Supplementary article data

Zoledronic acid in vivo increases in vitro proliferation of rat mesenchymal stromal cells

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Figure 4. Cell yield and maximum population doubling of rat MSCs. There were no statistically significant differences in the number of BM cells obtained from each experimental group (panel A) or in the maximum number of PDs obtained over a single passage (panel B) between different groups. Abbreviations: I: intact; F: fracture; P: placebo; Z: zoledronic acid; B: bolus; W: weekly.
Figure 5. Osteoblastic differentiation of rat MSCs. Representative photographs of ALP- and von Kossa-stained cell cultures after 2 weeks of induction culturing, showing that functional osteoblasts were successfully differentiated from MSCs of all experimental groups, regardless of treatment. Scale bar: 5 mm. Abbreviations as in Figure 4.

Figure 6. Quantification of osteoblastic differentiation capacity of MSCs. Osteoblastic differentiation was assessed by spectrophotometric quantification of ALP activity (panel A), by quantification of ALP-stained area (panel B), and by quantification of mineralized matrix (von Kossa staining) (panel C) for assessment of osteoblast function. MSCs from intact animals and animals with fracture, treated with bolus ZA, had statistically significantly higher ALP activity than MSCs from corresponding groups treated with ZA on a weekly basis. The average levels of ALP and von Kossa stainings were lower in weekly-treated animals than in other groups, but the differences were not statistically significant due to large variability. Data are presented as estimated marginal means with 95% confidence intervals. Mixed-models analysis with treatment group as fixed factor and animal identity as random factor; REML estimations and Sidak’s test were used for pairwise comparisons of the estimated marginal means. The intact and fracture groups were analyzed separately. *p < 0.05.