Quantification of Scar Tissue Formed Around Cranial Bone Grafts, and Its Reduction by Parathyroid Hormone Therapy

Doron Cohn Yakubovich[1], Uzi Eliav[2], Michal Rivlin[2], Ilan Kallai[1], Gadi Pelled[1,3], Dan Gazit[1,3], Zulma Gazit[1,3], and Gil Navon[2]

[1]Skeletal Biotech Laboratory, The Hebrew University of Jerusalem, Jerusalem, Israel, [2]School of Chemistry, Tel Aviv University, Tel Aviv, Israel, [3]Department of Surgery, Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, California, United States

Target audience

We are presenting an innovation to the MRI field, targeted to researchers and clinicians interested in the phenomenon of fibrotic scar tissue formation and its quantification.

Purpose

Bone loss in the craniofacial complex due to trauma or disease presents a major clinical challenge. Bone allografts composed of nonvital bone serve as a widely accepted solution - though often fail to integrate with host bone due to formation of fibrotic scar tissue. We have recently showed that daily short-time administration of parathyroid hormone (PTH) following allograft implantation promotes stem cell recruitment and differentiation, thereby inducing bone formation with improved osteo-integration at the implantation site[1]. We observed that PTH significantly down-regulates the pro-fibrogenic gene CCN2 of endogenous cells at the graft-host junction. To further examine the PTH effect on scar tissue formation by molecular and imaging approach, we have developed a unique MRI scanning protocol to measure collagen fiber deposition in the scar-bone suture area. **We hypothesize that this non-invasive, quantitative MRI technique for evaluating collagen deposition will confirm that PTH administration decreases collagenous scar tissue formation.**

Methods

Circular calvarial bone defects (5 mm in diameter) were created in FVB/n mice. The mice were implanted with decellularized allografts, with or without daily PTH treatment (40 μg/kg/day) and scanned using in-vivo micro-magnetic resonance imaging scanner (mMRI) 4 weeks post-surgery. Next, the animals were euthanized, the calvarial region was isolated and scanned ex-vivo with 360MHz mMRI – using magnetization transfer contrast (MTC) and double quantum magnetization transfer filter (DQF-MT) protocols. These methods were found to be sensitive to the presence of collagen. Furthermore, the intensity signal of the DQF-MT-weighted image is linearly dependent on the amount of collagen and is affected by the rigidity of the collagen fibrils and fibers[2]. The mMRI scans were followed by a micro-computed tomography (mCT) scan and histological analysis.

Results

In-vivo mMRI of animals that were not given PTH treatment demonstrated intense signal at the graft-bone surroundings. Ex-vivo MTC and DQF-MT-weighted MRI[2] scan of samples extracted from animals that were treated with PTH showed an enhanced signal by a factor larger than three, comparing to the control animals. This enhancement is attributed to changes in collagen. mCT scans ensured that the mMRI signal correlates to the pertinent anatomical site and demonstrated new bone formation at the allograft-host interface in the PTH-treated group. The presence of collagen fibers at the graft-host junction was confirmed by histological analysis.

Discussion

The enhancement of the ex-vivo MTC and DQF-MT MRI signal indicates augmented deposition of rigid collagen fibrils and fibers in PTH treated animals. Both methods gave clear images of the scar tissues. While MTC had the advantage of better SNR, the DQF-MT gave information about the amount and the rigidity of the collagen deposits. In the allograft only group, weak signals obtained by these methods are attributed to smaller and less rigid collagen fibrils and fibers. The rigid collagen can be attributed to new osteoid tissue formation, and the softer collagen can be correlated with scar tissue formation thus, the proposed approach enables differentiation between new bone formation and scar tissue. The in-vivo MRI scan supports this determination as the high signal may be ascribed to large water amounts or loosely packed collagen fibers characteristic of scar tissue. The enhanced new bone formation demonstrated by the mCT scan was possible due to the decrease in scar tissue formation.

Conclusion

This approach will enable physicians to assess the clinical outcome of surgical procedures intended to treat non-union fractures in a non-invasive manner as well as monitor a spectrum of other inflammatory conditions involving scar tissue formation, such as cirrhosis, renal glomerular fibrosis, asthma, and various autoimmune diseases.

Funding acknowledgment

This research was funded by NIH grant DE019902-04S.

References

1. Sheyn D, Cohn Yakubovich D, Kallai I, Su S, Da X, et al. (2013) PTH promotes allograft integration in a calvarial bone defect. Mol Pharm.
2. Eliav U, Komlosh ME, Basser PJ, Navon G (2013) Collagen Composition and content-dependent contrast in porcine annulus fibrosus achieved by using double quantum and magnetization transfer filtered UTE MRI. Magn Reson Med.