Sildenafil accelerates fracture healing in mice

INTRODUCTION:
Fracture healing is a complex, sequentially orchestrated process, including inflammation, mesenchymal cell condensation, chondrogenesis, angiogenesis and osteogenesis. Within this process, the vascularization of the tissue is a prerequisite for successful bone healing. Reduced vascularity at the fracture site, therefore, has been identified as one of the most significant parameters, accounting for delayed fracture healing and atrophic non-union formation. PDE5 catalyzes the breakdown of cGMP, one of the primary factors causing smooth muscle relaxation. Sildenafil is a selective inhibitor of phosphodiesterase-5 (PDE5). During the last few years, several studies have shown that sildenafil exerts also angiogenic actions through upregulation of distinct pro-angiogenic growth factors. These findings have been generalized to a variety of ischemic disease models. Previous studies have shown that the angiogenic and osteogenic factors VEGF and CYR61 are involved in the process of bone formation and fracture healing. There is complete lack of information, however, whether sildenafil is capable of influencing these growth factors and thus the process of fracture healing. Therefore, we herein aimed at determining a novel role of sildenafil in fracture healing. We hypothesized that sildenafil accelerates fracture healing through stimulation of growth factor expression and bone formation.

METHODS:
All animal procedures were performed according to the National Institute of Health guidelines for the use of experimental animals and were approved by the German legislation on the protection of animals. For the present study a total of sixty 12 to 14 week old CD-1 mice were used. Thirty mice were fed daily with 5mg/kg body weight (BW) sildenafil (Viagra®, Pfizer, Germany). Thirty vehicle (saline)-treated mice served as controls. Bone healing was studied in a murine closed femur fracture model using radiological, biomechanical, histomorphometric and protein biochemical analysis at 2 and 5 weeks after fracture. All data are given as means±SEM. After proving the assumption for normal distribution (Kolmogorov-Smirnov test) and equal variance (F-test), comparison between the two experimental groups was performed by Student’s t-test.

RESULTS:
Radiological analyses 2 weeks after fracture indicated an improved healing in sildenafil-treated animals (n=10). In these animals ossous bridging of the fracture gap was achieved earlier compared to non-treated controls (p<0.05) (n=10). After 5 weeks, the fractures in both groups were completely healed radiologically. Biomechanical analysis at 2 weeks after fracture healing showed a significantly higher bending stiffness in sildenafil-treated (n=10) animals compared to controls (n=10). At 2 weeks after fracture healing, the sildenafil-treated animals (n=10) showed a significantly smaller total callus area when compared to controls (p<0.05), indicating a more dominant intramembranous healing. After 5 weeks callus size was found decreased also in controls (n=10), indicating appropriate remodeling at this time point. In parallel, we observed a greater healing score in sildenafil-treated animals (n=10) at 2 weeks after fracture healing when compared to controls (p<0.05) (n=10). According to the radiological and biomechanical results, at 2 weeks bone tissue was found slightly increased in sildenafil-treated animals compared to controls. After 5 weeks, all animals of the two groups showed a comparable amount of bone tissue, i.e. ~90%. Accordingly, at 2 weeks cartilaginous tissue was found reduced after sildenafil treatment, while at 5 weeks the remaining amount of cartilage within the callus of sildenafil-treated animals was comparable to that of controls. After 2 weeks of fracture healing Western blot analysis of the callus tissue (n=5 each group) revealed that sildenafil did not affect the expression of VEGF. Of interest, CYR61 was found significantly increased compared to controls (p<0.05). In addition, expression of PCNA, indicating cell proliferation, and OPG, inhibiting osteoclastogenesis, were found slightly but not significantly increased in the callus of sildenafil-treated animals. In contrast, expression of RANKL, a stimulator of osteoclastogenesis, was slightly reduced after sildenafil treatment. Of interest, eNOS, which is also capable of promoting angiogenesis, was found significantly lowered to ~50% in sildenafil-treated animals when compared to vehicle-treated controls.

DISCUSSION:
We herein demonstrate for the first time that sildenafil treatment enhances bone healing by accelerating osseous fracture bridging, resulting in an increased biomechanical stiffness by a switch from endochondral towards intramembranous healing, which has been confirmed in a variety of ischemic disease models. Previous studies have shown that the angiogenic and osteogenic factors VEGF and CYR61 are involved in the process of bone formation and fracture healing. There is complete lack of information, however, whether sildenafil is capable of influencing these growth factors and thus the process of fracture healing. Therefore, we herein aimed at determining a novel role of sildenafil in fracture healing. We hypothesized that sildenafil accelerates fracture healing through stimulation of growth factor expression and bone formation.

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