P418

Globular Adiponectin Reverses Trabecular Osteopenia In Ovariectomized Rats

Shyamsundar Pal China[1], Subhashis Pal[1], Konica Porwal[1], Sapana Kushwaha[2], Anagha Gurjar[2], Sourav Chattopadhyay[2], Abhishek Singh[2], Sabyasachi Sanyal[2], Naibedya Chattopadhyay[1]

Endocrinology Division[1] & Biochemistry Division[2]
CSIR-Central Drug Research Institute, Lucknow-226031, India

Abstract

The skeletal effect of recombinant globular adiponectin (gAd) was tested in preclinical model of post-menopausal osteoporosis (ovariectomized rats). gAd restored trabecular bone microarchitecture in osteopenic rats up to the level of control.

Introduction

Adiponectin has recently been shown to influence skeletal metabolism. Over-expression of adiponectin gene suppresses bone resorption and increases trabecular bone mass[1]. Bone marrow stromal cells from adiponectin-null mice had reduced mineralization capability[2]. Adiponectin, signalling via the adiponectin receptor 1 (AdipoR1) stimulates osteoblast differentiation[3] and inhibits osteoclastogenesis[4]. The globular form of adiponectin (gAd) signals via AdipoR1 and stimulates osteoblast differentiation[5]. Based on these observations, we hypothesized that gAd could restore bone loss in osteopenic rats.

Recombinant gAd used in this study has the following features[6]:

- ❑ 18kD c-terminal globular domain, cloned from human genomic DNA.
- ❑ The protein activity is tested in C2C12 cell line for phosphorylation of AMPK and in MCF7 cell line for induction of ACC phosphorylation (Ser79).
- ❑ Expressed in *E.coli* as bacterial inclusion bodies and solubilized by alkaline shock.

Results: In vitro

Effect on osteoblast:

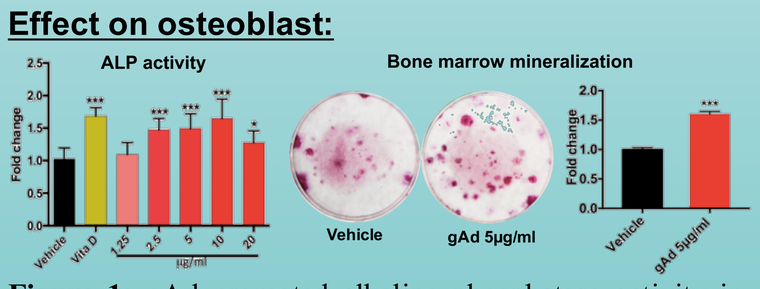

Figure 1: gAd promoted alkaline phosphatase activity in rat calvarial osteoblast and increased mineralization in bone marrow stromal cells.

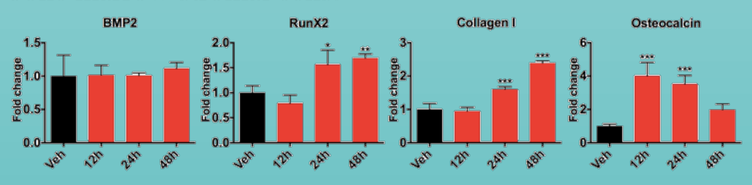

Figure 2: gAd (5µg/ml) increased osteogenic gene expression in calvarial osteoblast.

Effect on osteoclast:

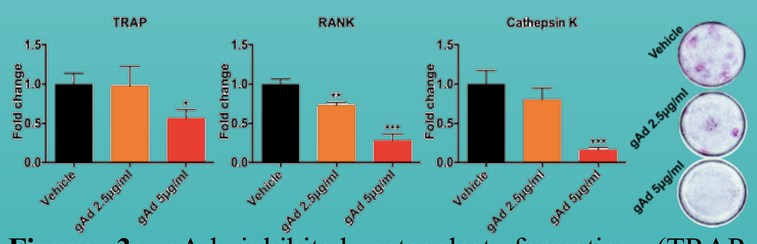

Figure 3: gAd inhibited osteoclast formation (TRAP staining) and decreased osteoclast marker expression in bone marrow-derived osteoclast progenitor cells.

References

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Results: In vivo

Adult Sprague-Dawley female rats (200±10g) were either sham-operated (ovary intact) or ovariectomized (OVX) and left untreated for 12 weeks to develop trabecular osteopenia.

Fracture model:
Drill hole fracture was performed on femur diaphysis with 0.8mm drill bit either in sham-operated or osteopenic animals. After 12days treatment with 0.9% saline (Veh) or gAd, animals were injected with 20mg/kg calcein (i.p.) and sacrificed after 24 hours.

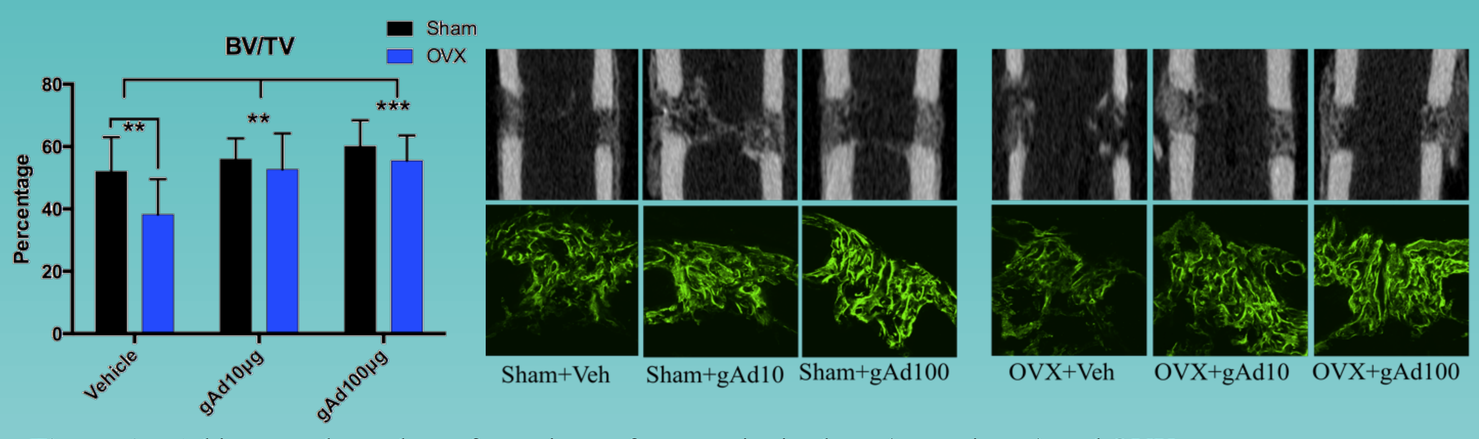

Figure 4: gAd increased new bone formation at fracture site in sham (ovary intact) and OVX rats.

Post-menopausal curative model:
After developing osteopenia, rats were randomized into following (Table1) groups (n=10/group) and further treated for twelve weeks.

Sham+Veh	Ovx+Veh	Ovx+PTH	Ovx+Aln	Ovx+gAd10	Ovx+gAd100
0.9% saline	0.9% saline	hPTH (1-34) 40µg/kg/day, 5 days/week, i.p	Alendronate 3mg/kg, orally	gAd 10µg/rat, i.p.	gAd 100µg/rat, i.p.

Table 1: Experimental plan▲

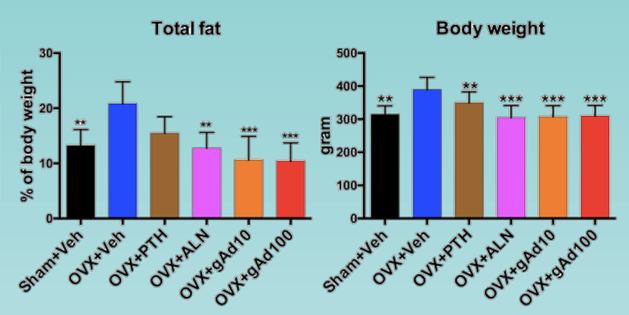

Figure 5: gAd significantly reduced body fat in OVX rats. ▶

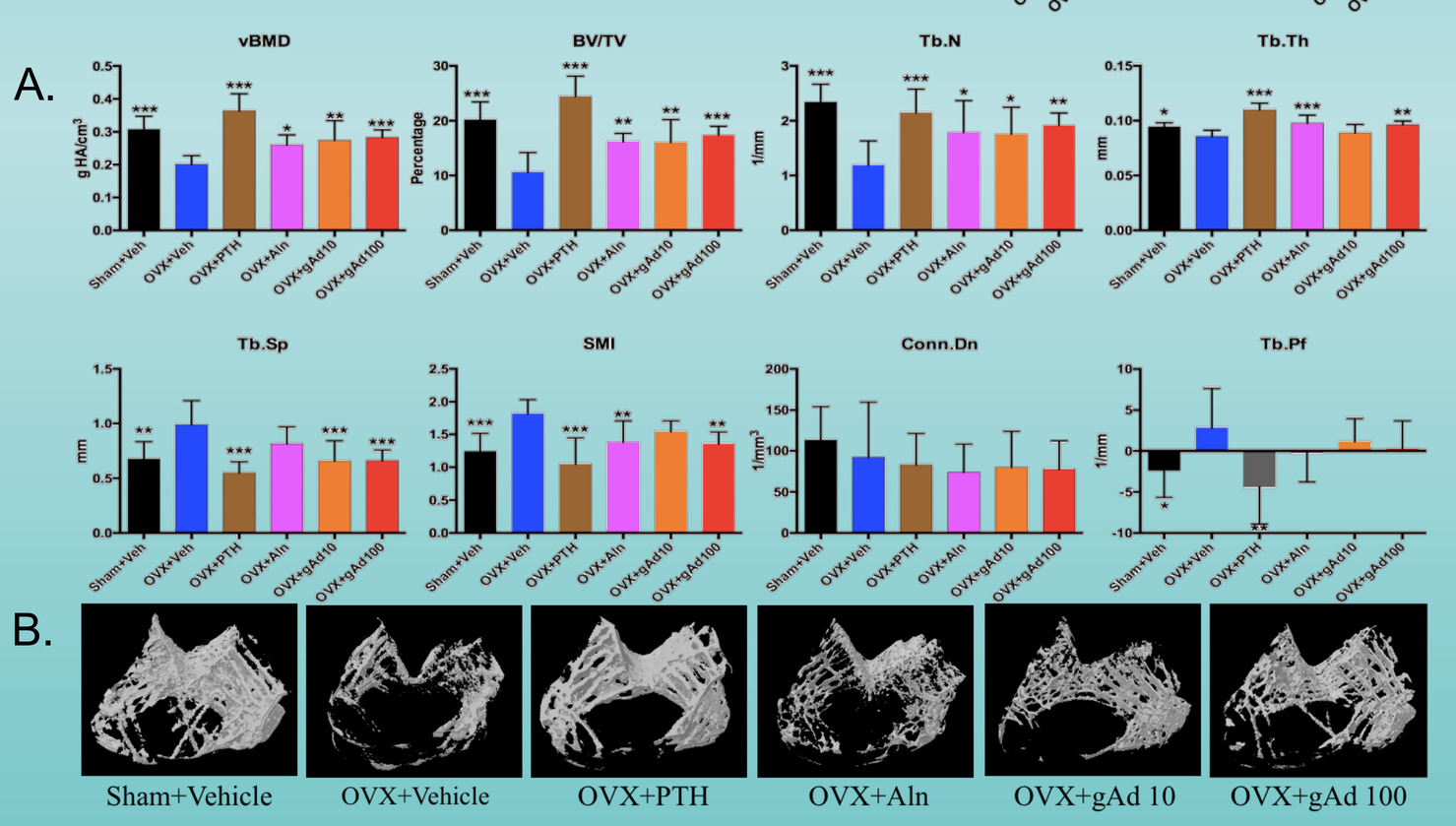

Figure 6: A. gAd restored femur trabecular micro-architecture in OVX rats. (vBMD: Volumetric bone mineral density; BV/TV: Bone volume/Tissue volume; Tb.N: Trabecular number; Tb.Th: Trabecular thickness; Tb.Sp: Trabecular separation; SMI: Structure model index; Conn.Dn: Connection density: Tb.Pf: Trabecular pattern factor) All groups were compared with OVX+Vehicle. **B.** Representative 3D images of femur trabecular bone.

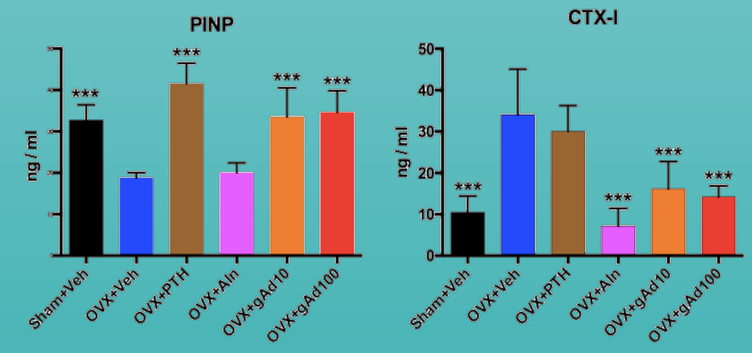

Figure 7: gAd increased serum PINP (bone formation marker) and reduced urinary CTX-1 (bone resorption marker).

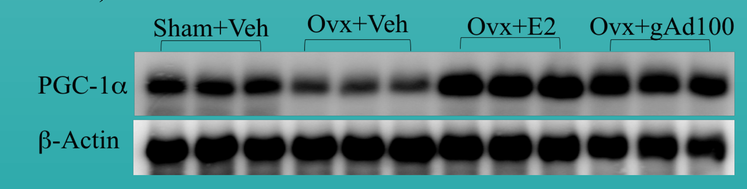

Figure 8: Supplementation of gAd (100µg) or Estrogen restored PGC-1alpha expression in bones of OVX animals to the level of Sham.

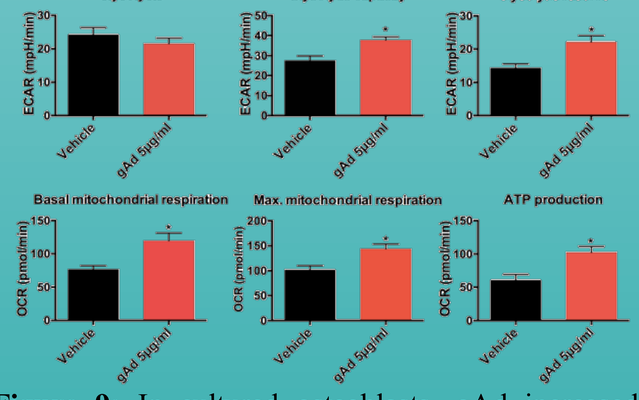

Figure 9: In cultured osteoblasts, gAd increased oxidative phosphorylation (aerobic respiration).

Conclusion

gAd accelerates fracture healing and improves bone micro-architecture in post-menopausal osteoporosis model likely via osteogenic and anti-resorptive modes. So, gAd can be a potential therapeutic candidate for postmenopausal osteoporosis.

Statistics: Data are expressed as mean±SEM and analysed using t test, non parametric one way ANOVA followed by Dunnett's multiple comparisons test. *P< 0.05, **P< 0.01, *** P< 0.001

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[None of the authors has any potential conflict of interest to declare]