Muscular Dystrophy causes delayed bone formation and remodeling during fracture repair

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Introduction

Many clinical and experimental observations have suggested that muscle plays a role in bone regeneration. Severe soft tissue damage following extreme trauma highly increases the risk of non-union, indicating that the presence of intact muscle is critical for bone repair. Soft tissue grafting and specifically muscle flaps are known to be beneficial for the treatment of open fractures and for decreasing the incidence of complications. Yet, it is not clear whether muscle tissue itself is involved or whether the muscle vasculature or other adjacent tissues have a higher impact on bone repair. To functionally assess the role of muscle in bone repair, we assessed fracture healing in Dmdmdx mice, which lack the dystrophin gene. Dmdmdx mice exhibit progressive muscle degeneration due to structural defects in muscle fibers. Although this phenotype is often associated with osteoporosis in human, mice carrying this mutation are not osteoporotic, allowing us to determine the unique effects of muscle degeneration on bone repair (1).

Methods

All protocols were approved by the Institutional Animal Care and Use Committee. Adult 12-16 week old wild type and Dmdmdx (Jackson Laboratory) male mice were anesthetized and a non-stabilized tibial fracture was created in the mid-diaphysis via three point bending (2, 3). Fracture calluses were collected at days 7, 14, 21 and 28 days post-fracture (n=6 per group), fixed, paraffin embedded and sectioned. Safranin-O and Trichrome staining were used to quantify callus, cartilage and bone volume via histomorphometry (2, 3).

Results

Callus size was comparable between wild type and Dmdmdx mice at day 7 post-fracture. Total bone volume (BV) and the proportion of bone in the callus (BV/TV) were decreased in Dmdmdx compared to wild type calluses (Fig.1). The volume of cartilage was also decreased in Dmdmdx compared to wild type calluses, although the difference was not statistically significant (p=0.06). By day 14, the callus size was still comparable between the two groups, with a continued decreased bone volume in Dmdmdx calluses (Fig.1). By day 14, there was more cartilage in the callus of mice Dmdmdx compared to wild type mice, which again lacked significance. By day 21, the total callus volume and total bone volume were increased in Dmdmdx compared to wild type mice. Moreover, callus and bone volumes were increased in Dmdmdx mice at day 21 compared to day 14, while callus and bone volumes were decreased in wild type mice. At day 21, the cartilage was almost completely removed in the callus of both wild type and Dmdmdx mice. By day 28, callus, bone and cartilage volumes as well as the proportions of bone and cartilage were comparable between Dmdmdx and wild type mice.

Discussion

Histomorphometric analyses indicate that bone deposition is delayed in the callus of Dmdmdx mice, since the volume of bone in the callus was decreased during the soft callus (day 7) and hard callus (day 14) phases of repair. During the remodeling phase of repair, the volume of bone began to decrease in the wild type calluses but continued increasing in the calluses of Dmdmdx mice, suggesting a delay in the remodeling of bone. Similar differences were observed for cartilage, with a delay in matrix deposition during the soft callus phase of repair and a delay in the removal of the matrix during the hard callus and remodeling phase of repair. These results illustrate that muscle defects in Dmdmdx mice impact the normal process of bone repair. Muscle defects in Dmdmdx mice may affect bone repair at various levels. As Dmdmdx mice are particularly susceptible to muscle injury, poor muscle regeneration capacities may compromise vascularization of the callus and consequently affect bone formation. Although blood vessel development is not altered in Dmdmdx mice, dystrophin-deficiency has also been reported in vascular smooth muscle cells, leading to possible direct or indirect effects on callus revascularization (4). In addition, dystrophic muscles are known to contain elevated numbers of inflammatory cells, specifically macrophages, contributing to muscle degeneration and potentially affecting the inflammatory phase of bone repair (5). These changes in the inflammatory environment may interfere with the early stages of fracture repair. These events may subsequently have an impact on the recruitment of osteoclasts and the remodeling phase of repair. In order to understand the impact of the Dmdmdx mutation on bone repair and the role of muscle in this context, current analyses aim to clarify which cell types are affected in these mice and the extent to which they participate in bone regeneration. The results will help determine whether muscle plays a protective role and/or participates actively in bone regeneration. Furthermore, this research also has relevance to patients with Duchenne muscular dystrophy, who often sustain fractures following minimal trauma. In these patients, post-fracture complications are devastating and often lead to permanent loss of function (6).

Figure 1: Histomorphometric analyses of total callus volume (TV), bone volume (BV), cartilage volume (CV) and proportions of bone (BV/TV) and cartilage (CV/TV) in the fracture callus of Dmdmdx and wild type mice at days 7, 14, 21 and 28 post-fracture (*p<0.05, Student-t test).

References
2. Colnot et al, Development. 2003 Sep; 130(17):4123-33

Acknowledgements

This work was supported by grants from the University of California San Francisco Academic Senate to C.C, and NIH/NIAMS R01 AR057344 to C.C and T.M.