Effect of inhaled beclomethasone dipropionate on saliva cortisol concentrations

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SUMMARY Serial saliva cortisol measurements were used to assess pituitary-adrenal function in a group of asthmatic children treated with beclomethasone dipropionate (400 µg daily). Asthmatic children who were not being treated with steroids and normal children were also studied for comparison. A diurnal cortisol rhythm was observed in all three groups. Early morning cortisol concentrations were significantly higher in the group treated with beclomethasone dipropionate than in the normal children; this may indicate a stress induced response to decreased morning peak expiratory flow. In both groups, plasma and salivary cortisol responses after adrenocorticotrophic hormone stimulation test were normal but peak cortisol concentrations showed a 7 fold increase over basal values in saliva compared with a three fold increase in plasma. Beclomethasone dipropionate does not suppress pituitary-adrenal function in children when used in recommended doses. Serial measurement of the salivary cortisol concentration is a simple, safe, and sensitive method for the routine monitoring of adrenal function in children treated with this steroid. Monitoring may be supplemented with an assessment of the adrenal response to adrenocorticotrophic hormone stimulation, if necessary.

Inhaled beclomethasone dipropionate is well established both as a substitute for systemic glucocorticoid treatment and as initial treatment for the asthmatic child.¹ The recommended daily dose of 400 µg is effective in controlling symptoms and seems to be free of side effects.² Several studies have reported the absence of adrenal suppression, and lack of inhibition of linear growth with this treatment.³⁻⁵

Vaz *et al* used the insulin tolerance test as a test of pituitary-adrenal reserve and found significantly lower basal and peak cortisol concentrations in asthmatic children compared with controls.⁶ Nevertheless, the cortisol response to adrenocorticotrophic hormone stimulation has been shown to reflect accurately hypothalamic-pituitary-adrenal function when compared with the results of an insulin tolerance test performed in a large group of patients with suspected pituitary-adrenal dysfunction.⁷

This paper describes the application of a recently developed, sensitive and specific salivary cortisol radioimmunoassay^{8 9} for the assessment of pituitary-adrenal function in a group of asthmatic children receiving inhaled beclomethasone dipropionate. The results are compared with those obtained from

normal children and a group of asthmatic children receiving non-steroid medication.

Patients and methods

Patients. Details of the three groups of children studied are shown in Table 1.

Group I

This group comprised normal children with no respiratory disease or any known endocrine dysfunction.

Table 1 *Number, sex, and age of children in study*

	Boys	Girls	Age (years)	
			Mean	Range
<i>Group I</i>				
Normal children	11	14	10.0	6-14
<i>Group II</i>				
Steroid treated children	10	8	10.1	7-13
<i>Group III</i>				
Non-steroid treated children	9	3	10.8	6-14

Table 2 Specificity of cortisol antiserum

Steroid	Cross reactivity (%)*
Cortisol	100
Prednisolone	64
11-Deoxycortisol	7.1
Corticosterone	4.0
Cortisone	0.7
11-Deoxycorticosterone	0.2
Dexamethasone	0.05
17 OH-progesterone	<0.01
Progesterone	<0.01
Oestradiol	<0.01
Testosterone	<0.01
Betamethasone	<0.01
Beclomethasone	<0.01

*Calculated as the amount of steroid required to displace 50% binding of labelled cortisol at the zero binding.

Group II

Group II, comprised asthmatic children receiving beclomethasone dipropionate (400 µg daily) by aerosol inhalation for at least four months before the study. Those who had received more than one short course of systemic glucocorticoid treatment during the previous year were excluded from the

study; none had systemic treatment within four months of starting the study. Children treated with topical glucocorticoids for skin conditions such as eczema were not included.

Group III

This group comprised asthmatic children who were not being treated with steroids but with cromoglycate and bronchodilators. None had previously received either systemic or topical glucocorticoid treatment.

Informed parental consent was obtained for all three groups of children and the study protocol was approved by the hospital ethics committee.

Methods. Samples of saliva were collected from all children between 7 am and 9 am, 4 pm and 6 pm, and 8 pm and 10 pm on two consecutive days to determine diurnal cortisol profiles. After mouth rinsing with tapwater, 2 ml saliva samples were collected by dribbling into a plain container. In addition, two normal children collected saliva samples at these times for five consecutive days.

Fig. 1 Cortisol concentrations in saliva samples collected daily from two normal children on five consecutive days.

Blood and saliva samples were collected simultaneously in groups II and III before and 60 minutes after adrenocorticotrophic stimulation using tetracosactrin (0.25 mg given intramuscularly). Plasma and saliva samples were stored at -20°C until assay.

Plasma and saliva cortisol concentrations were determined by direct radioimmunoassay using a method previously described.⁸ The cross reactivity of the cortisol antiserum with a selection of relevant steroids is shown in Table 2. In particular, betamethasone and beclomethasone which have fluoride and chloride radicals in the 9 carbon position of the steroid nucleus respectively, showed undetectable cross reactivity with the antiserum. Statistical analysis was performed using a two way analysis of variance.

Results

Daily profiles of saliva cortisol concentrations determined on five consecutive days in two normal children are shown in Fig. 1. A pronounced diurnal rhythm was consistently observed each day. Fig. 2 illustrates the mean (SEM) saliva cortisol concentrations throughout the day in all three groups studied. Each group showed a normal diurnal cortisol rhythm. Early morning cortisol concentrations were significantly higher in the steroid treated children (group II) compared with normal children ($P<0.03$). Saliva cortisol concentrations in samples collected in the afternoon and evening were similar in all three groups of children.

Fig. 2 Cortisol concentrations determined in saliva samples collected on two consecutive days from normal children (group I), steroid treated (group II), and non-steroid treated (group III) asthmatic children. The data are expressed as mean (SEM). The values denoted by the asterisk were significantly different ($P<0.03$).

Fig. 3 Plasma and saliva cortisol response to adrenocorticotrophic hormone stimulation (250 μg) in asthmatic children. The figures in parentheses indicate the numbers tested in each group.

The plasma and saliva cortisol responses to adrenocorticotrophic hormone stimulation in the two groups of asthmatic children studied are illustrated in Fig. 3. The data are expressed as the mean cortisol increment in relation to the basal cortisol concentration. There was no significant difference in the results obtained in both groups. Basal plasma and saliva cortisol concentrations were similar in both groups.

Discussion

Saliva steroid concentrations are independent of flow rate and reflect the 'free' or non-protein bound fraction in plasma.⁹ Changes which occur in plasma concentrations are rapidly reflected by similar patterns in saliva. Since saliva collection is easy and painless, the technique is ideally suited to the study of serial changes in steroid concentrations in children.

The value of serial saliva 17 OH-progesterone concentrations in monitoring control in children with congenital adrenal hyperplasia^{10 11} and saliva cortisol measurements in studying the development of circadian rhythm in infants has recently been reported.¹² In the present study a noticeable diurnal cortisol rhythm was observed in normal children and in both groups of asthmatic children. Early morning saliva cortisol concentrations were significantly raised in children treated with beclomethasone dipropionate compared with controls. Plasma adrenocorticotrophic hormone and cortisol secretory peaks increase in magnitude between 2 am and 8 am;¹³ the amplified morning cortisol response in steroid treated asthmatic children may be an appropriate response to stress. Presumably asthma in children treated with beclomethasone dipropionate was more severe than in those children receiving non-steroid medication, but objective data for comparison were not available for this study. Raised morning cortisol concentrations may be a useful marker of decreased morning peak expiratory flow rate characteristically observed in asthmatics.^{14 15}

The plasma and saliva cortisol response to exogenous adrenocorticotrophic hormone stimulation was similar in both groups of asthmatic children. It is accepted that recovery from pituitary-adrenal suppression may be delayed for at least a year after chronic, long term, high dose glucocorticoid treatment.¹⁶ In that situation, the cortisol response to adrenocorticotrophic hormone stimulation may be an unreliable indication of the capacity of the adrenal gland to respond to stress.¹⁷ Children treated with beclomethasone dipropionate were a defined group, however, who had not received chronic, long term systemic glucocorticoid treatment. Since this constitutes the majority of asthmatic children who require steroid medication, the short term adrenocorticotrophic hormone stimulation is a useful and reliable index of pituitary adrenal function.

In both groups of asthmatic children studied, peak cortisol concentrations showed a 7 fold increase over basal values in saliva compared with a threefold increase in plasma. This confirms data previously reported in adults;¹⁸ the discrepancy is probably due to the disproportionate increase in the plasma 'free' fraction of cortisol after adrenocorticotrophic hormone stimulation which is accurately reflected in saliva.

The cortisol response to adrenocorticotrophic hormone stimulation measured in saliva is thus a potentially more sensitive marker of adrenal suppression. The data obtained from this study indicate that children treated with inhaled beclomethasone dipropionate in recommended doses do not show

evidence of significant pituitary-adrenal suppression. The insulin tolerance test which is unpleasant and potentially dangerous, is an unjustified investigation of pituitary-adrenal reserve in most asthmatic children who require steroid medication.

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