Adrenocortical function in hospitalised patients with active pulmonary tuberculosis receiving a rifampicin-based regimen – a pilot study

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Objective. To assess whether adrenocortical function was compromised in patients with active tuberculosis (TB) during the first 5 days of therapy with either a rifampicin-based or ciprofloxacin-based regimen.

Design. Patients were randomised into two groups of 10 each. Adrenocortical function was compared in both groups by the measurement of biochemical indices, electrolytes, osmolality and pituitary-adrenocortical hormones. Adrenal reserve was assessed by intravenous 250 µg adrenocorticotropin hormone (ACTH) stimulation tests.

Setting. Department of Medicine, Johannesburg Hospital.

Subjects. Twenty hospitalised patients who were diagnosed with TB.

Outcome measures. Respiratory rate, pulse rate and blood pressure were recorded, and urinary sodium and osmolality were measured. Serum ACTH, cortisol, dehydroepiandros-terone-sulphate (DHEA-S) and aldosterone were assayed.

Results. None of the patients demonstrated biochemical evidence of overt adrenal insufficiency. There were no significant differences between the two groups before or during therapy for any biochemical indices, electrolytes, hormones or calculated osmolality. Mean basal cortisol concentrations were substantially elevated and DHEA-S levels were consistently subnormal, resulting in a high cortisol/DHEA-S ratio. In the ciprofloxacin group, cortisol responses to ACTH stimulation on day 1 were not significantly lower than on day 5. In the rifampicin group, cortisol concentrations decreased at each time point on day 5 compared with day 1 (p = 0.001). However, a significantly higher mean incremental rise from the basal cortisol concentration was measured on day 5 at 60 minutes (p = 0.04). In the entire cohort of 20 patients, 40% demonstrated an incremental cortisol rise of < 250 nmol/l after ACTH stimulation on day 1.

Conclusions. Rifampicin did not additionally impair adrenocortical function during the initial period of therapy. The high cortisol/DHEA-S ratio might be of clinical relevance.

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Tuberculosis (TB) is a major cause of morbidity and mortality in sub-Saharan Africa, especially in the southern region of the continent.[1] The escalating incidence of TB over the past decade is largely attributable to the devastating HIV epidemic.[2] HIV is an important risk factor for the reactivation of latent TB infection, and HIV seropositive persons who become co-infected with *Mycobacterium tuberculosis* rapidly progress to active TB.[3]

TB has a relatively high mortality rate in the first few days after diagnosis, despite initiation of treatment with antituberculosis drugs.[4] Postulated causes include late presentation with advanced systemic inflammatory response, pulmonary embolus secondary to hypercoagulability, or bacterial superinfection of damaged lungs. Another possible cause is hypoadrenalism secondary to tuberculous infection of the adrenal glands. Hypoadrenalism in TB is particularly relevant because cortisol deficiency could account for some of the sudden deaths in these patients.[5] A large study from South Africa published in 1986, before HIV became a serious clinical problem, found suboptimal responses to ACTH stimulation in 55% of patients with pulmonary TB.[6]

Standard TB therapy includes the antibiotic rifampicin, which is a potent hepatic enzyme inducer that might contribute to adrenal insufficiency by accelerating the catabolism of cortisol.[7] The objective of this prospective study was to assess whether adrenocortical function was compromised in patients with active pulmonary TB during the initial period of therapy with two different antituberculosis regimens, one of which contained rifampicin and the other ciprofloxacin.

Materials and methods

Patients

Twenty hospitalised patients diagnosed with sputum-positive TB were studied prospectively. Before admission none had

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received antituberculosis therapy. Patients who were pregnant or who were on treatment with corticosteroids or any drugs known to interfere with steroid metabolism, were excluded. Informed consent was obtained from all patients and the study was approved by the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg.

Design

Patients were randomised into two groups of 10 (5 men and 5 women in each group), and treated with a quadruple antituberculosis regimen containing either rifampicin or ciprofloxacin. In accordance with local guidelines, patients in the rifampicin group were given 300 mg daily if they weighed < 50 kg, and 450 mg if > 50 kg. Patients in the ciprofloxacin group received 500 mg twice daily, irrespective of weight. The remainder of the regimen comprised standard therapy (isoniazid, ethambutol and pyrazinamide), with doses adjusted individually for weight.

The study was designed to compare adrenocortical function in the two groups during the first 5 days of therapy with either rifampicin or ciprofloxacin. Clinical measures of respiratory rate, pulse rate and supine blood pressure were recorded before starting therapy. A urine sample was taken for measurement of sodium and osmolality. From day 1 to day 5, between 07h00 and 09h00, fasting blood samples were taken for the measurement of adrenocorticotropic hormone (ACTH), cortisol, dehydroepiandrosterone-sulphate (DHEA-S) and aldosterone. On days 1, 3 and 5, blood samples were also analysed for glucose, sodium, potassium, chloride, total CO_2, urea and creatinine.

In addition, on days 1 and 5, intravenous ACTH (250 µg) stimulation tests were performed. An indwelling intravenous cannula was inserted near the antecubital fossa and kept patent with heparinised saline, and a 30-minute rest period was allowed. Fasting blood samples were taken for basal measurements and thereafter 250 µg Synacthen (Novartis Pharma AG, Basle, Switzerland) was administered as an intravenous bolus. Additional blood samples were taken at 30 minutes and 60 minutes for measurement of cortisol, and a normal response was defined as an increment of > 250 nmol/l at 60 minutes, based on criteria recently recommended for acutely ill patients.[8]

Measurement of biochemical indices, electrolytes and osmolality

Plasma glucose levels were determined using a standard glucose oxidase method (normal range 3.0 - 6.0 mmol/l). Serum electrolytes (sodium, potassium, chloride, total CO_2, urea and creatinine) and urinary sodium were measured with a COBAS Integra 400 autoanalyser using reagents supplied by Roche Diagnostics GmbH, Mannheim, Germany. Respective normal ranges are as follows: sodium 135 - 147 mmol/l (serum), 60 - 200 mmol/l (urine); potassium 3.3 - 5.3 mmol/l;

chloride 99 - 113 mmol/l; total CO_2 18 - 29 mmol/l; urea 2.6 - 7.0 mmol/l; creatinine 60 - 120 µmol/l (men) and 60 - 100 µmol/l (women). The inter- and intra-assay coefficients of variation (CVs) for these assays were between 5% and 10%.

The following formula was used to calculate serum osmolality: 2 (sodium + potassium) + urea + glucose (normal range 280 - 295 mOsm/kg). Urine osmolality was measured with an automatic micro-osmometer (Roebling, Berlin, Germany), normal range 50 - 1 200 mOsm/kg.

The syndrome of inappropriate antidiuretic hormone (SIADH) secretion was defined by the combination of hyponatraemia (sodium < 130 mmol/l) with low serum osmolality (< 280 mOsm/kg), inappropriately high urine osmolality compared with serum osmolality, and urinary sodium > 20 mmol/l, in the absence of clinical volume depletion.[9]

Assays of pituitary-adrenocortical hormones

Serum ACTH, DHEA-S and aldosterone concentrations were measured by immunoassay using direct chemiluminometric technology and reagents supplied by Diagnostics Products Corporation, Los Angeles, Calif., USA. Respective fasting normal ranges are as follows: ACTH < 46 ng/l, DHEA-S 4.0 - 13.3 µmol/l (men) and 0.9 - 8.7 µmol/l (women) for age range 20 - 40 years, and aldosterone < 440 pmol/l (supine). Radioimmunoassay kits supplied by ICN BIO medicals, Inc., Costa Mesa, Calif., USA were used to measure serum cortisol (normal range 190 - 660 nmol/l). The inter- and intra-assay CVs for these assays varied between 5% and 10%.

Statistical analysis

Results were analysed using one-way analysis of variance (ANOVA) for repeated measures, followed by the two-tailed t-test for parametric data and Wilcoxon's rank sum test or signed rank test when distribution of the data was non-parametric. The effect of ACTH stimulation was analysed by comparison of the incremental rises in cortisol, which were calculated by subtracting the basal level from the peak level attained at 30 minutes and 60 minutes (Δ change). Results are expressed as mean ± standard error of the mean (SEM) and a value of $p < 0.05$ was considered significant.

Results

Clinical measurements

Table I shows clinical measurements for the 20 patients on admission to hospital. Before start of therapy there were no significant differences between the two groups in terms of mean age, weight, respiratory rate and pulse rate. Patients randomised to the ciprofloxacin group had significantly lower systolic blood pressure (BP) ($p = 0.03$) and diastolic BP ($p = 0.01$). One patient in this group refused to be tested for HIV status, and of the 19 patients tested, 1 patient was found to be

HIV seronegative and 18 were HIV seropositive. There were 9 documented HIV seropositive patients in each group. Mean baseline CD4 counts were not significantly different: rifampicin group 192 ± 83/µl and ciprofloxacin group: 171 ± 67/µl (normal range 500 - 2 010/µl). All 20 patients had improved clinically when they were reassessed on day 5.

Biochemical indices, electrolytes and osmolality before and during therapy

There were no significant differences *between* the groups treated with either rifampicin or ciprofloxacin for any of the variables measured (Table II). *Within* the rifampicin group, however, the mean glucose level on day 5 was significantly lower than on days 1 and 3 ($p = 0.03$). In both groups, mean serum sodium values were below the normal range and remained low during the period of study. Mean levels of potassium, chloride and total CO_2 were all normal and were not affected by either drug. Mean urea and creatinine concentrations were slightly higher in the rifampicin group throughout the study. Concentrations of both indices decreased from days 1 and 3 to day 5, significantly so for urea and creatinine in the rifampicin group ($p = 0.001$) and for creatinine in the ciprofloxacin group ($p = 0.001$).

Before starting therapy, 7 of the 20 patients (35%) were hyponatraemic (mean sodium 126 mmol/l), and all 7 patients had some features of SIADH. In this small subgroup, mean serum osmolality was 272 mOsm/kg, mean urine osmolality was 454 mOsm/kg and mean urinary sodium was 31 mmol/l.

Pituitary-adrenocortical hormones before and during therapy

There were no significant differences *between* the groups treated with either rifampicin or ciprofloxacin for any of the hormones measured (Table III). *Within* each group, however, mean ACTH levels fluctuated and were all higher during the therapy period than on day 1. This increase was significant in the rifampicin group ($p = 0.001$). Mean basal cortisol concentrations in both groups were substantially above the normal range throughout the study, possibly reflecting the stress of acute illness. Compared with the ciprofloxacin-treated patients, mean cortisol concentrations in the rifampicin group were all higher, but not significantly so. On rifampicin therapy, mean cortisol concentrations decreased, reaching significance on day 5 compared with days 1 and 2 ($p = 0.003$). Mean DHEA-S levels were consistently subnormal and a significant decline from day 1 through to day 5 was seen in the rifampicin group ($p = 0.007$). In the ciprofloxacin group, DHEA-S levels were also low without any significant change during the study. Mean aldosterone values in both groups were variable and all were within the normal range. Values were lowest in both groups on day 5, significantly so compared with day 1 in the rifampicin group ($p = 0.001$).

Cortisol responses to intravenous ACTH (250 µg) stimulation tests

Mean cortisol concentrations at baseline and after ACTH stimulation did not differ significantly at any time point on day 1 or day 5 *between* the rifampicin and ciprofloxacin groups (Fig. 1). There were no significant differences *within* the

Table II. Fasting serum biochemical indices, electrolytes and osmolality in two groups of patients before and during therapy (mean ± SEM)

	Rifampicin group ($N = 10$)			Ciprofloxacin group ($N = 10$)		
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
Glucose (mmol/l)	5.3 ± 0.4	5.4 ± 0.4	4.6 ± 0.3*	5.1 ± 0.5	5.0 ± 0.5	5.1 ± 0.4
Sodium (mmol/l)	129 ± 1	133 ± 1	133 ± 1	133 ± 2	133 ± 1	135 ± 1
Potassium (mmol/l)	3.7 ± 0.1	3.7 ± 01	3.9 ± 0.1	3.9 ± 0.1	3.7 ± 0.1	3.8 ± 0.1
Chloride (mmol/l)	99 ± 2	102 ± 1	102 ± 1	99 ± 2	102 ± 2	105 ± 2
Total CO_2 (mmol/l)	20 ± 1	20 ± 1	20 ± 1	22 ± 1	21 ± 1	20 ± 1
Urea (mmol/l)	4.5 ± 1.0	4.2 ± 0.7	3.1 ± 0.4†	3.2 ± 0.6	3.3 ± 0.3	2.7 ± 0.3
Creatinine (µmol/l)	84 ± 6	80 ± 6	73 ± 7‡	75 ± 6	74 ± 3	68 ± 2*
Calculated osmolality (mOsm/kg)	277 ± 2	283 ± 2	282 ± 2	283 ± 3	282 ± 2	287 ± 3

*$p = 0.03$ day 1, 3 v. day 5.
†$p = 0.001$ day 1, 3 v. day 5.
‡$p = 0.001$ day 1, 3 v. day 5.

Table III. Fasting serum concentrations of pituitary-adrenocortical hormones in two groups of patients before and during therapy (mean ± SEM)

	Rifampicin group (N = 10)					Ciprofloxacin group (N = 10)				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
ACTH (ng/l)	26 ± 5*	46 ± 20	30 ± 7	68 ± 26	34 ± 6	26 ± 4	62 ± 25	37 ± 9	66 ± 22	32 ± 8
Cortisol (nmol/l)	1 258 ± 180	1 232 ± 130	1 144 ± 86	1 098 ± 127	918 ± 97†	989 ± 124	1 090 ± 104	864 ± 113	953 ± 129	793 ± 102
DHEA-S (μmol/l)	2.7 ± 1.0	2.3 ± 0.8	1.8 ± 0.6	1.0 ± 0.2	1.0 ± 0.1‡	1.4 ± 0.3	1.5 ± 0.3	1.4 ± 0.4	1.5 ± 0.3	1.2 ± 0.2
Aldosterone (pmol/l)	372 ± 108	297 ± 86	393 ± 101	328 ± 117	135 ± 34§	144 ± 56	256 ± 112	212 ± 75	265 ± 183	138 ± 52

*p = 0.001 day 1 v. day 2, 3, 4, 5.
†p = 0.003 day 1, 2 v. day 5.
‡p = 0.007 day 1 v. day 2, 3, 4, 5.
§p = 0.001 day 1 v. day 5.

ciprofloxacin group on day 1 compared with day 5. However, *within* the rifampicin group, mean cortisol concentrations apparently decreased at each time point on day 5 compared with day 1 (*p* = 0.001). When expressed as an *incremental* rise from basal, however, levels were, in fact, greater on day 5 than on day 1 (30 minutes: 360 nmol/l (day 5) v. 158 nmol/l (day 1), and 60 minutes: 615 nmol/l (day 5) v. 348 nmol/l (day 1); *p* = 0.04). This was due to the basal cortisol level having declined significantly from 1 258 nmol/l on day 1 to 918 nmol/l on day 5 (*p* = 0.005). Applying the normal cortisol response, defined as an increment of > 250 nmol/l at 60 minutes,[8] 5 patients in the ciprofloxacin group and 3 in the rifampicin group were found to have inadequate adrenal reserve on day 1; these numbers had dropped to 2 in each group by day 5.

Discussion

The primary objective of this study was to establish whether adrenocortical function was compromised in patients with active pulmonary TB during the initial phase of treatment with a rifampicin-based regimen. To this end we measured basal serum levels of the pituitary-adrenocortical hormones – ACTH, cortisol,
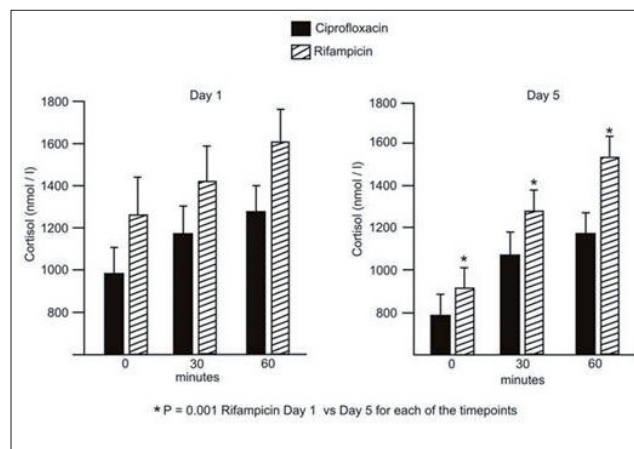

*Fig. 1. Serum cortisol responses following intravenous ACTH (250 μg) stimulation tests in two groups of patients with pulmonary TB treated with rifampicin (N = 10) and ciprofloxacin (N = 10). Results are expressed as mean ± SEM. Rifampicin day 1 v. day 5 *p = 0.001 for each of the time points.*

DHEA-S and aldosterone – and serum cortisol responses to intravenous ACTH stimulation tests.

Mean basal cortisol concentrations were substantially elevated, almost certainly reflecting the stress of the underlying medical condition, as in other acute respiratory illnesses.[10] In this context, it has been proposed that random serum cortisol levels above 938 nmol/l make adrenal insufficiency unlikely,[8] and this was clearly evident on day 1 for both our treatment groups. Although mean cortisol levels dropped below this threshold on day 5 in both groups, this probably reflected their improving clinical state owing to effective antituberculosis and supportive therapy, rather than deteriorating adrenocortical function itself.

DHEA-S concentrations, on the other hand, were consistently subnormal, probably as a result of the poor health status of the majority of patients in our study.[11] The discrepancy between high cortisol and low DHEA-S levels in our patients is interesting and might have clinical relevance. Reduced output of DHEA metabolites in patients with severe TB could be detrimental for several reasons.[12,13] Effective immunity to TB requires a T helper-1 (Th1) pattern of cytokine release and experimental animal studies have demonstrated that even a minor T helper-2 (Th2) component is associated with impaired immunity and a non-protective cytokine profile. Exposure of T-cells to glucocorticoids such as cortisol drives them towards a Th2 response, which may both reactivate and exacerbate TB. In contrast, DHEA and its derivatives have antiglucocorticoid effects *in vivo* and, in the experimental situation, have been shown to protect against shifts towards Th2 effects and to be associated with enhanced granuloma formation. In short, the high cortisol/DHEA-S ratio that occurred in our patients

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may be crucial to both the susceptibility and the pathology of disease in TB, and warrants further investigation.

In the rifampicin group, cortisol responses to ACTH stimulation tests were well above normal, except for 3 patients on day 1 and 2 patients on day 5. However, the dose of Synacthen (250 µg) administered was supraphysiological, with reduced sensitivity compared with a low dose (1 µg),[14] and this could have masked more extensive impairment of function produced by rifampicin. This might apply particularly to HIV-infected patients who are acutely ill.[15] Nevertheless, the greater incremental post-stimulation cortisol response at each of the time points on day 5 in this group of patients is an indication of unimpaired cortisol metabolism, although (as previously stated) their improved clinical state, owing to the effective antituberculosis therapy, may also have had some effect. Overall, in the entire cohort of 20 patients,[8] demonstrated relatively impaired cortisol responses to ACTH stimulation on day 1, using the cut-off level of < 250 nmol/l.[8] However, since the basal cortisol level in all but 3 cases was well above 938 nmol/l, the clinical relevance of this observation is uncertain. Furthermore, considerable ambiguity exists when defining the ideal cut-offs for diagnosing relative adrenal insufficiency after ACTH stimulation in different stress situations.[16]

It transpired that no subject demonstrated biochemical evidence of overt adrenocortical insufficiency in the entire group of 20 patients. In this regard, our finding is in keeping with the low incidence of primary hypoadrenalism reported in other recent African studies involving similar patient cohorts.[9,17] It is difficult to interpret the lower than expected mean levels of serum ACTH in both study groups on admission to hospital. Since pre-existing basal levels of ACTH were unknown, we cannot say whether the recorded values represented some increase from healthy baseline status. During subsequent days of sampling, transient elevations were clearly seen but levels had returned to normal by day 5. This observation is similar to a previous report of normal ACTH levels despite raised cortisol in acute pneumonia.[10]

Finally, the occurrence of SIADH was the commonest metabolic abnormality encountered in our patients, having been diagnosed in about one-third of cases on admission to hospital. This figure is higher than the 10% prevalence rate reported in the recent study by Kaplan *et al.*[9] and may be ascribed to most of our patients being HIV seropositive and having had more advanced disease, despite similar diagnostic criteria being employed. The mechanisms involved relate to increased total body water with decreased total body sodium as a consequence of enhanced or an abnormal pattern of ADH release.[18]

In conclusion, 40% of the entire cohort of 20 patients showed relatively impaired cortisol responses to ACTH stimulation on admission, but the significance of this is uncertain in view of extremely raised basal levels in most cases. During the first few days of therapy, rifampicin did not impair adrenal responses to conventional ACTH stimulation tests in this relatively small group of patients with active pulmonary TB, most of whom were HIV seropositive. Our finding, however, may not apply to occasional patients who have compromised adrenal function at the time of their initial admission to hospital or who are more acutely ill.

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