Gait Analysis of Model Mouse of Knee Osteoarthritis Induced by Surgical Destabilization of Medial Meniscus using Catwalk System

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[Material and Methods]
Total of 20 C57BL/6 mice (10 weeks old, female) were purchased. Fifteen mice were surgically treated with DMM in their left knees and five mice were sham operated in their left knees as well. The animal experiments were conducted under the approval of the Animal Care and Use Committee of Chiba University, Japan.

For histological evaluation of OA changes, DMM mice were euthanized at 2, 4, 8, and 12 weeks post surgery, five animals at each time point were used. Sham operated mice were euthanized after 12 weeks. Specimens were stained with hematoxylin and eosin (H&E) and safranin-O. OA changes were evaluated by the grading score of OARSI.

Gait analysis was performed employing CatWalk just before surgical induction, 1, 2, 4, 8, and 12 weeks post surgery. In CatWalk, twenty-one parameters regarding the gait behavior are automatically calculated from each run. The basic parameters consist of time of stand, paw print intensity, and paw print area. Additionally, other parameters are calculated using basic parameters, such as swing speed, gait cycle. To correct age and individual difference, each parameter of left knee was divided with right knee and the left/right ratio was compared between each time point. Comparisons of each parameter of the gait analysis between each time point were made by one-factorial ANOVA. Where significant effects were found, subsequent comparison were performed using Scheffe’s test.

[Results]
OA characteristic were observed from 4 weeks after DMM. The OA change progressed time-dependently and OARSI score also increased in time. Sham operated mice did not develop OA even after 12 weeks.

Gait analysis using CatWalk showed significant difference in 4 parameters, i.e. Duty Cycle, Swing Speed, Time of Stand Phase, and Single stand phase, on 12 weeks post surgery compared with the other time-points. There was no significant difference in the earlier time points and the rest of the parameters. Duty cycle, the ratio of stand phase of whole step cycle, was significantly smaller in the DMM limb compared to contralateral hind limb. Swing speed, the speed of the paw during the swing phase, was slower in the DMM limb than contralateral hind limb on 12 weeks. Stand phase and Single stand phase was significantly shorter in the DMM limb compared to contralateral hind limb. Duty cycle tended to be lower than the earlier time point at 8 weeks after surgery but they were not statistically significant.

[Discussion]
From the gait analysis using CatWalk method, the DMM model demonstrated clear behavioral responses in the OA knee compared with contralateral knee after 12 weeks. The difference of gait pattern clearly shown by four parameters assumedly reflects the pain-related gait behaviours. Decreased duty cycle, swing speed, time of stand phase, and single stand phase reflected the limping gait due to unwillingness of the animals to bear weight on the DMM limb. Although pathological changes corresponding to early osteoarthritic changes were histologically proved at 4 weeks post DMM, behavioural changes emerged as late as 12 weeks. This time lag would be understandable that minor osteoarthritic changes did not necessarily mean symptomatic change. It has been unclear which pathological changes were most responsible for OA symptom but our data suggested that full depth erosion might be the most responsible change. This is the first report that showed validity of the gait analysis using CatWalk system in mice OA models induced by DMM. With the use of this assessment system, efficacy of DMOADs will be examined effectively. CatWalk analysis has greater advantage than histological examination because mice did not need to be necropsied. Thus it can be applied repeatedly to the same mice. This specialty enables to assess not only efficacy of DMOADs but also the proper timing of DMOADs introduction.

[Conclusion]
CatWalk method is an effective tool to assess pain behavior of surgical induced mouse OA model.

[Significance] This study demonstrated that CatWalk is a useful tool to assess pain-related behavior of mouse OA model induced by DMM. Using this method, improvement in understanding OA pathology and pharmacology can be expected.