Serum PINP is a Sensitive Marker of Bone Formation in Mouse Ovariectomy Model

Jukka P Rissanen1, ZhQi Peng1, Mari I Suominen1, Christiane Otto5, Tim Wintermantel2 and Jussi M Halleen1
1Pharmatest Services Ltd, Turku, Finland; 2Baycr Schering Pharma AG, Berlin, Germany
jukka.rissanen@pharmatest.fi

Introduction
Serum procollagen I N-terminal propeptide (PINP) (1) is a widely used bone formation marker in humans (2). We have previously shown that PINP is a sensitive marker of bone formation in rat ovariectomy (OVX) model, allowing the use of PINP in practical efficacy studies of new osteoporosis drug candidates (3). Here we have studied serum PINP as a bone formation marker in mouse OVX model.

Materials and methods

Animal experimentation: Three-month-old female mice from two different strains, C57Black/6J and C3H/HeN, were included in the study. Three study groups (n=12/group) were included for both strains as follows: 1) Sham-operated control group receiving vehicle; 2) OVX group receiving estradiol (E2); 3) USP saline vehicle (n=12/group). After 6 surgery weeks the animals were sacrificed and the uterus weights were determined.

Bone turnover markers: PINP (Pharmatest, Turku, Finland; IDS Ltd, Boldon, UK) was determined as a marker of bone formation and C-terminal cross-linked telopeptides of type I collagen (ICTx; Rat, Lap, Ym, 035 Ltd) as a marker of bone resorption. Blood was collected from the saphenous vein after six hour fasting. PINP and ICTx were measured from serum samples obtained before the operations and at days 2, 6, 10 and 14. Relative marker values were calculated for each individual animal at each time point by dividing the values obtained from each animal with values obtained from the same animal before the operations.

Bone parameters: Mineral density and cross-sectional dimensions of trabecular and cortical bone were measured from metaphysis and diaphysis of left tibia using pQCT scanning before surgery operations and at the end of the study phase. Relative bone parameters were calculated for each individual animal at the end of the study by dividing the values obtained from each animal with values obtained from the same animal before the operations.

Statistical analysis: Statistical analysis was performed with ANOVA for follow-up measurements and either ANCOVA or Kruskal-Wallis test for end-point measurements after checking assumptions for normally and homogeneity of variances. Dunnet’s test or Mann-Whitney test were utilized for pairwise comparisons.

Uterus weight and bone parameters

Figure 1. Uterus weight and bone parameters at 6 weeks after OVX. A) Uterus weight in C57Black/6J mice; B) Uterus weight in C3H/HeN mice; C) Total BMD in C57Black/6J mice as determined by pQCT analysis from metaphysis; D) Total BMD in C3H/HeN mice as determined by pQCT analysis from metaphysis; E) Total bone area in C57Black/6J mice as determined by pQCT analysis from metaphysis; F) Total bone area in C3H/HeN mice as determined by pQCT analysis from metaphysis.

Serum CTX

Figure 2. Changes of serum CTX levels after OVX. A) Change of serum CTX levels in C57Black/6J mice at 2, 6, 10 and 14 days after the operations; B) Change of serum CTX levels in C3H/HeN mice at 2, 6, 10 and 14 days after the operations. The results of the sham group and the OVX+E2 group were compared with the results of the OVX group receiving vehicle. *p<0.05, ***p<0.001.

Serum PINP

Figure 3. Changes of serum PINP levels after OVX. A) Change of serum PINP levels in C57Black/6J mice at 2, 6, 10 and 14 days after the operations; B) Change of serum PINP levels in C3H/HeN mice at 2, 6, 10 and 14 days after the operations. The results of the sham group and the OVX+E2 group were compared with the results of the OVX group receiving vehicle. *p<0.05, ***p<0.001.

Summary

In both used mouse strains, uterus weights and total BMD decreased significantly by OVX, which was prevented by treatment with 17β-estradiol, demonstrating successful estrogen depletion and osteopenia by OVX.

OVX increased significantly PINP values at days 6, 10 and 14 in both strains, which was prevented by treatment with 17β-estradiol. Similar effects were observed for CTX only at day 14 in C57Black/6J mice.

Serum PINP is a more sensitive bone turnover marker than serum CTX in mouse OVX model.

Conclusions

These results demonstrate that serum PINP is a sensitive marker of bone formation that describes changes in bone homeostasis in mouse OVX model.

PINF values change rapidly after OVX, and short-term changes in PINP predict long-term changes in BMD in mouse OVX model.

Acknowledgements

We thank Anna Lisaustern, Johanna Rantanen and Suli Sutant for their skilful technical assistance.

References

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