Pharmacological manipulation of CCR7 prevents bony growth imbalance following physeal injury in mice

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INTRODUCTION: Growth plate (GP) is responsible for longitudinal bone growth. However, it is the weakest structure in the skeleton of a child and a frequent site of an injury or fracture, leading to progressive skeletal growth imbalance and deformity, and significant physical problems. Current treatment involves surgical resection of the bar and replacement with an interpositional material to preserve normal growth in the remaining physis. However, these procedures are invasive and cause substantial burden to the child and their family. Chemokines are low-molecular-weight proteins of 8-10 kDa and are known to play a crucial role in recruiting stem cells or precursor cells. We previously reported that depletion of C-C chemokine receptor type 7 (CCR7) ameliorated growth imbalances after physeal injury in a mouse model (1). Therefore, we hypothesized that pharmacological manipulation of CCR7 could prevent growth disturbances following growth plate injury. The purpose of current study was to show that exogenous administration of an anti-CCR7 monoclonal antibody could modulate and suppress growth disturbances after physeal injury, as a proof-of-concept study.

METHODS: All experimental procedures fully complied with the related laboratory animal regulations. GP injury was made in the left proximal tibial GP in 3-week-old mice as previously reported (2). Briefly, under general anesthesia, the skin over the proximal tibia was incised with a scalpel and opened with forceps to render the tibiae visible. The proximal tibia GP was entirely pierced in a lateral-medial direction with a 25-G needle. The right tibial GPs were left unoperated and served as controls. Mice were administered either anti-CCR7 monoclonal antibody (CCR7 Ab) or control antibody (CTRL Ab) every 3–4 days for two weeks. The tibiae were analyzed at 1, 2, 3 and 5 weeks postoperatively and analyzed at 5 weeks. The length of tibiae was measured macroscopically using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and sections of the physeal injury were analyzed histologically and immunohistochemically. Bone volume of the physeal bridge at the injury site was measured using micro-CT. Quantitative real-time reverse transcriptase-PCR (qPCR) was performed by ΔΔCt method. Data was normalized to the average mRNA level at day 0 (set at 1). Physeal cartilage from 5-day-old mice was harvested and cultured as described in a previous protocol (3) and subjected to qPCR analysis and RNA sequence following 24 hours of antibody treatment. All results were statistically compared using unpaired t-tests to evaluate differences between pairs of groups. Significance was accepted with a p value < 0.05. All statistical analyses were done using statistical software JMP Pro 17.0 (SAS Institute, Cary, NC, USA).

RESULTS: The drop ratio, defined as the difference between the contralateral and injured tibial lengths divided by the contralateral tibial length, was significantly lower in CCR7 Ab (mean ± standard deviation; 3.09 ± 1.09% in CTRL Ab vs 1.02 ± 0.59% in CCR7 Ab at 2 weeks, $p < 0.01$; 3.57 ± 0.61% in CTRL Ab at 3 weeks vs 0.99 ± 0.50% in CCR7 Ab, $p < 0.01$; 3.43 ± 0.99% in CTRL Ab vs 1.89 ± 1.02% in CCR7 Ab at 5 weeks, $p = 0.02$, Figure 1A-D). Bone volume of the physeal bridge in CCR7 Ab was significantly lower than that in CTRL Ab at 1, 2, 3 and 5 weeks (mean ± standard deviation, 150.67 ± 13.29 HU in CTRL Ab vs 136.00 ± 8.07 HU in CCR7 Ab at 1 week, $p = 0.01$; 176.00 ± 11.68 HU in CTRL Ab vs 160.17 ± 4.86 HU in CCR7 Ab at 3 weeks, $p = 0.01$; 190.67 ± 6.31 HU in CTRL Ab 177.57 ± 22.30 HU in CCR7 Ab at 5 weeks, $p = 0.02$, Figure 1E-K). Histological examination revealed that CTRL Ab showed a denser and more continuous bony bar (Figure 2A-C), while the bony bar in CCR7 Ab showed rather sparse, unstable and discontinuous at 5 weeks (Figure 2D-F). The osteogenic markers such as Sox9 and Osterix in physeal chondrocytes following 24 hours of antibody treatment were significantly downregulated in CCR7 Ab, in contrast the expression of Runx2, Type X collagen, VEGF and BMP-2 was almost equal between CCR7 Ab and CTRL Ab (Sox9: 1.00± 0.12 in CTRL Ab vs 0.60 ± 0.11 in CCR7 Ab, $p < 0.01$; Osterix: 1.00 ± 0.19 in CTRL Ab vs 0.56 ± 0.10 in CCR7 Ab, $p < 0.01$). In the RNA sequencing analysis, KEGG pathway analysis of differentially expressed genes revealed enrichment of terms related to inflammatory and cytokine responses, MAPK signaling, extracellular matrix organization, and metabolic/hormone synthesis pathways. Gene Ontology enrichment analysis identified “blood vessel endothelial cell proliferation involved in sprouting angiogenesis” as the most significant biological process. Normalized expression levels of the genes associated with this term were displayed in a heatmap, with the CTRL Ab set as 1.0 (Figure 3). Among these genes, Hmox1 showed upregulation consistent with a pro-angiogenic role, whereas Ngfr and Il12b were inhibitory, and Bmper exhibited biphasic reports.

DISCUSSION: The present study demonstrated that exogenous administration of an anti-CCR7 monoclonal antibody could modulate and suppress growth disturbances after physeal injury. Since surgical resection of physeal bar may affect the viability of remaining cartilage, we must pay much attention to decision of additional non-surgical intervention. The treatment strategy to inhibit physeal bridge formation by manipulation of CCR7 may be future directions after the growth plate injury.

Significance: Pharmacological manipulation of CCR7 prevents growth disturbances following growth plate injury.

REFERENCES: (1) Y. Sakai et al. Sci Rep. 2024 (2) Y. Hosokawa et al. BMC Musculoskelet Disord. 2024 (3) M. Gosset et al. Nat Protoc. 2008

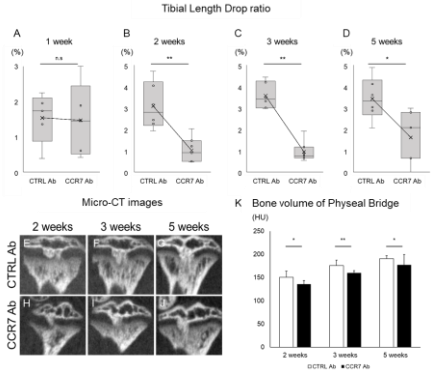

Figure 1. Micro-CT analysis (n = 6/CTRL Ab, n = 7/CCR7 Ab). Comparison of tibial length drop ratio after physeal injury. Micro-CT images showing bony bar formation within the physeal injury (E-J). Bone volume of the bony bar (K). Scale bar: 1 mm.

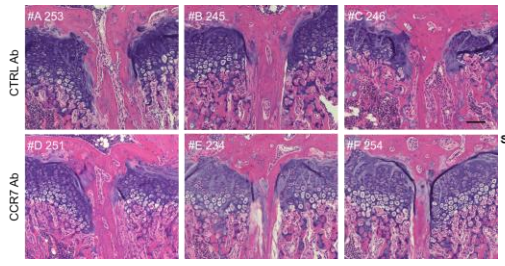

Figure 2. Histology of physeal bridge formation after injury at 5 weeks. Histology showed differences in bony proportion of physeal bridge in CCR7 Ab (A-C), and CTRL Ab (D-F) at operated side. Scale bar: 0.1mm.

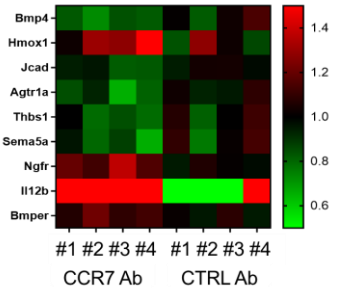

Figure 3. RNA sequence Heatmap of normalized expression of angiogenesis-related genes.