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

N H Birkebæk, G Esberg, K Andersen, O Wolters, 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 (PIIINP). 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), PIIINP 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), PIIINP 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 PIIINP 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.

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 (PIIINP), 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 β2 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 (0.78-1.2 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.
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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, Farmos Diagnostica, Ouluunso, 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 μg/l (p=0.03), and PIIINP from 8·8 to 6·7 μg/l (p=0·0002) during treatment with budesonide. PICP was reduced from 268 to 215 μg/l (p=0·01), and PIIINP from 8·7 to 5·6 μg/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 μg/l) was more marked than during budesonide treatment (2·1 μg/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.

![Graph showing changes in serum osteocalcin, PICP, and PIIINP during different treatments.]

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

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.\(^2\)\(^3\) 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.\(^4\)

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.\(^5\) 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.

adults have shown that serum concentrations of osteocalcin and PICP and of excretion of uPYR and uPYR crosslinks exhibit circadian rhythms with decreases during the morning, increases in the afternoon, and reaching peak levels during the night.\(^6\)\(^−\)\(^10\) 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
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Bone and collagen turnover in asthmatic children. The suppressive effect of beclometasone dipropionate seems 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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