J Microbiol Immunol Infect
2000;33:25-28

Acute effect of glucocorticoid treatment on serum osteocalcin levels in asthmatic children

Jin-Shuh Jan, Wei-Fong Wu

Department of Pediatrics, Taipei Municipal Jen Ai Hospital, Taipei, Taiwan, ROC

Received: June 15, 1999 Revised: August 10, 1999 Accepted: August 18, 1999

The serum levels of osteocalcin (OC), a sensitive and specific biochemical marker of bone formation, were measured in 36 asthmatic children before and after intravenous administration of glucocorticoid (GC), methylprednisolone (1 mg/kg/6 h). A statistically significant ($p < 0.005$) decrease in OC level (13.5 - 2.7 μg/L) after the completion of 1-day treatment. In patients who received 2-day treatment, the OC levels further declined and reached about 20% of the pretreatment levels. Blood samples collected from patients who received 3-day treatment showed serum OC had declined to an even lower level after the completion of therapy (11.5-1.8 μg/L). An inverse relationship was found between the OC concentration and the duration of GC therapy. GC therapy induced a minor, significant decrease of serum alkaline phosphatase level but did not influence serum calcium level. A tendency toward decrease of serum phosphate level was also noted during GC treatment. An acute effect of GC therapy on the suppression of osteoblasts was biochemically revealed by the finding of reduced serum OC levels. The results suggest that early change in serum OC may be a useful indicator for patients at high risk of bone loss.

Key words: Osteocalcin (OC), methylprednisolone, asthmatic children

Osteocalcin (OC), also known as bone Gla protein, is a Ca-binding and vitamin K-dependent protein of 49 amino acids with three γ-carboxyglutamic residues [1]. It is the most abundant noncollagenous protein in mature bone matrix and constitutes 1% to 2% of the total bone protein or 20% to 25% of the noncollagenous bone protein. After maturation and secretion by bone cells, OC remains predominantly bound to the hydroxyapatite matrix in the bone, while about 1% of newly synthesized OC is released and circulates in the blood stream [2]. Measurement of serum OC levels has been suggested to be a more specific biochemical index of bone formation compared to other more-established indices such as urinary hydroxyproline and total serum alkaline phosphatase activity [3]. Serum levels of OC are used in the management of patients with metabolic bone disorders, including osteoporosis, Paget's disease, hyperparathyroidism, and diseases related to excess glucocorticoids (GC).

Radiography of the ribs and histomorphometric analysis of the iliac crest bone in patients with excess levels of GC have demonstrated both increased bone resorption and decreased bone formation. Increased bone resorption has been attributed to decreased intestinal calcium absorption, increased urinary calcium excretion, and enhanced parathyroid hormone secretion [4]. However, GC's inhibition of bone formation is due to a direct effect of GC on osteoblasts. OC, which reflects osteoblast activity and the formation of new bone tissue, may be an indicator of this inhibition. In this study, we measured levels of serum OC in asthmatic children before, during, and after intravenous methylprednisolone treatment and examined the relationship between serum OC concentration and the duration of treatment.

Patients and Methods

From December 1994 to August 1995, a total of 36 children who had just experienced attacks of asthma, were treated with intravenous GC methylprednisolone (1 mg/kg/6 h). Their mean age was 5 years old, ranging from 1 to 12 years old. There were 20 boys and 16 girls. None of the patients had a history of endocrine, hepatic, renal, or other diseases known to affect bone metabolism. None of the patients had received GC treatment within two months prior to admission. Routine serum chemistry and hematological evaluations, including creatinine, were made at the time of admission.

Blood samples were taken just before GC therapy

Corresponding author: Dr. Jin-Shuh Jan, Department of Pediatrics, Taipei Municipal Jen Ai Hospital, 10, Sec. 4, Jen-Ai Road, Taipei, Taiwan, ROC.

Table 1. Clinical and laboratory data of 36 asthmatic children before IV methylprednisolone treatment

	Mean ±SD	Range
Age (years)	5.2 ± 2.1	1.2 - 12.1
Sex (M:F)	20 : 16	
WBC ($10^3/mm^3$)	8.18 ± 4.21	2.71 - 13.40
Eosinophil (%)	1.1 ± 0.8	0 - 12
BUN (mg/dL)	12.0 ± 3.1	9 - 17
Cr (mg/dL)	0.44 ± 0.08	0.3 - 0.7
AST (U/L)	38 ± 9	22 - 84
ALT (U/L)	34 ± 5	17 - 33
IgE (IU/mL)	405 ± 201	38 - 1630

Abbreviation: WBC = white blood cell; BUN = blood urea nitrogen; Cr = creatinine; AST = aspartate aminotransferase; ALT = alanine aminotransferase

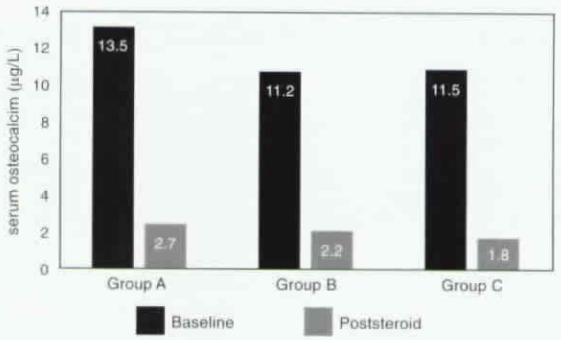

Fig. 1. Serum OC level in three asthmatic groups before and after methylprednisolone treatment. The duration of treatment in group A, B and C were 1, 2 and 3 days, respectively.

and the day after the completion of treatment. The treatment with GC was stopped after 1, 2, or 3 days depending on the patient's clinical improvement. These patients were then divided into three groups depending on the number of days of treatment with GC. There were 10 patients who had received 1 day of GC treatment (group A), 12 patients who had received 2 days of treatment (group B), and 14 patients who had received 3 days of treatment (group C). The serum levels of OC just before treatment and on the day after completion of treatment were analyzed with a monoclonal antibody-based fluorometric assay (Pharmacia CAP System). This method is an *in vitro* test system for quantitative measurement of intact OC. The serum concentrations of calcium and phosphate were determined by automated methods. The serum values of total alkaline phosphatase were measured by an enzymatic method.

Results

The results of routine serum biochemistry and hematological evaluations of the 36 asthmatic children before GC therapy are shown in table 1. All of the patients had normal renal function. The mean level of serum Ig E was higher than normal, and some patients had an elevated eosinophil count.

The levels of serum OC were not significantly different among the three groups before therapy (Fig. 1). On the day after completion of intravenous methylprednisolone therapy, a decrease of serum OC was found in all 10 patients of group A. The 12 asthmatic children who received 2 days' therapy showed further declines in OC to about 20% of the pretreatment level. The patients who received 3 days of treatment showed reduction of OC to an even lower level. Figure 2 shows the progressive decline in OC with increasing duration of GC therapy.

Table 2. Mean concentrations of serum OC, calcium, phosphate, and alkaline phosphatase in asthmatic patients before and after IV methylprednisolone treatment

		Group A	Group B	Group C
OC (μg/L)	Baseline	13.5 ± 5.4	11.2 ± 4.8	11.5 ± 4.6
	Post-treatment	2.7 ± 3.2[a]	2.2 ± 1.9[b]	1.8 ± 1.5[b]
Calcium (mM/L)	Baseline	9.9 ± 0.7	9.9 ± 0.3	10.0 ± 0.7
	Post-treatment	9.9 ± 0.8	9.9 ± 0.6	9.9 ± 0.8
Phosphate (mM/L)	Baseline	5.2 ± 1.1	5.0 ± 1.1	5.7 ± 1.4
	Post-treatment	4.2 ± 1.1	4.1 ± 1.0	4.2 ± 0.9
Alkaline phosphatase (U/L)	Baseline	581 ± 96	574 ± 140	539 ± 123
	Post-treatment	472 ± 196[a]	450 ± 80[a]	408 ± 79[a]

Group A represents patients who completed treatment after one day; group B after 2 days; and group C after 3 days. Data are expressed as mean values ±SD, *p* value by Wilcoxon Signed-ranks test.

[a] $p < 0.001$

[b] $p < 0.0001$

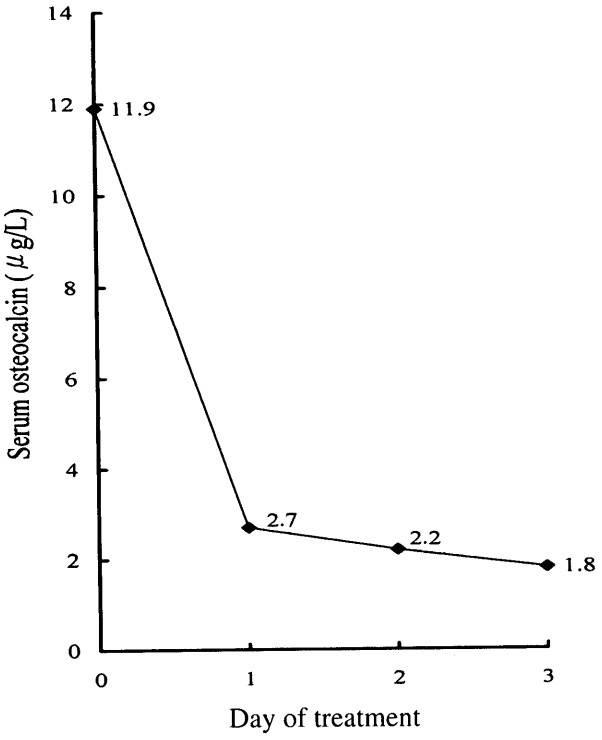

Fig. 2. Serum OC concentrations in patients with asthma before (n = 36) and after methylprednisolone treatment, for one (n = 10), two (n = 12) and three (n = 14) days.

During GC therapy, the serum concentration of alkaline phosphatase decreased significantly in all three groups. We also observed a tendency toward a decrease of serum phosphate. However, serum calcium levels remained unchanged before and after therapy (Table 2).

Discussion

The use of intravenous methylprednisolone in the treatment of severe asthmatic children provides significant therapeutic advantages [5]. Some asthmatic children may require long-term administration of GC. Among the many adverse effects of GC, retardation of growth is commonly observed and is particularly unpleasant for children. Osteoporosis is a serious clinical side-effect. It is important to find the early signs of growth retardation in order to protect children from development of irreversible short stature. The mechanisms whereby GC induces osteoporosis are multifactorial. On one hand, excess GC induces a reduction of bone formation because of its direct effect on osteoblast function; on the other hand, excess GC is thought to be associated with the enhancement of bone resorption [4]. However, the greatest contribution of GC excess to bone loss has been suggested to occur through osteoblast suppression [6].

Most previous studies on the potential adverse effects of GC have focused on the effect of GC on pituitary-adrenal function. However, the suppression of osteoblast activity is one of the major adverse effects of GC treatment. OC is synthesized and secreted only by bone cells of the osteoblastic phenotype [2]. OC has been evaluated in as a biochemical marker of osteoblast activity in many previous studies [7]. Previous studies have reported increased serum OC in patients with Paget's disease, acromegaly, primary and secondary hyperparathyroidism, postmenopausal osteoporosis, streak gonad syndrome, severe impairment of renal function, and a syndrome characterized by increased bone remodeling as a reparative process [7-12]. Decreased levels of serum OC have been found in patients with hypoparathyroidism, growth hormone deficiency, and in children with diabetes mellitus type 1 [13,14].

GC may induce a reduction of bone formation due to the suppression of osteoblastic activity. Biochemically, the degree of this suppression is specifically indicated by extent of reduced serum OC, a major osteoblast product. In this study we evaluated the direct effect of GC on serum OC. We found that the value of OC was significantly reduced as early as 24 h after GC therapy. This finding is supported by two previous studies although the number of patients in both of those studies was relatively small. Lem *et al* reported OC was decreased that immediately after GC-pulse therapy and returned to baseline levels 3 and 6 weeks after therapy [15]. Chertok *et al* noted that serum OC was a good biochemical marker of osteoblastic activity [16]. In general, OC is elevated in conditions characterized by increased bone turnover and the extent of elevation is correlated with serum alkaline phosphatase levels. Bromn *et al* indicated that OC is the only serum parameter that discriminates between high and low turnover osteoporosis as determined histomorphometrically.

Serum total alkaline phosphatase has been used as a marker of bone metabolism since Robison first suggested that osteoblasts were a source of the enzyme in serum. However, difficulties in interpreting the meaning of this marker are encountered when concomitant disease occurs as serum alkaline phosphatase originates not only from bone but also from liver, intestine, malignant tumor and other sources. Serum total alkaline phosphatase is the most commonly used marker of bone formation, but it lacks high sensitivity and specificity. It is not correlated with OC in a series of physiological and pathological conditions. In the present study, we found substantially reduced alkaline phosphatase levels in patients who had received

GC therapy. This result is in accordance with the findings of Ekenstam's study [18]. A highly significant, though more gradual decline in total and in bone alkaline phosphatase levels with GC therapy was reported by Prummel *et al* [19]. Serum OC and alkaline phosphatase could represent different aspects or different phases of osteoblast function, which are not inhibited to the same extent by GC. In contrast to the results of this study, serum alkaline phosphatase did not decrease significantly after GC therapy in Lem *et al*'s study [15]. Pederson also found no significant influence on alkaline phosphatase in asthmatic children treated with prednisolone [20]. The unaffected values of serum alkaline phosphatase might reflect the relative insensitivity of this marker of bone metabolism in children.

Gundberg's study illustrated that serum OC levels exhibited a circadian rhythmicity in normal men and women [21]. OC fell during the morning, rose in the afternoon and early evening, and reached a peak nocturnally. Men had higher levels of OC than women. The serum levels of OC level have been reported to increase with age, height, and bone age until age 12 to 13 years in girls and 14 to 15 years in boys [22]. Other factors may play a role in the variation in serum OC. It's clearance decreases in patients with severe impairment of renal function [13]. In addition, 1,25-dihydroxyvitamin D3 is known to regulate its synthesis [23]. Finally, age, sex, height, hormonal status and/or drug consumption are all important biological factors when measuring OC homeostasis.

In this study, the acute effect of GC on the suppression of osteoblast activity was well demonstrated by the finding of reduced serum OC. OC is synthesized only by osteoblasts. The metabolic clearance of OC is mainly dependent on renal function, and serum half-life of OC is approximately 20 min in men. These qualities make OC a specific, sensitive, and rapidly identifiable marker of osteoblastic activity. There are significant correlations between OC and histomorphometric parameters of bone formation. Impaired bone formation may have an important role in GC-induced osteoporosis, and early changes in serum OC may be a useful indicator of those patients at risk for severe bone loss. We therefore recommend monitoring the levels of serum OC when GC is administered for a long period.

References

1. Poser JW, Esch TS, Ling NC, Price PA. Isolation and sequence of the vitamin K-dependent protein from human bone. J Biol Chem 1980;255:8685-91.
2. Price PA, Williamson MK, Lothringer JW. Origin of the vitamin K-dependent bone protein found in plasma and its clearance by kidney and bone. J Biol Chem 1981;256:12760-6.
3. Delmas PD. Biochemical markers of bone turnover for the clinical assessment of metabolic bone disease. Endocrinol Metab Clin North Am 1990;19:1-18.
4. Lukert BP, Raisz LG. Glucocorticoid-induced osteoporosis: pathogenesis and management. Ann Intern Med 1990;112:352-64.
5. Younger RE, Gerber PS, Herrod HG, Cohen RM, Crawford LV. Intravenous methylprednisolone efficiency in status asthmaticus of childhood. Pediatrics 1987;80:225-30.
6. Smith R. Corticosteroid and osteoporosis. Thorax 1990;45:573-8.
7. Slovik DM, Gundberg CM, Neer RM, Lian JB. Clinical evaluation of bone turnover by serum osteocalcin measurements in a hospital setting. J Clin Endocrinol Metab 1984;59:228-30.
8. Wilkinson MR, Wagstoffe C, Posen S. Serum osteocalcin concentrations in Paget's disease of bone. Arch Intern Med 1986;146:268-71.
9. Marazuela M, Astigarraga B, Tabuenca MJ, Estrada J, Marin F, Lucas T. Serum bone Gla protein as a marker of bone turnover in acromegaly. Calcif Tissue Int 1993;52:419-21.
10. Delmas PD, Wahner HW, Mann KG, Riggs BL. Assessment of bone turnover in post menopausal osteoporosis by measurement of serum bone gla protein. J Lab Clin Med 1983;120:470-6.
11. Zseli J, Bosze P, Lakatos P. Serum bone Gla protein in streak gonad syndrome. Calcif Tissue Int 1991;48:387-91.
12. Delmas PD, Wilson DM, Mann KG, Riggs BL. Effect of renal function on plasma levels of bone Gla protein. J Clin Endocrinol Metab 1983;57:1028-30.
13. Kruse K, Kracht U. Evaluation of serum osteocalcin as an index of altered bone metabolism. Eur J Pediatr 1986;145:27-33.
14. Johansen JS, Jensen SB, Riis BJ, Rasmussen L, Zachmann M, Christiansen C. Serum bone Gla protein: a potential marker of growth hormone deficiency and the response to growth hormone therapy. J Clin Endocrinol Metab 1990;71:122-6.
15. Lems WF, Jacobs JW, Brink VD. Transient decrease in osteocalcin and markers of type 1 collagen turnover during high-dose corticosteroid pulse therapy in rheumatoid arthritis. Br J Rheumatol 1993;32:787-9.
16. Chertok P, Leroux JL, Blotman F, Colette C. High-dose intravenous methylprednisolone treatment and serum bone Gla protein. Arthritis Rheum 1991;11:1478-81.
17. Triffitt JT. The special protein of bone tissue. Clin Sci 1987; 72:399-408.
18. Ekenstam E, Stalenheim G, Hailgren R. The acute effect of high dose corticosteroid treatment on serum osteocalcin. Metab 1988;37:141-4.
19. Prummel MF, Wiersinga WM, Lips P, Sanders GT, Sauerwein HP. The course of biochemical parameters of bone turnover during treatment with corticosteroids. J Clin Endocrinol Metab 1991;72:382-6.
20. Wolthers OD, Riis BJ, Pedersen S. Bone turnover in asthmatic children treated with oral prednisolone or inhaled budesonide. Pediatr Pulmol 1993;16:341-6.
21. Gundberg CM, Markwitz ME, Mizruchi M, Rosen JF. Osteocalcin in human serum: a circadian rhythm. J Clin Endocrinol Metab 1985;60:736-9.
22. Tarallo P, Henny B, Fournier B, Siest G. Biological variations and reference limits. Scand Clin Lab Invest 1990;50:649-55.
23. Price PA, Baukol SA. 1,25-dihyoxyvitamin D3 increases synthesis of the vitamin K-dependent bone protein by osteosarcoma cells. J Biol Chem 1980;255:11660-3.