Bloodspot cortisol in mild asthma: the effect of inhaled corticosteroids

Iolo J M Doull, Stephen J Donovan, Peter J Wood, Stephen T Holgate

Abstract

Bloodspot cortisol, where finger pricked blood is applied to blotting paper, is suitable for repeated measurements in the home environment. The use of bloodspot cortisol measurements in children with asthma and the effect of inhaled corticosteroids on daytime cortisol concentrations were assessed. Twenty children with mild asthma were randomised to receive double blind either placebo or beclomethasone dipropionate 200 μg twice daily. Blood was taken by finger prick at home on waking, and treatment administered. Blood was then taken one hour after treatment, at lunchtime, and in the evening. The area under the curve (AUC) for the four time points was calculated as a composite index of daytime cortisol.

Mean (SEM) bloodspot cortisols fell progressively over the day from 199·2 (15·6) nmol/l to 58·4 (8·9) nmol/l. Cortisol in the group treated with beclomethasone dipropionate was lower at all time points, but was significant only after treatment (mean (SEM) 120·9 (14·3) *v* 177·5 (21·0) nmol/l) and at lunchtime (mean (SEM) 82·7 (12·4) *v* 128·9 (12·6) nmol/l). AUC for the beclomethasone dipropionate treated group was also significantly decreased (mean (SEM) 317 (31·4) *v* 446 (29·7)). Beclomethasone dipropionate at a dose of 400 μg/day significantly suppresses the daytime cortisol profile.

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With the recognition of asthma as an inflammatory disease has come a greater use of anti-inflammatory treatment, commonly inhaled corticosteroids. Inhaled corticosteroids are well recognised to have systemic side effects including decreased growth in childhood,[1] impaired bone metabolism,[2] skin thinning with purpura,[3] and suppression of the hypothalamo-pituitary-adrenal axis.[4]

Detectable adrenal suppression is dependent not only on the on treatment and dosage, but crucially the method of assessing function. Dynamic tests of adrenal function such as those using tetracosactrin (Synacthen, Ciba)[5], metyrapone,[6] or insulin stress,[7] or measurements of 24 hour urinary cortisol excretion,[8] have shown little suppression of pituitary adrenal axis on up to 800 μg/day of corticosteroid. However integrated overnight serial plasma cortisol concentrations do show significant suppression at doses as low as 400 μg/day of beclomethasone dipropionate when compared with controls,[8] and it is likely this is a more sensitive index of adrenal suppression. A major drawback of serial plasma cortisol measurement is the need for either repeated venesection or a patient indwelling cannula – effectively limiting patients to the hospital environment. It is also unclear what effect a dose of inhaled corticosteroid has in the short term on the pituitary-adrenal axis.

The purpose of this study was to measure the effect of a conventional dose of beclomethasone dipropionate in children had on daytime cortisol profile, and determine whether there was an effect on cortisol concentrations immediately after treatment administration.

Subjects and methods

SUBJECTS

Twenty subjects with mild asthma participating into a larger study on the effect of inhaled steroids on viral induced wheezing episodes were recruited into the study. All had either five or more wheezing episodes in the preceding year or an episode of wheezing lasting for three days or more in the preceding year. Exclusion criteria at entry included the use of inhaled or use of oral corticosteroids immediately before enrolment, or severe respiratory disease such as cystic fibrosis. Subjects were then randomised in a double blind manner to receive either beclomethasone dipropionate 200 μg twice daily or placebo, as dry powder via a diskhaler (Allen and Hanbury). At the time of testing all subjects had been taking the steroid or placebo for a minimum of six months and none had received any oral steroids in the preceding six months. All participants completed baseline spirometry with a dry wedge spirometer.

Subjects were shown the use of the 'Soft Touch' (Boehringer Mannheim UK) bloodletter, and application of blood onto filter paper. Only children happy to let blood themselves were entered into the study. They were asked to apply blood in duplicate to prescribed areas of the filter paper four times during the day: once immediately on getting up in the morning, one hour after taking their treatment, at midday, and in the evening before their treatment. The children were asked to inhale their medication at their usual time soon after arising. The four blood tests were then repeated on a separate day within a two week period. The samples were kept dry before analysis.

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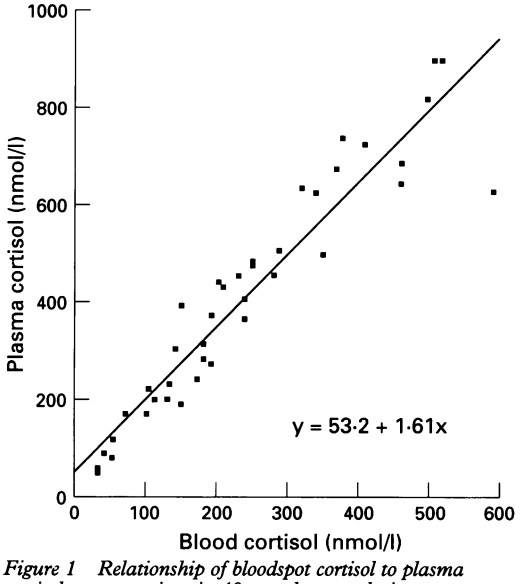

Figure 1 Relationship of bloodspot cortisol to plasma cortisol concentrations in 40 samples; correlation coefficient=0·96.

BLOODSPOT CORTISOL ASSAY

Bloodspot cortisol assays were performed by the method described by Wood *et al.*[9] Discs of 6 mm diameter were punched out of the filter paper containing the dried blood. These were reacted with ^{125}I-labelled cortisol and a limited amount of sheep anticortisol antiserum. Incubation at pH 4·0 removed interference by cortisol binding globulin and permitted direct analysis of samples. The bound and free fractions were separated by adding dextran coated charcoal, enabling the removal of the aliquot of bound fraction after centrifugation.

PREPARATION OF STANDARDS

Standards were prepared using time expired transfusion blood, which was centrifuged and the plasma separated from the red cells. Plasma was stripped by stirring with charcoal (20 g/100 ml plasma) for 24 hours at 4°C. Charcoal was removed by centrifuging at 3000 rpm for 15 minutes and then at 25 000 for one hour. Red cells were resuspended in isotonic saline, centrifuged, and the saline wash discarded. Stripped plasma and red cells were then recombined at a ratio of 45% cells to 55% plasma to give cortisol free blood. Ethanolic cortisol standard was dried down, and taken up in cortisol free blood to give a range of standards from 15·6 to 1000 nmol/l blood. Standards were spotted onto filter paper and allowed to dry.

ACCURACY OF BLOODSPOT CORTISOL

Bloodspot cortisol was validated against plasma cortisol in 40 sequential routine paediatric and adult cortisol samples. Before separation of the plasma, whole blood samples were spotted onto filter paper. Plasma cortisol measurements were by standard methods.

ANALYSIS OF DATA

For each time point the mean of the two separate days sampling was used for all analysis. As an integrated function of the bloodspot cortisols throughout the day, the area under the curve (AUC) for the bloodspot cortisol for each group was calculated by adding trapezoids. Analysis was by the Statistical Programme for Social Sciences (SPSS); the χ^2 test was used for sex distribution. The bloodspot cortisol concentrations and AUC in the study group were log transformed to normalise the distribution, and Student's unpaired t test used to compare the log transformed means. Multiple linear regression was used to measure association between continuous variables. The relationship between plasma cortisol and bloodspot cortisol was investigated by linear regression. Results are presented as mean (SEM).

Permission for the study was obtained from the ethical committee of the Southampton University teaching hospitals. The children gave informed assent and the parents informed written consent.

Results

ACCEPTABILITY

Twenty seven children agreed to the study but only 25 found themselves able to prick themselves to produce blood. Nine blotting papers (five children) were totally rejected as either there was insufficient blood on the card to take a 6 mm sample or the samples were not in duplicate. Thus the samples from 20 children were suitable for analysis.

BLOODSPOT CORTISOL ASSAY

The coefficient of variation for the method was less than 10% between 100 and 1000 nmol/l for the single day paired samples. Bloodspot cortisol showed excellent correlation (fig 1) with plasma cortisol (plasma cortisol=1·61×bloodspot cortisol+53·2 (SD about regression line 77·8); r=0·961).

BASELINE CHARACTERISTICS

Baseline characteristics of the 20 children able to supply samples suitable for analysis are presented in table 1. There were 11 boys and nine girls with a mean age of 8·5 years (range 7·1–9·9), and all children were prepubertal. Eight children were receiving beclomethasone dipropionate and 12 were receiving placebo. There were no significant differences in sex

Table 1 Comparison of placebo and beclomethasone dipropionate groups: baseline characteristics

Characteristic	*Placebo (n=12)*	*Beclomethasone dipropionate (n=8)*	*p Value*
Boys/girls	6/6	5/3	0·67*
Mean (SEM) age (years)	8·4 (0·25)	8·9 (0·26)	0·11
Mean (SEM) baseline mean FEV_1 (% predicted)	86·0 (3·4)	91·0 (4·7)	0·40
Mean (SEM) height (cm)	129·4 (1·7)	134·5 (2·2)	0·09
Mean (SEM) weight (kg)	27·1 (1·0)	33·2 (2·2)	0·029
Mean (SEM) surface area (m^2)	0·98 (0·021)	1·11 (0·048)	0·01

FEV_1=forced expiratory volume in one second.
*Fisher's two tail test.

Table 2 Geometric mean (and 95% confidence interval (CI) of ratio of means) of bloodspot cortisol on placebo or on inhaled beclomethasone dipropionate (400 μg/day)

Geometric mean cortisol (nmol/l)	*Placebo*	*Beclomethasone dipropionate*	*Ratio of means (95% CI of ratio)*	*p Value*
Arising	200·4	168·6	1·18 (0·93 to 1·68)	0·312
One hour after inhalation	165·8	116·0	1·43 (1·0 to 2·04)	0·05
Midday	121·6	74·2	1·24 (1·03 to 2·51)	0·026
Evening (before treatment)	53·2	39·4	1·13 (0·69 to 1·51)	0·357
AUC	445·5*	316·9*	128·6 (27·4 to 229·8)*	0·011

*For AUC mean, mean difference and 95% CI of the difference.

distribution, age, forced expiratory volume in one second, or height between the treatment groups. However, the beclomethasone dipropionate group were significantly heavier and had significantly greater surface area than the placebo group.

EFFECT OF INHALED CORTICOSTEROIDS

Mean (SEM) cortisol concentrations fell significantly over the course of the day from 199·2 (15·6) nmol/l on arising to 58·4 (8·9) nmol/l in the evening. Mean cortisols for the four times during the day both on beclomethasone dipropionate and on placebo are presented in table 2. Mean cortisol concentrations were lower in the beclomethasone dipropionate treated group compared with the placebo treated group at all four time points, but only reached significance at the collection one hour after inhalation of treatment and at the midday collection (fig 2). When the AUC was used as an integrated function of daytime levels, again the beclomethasone dipropionate group had significantly lower concentrations than the placebo group (316·9 (31·4) *v* 445·5 (29·7), p=0·011). There was no significant difference in cortisols at any of the four time points or in the AUC between the sexes. There was no significant correlation between the AUC and height, weight, or surface area of the children.

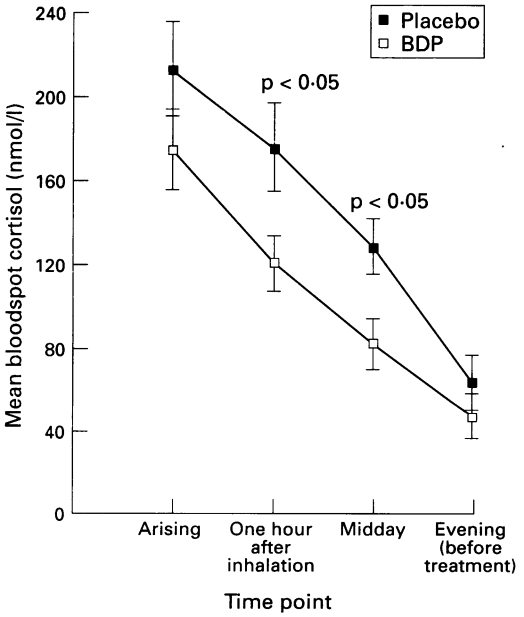

Figure 2 Mean (SEM) daytime bloodspot cortisol concentrations; BDP=beclomethasone dipropionate.

Discussion

We have shown in a group of prepubertal children that blood letting by the Soft Touch is an acceptable means of collecting bloods in the 'physiological' conditions of normal life. Furthermore we have shown that cortisol concentrations one hour after inhalation of beclomethasone dipropionate of 200 μg are significantly decreased, and that integrated daytime cortisols are significantly decreased on a conventional dose of beclomethasone dipropionate of 400 μg/day. Our groups were well matched, with the only difference by chance between the two groups being that the group on beclomethasone dipropionate were significantly heavier and had greater surface area. These differences are if anything likely to mask significant suppression of the pituitary axis in the beclomethasone dipropionate group. Thus the differences we observed are likely to be due to the treatment and not the disease. Tests of pituitary-adrenal function on inhaled corticosteroid treatment have given apparently contradictory results. Many studies have shown no significant adrenal suppression on a daily dose of 400 μg of inhaled corticosteroid,[5 10–14] or even up to 800 μg/day.[15 16] Others have shown suppression on daily doses ranging from 300 μg to 800 μg.[4 17–19] However, many of these studies have used relatively insensitive stimulation tests, or the even less sensitive 8.00 am cortisol concentration. It is now accepted that integrated tests of adrenal function are superior to stimulation testing, either timed urinary cortisol measurements[20] or preferably serial plasma cortisol measurements.[21]

Integrated plasma cortisol studies, where subjects are admitted overnight and blood sampled every 20 minutes via an indwelling cannula for measurement of plasma cortisol, have shown diminished adrenal function on inhaled corticosteroids. A cross sectional study in 19 asthmatic children, 12 of whom were receiving inhaled beclomethasone dipropionate, showed a dose dependent decrease in integrated overnight plasma cortisol.[4] The same group have since shown similar results, again for overnight sampling, in a prospective crossover study comparing beclomethasone dipropionate and budesonide in 12 asthmatic children. Both beclomethasone dipropionate and budesonide significantly decreased overnight integrated plasma cortisol by greater than 30% compared with baseline.[19] Both of these studies have been restricted to overnight cortisols due to the difficulties in collecting daytime samples. The only study to measure serial daytime samples does not have a control group for comparison,[22] a difficulty we have avoided by our placebo controlled design. We have also been able to demonstrate the significant suppression seen after a single inhalation of 200 μg of corticosteroid.

A major difficulty with serial plasma cortisol testing is the need for an indwelling cannula, so confining the subjects to the relatively non-physiological setting of the hospital environment. Using the Soft Touch bloodletter and collection on to filter paper, the children were able to lead their normal lives in their own

home environment. Furthermore our sampling immediately on waking is likely to be of greater physiological significance than conventional 8.00 am cortisol collection, where children are often woken early to be brought to hospital for formal venesection.

A possible alternative to plasma cortisol would be salivary cortisol measurement, which has also been shown to be suppressed during the daytime in children with asthma receiving inhaled corticosteroids.[23] The disadvantage of salivary cortisol is the need to rapidly freeze samples to −20°C, so diminishing its appeal for serial measurements outside the hospital environment. We feel bloodspot cortisol is superior because, as long as it is kept dry, the sample may be stored for up to three months before analysis (unpublished). Measurement of bloodspot cortisol combines both simplicity of collection and ready acceptability to most children. It is clear that sensitive indices of the pituitary-adrenal axis are suppressed in children receiving inhaled corticosteroids. However, the functional significance of suppression of biochemical indices of pituitary-adrenal axis function remains unclear. It is salient to note that despite the widespread use of inhaled corticosteroids only two cases of resultant acute adrenal insufficiency have been reported, in one adult[24] and one child.[25] We feel that until there is a clearer understanding of the long term side effects of inhaled corticosteroids it is important to document their potential adverse effects, but that for the time being they must remain the mainstay of treatment for the child with moderate to severe asthma.

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