RENAL PLASMA FLOW IN RHESUS MONKEYS INFECTED WITH *PLASMODIUM KNOWLESI*

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INTRODUCTION

The pathophysiological changes in patients with malaria are complex and involve many organs. Renal involvement sometimes occur and can vary from case to case. Impaired renal function has been reported in a few patients, while it was normal in uncomplicated cases of *P. falciparum* malaria (Sitprija *et al.*, 1966). Glomerulonephritis and acute renal failure due to acute tubular necrosis has been reported in patients with falciparum malaria (Stone *et al.*, 1972; Hartenbower *et al.*, 1972; Berger *et al.*, 1967; Rosen *et al.*, 1968). The underlying mechanisms causing these pathophysiological changes have not been fully elucidated. It has been suggested that disturbances in the renal microcirculation are responsible for acute renal failure, from the observations of a marked reduction in perfusion of the kidney of rhesus monkeys infected with *P. knowlesi* (Chongsuphajaisiddhi, 1966). In order to investigate this problem, the present study was undertaken to measure renal plasma flow by using ^{125}I-sodium orthoiodohippurate as a tracer in monkeys infected with *P. knowlesi.*

MATERIALS AND METHODS

The studies were performed on 6 rhesus monkeys (*Macaca mulatta*), Thai strain, weighing between 2.2 and 5.5 kg. Monkeys were anesthesized with intravenous injection of Nembutal sodium (30 mg/kg). Each monkey was injected intravenously with 10 microcuries of ^{125}I-sodium orthoiodohippurate (^{125}I-OIH). Blood samples were taken at 10, 15, 20, 25, 30, 40, 50 and 60 minutes after injection. Plasma samples were assayed for radioactivity in a well type scintillation counter and expressed as counts per minute per milliliter. Each monkey first served as the control monkey. Then it was infected with *P. knowlesi* malaria by intravenous injection of 1-2 ml of whole blood containing about 1-2 million trophozoites from a donor monkey. When the parasitemia was high, the studies were repeated.

The plasma radioactivity (expressed as counts per minute per ml) was plotted against time on the semilog paper as illustrated in Fig. 1. A close fit to the observations was obtained by using the equation :-

$$C_t = A.e^{-\gamma_a^t} + B.e^{-\gamma_b^t} \quad \ldots\ldots\ldots (1)$$

Where A, B and γ_a, γ_b were the intercepts on the ordinate and the slopes of these 2 curves respectively. Fractional disappearance rates γ_a and γ_b were determined from half time (t_a and t_b) of the lines by the equation:-

$$\gamma_a = \frac{\ln 2}{t_a} \text{ and } \gamma_b = \frac{\ln 2}{t_b} \quad \ldots\ldots\ldots (2)$$

From these values, the volumes, flow rates and fractional rate constants could be determined by using an open 2 compartment system as shown in Fig 1. Let I designated dose given, V_1 and V_2 were the initial and exchangeable volumes respectively and F_{12} and F_{21} designated the intercompartmental flow rates This model required that F_{12} and F_{21} be equal in magnitude but differing only in sign. V_3 was used to designate the end volume, in this case the kidney and F_{13} designated the flow

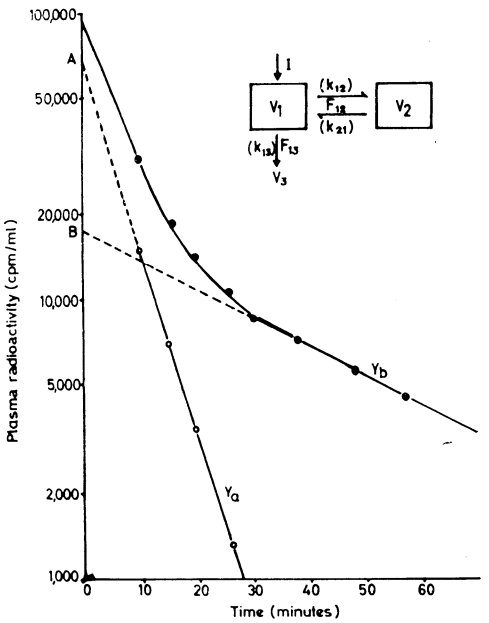

Fig. 1—Plasma disappearance curve of ^{125}I-OIH, it resolved into 2 curves with slopes y_a and y_b and with intercepts A and B. A model of the open two-compartment mamillary system is shown on the right.

from V_1 into V_3, i.e., renal plasma flow. Fractional intercompartmental rate constants were designated k_{12}, k_{21} and k_{13}. All these parameters were calculated by the following formulae of Sapirstein *et al.*, (1955).

$$F_{13} = \frac{I\,\gamma_a\,\gamma_b}{A\gamma_b + B\gamma_a} = \frac{I \times 0.693}{A\,t_a + B\,t_b} \quad \ldots\ldots (3)$$

$$V_1 = \frac{I}{A + B} \quad \ldots\ldots (4)$$

$$F_{12} = \frac{V_1\,(A\,\gamma_a + B\,\gamma_b)}{A + B} - F_{13} \quad \ldots\ldots (5)$$

$$V_2 = \frac{F_{13}\,F_{12}}{V_1\,\gamma_a\,\gamma_b} \quad \ldots\ldots (6)$$

$$k_{12} = \frac{F_{12}}{V_1} \quad \ldots\ldots (7)$$

$$k_{21} = \frac{F_{21}}{V_2} \quad \ldots\ldots (8)$$

$$k_{13} = \frac{F_{13}}{V_1} \quad \ldots\ldots (9)$$

As V_1+V_2 was the product of the mean transit time ($\bar{t}$) of the tracer in the organ and renal plasma flow (F_{13}). Therefore $\bar{t}$ could be obtained from:-

$$t = \frac{V_1 + V_2}{F_{13}} \quad \ldots\ldots (10)$$

Statistical analysis of all data was done using comparison of paired data by the t-test, difference method. The significance of the correlation coefficient (r) was estimated using the relationship $t_{(n-2)} = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$

RESULTS

Results of the renal plasma flow (F_{13}) and intercompartmental flow rate (F_{12} or F_{21}) in the normal and infected monkeys are shown in Table 1. The mean values of renal plasma flow in the infected group, either expressed as ml/min or ml/min/kg, were significantly lower than those of the normal group ($p < 0.05$). However, the plasma flow rates from V_1 to V_2 (F_{12}) or vice versa (F_{21}) were not significantly different ($p > 0.05$) between these 2 groups of monkeys.

Table 2 shows the initial and exchangeable volumes (V_1, V_2), fractional intercompartmental rate constants (k_{12}, k_{21}, and k_{13}) and the mean transit time ($\bar{t}$) of these monkeys. There was no alteration of V_1, V_2, $V_1 + V_2$, k_{12} and K_{21} in the infected group. However, as the renal plasma flow was diminished, this resulted in a significantly reduced ($p < 0.05$) rate constant (k_{13}) from V_1 to the kidney and a slightly but not significantly elevated mean transit time ($p > 0.05$) in the infected group. The parasitemia showed a reverse relationship with F_{13}, when expressed as ml/min/kg ($a = 13.46$, $b = -0.041$, $r = -0.814$, $p < 0.05$) and a direct relationship with the mean transit time

Table 1

The hematological values, renal plasma flow (F_{13}) and the intercompartmental flow rates (F_{12} or F_{21}) in normal and *P. knowlesi*-infected monkeys.

No.	B.W. (kg)	Hb (g%)	Ht (%)	F_{13}		F_{12}		Parasitized RBC 1000^{-1} RBC
				(ml/min)	(ml/min/kg)	(ml/min)	(ml/min/kg)	
Normal monkeys (n = 6)								
3	3.7	9.8	33	45.41	12.27	37.5	10.14	—
4	2.6	12.6	38	42.62	16.39	22.0	8.46	—
5	2.3	11.7	38	36.25	15.76	21.9	9.52	—
6	3.7	12.3	39	68.94	18.63	23.1	6.24	—
7	2.9	12.5	42	30.55	10.53	27.0	9.31	—
8	5.3	10.8	37	65.19	12.30	43.7	8.25	—
mean	3.4	11.6	38	48.16	14.31	29.2	8.65	—
SD	1.1	1.1	3	15.57	3.08	9.2	1.37	—
Infected monkeys (n = 6)								
3	3.5	9.3	30	30.86	8.82	18.1	5.17	96
4	3.0	11.3	32	6.08	2.03	19.7	6.57	299
5	2.2	10.6	35	30.42	13.83	14.4	6.52	22
6	3.9	11.4	35	55.64	14.27	27.3	7.00	77
7	3.0	11.5	39	20.02	6.67	21.5	7.17	128
8	5.5	9.8	32	29.15	5.30	85.8	15.60	94
mean	3.5	10.6	34	28.70	8.49	31.1	8.01	—
SD	1.1	0.9	3	16.25	4.84	27.1	3.79	—

Table 2

The initial and exchangeable volumes (V_1 and V_2), fractional intercompartmental rate constants (k_{12}, k_{21}, k_{13}) and mean transit time ($\bar{t}$) in normal and *P. knowlesi*-infected monkeys.

No.	V_1		V_2		k_{12}	k_{21}	k_{13}	$\bar{t}$
	(ml)	(ml/kg)	(ml)	(ml/kg)				(min^{-1})
Normal monkeys (n = 6)								
3	584	157.8	675	182.4	0.064	0.056	0.077	27.73
4	607	233.5	454	174.6	0.036	0.048	0.070	24.89
5	449	195.2	443	192.6	0.048	0.049	0.080	24.61
6	1005	271.6	315	85.1	0.023	0.073	0.069	23.72
7	484	166.9	456	157.2	0.056	0.059	0.063	30.77
8	656	123.8	685	129.2	0.066	0.063	0.099	20.57
mean	631	191.5	505	153.5	0.048	0.058	0.076	25.38
SD	199	53.9	146	40.3	0.016	0.009	0.012	3.50
Infected monkeys (n = 6)								
3	883	252.3	356	101.7	0.020	0.050	0.034	40.15
4	429	143.0	640	213.3	0.046	0.031	0.014	175.82
5	701	318.6	786	357.4	0.020	0.018	0.043	48.88
6	777	199.2	664	170.3	0.035	0.041	0.071	25.90
7	473	157.7	579	193.0	0.045	0.037	0.042	55.55
8	1230	223.6	1556	282.9	0.070	0.055	0.024	95.57
mean	749	215.7	764	219.8	0.039	0.038	0.038	73.65
SD	294	64.7	413	89.5	0.018	0.013	0.019	55.25

($a = 12.767, b = 0.510, r = 0.874\ p < 0.05$). There was no other relationship between parasitemia and F_{12}, V_1, k_{12}, k_{21} or k_{13}.

DISCUSSION

The renal plasma flow is usually determined by the conventional continuous - infusion technique with para-aminohippurate (PAH). Recently single injection techniques with radioisotopes such as ^{125}I-orthoiodohippurate (^{125}I-OIH) or ^{131}I-hippuran have gained wide popularity due to their technical simplicity and lack of untoward effects. Many studies have shown that the renal plasma flow obtained by the radioisotopes techniques correlated favourably with the classic clearance technique of PAH and the direct renal blood flow measurement (Gott *et al.*, 1962; Blaufox *et al.*, 1963; Stokes and Ter-Pogossian, 1964). In the present study, ^{125}I-OIH was used for measurement of renal plasma flow, volume of distribution and fractional rate constants in the normal and *P. knowlesi*–infected monkeys. A mean value $\pm$ one standard deviation of the renal plasma flow in 6 normal monkeys was found to be 14.31 ± 3.08 ml/min/kg. This result was in accordance with that of 11.9 ml/min/kg (9.5 – 14.4 ml/min/kg) obtained by the PAH clearance technique in 8 unanesthesized rhesus monkeys (Weigel *et al.*, 1966). These findings indicated the validity of the methods used in the present study.

The present study showed that the renal plasma flow (F_{13}) was significantly reduced in the *P. knowlesi*–infected monkeys. As V_1 and V_2 were slightly but not significantly elevated, which resulted in a significantly reduced k_{13} and an elevated $\bar{t}$. All these findings indicated that the renal plasma flow, the rate constant from intravascular compartment to the kidney were depressed and a prolonged mean transit time in monkeys infected with *P. knowlesi*. These findings were in accordance with reports in patients with *P. falciparum* with severe renal complications. A decrease in endogenous creatinine and PAH clearance was observed in 3 azotemic patients with severe falciparum malaria (Sitprija *et al.*, 1967). A selective renal angiographic technique demonstrated diminished cortical renal blood flow in one *P. falciparum* patient with acute renal failure (Arthachinta *et al.*, 1974). The total renal blood flow was found to be diminished in 2 out of 3 *P. falciparum* patients with acute renal failure by using ^{133}Xe (Sitprija *et al.*, 1977). Mice infected with *P. berghei* also showed the absence or low excretion of phenolsulfonaphthalein with an elevated blood urea nitrogen (Miller *et al.*, 1968). As there were no tubular pathological changes in these infected mice, this finding would implicate reduction of blood flow in the proximal tubules (Miller *et al.*, 1968).

Monkeys infected with *P. knowlesi* usually develop hemoglobinuria, oliguria and finally acute renal failure (Splangler *et al.*, 1978). Renal tubular necrosis and hemoglobin casts are consistent findings in these infected monkeys (Taliaferro *et al.*, 1937; Menon, 1939; Rosen *et al.*, 1968). The cause leading to renal failure in malaria is not well established. As tubular obstruction by hemoglobin casts have never been demonstrated, inadequate renal perfusion may be an important contributing factor in the pathogenesis of this lesion. Findings of significantly reduced GFR in monkeys infected with *P. knowlesi* supported this hypothesis (Areekul and Chantachum, 1984). It is possible that inadequate renal perfusion is secondary to the decrease in renal blood flow and if not treated properly, it may result in acute tubular necrosis. This mechanism has been proposed to occur in patients with *P. falciparum* who developed acute renal failure with oliguria and urea retention (Sitprija, 1970).

Several factors could be responsible for the decreased renal blood flow in malaria. The

findings of a reverse relationship between parasitemia and renal plasma flow in the present study indicated that the reduction in renal perfusion secondary to renal vasoconstriction might be the effect of malaria. Constriction of the renal arteries and arterioles has been shown in *P.knowlesi* infected monkeys particularly in shock and this was reversed by adrenergic blocking agents (Chongsuphajaisiddhi, 1966). In 2 patients with transient renal failure, dibenzyline intravenous injection resulted in increased urine flow and sodium excretion, suggesting the important role of renal vasoconstriction as a cause of decreased renal perfusion (Sitprija, 1970). Afferent arteriolar constriction decreased not only the rate of renal blood flow but also decreased the glomerular pressure, both decreasing the GFR. This has been illustrated by the finding that stimulation of the vasoconstrictor nerve activity to the kidneys in experimental animals resulted in reducing renal blood flow, GFR and urine flow (Selkurt, 1963).

SUMMARY

Renal plasma flow was studied in six rhesus monkeys before and during infection with *Plasmodium knowlesi* using ^{125}I-sodium orthoiodohippurate (^{125}I-OIH) as a tracer. The mean renal plasma flow and the rate constant from the intravascular compartment to the kidneys were significantly reduced in the infected monkeys. As both intravascular and extravascular compartments were slightly but not significantly elevated, which resulted in the prolonged mean transit time of ^{125}I-OIH in monkeys infected with *P. knowlesi*. The parasitemia showed a reverse relationship with the renal plasma flow and a direct relationship with the mean transit time. These findings indicated that the renal plasma flow in the infected monkeys was depressed in proportion to the number of *P. knowlesi* parasites. The mechanism of reduced renal plasma flow was probably due to renal vasoconstriction.

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