Meclozine ameliorates osteogenic mineralization of bone marrow-derived mesenchymal stem cells using a mouse model of achondroplasia

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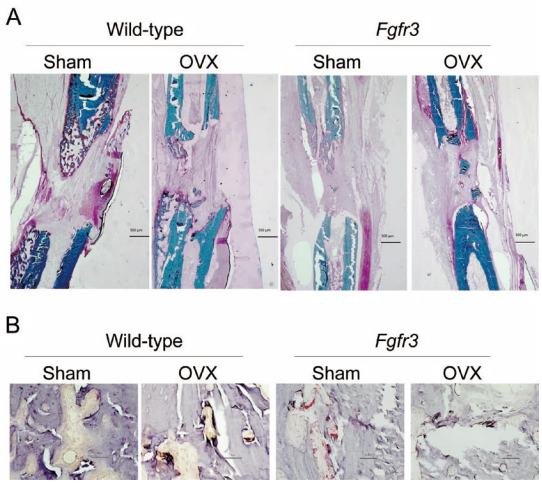
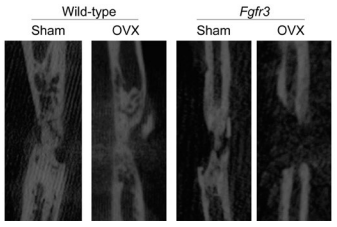
INTRODUCTION: Achondroplasia (ACH) is a common skeletal dysplasia with shorten-limbed short stature caused by gain-of-function mutations in the fibroblast growth factor receptor 3 (FGFR3) gene. Limb lengthening surgery is a treatment option for short stature of adolescent patients with ACH. The 10 cm can be gained in one long bone and the treatment duration is shorter than that in other diseases because the bone regeneration is faster in adolescent patients with ACH. However, the ability of bone regeneration remains unknown in older patients with ACH. Recently, several kinds of FGFR3 inhibitors have been developed for the treatment of short stature associated with ACH including meclozine[1)], which has been used as an anti-motion sickness for more than 50 years. We previously demonstrated that new bone formation after tibial lengthening is upregulated in a mouse model of ACH (*Fgfr3*^{ach} mice) at the age of 4 weeks[2)]. The purpose of present study is to evaluate the ability of new bone formation in *Fgfr3*^{ach} mice aged 16 weeks with or without ovariectomy (OVX), and to evaluate the effects of meclozine on osteogenic mineralization using bone marrow-derived mesenchymal stem cells from each mouse.
METHODS: All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee in our institute. We employed female wild-type mice and *Fgfr3*^{ach} mice (FVB background). We performed OVX and tibial lengthening surgeries at the age of 8 and 16 weeks, respectively. A 5-day-latency period was followed by the lengthening at the rate of 0.2 mm/day for 9 days. At the 7th day after completion of lengthening, lengthened tibiae were subjected to histological analysis using Villanueva Goldner and tartrate-resistant acid phosphatase (TRAP) stainings. Bone volume (BV)/lengthened area, osteoid volume (OV)/lengthened area, and number of osteoclasts were measured. At the 28th day, the individual mice were subjected to micro-computed tomography (micro-CT) scans, and BV in the lengthened space was calculated. Finally, bone marrow-derived mesenchymal stem cells (BMSCs) were harvested from both mice at the age of 16 weeks with or without OVX, and cultured with each concentration of meclozine. Alizarin red staining was quantified after 21 days. Results are expressed as mean ± SD. Statistical analysis was carried out using one-way ANOVA post hoc bonferroni with significance set at $p < 0.05$.
RESULTS: Histological analysis revealed that BV and OV in the lengthened area were reduced in *Fgfr3*^{ach} mice than wild-type mice (Sham *Fgfr3*^{ach} mice, n = 5 vs. Sham wild-type mice, n = 11: BV: 0.58 ± 0.045 vs. 1.27 ± 0.43, $p < 0.005$, OV: 0.98 ± 0.084 vs. 1.27 ± 0.43, $p = 0.160$) and further deteriorated after OVX in *Fgfr3*^{ach} mice (OVX *Fgfr3*^{ach} mice, n = 6 vs. Sham *Fgfr3*^{ach} mice, n = 5: BV: 0.23 ± 0.082 vs. 0.58 ± 0.045, $p < 0.005$, OV: 0.27 ± 0.12 vs. 0.98 ± 0.084, $p < 0.005$) (Fig. 1A). The number of osteoclasts were increased after OVX in both mice (OVX *Fgfr3*^{ach} mice, n = 6 vs. Sham *Fgfr3*^{ach} mice, n = 5: 13.0 ± 4.3 vs. 9.4 ± 3.7, $p = 0.170$, OVX wild-type mice, n = 11 vs. Sham wild-type mice, n = 11: 14.4 ± 3.3 vs. 8.1 ± 3.5, $p < 0.005$) although there was no statistical difference between both mice (Fig. 1B). In micro-CT analysis, BV was also reduced in combination of *Fgfr3*^{ach} mice and OVX mice (Sham *Fgfr3*^{ach} mice, n = 8 vs. Sham wild-type mice, n = 12: 0.42 ± 0.083 vs. 0.65 ± 0.096, $p < 0.005$; OVX *Fgfr3*^{ach} mice, n = 8 vs. Sham *Fgfr3*^{ach} mice, n = 8: 0.15 ± 0.035 vs. 0.42 ± 0.083, $p < 0.005$) (Fig. 2). Alizarin red staining was significantly reduced in *Fgfr3*^{ach} mice and further deteriorated in OVX mice (Fig. 3). The 20 μM of meclozine significantly enhanced alizarin red staining of both mice with or without OVX (untreated *Fgfr3*^{ach} mice, n = 7 vs. meclozine-treated *Fgfr3*^{ach} mice, n = 7: OVX: 37.14 ± 10.21 vs. 139.4 ± 6.85, $p < 0.005$, Sham: 53.86 ± 14.23 vs. 141.6 ± 6.29, $p < 0.005$).
DISCUSSION: Unlike the 4-week-old mice, new bone formation was reduced in *Fgfr3*^{ach} mice aged 16 weeks and further aggravated by OVX. Thus, activated FGFR3 may deteriorate bone regeneration in older age and after menopause in human. Osteogenic mineralization using BMSCs was also downregulated by combination of *Fgfr3*^{ach} and OVX mice. Meclozine dramatically improved the alizarin red staining of BMSCs in *Fgfr3*^{ach} mice equal to wild-type mice. Since a previous study demonstrated that meclozine enhanced the bone mineralization after OVX in wild-type mice[3)], poor bone mineralization of bone lengthening in OVX *Fgfr3*^{ach} mice would be also reversed by meclozine. As a limitation in the current study, we did not perform meclozine administration during tibial lengthening in *Fgfr3*^{ach} mice with or without OVX. Further study is needed to evaluate the effect of meclozine on new bone formation and bone mineralization using *Fgfr3*^{ach} mice.
CLINICAL RELEVANCE: Bone regeneration would be downregulated in older or postmenopausal patients with ACH. Meclozine has a potential to ameliorate osteoporosis associated with ACH.
REFERENCES: 1) Matsushita M, Esaki R, Mishima K, et al. (2017). Sci Rep; 7:7371. 2) Osawa Y, Matsushita M, Hasegawa S, et al. (2017). Bone; 105:42. 3) Guo J, Li W, Wu Y, et al. (2017). Front Pharmacol; 8:693.
IMAGES:

Fig. 1



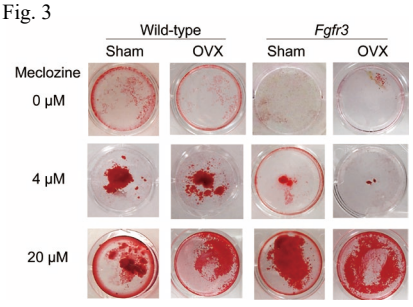
Histological images of lengthened area after 7 days of lengthening completion. (A) Villanueva Goldner staining. Green signals show calcified bones. Red signals show osteoid bones. (B) Tartrate-resistant acid phosphatase (TRAP) staining. Purple signals show osteoclasts.

Fig. 2



Micro-CT images after 28 days of lengthening completion.

Fig. 3



Osteogenic mineralization of BMSCs. Meclozine significantly enhanced alizarin red