INTRODUCTION:

Intermittent parathyroid hormone (PTH) treatment enhances bone volume in osteoporosis patients and thus it is considered to be one of the promising directions of treatment. Recent observations on the transgenic mice expressing constitutively active PTH/PTHrP receptor indicate that PTH signaling increases cancellous bone while it reduces cortical bone. These data suggest that PTH signal may have opposite effects in different sites. However, the downstream events which are involved in the differential PTH activation of bone formation are still unclear.

Osteopontin (OPN) exists as a non-collagenous bone matrix protein and cytokine. OPN is expressed in both osteoblasts and osteoclasts. Our previous observations revealed that OPN is required for PTH induced bone resorption in organ cultures of bone. However, the role of OPN in PTH-induced bone resorption and bone formation in vivo is not fully understood. We hypothesized that OPN could be one of the target molecules that may be involved in PTH actions to increase bone in vivo. Therefore, we examined the effect of PTH on the bone metabolism in OPN-deficient mice in comparison to its effect on bone in wild type mice.

METHODS:

The OPN-deficient mice were produced as described by Rittling et al.. The protocol was approved by the institutional guidelines for animal welfare of this university. 7 week-old female mice with either OPN-deficient or wild type genotype with 129 strain background were subjected to daily subcutaneous injections of human PTH (1-34) at 80μg/kg/day for 5 days per week for 4 weeks. 24 hours after the last injection, the mice were anesthetized and sacrificed. For measurements of bone mineral density (BMD) the right femora were scanned using dual-energy X-ray absorptiometry (DEXA). The bone volume in cancellous and cortical envelope was subsequently quantified by using two dimensional μ-CT. Urinary deoxypyridinoline (Dpyr) levels during the last 24 hours were measured by using an ELISA system kit.

Statistical analysis was performed by Mann-Whitney’s U test. A P value < 0.05 was considered to be statistically significant.

RESULTS:

DEXA analyses indicated that the total BMD in the whole femora was not increased by the treatment with PTH in wild type. In the absence of OPN, however, BMD of the whole femora was increased significantly by PTH treatment (by about 14%). OPN-deficiency further enhanced the PTH-dependent increase in cancellous bone volume (by about 77%) more than the increase observed by PTH treatment in wild type mice (by about 58%). PTH did not alter cortical bone area and cortical bone thickness, while it reduced bone marrow area (by about 12%) in wild type mice. In sharp contrast to such inability of cortical bone to respond to PTH in wild type mice, OPN-deficient mice showed increase in cortical bone area (by about 16%) and cortical bone thickness (by about 13%), and decrease in bone marrow area in response to PTH treatment for 4 weeks. PTH treatment increased N.Oc (about 2 fold) in wild type mice as well as in OPN-deficient mice. However, when osteoclast number was normalized per bone surface (N.Oc/BS) and osteoclast surface (Oc.S/BS) in the cancellous bone, PTH treatment increased these parameters in wild type mice but not in OPN-deficient mice. PTH treatment increased MAR (by about 30%) as well as BFR (by about 30%) in cancellous bone in wild type mice. In OPN-deficient mice, basal levels of the two bone formation parameters were similar to those basal levels in wild type mice. However, OPN-deficiency significantly augmented PTH enhancement of MAR (by about 40%) and BFR (by about 100%) in cancellous bone. In cortical bone, PTH treatment enhanced MAR (by about 45%) and BFR (by about 81%) in the endosteal region, and decreased MAR as well as BFR in the periosteal region in wild type mice. In contrast, PTH enhanced MAR (by about 20%) and BFR (by about 100%) in the periosteal region in the OPN-deficient mice. Such enhancement was not observed in the endosteal region. Furthermore, OPN-deficiency also enhanced mineralized nodule formation in vitro. In contrast to the OPN-deficiency enhancement of the PTH effects on bone formation parameters in vivo as well as in vitro, the levels of PTH-enhancement on Oc.N in vivo, excretion of Dpyr in urine, and osteoclast cell development in vitro were similar in wild type and OPN-deficient mice.

DISCUSSION

In this experiment, we demonstrated that OPN-deficiency induced PTH activation of cortical bone formation and potentiated PTH activation of cancellous bone formation. Osteoblastic bone formation induced by the intermittent PTH treatment was clearly increased in OPN-deficient mice rather than wild type mice. In contrast, osteoclast number and systemic osteoclastic bone resorption were similar in wild type and OPN-deficient mice. These data indicated that intermittent PTH treatment-induced bone gain in OPN-deficient mice was due to enhancement of osteoblastic activity rather than influence on the osteoclastic activity. Intermittent PTH treatment has been shown to reduce cortical bone loss at the specific site when used to treat osteoporosis. Thus, inhibition of the OPN function can lead to more effective intermittent PTH treatment for severe osteoporosis.

Our data indicated that OPN-deficiency specifically augments the effect of PTH on cortical bone formation in vivo as well as in vitro. Therefore, the long-standing question about how cortical bone differs from cancellous bone with regard to their responses to PTH could be explained at least in part to the presence of OPN.