Change in C-terminal cross-linking domain of type I collagen in urine, a new marker of bone resorption, during and after gonadotropin-releasing hormone agonist administration

Michiyoshi Taga and Hiroshi Minaguchi
Department of Obstetrics and Gynecology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, 236 Japan
(Correspondence should be addressed to M Taga)

Abstract

Objective: The major side effect of GnRH agonist (GnRHa) therapy is the reduction of bone mass. To analyze bone resorption by GnRHa, we measured the urinary excretion of C-terminal telopeptides of type I collagen (CTX), a new marker of bone resorption.

Methods: We used a new ELISA for CTX (CrossLaps) in a sample of 18 premenopausal women with leiomyoma who were treated with daily administration of 400 μg nafarelin or 900 μg buserelin for 16 weeks.

Results: Urinary CTX excretion increased significantly during GnRHa treatment and then decreased at 12 and 24 weeks after the cessation of GnRHa therapy. Whereas the excretory profile of CTX during GnRHa therapy was almost similar to that of pyridinoline (Pyr) or deoxypyridinoline (D-Pyr), both biochemical markers of bone resorption, the magnitude of the change in CTX was significantly greater than that in Pyr or D-Pyr.

Conclusions: These results indicate that CTX could be a more sensitive marker for bone resorption than the currently used biochemical markers, and that CrossLaps ELISA is useful for therapeutic monitoring during and after GnRHa treatment.

European Journal of Endocrinology 137 167–171

Introduction

While the advent of several gonadotropin-releasing hormone agonists (GnRHa) has enabled the effective treatment of patients with endometriosis or leiomyoma, such therapy poses a potential risk to the skeleton because GnRHa reduces the serum estrogen concentration to a postmenopausal level, resulting in accelerated bone loss (1). The hypoestrogenism is characterized by increased bone turnover in which bone resorption is markedly accelerated. Increased levels of biochemical markers of bone metabolism such as urinary calcium, hydroxyproline, pyridinoline (Pyr), and deoxypyridinoline (D-Pyr) have been recognized during GnRHa treatment (2). Finkelstein et al. (3) also reported that urinary excretion of free pyridinolines increased significantly during nafarelin treatment. Although it has been reported that urinary Pyr and D-Pyr are sensitive markers of bone resorption in various metabolic bone diseases characterized by increased bone turnover, including osteoporosis, the clinical use of these sensitive markers of bone resorption is limited by the HPLC technique, which is long and tedious (4). There is a clear need for more convenient assays.

Recently, the assessment of bone resorption with a new marker of collagen degradation, C-terminal telopeptide of type I collagen (CTX; CrossLaps), has been developed. CrossLaps ELISA is a new ELISA for quantitative determination of a type I collagen-specific sequence in human urine. Bonde et al. (5) evaluated the CrossLaps technique, and concluded that the assay presents a useful tool for quantifying the type I collagen degradation product in urine. Garnero et al. (4) also reported the assessment of bone resorption using CTX in patients with metabolic bone disease. However, there has been no report of an assessment of bone resorption by CTX during GnRHa treatment, although recently Marshall et al. (6) reported the effect of GnRHa therapy on N-telopeptides. In this study, in order to evaluate more precisely the effect of GnRHa administration on bone resorption, we measured the levels of CTX excretion during and after GnRHa administration for the treatment of leiomyoma and compared these levels with those of Pyr and D-Pyr.

Materials and methods

Patients

Eighteen otherwise healthy premenopausal women with leiomyoma, aged between 28 and 53 years of age...
(44.6±6.1, mean±S.D.), were treated with daily intra-nasal administration of 400 μg nafarelin (Syntex, Palo Alto, CA, USA) or 900 μg buserelin (Hoechst, Germany) for 16 weeks. Nafarelin was given in 200 μg doses twice a day and buserelin in 300 μg doses three times a day. Patients were then followed for another 24 weeks without medication. All had regular menstrual cycles and no disease which would affect bone metabolism. They were in good health, with normal renal function (serum creatinine < 1.5 mg/dl), and had not received hormonal therapy for leiomyoma within the previous year. The patients were randomly allocated to two treatment groups (nafarelin group, n=9 and buserelin group, n=9). All gave written informed consent to participate in the study.

**Assay of parameters**

Urine samples for biochemical marker measurements were obtained just before the initiation of treatment, at week 8 and week 16 of treatment, and 12 and 24 weeks after the cessation of treatment. The methodology for assays of all biochemical values except CTX was standard. Pyr and D-Pyr were assayed according to previously published methods using HPLC (7). Urinary creatinine was measured by standard laboratory procedures.

Urinary excretion of CTX was measured by ELISA according to the instruction leaflet issued by Osteometer A/S, Denmark, using CrossLaps ELISA by modifying the method of Garnero et al. (4). Antibody to CrossLaps was obtained by immunizing rabbits with the amino acid sequence specific for part of the C-terminal telopeptide of the α1 chain of type I collagen (Glu-Lys-Ala-His-Asp-Gly-Gly-Arg) (eight amino acids) conjugated to bovine serum albumin by a two-step carbodimide procedure. The within-assay and total coefficients of variation were <13% in the concentration range of the calibration curve. Duplicate measurements were performed for each urine sample. Data were corrected for creatinine excretion determined enzymatically using picric acid, and expressed as mg/dl. All assays were performed by the assay laboratory of Fuji Revio (Tokyo, Japan) at one time without knowledge of the details of each sample.

**Measurement of bone mineral density**

Bone mineral density (BMD) in the lumbar spine (L2–L4) was longitudinally measured in each patient just before the initiation of treatment, at week 16 of treatment, and week 24 after the cessation of treatment, using dual energy X-ray absorptiometry (DXA) (XR-26, Norland), with an accuracy of 1.0% and coefficient of variation of 1.0%. However, in five of the patients, BMD at 24 weeks after the cessation of treatment was measured by DXA using QDR-2000 (Hologic) instead of XR-26 because our departmental DXA apparatus had been changed during the study. As it is not possible to exchange BMD data between these two different DXA apparatuses, we analyzed BMD data only for 13 patients at 24 weeks after the cessation of treatment.

**Statistical analysis**

The P values indicate the significance level of the difference between means at each time point. Data are presented as the mean±standard deviation (S.D.).
Results

There was no significant difference between the nafarelin group and buserelin group in the efficacy of treatment for leiomyoma.

Change in Pyr, D-Pyr and CTX

Figures 1–3 illustrate the changes in urinary Pyr, D-Pyr and CTX during and after GnRHa treatment. All the markers of bone resorption increased significantly during the GnRHa treatment and returned to the pretreatment level at 12 and 24 weeks after the withdrawal of treatment. The levels of urinary CTX excretion markedly increased during the treatment, with the highest point being at week 16 of treatment, and decreased after the suspension of GnRHa treatment (Fig. 3). There were significant ($P<0.05$) increases in CTX at week 8 and week 16 of nafarelin or buserelin administration compared with pretreatment values. Figure 4 compares the percentage increments against the pretreatment values of Pyr, D-Pyr and CTX during and after GnRHa therapy. The percent increase in CTX was significantly greater than those in Pyr and D-Pyr at both week 8 ($P<0.05$) and week 16 ($P<0.01$) of treatment.

Change in bone mineral density

As shown in Fig. 5, BMD in the lumbar spine decreased significantly (3.8%, $P<0.01$) after 16 weeks of treatment with 400 $\mu$g/day nafarelin or 900 $\mu$g/day buserelin. While BMD increased slightly after withdrawal of treatment, it remained less than (2.2%) the pretreatment value, which was not significant compared with the pretreatment value (Fig. 5).

Discussion

Type I collagen accounts for more than 90% of the organic matrix of bone and is synthesized primarily in bone (8). During renewal of the skeleton, type I collagen is degraded, and small peptide fragments are excreted in the urine. One of these fragments, which is specific for type I collagen, is measured in the CrossLaps ELISA (5).
Bonde et al. (5) showed that the CrossLaps ELISA performs well with respect to precision, recovery and dilution of urine samples. CTX is reported to be significantly increased in patients with metabolic bone disease characterized by increased bone turnover (4). Thus measurement of the excretion of CTX in urine provides a direct and very sensitive index of the bone resorption rate because its excretion may be more specific for bone collagen. This new immunoassay appears to be a convenient and highly sensitive index of bone resorption by which to detect the increase in bone turnover associated with estrogen deficiency.

Biochemical markers of bone resorption usually increase when GnRHa administration strongly reduces the serum level of estrogen. As expected, GnRHa treatment in our study resulted in a significant increase in biochemical values for bone resorption, such as urinary excretion of Pyr, D-Pyr and CTX. We found that CTX, Pyr and D-Pyr excretion increased as early as 8 weeks after patients began receiving GnRHa, continued to increase during GnRHa administration, and decreased after the suspension of treatment. In this study we demonstrated for the first time that CTX increased during the administration of GnRHa and then decreased after the cessation of treatment. Although the profiles of the changes in Pyr and D-Pyr excretion during and after GnRHa treatment were similar to those of CTX, the percentage increase in CTX was significantly greater than that in Pyr or D-Pyr, which are well-established markers of bone resorption, suggesting its potential as a more specific indicator of bone degradation than other previously reported indicators. These findings lead us to the conclusion that excretion of the C-terminal cross-linking domain of type I collagen would be more sensitive than the total excretion of Pyr to detect increased bone resorption caused by estrogen deficiency, which is in agreement with the conclusions of Garnero et al. (4). The urinary concentration of cross-linked N-telopeptide of type I collagen (NTX) has also been reported to be a sensitive and specific marker of bone resorption (9). Rosen et al. found NTX to be responsive to bone resorption and to reflect the changes in bone turnover more accurately than Pyr or hydroxyproline (10). Marshall et al. (6) reported that measurement of increases in NTX may provide a tool to predict decreases in BMD during GnRHa therapy.

The hypoestrogenic uncoupling of the osteoblast–osteoclast effect on the bone remodeling unit causes an increase in osteoclast activity. The lack of complete recovery of bone mass in the 24-week period following suspension of GnRHa therapy in our present study confirms the result of our previous study (1), indicating that relatively long periods of bone metabolic change might be needed to alter the actual BMD. The change in osteocalcin during administration of nafarelin in our previous study (1) was not as pronounced as that in CTX in our current study. The greater increase in CTX than in osteocalcin during GnRHa treatment suggests an imbalance between bone resorption and bone formation, which is responsible for the accelerated rate of bone loss. Because an increase in bone resorption is the primary event after estrogen deficiency, a sensitive marker of bone resorption would be valuable in the analysis of the effect of GnRHa treatment on bone turnover. It is clinically important to identify patients who are susceptible to bone loss after any kind of GnRHa treatment, especially those with leiomyoma, as they are usually at an age close to menopause and reduction of bone mass by GnRHa therapy after the age of 45 may lead to postmenopausal osteoporosis. Thus measurement of CTX may be valuable and useful in the bone-metabolic monitoring of patients treated with GnRHa.

In conclusion, using a new biochemical marker of bone resorption, C-terminal cross-linking domain of type-I collagen (CrossLaps), we have confirmed the increase in bone resorption in a hypoestrogenic state induced by GnRHa. Because CTX is a more sensitive marker than Pyr or D-Pyr, we believe that its assessment will be useful in the management of bone metabolism during and after the administration of GnRHa.

References

1 Taga M & Minaguchi H. Reduction of bone mineral density by gonadotropin-releasing hormone agonist, nafarelin, is not completely reversible at 6 months after the cessation of administration. Acta Obstetrica et Gynaecologica Scandinavica 1996 75 162–165.

Received 18 September 1996
Accepted 10 April 1997