PITUITARY-ADRENAL FUNCTION IN UNCOMPLICATED FALCIPARUM MALARIA

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Abstract. To investigate pituitary-adrenal function in acute uncomplicated falciparum malaria, we performed an overnight dexamethasone suppression test in 13 Vietnamese adults with acute malaria and 6 healthy controls. After blood samples were taken for serum cortisol and plasma ACTH at 23.00 hours on the admission day, 1 mg dexamethasone was given and further samples were taken at 08.00, 16.00 and 23.00 hours the next day. The patients received conventional antimalarial and supportive treatment. Baseline plasma ACTH concentrations in the patients [3.9 (0.2-41.2) pmol/l] and controls [3.4 (1.1-4.3) pmol/l] were similar (p=0.51), and exhibited a similar fall after dexamethasone to 0.6 (0.2-2.5) and 0.9 (0.7-1.6) pmol/l at 08.00 hours respectively (p<0.03 *vs* 23.00 hour values). Serum cortisol levels before dexamethasone were higher in the patients than in the controls [456 (102-821) *vs* 145 (64-183) nmol/l respectively; p=0.007] and the overnight fall was less in the patients [208 (26-340) and 23 (15-46) nmol/l at 08.00 hours respectively; p<0.001 *vs* 23.00 hour values and between groups]. Between 08.00 and 23.00 hours, plasma ACTH and serum cortisol remained suppressed in the controls. In the patients, the serum cortisol continued to fall progressively towards control values. These data suggest that there is a raised set point for cortisol inhibition of ACTH secretion but normal corticotrophin responsiveness to dexamethasone in uncomplicated malaria. A raised serum cortisol after dexamethasone in the patients might reflect the combination of a prolonged cortisol half-life and the stimulatory effects of cytokines on the adrenal cortex, with a consequent protective effect against complications such as hypoglycemia.

INTRODUCTION

In view of complications such as complications of malaria such as hypoglycemia, hypotension and hyponatremia (Warrell *et al*, 1990), adequate adrenocortical function is an important part of the response to malaria infection. We have found previously that evidence of both primary and secondary adrenal insufficiency can be found in patients with severe falciparum malaria (Davis *et al*, 1997). Basal serum cortisol and ACTH concentrations were inappropriately low in adult Vietnamese patients with complicated *Plasmodium falciparum* infections, and there was an attenuated ACTH response to corticotrophin releasing hormone (CRH) (Davis *et al*, 1997). In other severe illnesses including sepsis, serum cortisol and ACTH levels are, by contrast, usually high, and the response to CRH is preserved (Reincke *et al*, 1993). Hypothalamic-pituitary-adrenal (HPA) activation in non-malarial severe illness might be due to the effects of cytokines, the noradrenergic system, hypersecretion of CRH-like peptides and/or pituitary resistance to glucocorticoids (Reincke *et al*, 1993; Van den Berghe, 2000). These factors may also operate in malaria (Davis *et al*, 1997), but could be offset by other malaria-specific effects, such as sequestration of parasitized erythrocytes within the hypothalamic-

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pituitary microvasculature and/or parasite production of a somatostatin-like peptide on corticotrophin function (MacPherson *et al*, 1985; Pan *et al*, 1987), which reduce stress-associated HPA axis activation.

Uncomplicated malaria is, by definition, neither associated with a high parasitemia nor significant microvascular sequestration (Warrell *et al*, 1990; Davis *et al*, 1999). Therefore, malaria-specific factors that attenuate HPA axis function should be minimal. Published data relating to HPA function in uncomplicated malaria are few. Brooks *et al* (1969) found raised plasma 17-OH corticosteroids but normal diurnal variation in patients who were likely to be non-severe, but no studies specifically assessing corticotrophin function have been performed.

We hypothesized that corticotrophin resistance, either in the form of an altered set-point for inhibition by basal serum cortisol concentrations or reflecting a more generalized hyporesponsiveness, is a part of the effects of acute uncomplicated malaria on HPA function. We tested this hypothesis by performing 1 mg dexamethasone tests in well-characterized Vietnamese patients and matched controls.

MATERIALS AND METHODS

Subjects

We studied 13 Vietnamese patients aged 21 to 45 years (Table 1) who had been admitted to Cho Ray Hospital, Ho Chi Minh City or to Lamdong Provincial Hospital, Dalat City. No patient had concomitant illness or had received recent corticosteroid therapy. All patients had uncomplicated falciparum malaria (Warrell *et al*, 1990), and were fully conscious and orientated, with neither significant hepatic dysfunction (defined as serum bilirubin and transaminase concentrations more than twice the upper limit of the reference range) nor renal impairment (oliguria and serum creatinine concentration >250 μmol/l). None of the patients were anemic (venous hematocrit <21%) nor hyperparasitemic (parasite density >250,000/μl whole blood). Plasma glucose concentrations were all >4.2 mmol/l.

Six healthy Vietnamese volunteers aged 24 to 45 years were also recruited to undergo dexamethasone suppression testing (Table 1). These controls were matched as closely as possible to the 13 patients for age, gender and body weight. In addition, we had access to morning (08.00 hours) unsuppressed serum cortisol and plasma ACTH data from a further 6 healthy controls studied previously (Davis *et al*, 1997; Table 1). None of the 12 controls were slide positive for malaria and all had normal hepatorenal function on conventional biochemical testing. Each subject (patients and controls) gave informed consent to study procedures which were approved by the Director and the Ethics Committee of Cho Ray Hospital, Vietnam.

Methods

Each of the 13 patients was assessed fully at presentation, including a blood smear for confirmation of the diagnosis by microscopy, and routine hematological and biochemical tests. Treatment with oral artesunate or artemisinin was commenced or continued, rehydration was instituted, and antipyretic and antiemetic drugs were given as required. At 23.00 hours on the admission day, a venous blood sample was taken and 1 mg dexamethasone given by mouth. Further venous samples were taken at 08.00, 16.00 and 23.00 hours the following day. Patients were assessed clinically at each sampling time point, and oral temperature, pulse and blood pressure were measured every 4 hours. Each of the 6 control subjects who had an overnight dexamethasone test were studied using an identical protocol to that of the patients.

All blood samples were taken into tubes kept on wet ice. After prompt centrifugation, separated sera and plasma were stored and transported below -20°C until assayed. Serum cortisol and plasma ACTH were measured by fluorometric enzyme immunoassay (Stratus, Baxter Diagnostics, Deerfield. Illinois, USA) and chemiluminescence immunometric assay (Nichols Institute, San Capristrano, California,

USA) respectively (Davis *et al*, 1997). Interassay precision was <10% and <5% for ACTH and cortisol respectively over the range of concentrations encountered in the present study.

Statistical analysis was by nonparametric methods (SPSS; SPSS Inc, Chicago, Illinois, USA). Two-sample comparisons were by Wilcoxon-Mann-Whitney tests and comparisons of multiple related samples by Friedman test. Associations between variables were assessed using the Spearman's rank correlation co-efficient. A two-tailed significance level was used. Data are presented as median and [range].

RESULTS

Clinical course

At study entry, the patients had higher serum aspartate transaminase, bilirubin and creatinine concentrations than the controls (p=0.035, 0.002 and 0.035 respectively; Table 1) and had a lower venous hematocrit. There were no adverse events during the 24-hour study period in either patients or controls. In particular, none of the patients developed hyperpyrexia (oral temperature >40°C), hypoglycemia (plasma glucose <3.0 mmol/l) or hypotension (systolic blood pressure <90 mm Hg or diastolic blood pressure <60 mm Hg) during the period of blood sampling. All patients responded to antimalarial treatment, comprising three days of an artemisinin drug and single-dose mefloquine on day 3, and were discharged afebrile and aparasitemic within 4 days of admission.

Plasma ACTH

Plasma ACTH concentrations at 23.00 hours on the day of admission were similar in the patients [3.9 (0.2-41.2) pmol/l] and 6 controls [3.4 (1.1-4.3) pmol/l; p=0.51; Fig 1]. After dexamethasone administration, there was a significant and similar overnight fall in both groups to 08.00 hours values on day 2 of 0.6 (0.2-2.5) and 0.9 (0.7-1.6) pmol/l respectively (p<0.03 *vs* 23.00 hours values on day 1 in each case). In both case and control groups in the present study, the 08.00 hour concentrations were significantly lower than those in the 6 other healthy young unsuppressed Vietnamese adults [Davis *et al*, 1997; 3.5 (1.9-13.4) pmol/l; p<0.002; Fig 1]. One patient had an initial plasma ACTH of 41.2 pmol/l, well above the control range, but this had fallen to 2.3 pmol/l by 08.00 hours. In the patient group as a

Table 1

Details of the patients and healthy volunteer controls. The first two columns contain data from subjects undergoing overnight dexamethasone suppression testing. The third column has details of 6 healthy controls from whom a single morning blood sample was taken without prior suppression with dexamethasone (from Davis *et al*, 1997). Unless otherwise stated, median and range.

	Patients	Controls	Unsuppressed controls
Number	13	6	6
Age (years)	28 (21-45)	33 (24-45)	41 (25-50)
Males (%)	77	50	33
Body weight (kg)	48 (42-61)[a]	54 (44-63)	52 (48-73)
Oral temperature (°C)	38.5 (36.5-40.2)[b]	36.8 (36.5-37.0)	36.7 (36.2-37.1)
Venous hematocrit (%)	31.0 (27.0-40.0)[a]	38.0 (34.0-44.5)	36.5 (32-42)
Parasitemia (/μl)	10,320 (50-228,500)	-	-
Serum creatinine (μmol/l)	97 (70-132)[a]	84 (53-106)	64 (46-97)
Serum aspartate transaminase (U/l)	28 (24-34)[a]	24 (18-31)	25 (15-31)
Serum bilirubin (μmol/l)	29 (15-34)[b]	10 (8-15)	6 (4-8)

[a]p<0.05, [b]p<0.01 *vs* controls as a single group (n=12).

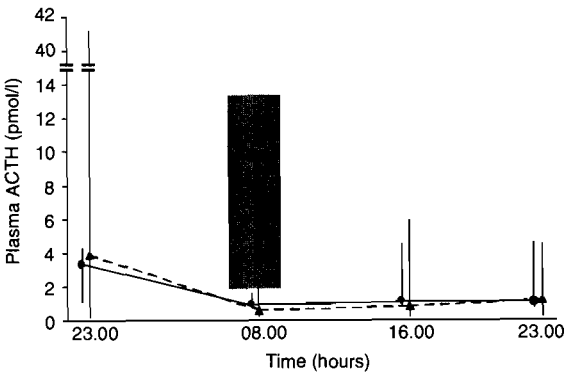

Fig 1–Median and range (vertical bars) for plasma ACTH in 13 patients (▲----▲) and 6 controls (●——●) undergoing a low-dose dexamethasone test. Dexamethasone (1 mg) was given immediately after the first 23.00-hour sample. The shaded area represents the previously published range of 08.00-hour values for 6 healthy Vietnamese controls who did not receive dexamethasone (see text).

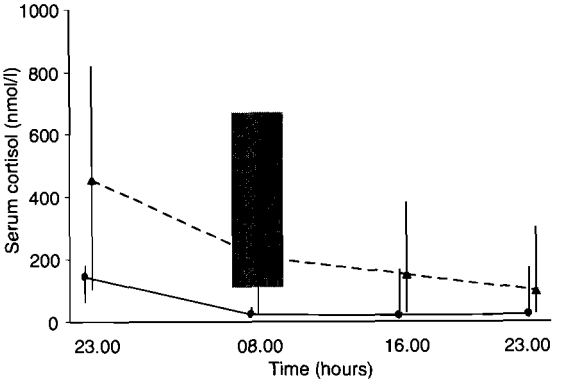

Fig 2–Median and range (vertical bars) for serum cortisol in patients (▲----▲) and 6 controls (●——●) undergoing a low-dose dexamethasone test. The shaded area represents the previously published range of 08.00-hour values for 6 healthy Vietnamese controls who did not receive dexamethasone (see text).

whole, there was no correlation between plasma ACTH concentrations at 23.00 hours on day 1 and those at 08.00 hours on day 2 (r_s=-0.19; n=13; p=0.53).

Plasma ACTH concentrations remained stable from 08.00 to 23.00 hours (p=0.28 by Friedman test) in the control group but there was a small but significant increase in the patients [1.2 (0.2-4.5) pmol/l at 23.00 hours on day 2; p=0.012 by Friedman test *vs* 08.00 and 16.00 hour values; Fig 1].

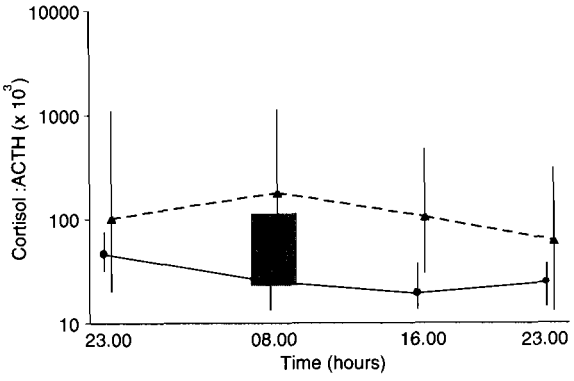

Fig 3–Semi-logarithmic plot of median and range (vertical bars) for serum cortisol:plasma ACTH ratio in patients (▲----▲) and 6 controls (●——●) undergoing a low-dose dexamethasone test. The shaded area represents the range calculated from previously published values for 08.00 hour serum cortisol and plasma ACTH in 6 healthy Vietnamese controls who did not receive dexamethasone (see text).

Serum cortisol

Serum cortisol levels before dexamethasone were significantly higher in the patients than in the controls [456 (102-821) *vs* 145 (64-183) nmol/l respectively; p=0.007; Fig 2]. There was also an overnight fall in both groups to 208 (26-340) and 23 (15-46) nmol/l respectively on 08.00 hours on day 2 (p<0.001 *vs* 23.00 hours values on day 1 in each case; Fig 2). However, whereas 08.00 hours concentrations on day 2 in the controls were significantly lower than in the six unsuppressed volunteers [Davis *et al*, 1997; 190 (110-676 nmol/l, p<0.001], the concentrations in most of the patients at this time were within the unsuppressed control range (p=0.45).

Serum cortisol concentrations remained suppressed but stable from 08.00 to 23.00 hours (p=0.39 by Friedman test) in the control group but there was a significant further fall in the

patients to 208 (26-340) pmol/l at 16.00 hours and 97 (26-301) pmol/l at 23.00 hours on day 2 (p=0.006 by Friedman test *vs* 08.00 hour values in each case; Fig 2). In the present study, the percentages of patients who had a serum cortisol below 5 mg/dl (138 nmol/l), the conventional cut point for a normal response (Zimmerman and Coryell, 1987), at 08.00, 16.00 and 23.00 hours were 42%, 50% and 67% respectively. For the controls, the equivalent percentages were 100%, 83% and 83% respectively.

Serum cortisol: plasma ACTH ratio

In order to assess the serum cortisol concentration profiles in the two groups against the simultaneous plasma ACTH, we calculated a ratio of the two measures for each patient and time point. The results are summarized in a semi-logarithmic plot in Fig 3. The cortisol: ACTH ratio before dexamethasone in the patients was significantly higher than that in the controls [102 (20-1095) *vs* 47 (32-76) x 10^{-3} respectively; p=0.039]. At 08.00 hours on day 2, the median ratio had doubled in the patients but had halved in the controls (Fig 3). The ratio then fell progressively in the patients to below baseline at 23.00 hours [62 (13-305) x 10^{-3}] but remained stable in the controls.

DISCUSSION

The present data and those we have published previously (Davis *et al*, 1997) highlight key changes in HPA function as falciparum malaria progresses from a mild to a severe infection. Our present results provide evidence that there is an altered 'set point' for cortisol inhibition of ACTH secretion by corticotrophins in uncomplicated malaria. Pre-dexamethasone serum cortisol concentrations in our patients were raised compared to controls but plasma ACTH concentrations were inappropriately normal. This may constitute an important initial protective effect against complications, such as hypoglycemia, that result from relative hypocortisolism. Despite this basal resetting of corticotrophin function, overnight pituitary corticotrophin suppression occurred after 1 mg dexamethasone in our patients. This suggests that there was adequate absorption of replacement-dose dexamethasone and argues against generalized corticotrophin resistance in our patients. We infer that the secondary adrenal insufficiency associated with severe malaria (Davis *et al*, 1997) develops only when complications are well established. Whether there is an intervening period of increased and unsuppressible ACTH secretion, such as found in other severe illnesses requiring intensive care (Renicke *et al*, 1993), is unknown but seems unlikely. There was no association between plasma ACTH concentrations at 23.00 hours on day 1 and 08.00 hours on day 2 in our patients, suggesting that those with the greatest baseline HPA activation were not necessarily the most resistant to dexamethasone suppression.

Despite the normal plasma ACTH response to dexamethasone, and in contrast to the changes observed in our controls, serum cortisol concentrations at 08.00 hours were suppressed in only the minority (31%) of our patients. Serum cortisol did not fall overnight in parallel with plasma ACTH in our patients and the cortisol:ACTH ratio had increased rather than decreased at 08.00 hours. One of the likely contributing factors to these changes is impaired serum cortisol metabolism, a suggestion advanced by Brooks and coworkers (1969) in a report of patients who, from available data, appeared mostly non-severe. Although measures of liver function were not presented, these authors found increased plasma 17-hydroxycorticosteroid concentrations and attributed them to malaria-associated hepatic dysfunction and consequently reduced cortisol metabolism (Brooks *et al*, 1969).

We have observed recently that, in Vietnamese patients with severe malaria, there was a prolonged serum cortisol half-life (median 4.6 *vs* 1.6 hours in controls) which correlated positively with serum bilirubin (Davis *et al*, 1997). Although none of the patients in the present study was jaundiced clinically, their serum aspartate transaminase and bilirubin concentrations were significantly greater than those

in the control group. It is, therefore, possible that mild degrees of hepatic dysfunction in malaria augment serum cortisol concentrations through reduced metabolism. The continued linear fall in median serum cortisol at 16.00 and 23.00 hours in the presence of plasma ACTH concentrations that were low but tending to increase is also consistent with a prolonged cortisol half-life in uncomplicated malaria. In a review of 53 studies using the 1 mg dexamethasone suppression test, Zimmerman and Coryell (1987) found, similarly to our control group, that the percentages of normal subjects with a serum cortisol below 5 μg/dl (138 nmol/l) at 08.00, 16.00 and 23.00 hours were relatively constant at 96.4%, 92.6% and 93.7% respectively. In the present study, the equivalent percentages in the patients with malaria showed a progressive increase over the same time from 42% to 67%.

The pattern of serum cortisol concentrations observed over the 24 hours of the study in our patients is, however, unlikely to be explained fully by the combination of an altered corticotrophin set point and prolonged cortisol half-life. In the 15 hour period between 08.00 and 23.00 on day 2, for example, the median serum cortisol fell by approximately 50% while the plasma ACTH remained suppressed in most patients. To account for this observation, the cortisol half life would have to be well in excess of the 4.6 hours that has been estimated for patients with much more severe illness (Davis *et al*, 1997). An alternatively explanation is that adrenocortical function is mediated by factors acting independently of, or synergistically with, ACTH in acute malaria. We have previously confirmed that, consistent with studies in sepsis (Spath-Schwalbe *et al*, 1994), interleukin (IL) 6 has a stimulatory effect on ACTH secretion in severe malaria (Davis *et al*, 1997). The circulating concentrations of other interleukins, specifically IL-1 and IL-2, are increased in malaria (Warrell *et al*, 1990; Kwiatkowski *et al*, 1990) and have been shown to promote cortisol secretion directly in other contexts (Winter *et al*, 1990; Dunn, 1993). All our patients continued to receive full antimalarial and supportive therapy over the 24 hours of the study and most cleared their parasitemia during this time. It could be that cytokine-mediated stimulatory effects on the adrenal gland decrease in parallel with clinical improvement, thus explaining the progressive fall in serum cortisol:plasma ACTH ratio from 08.00 hours to levels below baseline at 15.00 hours in our patients. A specific examination of this hypothesis was beyond the scope of the present study.

The present data show that pituitary corticotrophin responsiveness is preserved in acute, uncomplicated falciparum malaria despite a raised set point for basal cortisol inhibition of ACTH secretion. This is closely analogous to calcium homoeostasis in the same clinical context. We recently reported that baseline ionized calcium concentrations in Malaysian patients with uncomplicated malaria were lower than in controls but that serum intact parathormone (PTH) concentrations were similar (Davis *et al*, 1998), consistent with an altered parathyroid set point. In accord with the plasma ACTH response to dexamethasone in the present patient group, a citrate-induced fall in serum ionized calcium in the Malaysian patients was associated with prompt and appropriate PTH secretion. Serum cortisol profiles after dexamethasone in the present patients might reflect the combination of a prolongation of the cortisol half-life secondary to mild hepatic dysfunction and the effects of cytokines on the adrenal cortex. Although we did not observe augmented ACTH release, these changes would help to maintain energy sources to vital organs and fluid and electrolyte balance (Van den Berghe, 2000), thus providing some defence against complications of falciparum malaria.

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