Analysis of early changes in subchondral bone and cartilage after destabilization of the medial meniscus in osteoporotic mice

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INTRODUCTION: It has been reported that meniscus injury and extrusion cause rapid progression of osteoarthritis (OA), spontaneous osteonecrosis of the knee (SONK), and subchondral insufficiency fractures of the knee (SIFK)[1)]. SONK and SIFK are apparently caused by the sudden increase in mechanical stress on the subchondral bone due to meniscus injury[2,3)]. Although osteoporosis appears to be involved in SIFK, its detailed pathogenesis is not clear. Therefore, the purpose of this study was to clarify whether SIFK occurs after meniscectomy and its pathogenesis using osteoporotic mice.

METHODS: All procedures were approved by the Institutional Animal Care and Use Committee of our university (approval number P200808). Senescence accelerated mouse prone 6 (SAMP6) mice that spontaneously develop osteoporosis, were used for the analysis, and CL57BL/6J mice were used as control. The destabilization of the medial meniscus (DMM) surgery or sham surgery was performed on both 16-weeks-old female SAMP6 mice and control mice. Mice were euthanized 3 days, 7 days, and 2 weeks after surgery to assess early subchondral bone changes (n=5/group for each timing point). Articular cartilage was evaluated using the Osteoarthritis Research Society International (OARSI) cartilage OA histopathology grading system, and subchondral bone was evaluated according to the subchondral bone scoring system[4)]. Briefly, the thickness of the subchondral bone plate and the region between the osteochondral junction and the bone marrow cavity was measured according to the definition. To evaluate bone morphology and bone volume / total volume (BV/TV), μCT was performed on each specimen using a μCT imager. Immunostaining for type 2 collagen and fluorescent immunostaining for cathepsin K were used to evaluate the cartilage and osteoclasts in the subchondral bone. Two-way analysis of variance was performed to compare multiple groups using the Tukey method as a post-hoc test; p-values < 0.05 were considered significant.

RESULTS SECTION: In the evaluation of basal level of bone volume, both histological and μCT evaluations showed significantly lower BV/TV in the SAMP6 group compared to the control group. The μCT analysis showed mild subchondral bone sclerosis in the control group 2 weeks after DMM surgery, while no obvious changes were observed in the SAMP6 group. In the histological analysis with Safranin-O-fast green staining, progressive cartilage degeneration, osteophyte growth, and thinning and irregularity of the subchondral bone were observed in the SAMP6 group 2 weeks after DMM surgery. Meanwhile, osteophyte growth with thickening of the subchondral bone and increased trabecular bone were observed in the control group. After sham surgery, there were no obvious histological changes in both SAMP6 and control groups at all timing points. The OARSI score in the SAMP6 group with DMM surgery was significantly higher than that of the control group at 7 days and 2 weeks postoperatively. In the evaluation of subchondral bone plate thickness, significant thickening was observed in the control group with DMM surgery at 2 weeks postoperatively, while obvious subchondral bone plate thickening was not observed in SAMP6 with DMM surgery group. Immunostaining evaluation of type 2 collagen also showed that the number of positive chondrocytes was significantly decreased in the SAMP6 with DMM surgery compared with the other groups at 2 weeks postoperatively. Fluorescent immunostaining evaluation of cathepsin K, the number of positive cells was significantly increased in the subchondral bone area of the SAMP6 mice with DMM surgery at 2 weeks postoperatively compared with other groups. These results are summarized in **Table 1**.

DISCUSSION: In this study, earlier OA changes was observed in SAMP6 mice after DMM surgery than control mice. Thickening of the subchondral bone plate was observed after DMM surgery in the control group, while it was not observed in the SAMP6 mice. Therefore, subchondral bone thickening is an adaptative response to the increased mechanical stress, and may contribute to delay cartilage degeneration. It is also suggested that the earlier OA progression without subchondral bone plate thickening may reflect a part of the pathogenesis of SIFK. In addition, significantly more cathepsin K-positive cells were observed in the subchondral bone, suggesting that osteoclast activation is involved in the pathological conditions of SIFK. Further research will be needed to elucidate the detailed pathogenesis of SIFK.

S**IGNIFICANCE/CLINICAL RELEVANCE**: Progression of OA was observed without association of subchondral bone plate thickening in osteoporotic mice after induction of meniscus instability and osteoclasts may be involved in the pathological condition. This research may help to elucidate the pathogenesis of SIFK.

REFERENCES:
1)Yamagami et al. Knee 2016, 2) Akamatsu et al. Acta Orthop 2012, 3) Furumatsu et al. Orthop Traumatol Surg Res 2019, 4) Nagira et al. Sci Rep 2020

TABLES:

	SAMP6						Control					
	DMM			Sham			DMM			Sham		
postoperative	3 days	7 days	2 weeks	3 days	7 days	2 weeks	3 days	7 days	2 weeks	3 days	7 days	2 weeks
cartilage degeneration	—	—	progressive	—	—	—	—	—	slight	—	—	—
osteophyte growth	—	slight	progressive	—	—	—	—	slight	progressive	—	—	—
subchondral bone plate thickening	—	—	—	—	—	—	—	—	thickening	—	—	—
type 2 collagen (positive cells in chondrocytes)	—	—	decrease	—	—	—	—	—	—	—	—	—
cathepsin K (positive cells in subchondral bone)	—	—	increase	—	—	—	—	—	—	—	—	—

Table 1: Summary of the results.