Bone and collagen turnover during treatment with inhaled dry powder budesonide and beclomethasone dipropionate

N H Birkebæk, G Esberg, K Andersen, O Wolthers, C Hassager

Abstract

Objective—To assess bone and collagen turnover in asthmatic children treated with dry powder budesonide from the Turbuhaler and dry powder beclomethasone dipropionate from the Diskhaler in a dose of 800 µg/day.

Subjects—Thirteen prepubertal children with asthma.

Design—Open crossover study with two treatment periods and treatment free run-in and wash-out periods. All periods were of two weeks' duration. At day 14 in each period blood samples were taken for assessment of serum osteocalcin, the carboxyterminal propeptide of type I collagen (PICP), and the aminoterminal propeptide of type III collagen (PIINP). At the same time urine was collected for assessment of creatinine corrected pyridinoline (uPYR/cr) and deoxypyridinoline (udPYR/cr) crosslinks.

Results—Osteocalcin concentrations were not influenced by any of the treatments. During budesonide treatment mean (SEM) PICP was reduced by 18% (8%) ($p=0.03$), PIINP by 24% (3%) ($p=0.0002$), uPYR/cr by 16% (6%) ($p=0.03$), and udPYR/cr by 21% (13%) ($p=0.12$). During treatment with beclomethasone dipropionate mean (SEM) PICP was reduced by 20% (6%) ($p=0.01$), PIINP by 36% (3%) ($p=0.0002$), uPYR/cr by 18% (4%) ($p=0.004$), and udPYR by 13% (5%) ($p=0.02$). The suppressive effect of beclomethasone dipropionate on PIINP was more marked than that of budesonide ($p=0.001$).

Conclusion—Treatment with dry powder budesonide and beclomethasone dipropionate 800 µg/day is associated with suppression of bone and collagen turnover. The suppression seems to be more marked during treatment with beclomethasone dipropionate. Long term effects and effects of lower doses of budesonide and beclomethasone dipropionate on bone and collagen markers needs further study.

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Keywords: budesonide, beclomethasone dipropionate, procollagen peptides, pyridinoline crosslinks.

We recently evaluated some new markers of bone and collagen turnover as measures of systemic effects on children treated with glucocorticosteroids. Serum concentrations of osteocalcin, a marker of bone formation, were

depressed during treatment with low doses of oral prednisolone but not during treatment with inhaled budesonide 200 and 800 µg from a pressurised metered dose inhaler with a spacer.¹ The aminoterminal propeptide of type III procollagen (PIINP), a serum marker of type III collagen synthesis, and the carboxyterminal propeptide of type I procollagen (PICP), a marker of type I collagen synthesis, were suppressed during treatment with prednisolone.² These findings suggest that valuable information about glucocorticosteroid induced effects on bone and collagen turnover may be obtained from studies of these serum markers.

Treatment with inhaled glucocorticosteroids using dry powder inhalers are increasingly preferred due to concern about possible adverse effects of the Freon driven metered dose inhalers on the environment. Budesonide delivered from the Turbuhaler and beclomethasone dipropionate delivered from the Diskhaler are widely used dry powder delivery devices. The aim of the present study was to evaluate whether budesonide 800 µg/day delivered from the Turbuhaler and beclomethasone dipropionate 800 µg/day delivered from the Diskhaler influence serum and urinary markers of bone and collagen turnover in children.

Subjects and methods

Twelve prepubertal boys and four prepubertal girls were studied. All suffered from mild asthma and needed treatment only with inhaled β_2 stimulants at the time of the study. Mean (range) age was 8.7 years (6–11.5 years), and mean body surface area was 1.0 m² (0.78–1.20 m²).

The study was an open crossover trial with periods of 14 days. In period 1 (run-in) and 3 (wash-out) no treatment was given. In periods 2 and 4 the children took dry powder budesonide from the Turbuhaler or dry powder beclomethasone dipropionate from the Diskhaler in a dose of 800 µg/day. Treatment order was allocated by means of a computerised randomisation scheme prepared in balanced blocks. The inhalations were taken twice daily at 8 am and 8 pm as two actuations of 200 µg. The children were carefully instructed in inhalation techniques. They were asked to rinse their mouth after the inhalations. As a measure of compliance, the number of delivered inhalations taken by each patient was counted. If compliance was less than 80% the patient was excluded from the study.

Department of
Paediatrics, Århus
University Hospital,
Århus, and
Department of
Paediatrics, Esbjerg
Central Hospital,
Esbjerg, Denmark
N H Birkebæk

Department of
Paediatrics, Århus
University Hospital,
Århus
G Esberg
K Andersen
O Wolthers

Center for Basic and
Clinical Research,
Ballerup
C Hassager

Correspondence to:
Dr N H Birkebæk,
Skovgårdsparken 44, 7080
Børkop, Denmark.

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Forced expiratory volume in one second was measured with a dry wedge spirometer (Vitalograph) at each visit. Peak expiratory flow was measured at home in the morning and in the evening with a mini-Wright peak flow meter. To avoid any influence of changes in pulmonary function on the markers of bone and collagen turnover children showing more than 15% variation in pulmonary function between the various periods or more than 25% day to day variation in peak flow were withdrawn from the study.

Samples of blood and urine were taken at the end of each period between 2 pm and 3 pm. The children were instructed to empty their bladder at roughly 11 am and to discard the urine. Afterwards they were asked not to urinate again until urine sampling at 2–3 pm. The blood was centrifuged within one hour after sampling. Serum and urine samples were stored at -80°C and batch assayed at completion of the study.

SERUM AND URINE ANALYSIS

The plasma concentration of osteocalcin was measured by a radioimmunoassay.³ Antiserum was raised in rabbits immunised with purified calf osteocalcin, and homogenous calf osteocalcin was used for standard and tracer. The intra-assay and interassay variations were less than 10%. The serum concentration of PICP was determined by a radioimmunoassay recently developed.⁴ The assay is based on PICP purified from the cell culture medium of human fetal fibroblasts; this PICP is cleaved as in vivo from collagen. The assay uses polyclonal rabbit antibodies. The intra-assay and interassay variations were less than 2% and 4%, respectively. The serum concentration of intact and high molecular weight PIIINP was determined by a commercial available radioimmunoassay (PIIINP RIA Kit, Farnos Diagnostica, Oulunsalo, Finland).⁵ The assay uses polyclonal rabbit antibodies. The antigen used is purified human PIIINP. The intra-assay and interassay variations were less than 3% and 5%, respectively. The urinary pyridinoline (uPYR) and deoxypyridinoline (udPYR) crosslinks were measured by spectrofluorometry after acid hydrolysis and separation on a reverse phase C18 column by high performance liquid chromatography as reported elsewhere.⁶ The intra-assay and interassay variations were 4% and 9% for uPYR and 6% and 12% for udPYR, respectively. The excretion of uPYR and udPYR were expressed in ratios of creatinine excretion (uPYR/cr and udPYR/cr) in order to correct for dilution and sampling time.⁶

STATISTICS

The Wilcoxon non-parametric test for paired differences was used for comparison of the data. The method of Hills and Armitage was used to test for period and carry over effects.⁷ Probability <0.05 was considered significant.

The study was approved by the national ethics committee. All children and their

parents signed informed consent before entering the study.

Results

One boy was withdrawn because of a 30% deterioration of lung function during period 3 (wash-out period). All other children had normal pulmonary function throughout the study. Two boys were excluded from analysis because of compliance was less than 80%. The characteristics of the 13 children who were included in the analysis and the three boys, who were excluded, were similar with regard to age and body surface area. No significant differences in the measured bone and collagen parameters between the run-in period and the wash-out period were found. No carry over and period effect were found. Compliance with the dosage regimen during budesonide treatment was 95% (86%–104%), and during beclomethasone dipropionate 96% (86%–104%).

No statistically significant differences in the measured bone and collagen parameters between the run-in period and the wash-out period were found. No carry over and period effects were found.

Mean and individual data are shown in figs 1 and 2. The nominal reductions of osteocalcin during treatment with budesonide and beclomethasone dipropionate were not statistically significant. Mean PICP was reduced from 270 to 221 $\mu\text{g/l}$ ($p=0.03$), and PIIINP from 8.8 to 6.7 $\mu\text{g/l}$ ($p=0.0002$) during treatment with budesonide. PICP was reduced from 268 to 215 $\mu\text{g/l}$ ($p=0.01$), and PIIINP from 8.7 to 5.6 $\mu\text{g/l}$ ($p=0.0002$) during treatment with beclomethasone dipropionate. Budesonide treatment reduced mean uPYR/cr from 0.25 to 0.21 mmol/mmol creatinine ($p=0.03$) and udPYR/cr from 0.037 to 0.029 mmol/mmol creatinine ($p=0.12$). Beclomethasone dipropionate treatment reduced mean uPYR/cr from 0.22 to 0.18 mmol/mmol creatinine ($p=0.004$) and udPYR/cr from 0.029 to 0.026 mmol/mmol creatinine ($p=0.02$). The reduction in PIIINP during treatment with beclomethasone dipropionate (3.1 $\mu\text{g/l}$) was more marked than during budesonide treatment (2.1 $\mu\text{g/l}$) ($p=0.001$).

Figure 3 shows the per cent reduction in the mean value of the measured bone and collagen parameters during budesonide and beclomethasone dipropionate treatment.

Discussion

Aside from treatment with glucocorticosteroids several factors may influence bone and collagen turnover in asthmatic children such as suppression of physical activity, psychological stress, and severity of the disease. In this short term study of children with mild asthma we tried to keep all variables except glucocorticosteroid treatment constant throughout the study, as we wanted to reduce possible influences of other variables. The finding of similar levels of bone and collagen turnover during the run-in and the wash-out periods separated by four weeks suggested that this was achieved. Studies in

Figure 1 Individual and mean values of serum osteocalcin, PICP, and PIIINP. Column 1: before budesonide treatment; 2: during budesonide treatment; 3: before beclomethasone dipropionate treatment; and 4: during beclomethasone dipropionate treatment.

adults have shown that serum concentrations of osteocalcin and PICP and of excretion of uPYR and udPYR crosslinks exhibit circadian rhythms with decreases during the morning, increases in the afternoon, and reaching peak levels during the night.⁸⁻¹⁰ In the present study the possible influence of circadian rhythms was minimised by the time of blood and urine sampling.

Osteocalcin is the most abundant non-collagenous protein of bone. In adults serum concentrations of osteocalcin correlate well with changes in osteoblast activity.¹¹ Our findings of no influence on osteocalcin from 800 µg dry powder budesonide and beclomethasone dipropionate are in agreement with our previous

study of inhaled budesonide in doses of 200–800 µg delivered from a metered dose inhaler.¹ Contradicting evidence has been found in studies in adults treated with doses of 400–2000 µg beclomethasone and from one study in children treated with 800 µg budesonide.^{12,13} In the latter study blood samples for analysis were taken after a 12 hour fast in the morning. It is not known to what extent fasting may influence serum concentrations of osteocalcin in children and it must be kept in mind that there is a high interassay difference in osteocalcin measurements.¹⁴ Furthermore, there are no data on the sensitivity of serum osteocalcin as a marker of osteoblast activity in children. In adults, most of the bone formation occurs as part of a continuous remodelling of bone and only to a limited extent as part of skeletal growth (modelling). In contrast, modelling is the major activity of the skeleton in children, who are increasing bone mass throughout childhood. Serum osteocalcin may be a less sensitive marker of the effect of inhaled glucocorticosteroids on osteoblasts mainly engaged in bone modelling in children.

Type I collagen is the most abundant collagen type in connective tissue and accounts for more than 90% of the organic matrix of bone.¹⁵ During the formation of type I collagen PICP is cleaved from the procollagen and released into the circulation. PICP is thus a marker of type I collagen formation. Serum PICP concentrations correlate with bone formation rates measured by histomorphometry

Figure 2 Individual and mean values of uPYR/cr and udPYR/cr crosslinks. Column 1: before budesonide treatment; 2: during budesonide treatment; 3: before beclomethasone dipropionate treatment; and 4 during beclomethasone dipropionate treatment.

Figure 3 Mean per cent changes of osteocalcin, PICP, PIIINP, uPYR/cr, and udPYR/cr during treatment with budesonide (white columns), and beclomethasone dipropionate (black columns). The statistical differences between budesonide and beclomethasone dipropionate are shown.

in bone biopsies.¹⁶ PYR and dPYR are crosslinking amino acids that are formed during maturation of collagen fibrils in bone. PYR and dPYR are released from bone during resorption, and excreted in the urine without undergoing catabolisation.¹⁷ Though PYR is present in tendon and cartilage it is thought that, due to a slow turnover rate, these tissues contribute only to a little extent to the urinary output of PYR. Thus, the concentration of PYR and dPYR in urine are related to the amount of bone resorbed and hence to bone turnover.^{17 18} The simultaneous assessment of PICP and PYR make it possible to estimate the balance between type I collagen formation and degradation. When coupling between the two activities is effective they should be changed into the same direction. The finding of reduced serum concentrations of PICP and reduced concentrations of uPYR and udPYR during treatment with 800 µg inhaled budesonide and beclomethasone dipropionate indicates that the treatments inhibit bone collagen turnover, which may be reset in a suppressed steady state condition. That is in accordance with our previous findings of suppressive effects of oral prednisolone on bone turnover.² PIIINP is released into the circulation when collagen type III, which accounts for 10–50% of body collagen, is synthesised.¹⁹ Serum values of PIIINP seem to be sensitive markers of collagen synthesis. Furthermore serum PIIINP concentrations correlate with growth rates.^{20 21} Therefore reduced concentrations of PIIINP during both budesonide and beclomethasone treatment may suggest an inhibition of growth.

The present data are in accordance with recent findings of suppressive effect of 800 µg inhaled budesonide from a metered dose inhaler on serum concentrations of PIIINP.²² However, the suppressive effect of beclomethasone on PIIINP concentrations was more marked than that of budesonide. This suggests that beclomethasone from the Diskhaler may be associated with a greater systemic activity than budesonide from the Turbuhaler. This may be due to variations in systemic bioavailability and systemic potency of the two drugs and administration devices.²³ A difference in the systemic activity between the two inhaled compounds has been shown in studies of the effect on the hypothalamic-pituitary-adrenal axis.²⁴

This study indicate that 800 µg dry powder inhaled budesonide and beclomethasone dipropionate per day suppress bone and collagen

turnover in asthmatic children. The suppressive effect of beclomethasone dipropionate seem to be more marked than that of budesonide. However, long term studies need to be made to clarify the clinical implications of these findings.

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