Short Communication

A Sensitive Gait Parameter for Quantification of Arthritis in Rats

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Abstract. Quantification of arthritis is helpful for investigating pain mechanisms of arthritis and for developing new drugs. We assessed and identified a feasible parameter for quantification of rat arthritis using a novel gait analyzing system. Knee-joint injection with small doses of \(\lambda\)-carrageenan decreased swing time ratio (STR, swing time of the non-treated hindlimb/swing time of the \(\lambda\)-carrageenan-injected hindlimb) in a dose-dependent manner. Intraperitoneal treatment with indomethacin restored the decreased STR dose-dependently. The arthritis could not be accurately quantified by swing time and swelling, common indices of arthritis. These results show that STR is a sensitive, reliable parameter for quantification of arthritis.

Keywords: arthritis, quantification, gait analysis

Severity of arthritis has been evaluated by pain-related behaviors, including behavioral scoring in an open field and struggle threshold of the knee extension (1–3). These parameters, however, have to be determined by an experimenter subjectively. Especially in the latter experiment, an experimenter has to hold an animal in hand. As immobilization stress modifies the pain threshold (4), it is highly likely that the parameter may vary between experimenters. Because gait disturbance is one of the representative signs of arthritic pain, a gait analysis may be a feasible, objective method to quantify arthritis (5). Swing is a period when the foot is off the ground and moves forward. A single hindlimb must solely bear the weight of the animal’s body during this period. Thus, swing time (ST) of the hindlimb may shorten when the opposite hindlimb is suffering from arthritic pain. On the other hand, rats adjust their walking velocity by changing ST (6), suggesting that gait disturbance may not be evaluated accurately when rats walk with varying velocity. Swing time ratio (STR) is a parameter obtained by dividing the ST of the non-treated hindlimb (affected ST) by the next ST of the contralateral compromised hindlimb (non-affected ST). This parameter would be affected by only minimal velocity changes. Thus, we hypothesized that STR might be a sensitive parameter to quantify gait disturbance.

In the present study, we made a rat model of arthritis by injecting \(\lambda\)-carrageenan into the right knee-joint and the relationship between the change in ST of the non-treated hindlimb or STR and severity of arthritis was examined. Then, to reveal whether a therapeutic strategy could be successfully quantified by STR, the effect of indomethacin, a nonsteroidal anti-inflammatory drug, on the gait disturbance induced by \(\lambda\)-carrageenan was examined. A knee-joint diameter, another important parameter for evaluation of arthritis (7, 8) was also examined. From these results, we evaluated validity of STR as a parameter for quantification of arthritis.

Male Sprague-Dawley rats (SLC, Shizuoka), 10–13-week-old, weighing 330–410 g, were used. Each rat was put into a transparent, acrylic wheel (10 cm in width and 40 cm in diameter) that was revolving at 10 rpm, for 5 min/day for 5 days to become habituated to the apparatus and to keep walking in it. On the experimental day, each rat was made to keep walking for 60 s in the revolving wheel after the foot bottom of each hindlimb was marked with red ink. The walking behavior was video-recorded below with a high-speed camera (GT-
For induction of arthritis, each rat was anesthetized with ether and $\lambda$-carrageenan (Wako Pure Chemical, Osaka) was injected into the right knee-joint at a dose of 2.5, 7.5, or 25 $\mu$g. The walking behavior was recorded for 60 s before and 1, 2, 3, 4, 5, 6, and 24 h after the administration. For the evaluation of therapeutic effects, indomethacin (1 or 10 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) was administered into the abdominal cavity immediately after the video recording 2 h after the injection with $\lambda$-carrageenan (25 $\mu$g). The knee-joint diameter was measured just after each walking with calipers while the joint was held in extension (8). In each step cycle, swings of the both hindlimbs were automatically extracted and their ST was calculated. STR in each gait cycle was calculated as follows:

$$\text{STR} = \frac{\text{ST of non-treated hindlimb}}{\text{ST of compromised hindlimb}}$$

Then averaged ST of the non-treated hindlimb and STR during a 60-s session were obtained. These motion analyses were performed with GAIT® (Noveltec).

All the experiments with animals complied with the standards in the guidelines of the University Animal Care and Use Committee at Tokyo University of Agriculture and Technology.

All results were expressed as means ± S.E.M. The differences in body weight among all groups and the differences in the knee-joint diameter 2 h after $\lambda$-carrageenan (Carr) into the right knee-joint. Values are expressed as means ± S.E.M. of 6 animals. Tables show the F and P values of group differences, time, and group × time interaction revealed by repeated measures of a two-way analysis of variance.
carrageenan in the experiment of the effect of indomethacin against \( \lambda \)-carrageenan-induced arthritis were evaluated by a one-way analysis of variance. Effects of injection with \( \lambda \)-carrageenan into the right knee-joint and intraperitoneal treatment with indomethacin were evaluated by a two-way (with repeated measures) analysis of variance. The criterion for statistical significance was considered to be \( P < 0.05 \).

There were no statistical differences in body weight among all groups (F(5, 30) = 0.206, \( P = 0.9575 \)). After injection with \( \lambda \)-carrageenan into the right knee-joint, the knee-joint diameter increased and STR decreased in a dose-dependent manner (Fig. 1: A and C). Although ST of the non-treated hindlimb was changed significantly by injection with \( \lambda \)-carrageenan at 7.5 and 25 \( \mu \)g, it shortened obviously only at the highest dose of 25 \( \mu \)g (Fig. 1B).

There were no significant differences in the right knee-joint diameters of 3 groups when measured right before administration of indomethacin (F(2, 15) = 0.964, \( P = 0.4039 \), Fig. 2A). Treatment with indomethacin showed no effect on the increased diameter of the affected knee-joint even at 10 mg/kg. The shortened ST of the non-treated hindlimb was restored significantly only when indomethacin was administered at 10 mg/kg (Fig. 2B). On the other hand, the decreased STR was
restored by indomethacin in a dose-dependent manner (Fig. 2C).

Rats adjusted their walking speed by changing stride time and length. Stride time adjustments were achieved by changing ST, suggesting that ST may vary depending on rat walking velocity (6). Rats ran 5 – 10 steps continuously but stopped shortly in a revolving wheel. Thus, ST of the non-treated hindlimb may not be a sensitive parameter for evaluating gait disturbance. STR was obtained by normalizing the affected ST by the next non-affected ST. Thus, the influence of walking velocity may be minimal. It is highly possible that the stance time, which is the portion of the limb motion cycle when the limb is in contact with the ground, may shorten in the affected limb to reduce loading (9). As mentioned above, however, rats often stopped shortly in the revolving wheel. During abeyance, their bodies were supported by 4 feet. This “double support time” may hamper precise quantification of gait disturbance.

It is worthy to note that STR was restored by intraperitoneal treatment with indomethacin, although the knee-joint diameter was still unchanged. Indomethacin may recover STR by alleviation of pain without bringing the swelling down. An inhibition of local cyclooxygenases contributes to the anti-inflammatory action of indomethacin. Besides the inhibition of local cyclooxygenases, the inhibition of central cyclooxygenases is the mechanism for the analgesic action of indomethacin (10). The difference of the pharmacological point(s) of action may be one of the causes of the different potency of analgesic and anti-inflammatory effects.

In conclusion, the present results suggest the STR is a sensitive and reliable parameter for quantifying acute arthritis and therapeutic effect of drugs.

References