Changes in markers of bone metabolism during dexamethasone treatment for chronic lung disease in preterm infants

P C Ng, C W K Lam, G W K Wong, C H Lee, P S Cheng, T F Fok, I H S Chan, E Wong, K Cheung, S Y Lee

Aim: To characterise the change in serum and urinary bone markers in the early postnatal period, and to assess the effect of systemic corticosteroid on bone metabolism in preterm infants.

Methods: Bone formation was quantified by measurement of serum concentrations of bone specific alkaline phosphatase (BALP) and osteocalcin. Bone resorption was measured by monitoring creatinine adjusted urinary deoxypyridinoline (Dpd) concentration. Blood and urinary samples were collected from corticosteroid treated infants \( n = 19 \) immediately before the start \( (T_{\text{pre}}) \), three weeks after the start \( (T_{\text{end}}) \), and two \( (T_{\text{pre}1}) \) and four weeks \( (T_{\text{pre2}}) \) after the end of the dexamethasone course. Untreated patients \( n = 30 \) had specimens taken at week 3 \( (T_{\text{wk3}}) \), 6 \( (T_{\text{wk6}}) \), 8 \( (T_{\text{wk8}}) \), and 10 \( (T_{\text{wk10}}) \) of postnatal age.

Results: Serum concentrations of BALP and osteocalcin at \( T_{\text{pre}} \) were significantly lower than pretreatment levels and the levels at the corresponding time point \( (T_{\text{pre2}}) \) of the non-treatment group. In contrast, urinary Dpd concentration at \( T_{\text{pre2}} \) was not significantly decreased compared with the pretreatment level. However, it was significantly lower than the urinary Dpd concentration at \( T_{\text{end}} \) of the non-treatment group. The rate of increase in lower leg length was significantly higher in the non-treatment group between weeks 3 and 6 than in the corresponding period during dexamethasone treatment in the corticosteroid group.

Conclusion: Systemic corticosteroid causes appreciable suppression of serum BALP and osteocalcin and, to a lesser extent, urinary Dpd. The results suggest that corticosteroid inhibits bone growth mainly by decreasing bone formation.

PATIENTS AND METHODS

Study population

Nineteen preterm VLBW infants who were admitted to the neonatal unit at Prince of Wales Hospital, Hong Kong between September 1997 and January 1999 and received systemic dexamethasone for treatment of BPD, were prospectively enrolled in the study. In addition, 30 VLBW infants who did not receive corticosteroid treatment within the same study period were used for comparison. Preterm infants with major congenital or skeletal abnormalities or chromosomal disorders, and those who received postnatally inhaled corticosteroid were excluded.

Processing of specimens and anthropometric measurements

Blood and urinary samples were collected from corticosteroid treated infants immediately before the start \( (T_{\text{pre}}) \), three weeks after the start \( (T_{\text{end}}) \), and two \( (T_{\text{pre1}}) \) and four weeks \( (T_{\text{pre2}}) \) after the end of dexamethasone treatment. Untreated patients had blood and urinary specimens taken at week 3 \( (T_{\text{wk3}}) \), 6 \( (T_{\text{wk6}}) \), 8 \( (T_{\text{wk8}}) \), and 10 \( (T_{\text{wk10}}) \) of postnatal age. Serum BALP was quantified by precipitation with wheatgerm lectin, and serum osteocalcin by fluorescence enzyme immunoassay (Pharmacia Upjohn Diagnostics AB, Uppsala, Sweden). Urinary Dpd was measured by chemiluminescence enzyme immunoassay (ACS analyser, Chiron Diagnostics Corp, Norwood, Massachusetts, USA), and urinary creatinine.

Abbreviations: BALP, bone specific alkaline phosphatase; Dpd, deoxypyridinoline; VLBW, very low birth weight; BPD, bronchopulmonary dysplasia.
by the Jaffe (kinetic) method (Hitachi 911 autoanalyser, Roche Diagnostics Corp, Indianapolis, Indiana, USA). The interassay coefficients of variation for serum BALP and osteocalcin and urinary Dpd and creatinine were: 4.1% at 83 U/l, 5.6% at 37.8 ng/l, 6.7% at 23.7 nmol/l, and 4% at 7.51 mmol/l respectively. Urinary Dpd concentrations were subsequently corrected for creatinine concentration. All blood and urinary specimens were collected in the morning between 0800 and 1200, and coincided with the weekly measurements of haemoglobin and liver function in order to minimise disturbance of these infants. Lower leg length was also measured weekly at the bedside using a neonatal knemometer.10

**Dexamethasone dose**

The decision to start systemic dexamethasone treatment was made by the attending neonatologists. The neonatal unit guidelines were: (a) ventilator dependence or oxygen requirement of more than 40% after 2 weeks of age; (b) absence of any treatable cause that might prevent successful weaning, such as infection or patent ductus arteriosus; (c) absence of any major contraindication for starting corticosteroid treatment, such as severe hypertension, uncontrolled hyperglycaemia, massive gastrointestinal haemorrhage, or recent abdominal surgery. Each infant was given a three week dose tapering course of dexamethasone (dexamethasone sodium phosphate; Weimex Pharma, Rastatt, Germany) starting with 0.6 mg/kg/day in the first week. The initial dose was then halved and quartered in the second, and third week of treatment respectively.2 Dexamethasone was given as a bolus intravenous injection in the morning.

**Enteral and parenteral nutrition**

Preterm VLBW infants were routinely started on intravenous dextrose after birth to prevent hypoglycaemia. Parenteral nutrition, 6% TrophAmine (McGaw Inc, Irvine, California, USA) and 20% Intralipid (Kabi Pharmacia AB, Stockholm, Sweden), was started on day 3 of life. Oral milk feeds were usually introduced between day 5 and 7, and the quantity of milk was cautiously increased by 0.5–1 ml/hour/day depending on tolerance. VLBW infants were fed mothers’ milk whenever possible, but preterm commercial milk formula could also be used if the parents preferred. They were closely monitored for the occurrence of vomiting, abdominal distension, and volume of gastric residuals. Strict guidelines were provided for neonatologists with regard to stopping and restarting of enteral feeding and hyperalimentation. Oral vitamin D (400 IU/day) and oral sodium hydrogen phosphate supplements (1 mmol/kg/day in six divided doses) were started once the infant was tolerating full enteral feeding. Our unit policy is to maintain the plasma phosphate concentration at 1.8–2.2 mmol/l.

**Ethics**

Ethical approval for the study was obtained from the clinical research ethics committee of the Chinese University of Hong Kong. Informed parental consent was obtained for each case.

**Statistical analysis**

The descriptive statistics on clinical details and concentrations of serum and urinary bone markers are expressed as median and interquartile range. Mann-Whitney U test or $\chi^2$ test was used to compare the clinical characteristics, anthropometric variables, initial rate of increase in lower leg length, and concentrations of serum and urinary bone markers before dexamethasone treatment ($T_{d-pre}$) and at the corresponding time point in the non-treatment group ($T_{d-pre}$). Multilevel models (mixed effects models)11 were used to assess the longitudinal change in growth of the lower leg and concentrations of serum and urinary bone markers at different stages of dexamethasone treatment in the corticosteroid group, and at different time points in the non-treatment group. Moreover, these models were also used to compare the concentrations of the bone markers at the corresponding time points between infants who received dexamethasone and those who did not ($T_{d-pre}$ $T_{d-post}$ $T_{d-post}$ and $T_{d-post}$ $T_{d-post}$). Multilevel modelling is an extension of the ordinary least squares regression, but takes into account the within and between subject heterogeneity.11 For longitudinal data, multilevel models allow for measurements made at unequal intervals and with a varied number of measurements—that is, subjects who may have one or several measurements. The models were fitted by using the restricted iterative generalised least square algorithm of MLn for Windows software.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics of very low birthweight infants, one group of which received corticosteroid (n=19) and the other group did not (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corticosteroid group (n=19)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>27 (26.7–29.2)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1038 (884–1192)</td>
</tr>
<tr>
<td>Male to female ratio (n)</td>
<td>12 (63%) : 7 (37%)</td>
</tr>
<tr>
<td>Antenatal dexamethasone (doses)</td>
<td>2 (2–4)</td>
</tr>
<tr>
<td>Duration between the last dose of antenatal dexamethasone and delivery (h)</td>
<td>52 (15–115)</td>
</tr>
<tr>
<td>Mode of delivery: Caesarean section : instrumental : normal</td>
<td>9 (47%) : 0 (0%) : 10 (53%)</td>
</tr>
<tr>
<td>Apgar scores 1 min</td>
<td>5 (4.0–7.3)</td>
</tr>
<tr>
<td>5 min</td>
<td>9 (8.0–9.0)</td>
</tr>
<tr>
<td>Age at start of postnatal dexamethasone treatment (days)</td>
<td>25 (17–37)</td>
</tr>
<tr>
<td>Age at start of multimitan preparation—Vimax (days)</td>
<td>35 (25–53)</td>
</tr>
<tr>
<td>Age at start of oral phosphate supplement (days)</td>
<td>33 (25–54)</td>
</tr>
<tr>
<td>Age at which full enteral feeding achieved (days)</td>
<td>33 (25–53)</td>
</tr>
<tr>
<td>Duration of mechanical ventilation (days)</td>
<td>28 (13–59)</td>
</tr>
<tr>
<td>Duration of oxygen dependency (days)</td>
<td>37 (22–63)</td>
</tr>
<tr>
<td>Duration of hospital stay (days)</td>
<td>103 (84–118)</td>
</tr>
<tr>
<td>Outcome</td>
<td>Alive : dead</td>
</tr>
</tbody>
</table>

Where applicable, results are median (interquartile range). *p<0.05, **p<0.001 compared with corticosteroid group.
The rest of the statistical tests were performed by SPSS for Windows (Release 9.0 SPSS Inc, Chicago, Illinois, USA). The statistical analysis was performed on raw, square root, and logarithmically transformed results where appropriate in order to correct the skewness of the data. The level of significance was set at 5% in all comparisons.

RESULTS

Nineteen VLBW infants who required dexamethasone for treatment of BPD were enrolled in the study. All infants completed a full course, and their results were compared with those of 30 VLBW infants who did not receive the drug. Table 1 summarises the clinical characteristics of the corticosteroid and non-treatment group. More infants in the non-treatment group were born by caesarean section ($p < 0.05$). Corticosteroid treated infants had significantly lower gestational ages at birth ($p < 0.001$) and longer duration of mechanical ventilation ($p < 0.001$), oxygen supplementation ($p < 0.001$), and hospital stay ($p < 0.001$).

The median age at which dexamethasone treatment was started was 25 days ($T_{d-pre}$), and this time point corresponded closely to week 3 of postnatal age ($T_{wk-3}$) in the non-treatment group. Table 2 summarises the changes in concentrations of serum and urinary bone markers over the first 10 weeks of life. Figures 1–4 show the concentrations of serum BALP and osteocalcin, creatinine adjusted urinary Dpd concentrations, and rate of increase in lower leg length respectively at various time points.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Before treatment ($T_{d-pre}$)</th>
<th>End of treatment ($T_{d-end}$)</th>
<th>2 weeks after end of treatment ($T_{d-post 2}$)</th>
<th>4 weeks after end of treatment ($T_{d-post 4}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALP (U/l)</td>
<td>307 (212–389)</td>
<td>177 (126–268)</td>
<td>266 (195–367)</td>
<td>312 (192–340)</td>
</tr>
<tr>
<td>Osteocalcin (ng/l)</td>
<td>15.8 (11.1–21.0)</td>
<td>7.7 (4.9–13.6)</td>
<td>12.8 (8.6–19.3)</td>
<td>14.7 (10.2–17.2)</td>
</tr>
<tr>
<td>Dpd creatinine adjusted (mMol/l)</td>
<td>2.1 (2.0–2.4)</td>
<td>1.0 (0.9–1.3)</td>
<td>4.5 (3.5–5.6)</td>
<td>31.8 (10.3–40.3)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Results are median (interquartile range). BALP, bone specific alkaline phosphatase; Dpd, deoxypyridinoline.

### Figure 1

Serum bone specific alkaline phosphatase (BALP) concentrations (U/l; square root) in very low birthweight infants receiving corticosteroid or not over the study period. The asterisks indicate significant increase or decrease in serum BALP concentration compared with the pretreatment ($T_{d-pre}$) or week 3 ($T_{wk-3}$) levels within the same group. The parentheses indicate a significant difference in serum BALP concentration between the two groups when the corresponding time periods are compared. Results are expressed as mean and SEM.

### RESULTS

Nineteen VLBW infants who required dexamethasone for treatment of BPD were enrolled in the study. All infants completed a full course, and their results were compared with those of 30 VLBW infants who did not receive the drug. Table 1 summarises the clinical characteristics of the corticosteroid and non-treatment group. More infants in the non-treatment group were born by caesarean section ($p < 0.05$). Corticosteroid treated infants had significantly lower gestational ages at birth ($p < 0.001$) and longer duration of mechanical ventilation ($p < 0.001$), oxygen supplementation ($p < 0.001$), and hospital stay ($p < 0.001$).

The median age at which dexamethasone treatment was started was 25 days ($T_{d-pre}$), and this time point corresponded closely to week 3 of postnatal age ($T_{wk-3}$) in the non-treatment group. Table 2 summarises the changes in concentrations of serum and urinary bone markers over the first 10 weeks of life. Figures 1–4 show the concentrations of serum BALP and osteocalcin, creatinine adjusted urinary Dpd concentrations, and rate of increase in lower leg length respectively at various time points.

### Within group comparison

**Corticosteroid group**

At the end of the three week course of dexamethasone treatment ($T_{d-end}$), the markers of bone formation, serum BALP and osteocalcin concentrations, were significantly lower than pretreatment ($T_{d-pre}$) levels ($p < 0.0005$ and $p < 0.0001$ respectively). Serum concentrations of BALP and osteocalcin at $T_{d-end}$ and $T_{d-post 4}$ returned promptly to pretreatment values (figs 1 and 2). In contrast, urine Dpd concentration was only slightly lower at the end of the dexamethasone course, and thereafter
and 10 (Twk-10) were significantly raised compared with the level at Twk-3 (p < 0.05, p < 0.05, and p < 0.0001 respectively; fig 3). The rate of increase in lower leg length were significantly increased in the period after treatment (Td-pre–Td-end and Td-post2–Td-post4) compared with during treatment (T d-pre–Td-end) (p < 0.05 and p < 0.005, respectively; fig 3). In addition, rates of increase in lower leg length were significantly raised compared with that at week 3 (Twk-3) levels within the same group. The parentheses indicate a significant difference in serum osteocalcin concentration between the two groups when the corresponding time periods are compared. Results are expressed as mean and SEM.

Non-treatment group
Serum BALP and osteocalcin and urinary Dpd concentrations increased significantly with advancing postnatal age. Serum BALP concentrations at week 6 (T wk-6) and 8 (T wk-8) and 10 (T wk-10) were significantly raised compared with that at week 3 (T wk-3) (p < 0.05, p < 0.0001, and p < 0.0001 respectively; fig 1). Similarly, serum osteocalcin concentrations at week 8 (T wk-8) and 10 (T wk-10) were significantly raised compared with the week 3 (T wk-3) level (p < 0.05 and p < 0.005 respectively; fig 1). Moreover, urinary Dpd concentrations at week 6 (T wk-6), 8 (T wk-8), and 10 (T wk-10) were significantly increased compared with the level at T wk-1 (p < 0.05, p < 0.05, and p < 0.0001 respectively; fig 3). The rate of increase in lower leg length, however, did not change significantly over the first 10 weeks of life in the non-treatment group (fig 4).

Between group comparison
When the pretreatment concentrations of the bone markers of the corticosteroid group were compared with their corresponding levels at week 3 of postnatal age in the non-treatment group, only serum BALP was significantly higher in dexamethasone treated infants (p < 0.05). However, serum BALP concentration in the subsequent weeks (T wk-6 v T wk-3, T wk-10 v T wk-10) were significantly lower in the corticosteroid than in the non-treatment group (p < 0.0005, p < 0.001, and p < 0.0001 respectively; fig 1). Serum osteocalcin concentration was also significantly higher at T wk-3 and T wk-10 in the non-treatment group than at T wk-3 and T wk-10, in the corticosteroid group (p < 0.0001 and p < 0.05 respectively; fig 2). Urinary Dpd concentration at the end of dexamethasone treatment was significantly lower than the corresponding urinary concentration at week 6 of the non-treatment group (p < 0.01; fig 3). In addition, the rate of increase in the lower leg length was significantly higher in the non-treatment group between week 3 and 6 (T wk-3–Twk-6) than in the corresponding period (Td-pre–Td-end) of the corticosteroid group (p < 0.005). The mixed effects models also showed a significant difference in velocities in the periods after treatment (T d-end–T d-post2 v T wk-3–T wk-6 and T d-post2–T d-post4 v T wk-3–T wk-6) between the two groups (p < 0.005; fig 4). When the above comparisons are adjusted for gestational age and duration of mechanical ventilation, oxygen dependency, and hospital stay, all aforementioned significant results between the two groups remain unaffected.
DISCUSSION

Both markers of bone formation and resorption showed a progressive increase in concentration with advancing postnatal age in preterm infants. Bhandari et al. showed a progressive increase in the concentrations of serum C terminal propeptide of type I collagen and BALP in the neonatal period. Ward and his colleagues also showed a similar positive pattern with osteocalcin. Moreover, Naylor and his investigating team showed a significant increase in serum concentrations of osteocalcin and urinary pyridinium cross links in preterm infants in the first three weeks of life. The findings of Tsukahara et al., which were similar to our results, also showed that the mean concentrations of urine pyridinoline and Dpd at 30 days of postnatal age were significantly higher than those at day 7. Although Crofton and co-workers showed a significant trend of postnatal increase in markers of collagen synthesis, this was coupled with a decrease in concentrations of markers of collagen breakdown within the same period. The discrepancy in results on urinary bone markers between different studies may be attributable to the relatively small sample size in each study, the wide variation in concentrations of urinary markers, the wide margin of errors of spot urine specimens as these measurements were required to be standardised by correcting the urinary concentration with creatinine, and possibly also the influence of circadian variation in bone metabolism. Thus accumulating evidence, including our current study, indicates that markers of bone formation increase progressively with postnatal age, and the net increase in bone growth during the early neonatal period supports the notion that there is dynamic osteoblastic activity and rapid bone formation during this period.

As corticosteroid is of proven value in facilitating weaning from mechanical ventilation in preterm infants and is known to be more prevalent in patients of lower gestation, it is not possible to select a perfect control group without a randomised study, which is now no longer considered ethical to perform. Thus the best available approach is to compare the bone markers before and after dexamethasone treatment in corticosteroid treated patients, and also to compare the serum and urinary concentrations of bone markers of corticosteroid treated infants with those of non-treated infants with similar clinical characteristics and at comparable postnatal ages. Our results indicate that exposure of preterm VLBW infants to high dose systemic dexamethasone results in a significant decrease in serum concentrations of BALP and osteocalcin (fig 1 and 2), and suggest inhibition of bone formation. In vitro experiments have also shown considerable suppression of human osteoblastic cell function and DNA synthesis by dexamethasone and methylprednisolone. Similarly, the use of systemic corticosteroid in adult patients with rheumatoid arthritis has been associated with negative short term effects on bone formation, whereas markers of bone resorption remained unchanged or decreased. In contrast, Corton et al. in a recent study suggested an increase in plasma BALP concentration in preterm infants who received dexamethasone for treatment of BPD. However, only a small number of subjects (n = 14) were investigated, the response of BALP to corticosteroid was highly variable among patients, and the study only showed a non-significant positive trend. Although urinary Dpd concentration at the end of the dexamethasone course (T5-end) had not decreased significantly from the pretreatment level (T1pre), the concentration at T5-end was significantly lower than the corresponding level at week 6 (T6) in the non-treatment group (fig 3). The overall results therefore suggest that the use of systemic dexamethasone in preterm infants reduces bone turnover and metabolism.

In summary, we have shown that both serum concentrations of BALP and osteocalcin and urinary concentrations of Dpd increase significantly with advancing postnatal age, indicating an increase in bone turnover and rapid bone growth during the early neonatal period. In contrast, dexamethasone reduces cell proliferation, decreases differentiation of osteoblast progenitors, and disrupts the organisation of new osteoblast attachment to the osteoid surface. Hence, the negative effect of long term corticosteroid usage may cause a decrease in bone length, trabecular bone volume, and bone mineral content.

During the study, we also collected data on the daily nutritional intake including parenteral nutrition, daily milk consumption, and supplementation with vitamins and phosphate. There was no significant difference in the age at which full enteral feeding was achieved and the time at which oral vitamin and mineral supplements were introduced between the two groups (table 1). Changes in concentrations of bone markers in dexamethasone treated infants were, however, not affected by energy intake or supplementation with vitamins and phosphate. Thus the close temporal association between the change in levels of bone markers and the use of dexamethasone strongly suggests a causal relation.

In summary, we have shown that both serum concentrations of BALP and osteocalcin and urinary concentrations of Dpd increase significantly with advancing postnatal age, indicating an increase in bone turnover and rapid bone growth during the early neonatal period. In contrast, dexamethasone reduces cell proliferation, decreases differentiation of osteoblast progenitors, and disrupts the organisation of new osteoblast attachment to the osteoid surface. Hence, the negative effect of long term corticosteroid usage may cause a decrease in bone length, trabecular bone volume, and bone mineral content.

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Arch Dis Child Fetal Neonatal Ed 2002 86: F49-F54
doi: 10.1136/fn.86.1.F49

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