Δ x and P_s in 3 monkeys before, during and after infection with *P. knowlesi*.

No.	Days after infection	Ht (%)	Parasite (1000 RBC^{-1})	TER (%/hr)	Cl x 10^{-5} (ml/sec/100g)	outflux (mg/kg/min.)	As/Δ x x 10^5 (cm/100g)	Ps x 10^{-5} (cm/sec)	IVM (g/kg)	PV (ml/kg)
14	0	45	0	2.16	2.69	3.65	0.00032	0.00053	1.98	50.0
(5.01 kg)	4	45	0.1	7.62	9.89	12.10	0.00116	0.00197	2.06	46.8
	7	43	0.5	3.78	4.81	5.95	0.00057	0.00096	2.02	45.8
	13	35	184.2	12.54	15.96	19.67	0.00188	0.00319	1.92	45.8
	15	31	205.4	4.20	6.16	7.56	0.00072	0.00123	2.22	52.8
	18	28	97.8	6.18	10.22	11.90	0.00120	0.00204	2.44	59.6
	25	26	75.0	12.24	19.38	20.77	0.00228	0.00387	2.22	57.0
	32	23	144.0	3.84	6.33	6.86	0.00074	0.00126	2.31	59.3
	39	22	179.5	5.22	8.28	8.97	0.00097	0.00165	2.17	57.1
	60*	38	0	1.92	2.67	3.53	0.00031	0.00053	2.20	50.0
24**	0	45	0	3.54	4.113	4.59	0.00048	0.00082	1.88	41.8
(4.1 kg)	0	45	0	5.82	6.515	7.53	0.00077	0.00130	1.81	40.3
	7	43	3.5	6.12	6.293	7.19	0.00074	0.00125	1.63	37.0
	15	35	95.0	6.42	10.48	11.76	0.00123	0.00209	2.53	58.8
	18	24	398.6	12.48	16.21	16.48	0.00191	0.00324	1.92	46.8
31	0	45	0	2.70	3.43	4.62	0.00040	0.00068	2.15	45.7
(5.1 kg)	0	45	0	5.40	6.34	8.94	0.00075	0.00126	1.99	42.3
	7	44	2.1	6.30	8.38	10.92	0.00099	0.00167	2.20	47.9
	11	41	103.5	7.38	9.82	12.33	0.00115	0.00196	2.16	47.9
	14	31	390.0	9.72	14.20	18.41	0.00167	0.00284	2.37	52.6
	20	22	124.5	6.78	10.15	10.19	0.00120	0.00203	2.10	53.9
	50*	38	0	1.68	3.01	2.66	0.00036	0.00060	1.92	50.5

* After recovery from malaria.

** Died on day 19 after infection.

Plasma albumin concentration was determined by the method of Ingwersen and Raabo (1978). The hematocrit values were obtained after centrifuging the blood samples for 5 minutes at 10,000 g.

RESULTS

Results of alterations of plasma volume, intravascular mass (IVM), clearance (Cl), outflux and transcapillary escape rate (TER) of albumin, ratio of capillary surface area for diffusion and thickness ($A_s/\Delta X$), and capillary permeability coefficient (P_s) in 3 rhesus monkeys throughout the whole courses of *P. knowlesi* infection are shown in Table 1. Fig. 1 illustrates these values in monkey No. 14. As plasma volume was found to be increased while plasma albumin concentration decreased resulting in a higher intravascular mass of albumin during malarial infection.

About one week after infection, TER started to increase which resulted in elevated Cl, outflux, $A_s/\Delta X$ and P_s. As the parasitemia climbed up, all these values increased in parallel. The parasitemia showed a direct relationship with TER, $A_s/\Delta X$ and P_s as shown in Table 2. However, no other correlation between plasma volume or intravascular mass and all other parameters were observed in the present study. All increased values returned to the normal levels after treatment.

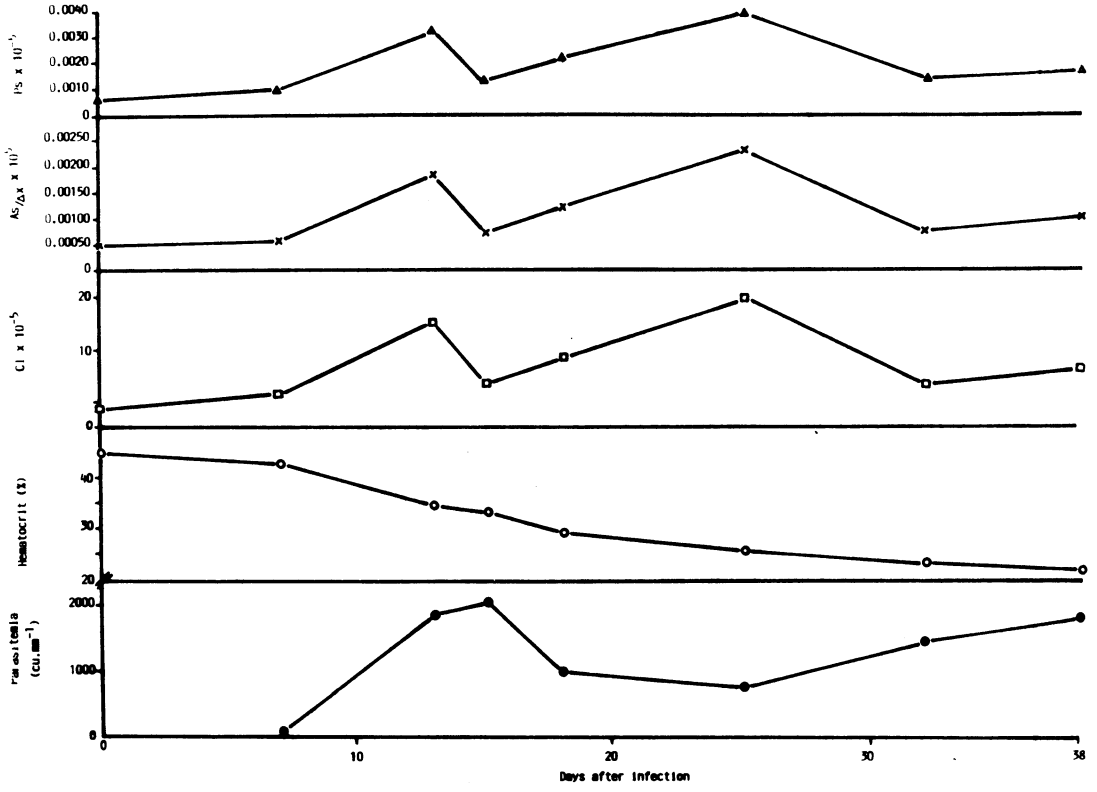

Fig. 1—Showing the alterations of Ht, Cl. $A_S/\Delta x$ and P_S and the parasitemia in monkey No. 14 throughout the whole course of *P. knowlesi* infection.

Table 2

Relationship between parasitemia, plasma volume and intravascular mass of albumin and other parameters (n = 22).

Relationship	Regression formula	r	P. value
(1). Parasitemia			
vs TER	$y = 6.1282 + 0.024\ x$	0.6320	< 0.01
vs Cl	$y = 4.6156 + 0.0162\ x$	0.6019	
vs $A_s/\Delta x$	$y = 0.00074 + 0.000002$	0.6291	
vs P_s	$y = 0.00123 + 0.000004$	0.6312	
(2). Plasma volume			
vs TER	$y = 4.84356 + 0.02522\ x$	0.04927	> 0.05
vs Cl	$y = -3.10469 + 0.22863\ x$	0.30499	
vs $A_s/\Delta x$	$y = -0.00036 + 0.000026\ x$	0.30257	
vs P_s	$y = -0.000346 + 0.000040\ x$	0.27870	
(3). IVM			
vs TER	$y = 5.1737 + 0.43766\ x$	0.02871	> 0.05
vs Cl	$y = -1.98352 + 4.95602\ x$	0.22687	
vs $A_s/\Delta x$	$y = -0.000096 + 0.000502\ x$	0.189881	
vs P_s	$y = -0.000402 + 0.000991\ x$	0.22691	

DISCUSSION

This study showed an increase in the capillary surface area and capillary permeability coefficient resulting in increased leakage of plasma albumin from the circulation into the extravascular space. These findings were in accordance with results reported in patients with *P. falciparum* and rhesus monkeys infected with *P. knowlesi* and *P. coatneyi* (Areekul *et al.*, 1984; 1985a; 1985b; 1985c).

The present study showed that plasma volume in the infected monkeys increased while plasma albumin concentration decreased with subsequent elevated intravascular mass of albumin. As there was no relationship between the increased plasma volume or IVM of albumin and TER, Cl, $A_s/\Delta X$ or P_s, the stretching pore area of the capillary membrane by the increased plasma volume or IVM as suggested by Wasserman *et al.*, (1955) did not occur in the infected monkeys.

Increased TER, Cl, $A_s/\Delta X$ and P_s were found in monkeys after infection for one week even with a very low infection rate, i.e., as low as 0.1 per 1000 red cells and then increased in parallel to the degree of parasitemia. The direct correlation between parasitemia and TER, Cl, $A_s/\Delta X$ and P_s indicated that malarial parasites probably play some role in causing increased capillary permeability in these monkeys.

Considerable interest has been aroused in the presence of endogenous substances in plasma which when activated induced pathological increase in capillary permeability in subjects with malarial infection. Pharmacologically active peptides and uncategorized permeability factor were detected in the serum of mice infected with *P. berghei* and

monkeys with *P. coatneyi*, respectively (Goodwin and Richards, 1969; Desowitz and Pavanand, 1967). A significant increase in bradykinin and enzyme kallikrein were also reported in monkeys with *P. knowlesi*, *P. inui* and *P. coatneyi* (Onabanjo and Maegraith, 1970; 1971). It has been postulated that both kinin and kallikrein were responsible for causing increased permeability of the capillary endothelial membrane (Maegraith and Fletcher, 1972). Other mechanisms that affect capillary endothelium especially in cerebral malaria has also been put forward. It has been observed that monocytes metabolizing arachidonic acid became vacuolated and produced free oxygen radicals when they phagocytose particles (Johnson *et al.*, 1976). These free oxygen radicals probably damaged the endothelium of the capillary membrane causing progressive loss of large molecules including albumin and other proteins from the blood vessels (Kontos *et al.*, 1980). Further studies on the mechanism of induced pathological increase in capillary permeability in subjects with malarial infection are in progress.

SUMMARY

The transcapillary escape rate and capillary permeability to albumin were studied in 3 rhesus monkeys throughout the whole course of *P. knowlesi* infection. The transcapillary escape rates started to increase on the first week after infection and were significantly higher than the control values during infection. Plasma volume was found to be increased while plasma albumin concentration decreased which resulted in the increased intravascular mass, outflux and clearance of albumin from the intravascular to the extravascular compartments. Increased $A_s/\Delta X$ and P_s during malarial infection indicated that the increased capillary permability was due to increased capillary surface area and the increased endothelial permeability. As there was no relationship between increased plasma volume or intravascular mass of albumin and $A_s/\Delta X$ or P_s, the increased capillary permeability in these infected monkeys could not be explained by the stretching pore phenomena. As these parameters show a close relationship to the parasitemia, therefore malarial parasites may play a role in causing increased capillary permeability.

REFERENCES

AREEKUL, S., KASEMSUTH, R. and KANAKAKORN, K., (1984). Studies on the transcapillary escape rate of fibrinogen and capillary permeability in patients with *Plasmodium falciparum* malaria. *Trop. Geogr. Med., 36* : 151.

AREEKUL, S., (1985 a). Studies on the transcapillary escape rate of albumin and capillary permeability in rhesus monkeys infected with *Plasmodium knowlesi* (in press).

AREEKUL, S., (1985 b). Transcapillary escape rate and capillary permeability to albumin in patients with *Plasmodium falciparum* (in press).

AREEKUL, S., (1985 c). *Plasmodium coatneyi*: Alterations of transcapillary escape rate and capillary permeability to fibrinogen in rhesus monkeys (in press).

DESOWITZ, R.S. and PAVANAND, K., (1967). A vascular permeability increasing factor in the serum of monkeys infected with primate malaria. *Ann. Trop. Med. Parasitol., 61* : 128.

GOODWIN, L.G. and RICHARDS, W.G., (1960). Pharmacologically active peptides in the blood and urine of animals infected with *Babesia rodhaini* and other pathogenic organisms. *Brit. J. Pharm. Chemother., 15* : 152.

INGWERSEN, S. and RAABO, E., (1978). Improved and more specific bromcresol green methods for the manual and auto-

matic determination of serum albumin. *Clin. Chim. Acta., 88* : 545.

JOHNSON, R.B., LEHMEYER, J.E. and GUTHRIE, L.A., (1976). Generation of superoxide anion and chemiluminescence by human monocytes during phagocytosis and on contact with surface bound immunoglobulin G. *J. Exp. Med., 143* : 1551.

KONTOS, H.A., WEI, E.P., POVLISHOCK, J.T., DIETRICH, W.D., MAGIERA, C. J. and ELLIS, E.F., (1980). Cerebral arteriolar damage by arachidonic acid and prostagandin G_2. *Science, 209* : 1242.

LANDIS, E.M. and PAPPENHEIMER, J.R. (1963). Exchange of substances through the capillary walls. *In:* Handbook of Physiology. Hamiltion, W.F. (Ed.) Section 2, Vol 2, p. 961. Amer. Physiol. Soc., Washington.

MAEGRAITH, B.G. and FLETCHER, A., (1972). The pathogenesis of mammalian malaria. *In:* Advances in Parasitology. Ben Dawes (Ed.), Vol 10, p. 49. Academic press, London.

MIGASENA, P. and MAEGRAITH, B.G., (1967). Pharmacological action of antimalarial drugs. Action of chloroquine and hydrocortisone on blood brain barrier in *Plasmodium knowlesi* malaria. *Trans. Roy. Soc. Trop. Med. Hyg., 61* : 6.

ONABANJO, A.O. and MAEGRAITH, B.G., (1970). Kallikrein as a pathogenic agent in *Plasmodium knowlesi* infection in *Macaca mulatta. Brit. J. Exper. Path., 51* : 523.

ONABANJO, A.O. and MAEGRAITH, B.G., (1971). Pathological lesions produced in the brain by kallikrein (kininogenase) in *Macaca mulatta* infected with *Plasmodium knowlesi. Ann. Trop. Med. Parasitol., 64* : 237.

SCHULTZ, H.E. and HERESMANS, J.P., (1966). *Molecular Biology of Human Proteins.* Vol. 1, Elsevier, Amsterdam.

WASSERMAN, K., LOEB, L. and MEYERSON, H.S., (1955). Capillary permeability to macromolecules. *Cir. Res., 3* : 594.