Connexin 43 Deficiency in Osteoblasts/Osteocytes Influences Murine Postnatal Shoulder Development

Shen, H1; Grimston SK2; Civitelli, R1; + Thomopoulos S1

1Department of Orthopaedic Surgery; 2Division of Bone and Mineral Disease, Department of Internal Medicine, Washington University, St. Louis, MO

ThomopoulosS@wudosis.wustl.edu

SIGNIFICANCE: Mechanical loading is necessary for the postnatal development of the shoulder [1-2]. Decreased loading due to muscle paralysis (e.g., due to neonatal brachial plexus palsy) leads to bony deformities and decreased joint function.

INTRODUCTION: The underlying mechanotransduction mechanisms driving joint development have not been fully characterized. Connexin 43 (Cx43) is the most abundant gap junction protein in the musculoskeletal system and has been implicated in osteoblast differentiation and mechanotransduction via cell-cell communication [3-7]. The objective of the current study was to use an osteoblast/osteocyte specific conditional knockout mouse model to investigate the role of Cx43 in postnatal shoulder joint development. We hypothesized that Cx43 deficiency in osteoblasts/osteocytes would impair the formation of the humeral head and the bony insertion of the rotator cuff.

METHODS: Transgenic Mice: Conditional knockout mice depleted in Cx43 (Gja1) in osteoblasts/osteocytes were generated as described elsewhere [5]. In brief, the osteoblast/osteocyte Cx43 cKO mice were produced by crossing homozygous Gja1flox/flox mice with mice expressing Cre recombinase under the control of a 2.3 kb α(I) collagen promoter fragment and only one allele of Gja1 (ColCre;Gja1flox). The resulting Cx43 cKO mice (ColCre;Gja1flox) and their WT equivalent (Gja1flox/flox) were sacrificed 14 and 28 days postnatal. Microcomputed Tomography (μCT): Mouse shoulders were dissected to obtain the entire supraspinatus muscle and tendon attached to the humerus. After fixation and dehydration, specimens were scanned (μCT 40, Scanco Medical AG, Switzerland) for supraspinatus (SS) muscle volume, SS tendon cross sectional area (CSA), humeral length, and indexes for trabecular and cortical bone micro-architecture. All μCT scanning was performed at 12 μm voxel, 45 kVp, 177 μA, and 250 ms integration time except for the trabecular bone, where the voxel resolution was 6 μm, and the integration time was 200 ms. Histology: After μCT scanning, the specimens were decalcified, embedded in paraffin, and sectioned at 5 μm. The resulting sections were stained with H&E and scanned. In brief, the osteoblast/osteocyte Cx43 cKO mice were depleted in Gja1 at 12 months. The resulting sections were stained with H&E and scanned. Histology: After μCT scanning, the specimens were decalcified, embedded in paraffin, and sectioned at 5 μm. The resulting sections were stained with H&E and scanned.

RESULTS: Cx43 deficiency in osteoblasts/osteocytes impairs cortical bone formation: Cx43 deficiency resulted in a significant reduction in cortical bone thickness (Cl.Th), bone area / total area ratio (B.Ar/T.Ar), bone mineral density (BMD) compared to WT. There was a significant increase in total area (T.Ar) and marrow area (Ma.Ar) in Cx43 cKO mice compared to their WT counterparts. Changes were evident at both 14 and 28 days (Fig. 1, Table 1 and 2). There was no difference in cortical area (Cl.Ar) between the WT and cKO mice at either age. Ablation of Cx43 in osteoblasts/osteocytes has little impact on trabecular bone microarchitecture: Trabecular bone indexes were compared between WT and Cx43 cKO mice in any trabecular bone indices at either 14 or 28 days (data not shown). Cx43 deficiency in osteoblasts/osteocytes hinders humeral growth: The humeral length of Cx43 cKO mice was significantly shorter than that of WT mice of 28 day old (Fig. 2). The growth plate of Cx43 cKO mice of either age appeared thinner than their WT counterparts (Fig. 3). Loss of Cx43 in osteoblasts/osteocytes affects adjacent tendon and muscle development: At day 14, there was a trend towards reduction of CSA in SS tendon (Fig. 4) but little change in SS muscle volume (Fig. 5) in Cx43 cKO compared to WT. By 28 days, there was a significant reduction of both SS tendon CSA and SS muscle volume in Cx43 cKO mice compared to WT (Fig. 4 and 5). No pathological changes were found histologically in SS muscle, tendon, or enthesis of Cx43 cKO mice.

DISCUSSION: Cx43 deficiency in osteoblasts/osteocytes significantly affected cortical bone architecture in the postnatal humerus, with thinner cortical bone, bigger bone marrow area, and larger diameter of diaphysis. The observed changes in cortical bone morphology are consistent with the phenotypes observed in the adult tibia [6]. Changes in the bone may reflect an imbalance between bone formation and resorption due to dysfunction in osteoblast cell-cell communication [4,5] and endocortical osteoclast activation [8]. Interestingly, ablation of Cx43 in osteoblast/osteocyte also affected humeral growth and growth plate formation, suggesting a secondary role of Cx43 in endochondral bone formation. Muscle forces are transmitted to bone across tendon. Our results indicate that, in addition to bone formation, Cx43 deficiency in osteoblasts/osteocytes also affected the development of the adjacent tendon and muscle. Further investigate is needed to determine if Cx43 is also depleted in SS tendon in this mouse model and how defects in bone architecture lead to impaired tendon and muscle formation. In conclusion, our results demonstrate that expression of Cx43 in osteoblasts/osteocytes is necessary for postnatal shoulder joint development.

ACKNOWLEDGEMENTS: The study was funded by a grant from the NIH (AR05580).

REFERENCES: