Inhibitory Effects of Steroid-Induced Myopathy on Muscle Fiber Conduction Velocity

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ABSTRACT. Muscle fiber conduction velocity (MFCV) has been reported to decrease in patients with myopathy. However, there has been no rerport regarding changes in MFCV in steroid-induced myopathy (SM). In this study, MFCV in rats with SM was compared with that in normal rats and a new technique for measuring MFCV in a single motor unit was established using the multi-point stimulation (MPS) technique. Ten eight-week-old male Wistar rats were treated with intramuscular injections of triamcinolone acetonide for two weeks (SM group). Ten eight-week-old normal male rats were reared for two weeks as the control group. After the right sciatic nerve and soleus muscle were exposed, an original four-channel surface electrode array was placed over the muscle belly parallel to the fibers. Several sites of the sciatic nerve were stimulated electrically using the MPS technique. MFCV was calculated from the distance of each electrode and each time lag between neighboring potentials. Twenty MFCV values of different motor units per each material were measured, and the values were averaged. There were significant differences in the mean MFCV values between the SM group and the control group. The number of MFCV values greater than 4.0 m/sec significantly decreased in the SM group, while the lowest values were the same as those in the normal group. The mean MFCV value in the SM rats decreased significantly compared to that in the normal rats. The decrease in the fast values of MFCV was in excellent agreement with the atrophy of type 2A fibers with a fast MFCV component in the SM rats.

Key words : muscle fiber conduction velocity — multi-point stimulation technique — rat — steroid-induced myopathy

Muscle fiber conduction velocity (MFCV) is the speed of a depolarization wave propagating on the sarcolemma from a neuromuscular junction to the bilateral ends of muscle fibers. MFCV has been reported to vary with the diameter and types of muscle fibers. MFCV has also been found to decrease as muscle fiber diameter decreases. Regarding muscle fiber type, MFCV has been observed to be faster in type 2 fibers (fast-twitch fibers) than in type 1 fibers.[1] In many human studies, MFCV has also been found to depend on myopathic changes or on muscle fatigue. MFCV has been reported to decrease in myopathic muscles, exhausted

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muscles after fatigue, and atrophied muscles after immobilization.[2-7]

Recently, many fundamental studies on MFCV in rats have been published, and they have revealed a wide range in MFCV values for measured muscles in normal rats. The results of these previous reports suggest that MFCV in rats also decreases in denervated muscles, disused ones, and exhausted ones after fatigue.[8-10] However, to the best of my knowledge, there have been no earlier reports concerning changes in MFCV in steroid myopathy rats.

To measure MFCV in most previous studies using rats, a number of muscle fiber action potentials were simultaneously evoked by direct electrical stimulation of an entire muscle.[8-10] With that method, it is impossible to measure the MFCV originating in each motor unit because of the simultaneous stimulation of many muscle fibers. The value obtained, therefore, reflects the average MFCV for the entire muscle. In the present study, I established a new method for measuring MFCV using the multi-point stimulation (MPS) technique, which has been used in the detection of single motor units in humans. To measure the MFCV originating in each motor unit in rats, the stimulus intensity was adjusted to activate a single nerve fiber, and several points were chosen using the MPS technique.[11] I also made a rat model of steroid-induced myopathy referring to a previous paper and measured the changes in MFCV using the new method.

MATERIALS AND METHODS

1. Production of steroid-induced myopathy

Injection of 5 mg/kg of triamcinolone acetonide (Kenacort A®) into the paraspinal muscles of ten eight-week-old male Wistar rats (SM group) was performed on consecutive days for two weeks.[12] They were reared for two weeks in animal quarters (314 mm width, 468 mm length, 201 mm height) where the temperature was kept at 25°C and the light of the room was switched on for 12 hours each day. They were allowed free access to a solid diet and water.

2. Setting of control group

Another group of ten eight-week-old Wistar rats were reared for two weeks without injection of medicine as the control group. The animal quarters and the environment were the same as for the SM group.

3. Measurement of MFCV

The animals were anesthetized intraperitoneally with 50 mg/kg of pentbarbital sodium (Nembutal®). The experiment was started after confirming with painful stimuli that an adequate depth of anesthesia had been induced. After the skin was incised extensively from the lateral area to the positerior area of the right hind limb, the sciatic nerve and soleus were exposed carefully without damage. The gastrocnemius was resected perfectly to prevent redundant volume conduction during the recording of single motor unit potentials (MUPs) from the soleus. The right hind limb was immobilized so that the ankle joint was kept at 0 degree to maintain constant tension in the muscles.

The room temperature was kept constantly at 25°C during the measurement of MFCV. The sciatic nerve was stimulated electrically using a pair of hook-like electrodes (Fig 1). Stimulation points were chosen using the MPS technique : several sciatic nerve sites were stimulated at the minimum intensity for evoking a single MUP. The stimulation intensity was increased gradually by 0.01 mA increments. The frequency was 1 Hz and the duration was 0.2 msec. At the moment a single MUP was identified, the stimulation switch was turned off and the waves were acquired.

The recording surface electrode array used in this study was an order-made original design (Unique Medical Co. Ltd). It employs an array of five silver surface electrodes attached to a silicon sheet 15 mm long, 10 mm wide, and 1 mm thick. Each electrode has a thin columnar shape and a diameter of 0.2 mm. The distance between electrodes is 2 mm (Fig 2). This four-channel surface electrode array was placed over the muscle belly

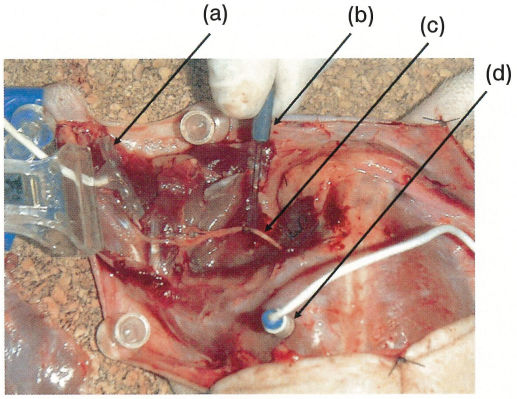

Fig 1. Anatomy of the right hind limb in a rat. (a) original surface electrode array, (b) a pair of hook-like electrodes for stimulation, (c) sciatic nerve, (d) grounded electrode.

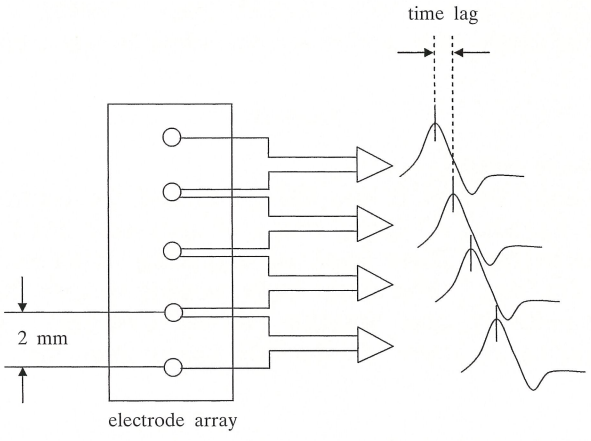

Fig 2. Schema of the four-channel surface electrode array used to measure MFCV. Circles represent the five silver surface electrodes. Waveforms on the right are single MUPs for which there was a time lag between neighboring waves.

parallel to the fibers of the soleus. A grounded electrode was placed on the ipsilateral thigh. Low and high frequency filters, 20 Hz- 2 KHz, respectively, were used to record single MUPs. Neuropack-4 (Nihon Kohden Co.) was utilized for the recordings, and SEN-3301 (Nihon Kohden Co.) was used for the stimulation device.

Single MUPs were detected from neighboring pairs of electrodes, four waves being recorded at a time from five electrodes. Single MUP propagation on the screen was observed in both the proximal and distal directions. Only those single MUPs that showed a time lag between neighboring waves were used to measure MFCV. Single MUPs that showed no time lag between neighboring waves were judged to be in a motor end-plate zone and were excluded from MFCV measurement. Each waveform peak on the screen was determined manually. A perpendicular line was drawn from the single MUP peak of each channel to the baseline. Intersection of the perpendicular line with the baseline was considered a coordinate point. The regression line of those points was calculated manually. MFCV was estimated from the slope of the line on the graph.[13] Twenty MFCV values belonging to different motor units were measured per each rat, and the values were averaged. After the measurement of MFCV values, a lethal dose of Nembutal® was injected into the animals intraperitoneally.

4. Data analysis

Mean MFCV values were compared between the SM group and the control group, and the distributions of MFCV values were also compared between the groups. The analysis of variance and Welch t-test were used for statistical analysis. Values were expressed as mean ± standard deviation (SD). Statistical significance was defined as a p-value of less than 0.05.

5. Permission for the study

Concerning the handling of the animals and the experiments, approval of the Animal Experimentation Committee of Kawasaki Medical School was obtained (approval number : 2004 ; 04-048, 2003 ; 03-043). All procedures carried out in the study were performed according to the ethical regulations.

RESULT

1. Changes in body weight

The mean body weights of the SM group and the control group before the injection of Kenacort A® (eight weeks of age) were 311 ± 19.4 g and 300 ± 13.9 g, respectively. There were no significant differences between the two groups at this time. The mean body weights became 206 ± 39.9 g and 378 ± 17.2 g, respectively, after injection (10 weeks of age). The mean body weight was significantly lower in the SM group than that in the control group.

2. Observation of the body and movement

Remarkable generalized wasting away of muscles and physical debility were observed in the SM rats. However, they did not show any localized

muscle atrophy typically present in paralyzed limbs. There were no significant differences in posture and bodily movement between the two groups, but the SM rats exhibited hypersensitive reactions to sound.

3. Comparison of MFCV

Figure 3 shows the typical waves obtained from an SM rat. Single MUPs without time lag were judged to be evoked in a single motor end-plate zone. Only single MUPs showing a time lag between neighboring waves were used to measure MFCV. The MFCV of the rat in Figure 3

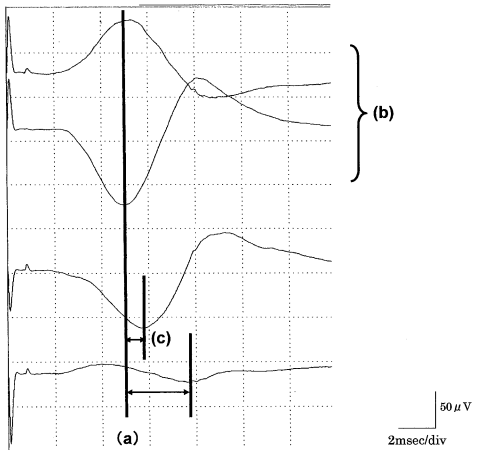

Fig 3. Typical single MUPs for MFCV measurement in the right soleus of a steroid-induced myopathy rat. Waveforms within the dotted box are single MUPs. (a) Peak latencies of single MUPs in end-plate zones, (b) end-plate zones, (c) time lags. Sweep velocity, 2 ms/division ; sensitivity, 50 μV/division. The MFCV measured from these single MUPs was 2.33 m/sec.

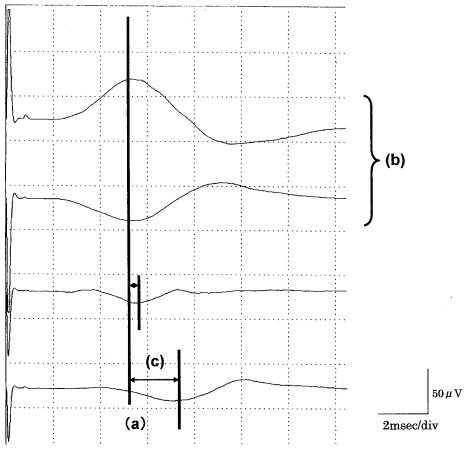

Fig 4. Typical single MUPs for MFCV measurement in the right soleus of a normal rat. (a) Peak latencies of single MUPs in end-plate zones, (b) end-plate zones, (c) time lags. Sweep velocity, 2 ms/division ; sensitivity, 50 μV/division. The MFCV measured from these single MUPs was 4.23 m/sec.

was 2.33 m/sec. Figure 4 shows the typical waves obtained from a control rat. Measurement of MFCV is also included in Figure 3, and the MFCV of this rat was 4.23 m/sec.

The mean MFCV value was 3.18 ± 0.40 m/sec in the SM group and 3.72±0.60 m/sec in the control group. There were significant differences in the mean MFCV values between the two groups.

TABLE 1. Comparison of MFCV values in the two groups

	mean MFCV	max MFCV	mini MFCV
steroid myopathy rat	3.18±.040 m/sec	4.35 m/sec	2.40 m/sec
	↕*		
normal rat	3.72±0.60 m/sec	5.78 m/sec	2.49 m/sec

*: $p<0.05$

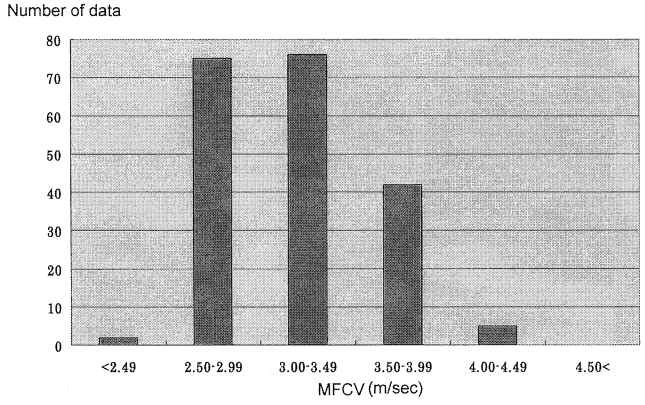

Fig 5. Distribution of MFCV values in steroid myopathy rats. The fastest MFCV value in the SM group was 4.35 m/sec and the slowest MFCV value was 2.40 m/sec. The number of MFCV values greater than 4 m/sec was only five in the steroid myopathy rats.

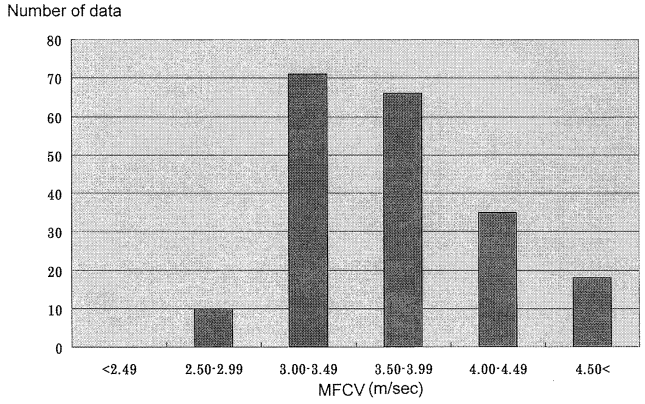

Fig 6. Distribution of MFCV values in the normal rats. The fastest MFCV value in the control group was 5.78 m/sec and the slowest was 2.49 m/sec. The number of MFCV values greater than 4 m/sec was 51 in the normal rats.

4. Distribution of MFCV

The fastest MFCV value in the control group was 5.78 m/sec and the slowest was 2.49 m/sec. The fastest MFCV value in the SM group, on the other hand was 4.35 m/sec and the slowest MFCV value was 2.40 m/sec (Table 1). The variance in the MFCV in the SM group was significantly smaller than that in the control group. In the latter, the number of MFCV values greater than 4 m/sec was 51, while in the SM group it was only 5 (Fig 5, 6).

DISCUSSION

Basic research regarding MFCV in humans began in the 1950s,[2,14] and velocities in normal adults were reported to vary widely. However, Stalberg measured the MFCV of the biceps brachii in healthy adults and reported values in a narrow range, mean 3.69 ± 0.71 m/sec. Lindstrom *et al* also reported that the normal value for the same muscles was in the narrow range of 3.5 to 4.8 m/sec.[3,16] Okajima *et al* measured the MFCV of the thenar muscle and found that it ranged from 2.5 to 5.0 m/sec.[17] Although the normal MFCV values have varied in different reports, most recent reports have indicated that the velocities are in the range of approximately 3.0 to 5.0 m/sec in human.[3,16,17]

There is a large variation in the MFCV of individual muscles in rats, since the distribution of the muscle fiber types in individual muscles is larger in the rat than in humans. Toikawa *et al* stated in their study that MFCV depended on muscle fiber types in the rat. The mean MFCV of the soleus, which contains predominantly type 1 fibers, was reported to be 2.17 ± 0.84 m/sec, while that of the tibialis anterior, which contains predominantly type 2 fibers, was 3.21 ± 1.77 m/sec. They concluded that muscles with a large proportion of type 2 fibers, known as fast-twitch muscles, had a fast MFCV speed.[10] In another report, the MFCV of the soleus was 2.4 ± 0.45 m/sec, while that of the EDL, which contains predominantly type 2 fibers, was 3.5 ± 0.42 m/sec.[8] Kondo *et al* reported faster MFCV values for the EDL of 4.0 ± 0.3 m/sec.[9]

The MFCV of the soleus was 3.72 ± 0.60 m/sec. This value was faster than the values reported previously except for that of Kondo *et al*, although the soleus is a slow-twitch muscle containing predominantly type 1 fibers. Generally, when nerve fibers have been micro-stimulated percutaneously, a nerve fibers located at the part nearest to the stimulation electrode has been reported to exit first.[18-21] It has also been reported that large nerve fibers exited by electrical stimulation more easily than small nerve fibers, when a nerve was stimulated directly.[18,19] The MFCV is fast in muscle fibers belonging to large motor units that are innervated by large nerve fibers. In the present study, since hook-like electrodes were used in addition to direct stimulation to the nerve, the area of stimulation was wide despite micro-stimulation. Therefore, it is suspected that the large nerve fiber surrounded by the stimulation electrode was excited, that muscle fibers belonging to the large motor unit were fired, and that the mean MFCV was faster. In future studies, it will be necessary to use a needle electrode to stimulate as narrow an area of the nerve as possible.

Besides the variation caused by muscle fiber types, MFCV is known to be proportional to the diameter of the muscle fibers, to decrease in exhausted muscles after fatigue, and to be affected by body temperature and muscle tension.[2-7] Toikawa *et al* reported that MFCV decreased in fatigued muscle in rats.[10] A decrease in MFCV compared with that in normal muscles was observed in disused muscles and denervated muscles in rats.[9,10] Takahashi *et al*[22] used bipedal rats to investigate the effect of muscular hypertrophy in the hind limbs following exercise, and reported a significant increase in MFCV. However, to the best of my knowledge, there have been no earlier reports using myopathy model animals to examine the effect on MFCV. In the present study, SM rats were produced by the injection of Kenacort A®. MFCV in the soleus of these rats was measured, and was compared with that of the normal rats.

The MPS technique was adopted as the method of electrical stimulation for the rats. The MPS technique was originally developed as a method for the detection of single motor units in humans, and has been specifically utilized for motor unit number estimation (MUNE). Various single MUPs can be obtained by micro-stimulation of several sites along a nerve. Since single MUPs could be acquired from a four-channel surface electrode array placed over the rat muscles, MFCV was calculated by the propagation of single MUPs on the screen. Consequently, the MFCV was found to decrease significantly in SM rats as compared with that in normal rats. It is also suggested that muscle fibers with a fast MFCV component may be damaged in SM rats, since the number of MFCV values greater than 4.0 m/sec was significantly decreased.

There have been many previous reports about production of SM in rats. The dose of Kenacort A® used in the present research was the same as that mentioned in previous reports.[23,24] The production of experimental SM using the same dose of Kenacort A® was ascertained pathologically in that report. Although histological changes were not examined in this study, generalized wasting away of muscles, the weight reduction and physical debility accord well with SM.[23,25] In feature studies, the relationship between pathological changes and MFCV must be examined.

Nakago *et al* reported that there were serial changes in the distribution of muscle fiber types of the vastus intermedius in rats treated with a steroid.[25] Muscle atrophy occurred predominantly in type 2A fibers after 10 days of steroid injection. After 20 days of steroid injection, type 1 fibers also became atrophied. Although the soleus is known to be mainly composed of type 1 fibers, some type 2A fibers exist in the muscle. Type 2A fibers may also become atrophied preferentially in the soleus, followed by atrophy of type 1 fibers. The slow MFCV observed in the present study may have been based on type 2A atrophy in the soleus.

To estimate the number of motor units in some specified muscles in humans, F-waves are sometimes also used along with the MPS technique. F-waves evoked by small stimulus intensity are believed to reflect single motor unit activity.[26,27] We have already reported that MFCV in a single motor unit could be measured by recording F-waves evoked by weak stimulation in humans, and that the obtained MFCV values were similar to previously reported values.[28] However, it is difficult to record F-waves

stably in rats, and recording of F-waves evoked by the MPS technique is more difficult. Therefore, in the present study, I decided to record directly the single MUPs evoked by the MPS technique to measure the MFCV in a single motor unit. Although the waves evoked by micro-stimulation of several sites along the sciatic nerve were judged as single MUPs by their different waveforms, latencies, and amplitudes, it was not firmly established whether they reflect single motor units. However, there have been no other reports describing the characteristics of single motor units evoked by the MPS technique in rats. Since the waves I recorded in this study had a smaller amplitude and shorter duration than the previously reported waves obtained by direct stimulation of the muscle, it seems that these waves reflect at least smaller numbers of motor units. Further studies are required to investigate the characteristics of single motor units in rats to clarify whether the waves evoked by the MPS technique reflect single motor units.

In conclusion, MFCV in a single motor unit could be measured in rats by recording single MUPs evoked by the MPS technique. This is the first report of use of the MPS technique to measure MFCV values directly. Several single MUPs with different waveforms, latencies, and amplitudes could be recorded by the MPS technique. We expect that this technique will also become widely used clinically. Further studies are required to investigate whether or not the MFCV recorded by the present technique imply slow components.

The mean MFCV in the SM rats was found to decrease significantly compared with that in the normal rats. In addition, the number of MFCV values greater than 4.0 m/sec significantly decreased in the rats with SM, while the lowest values were the same as those in the normal rats. These results are in excellent agreement with the observation that type 2A fibers with the fast component of MFCV atrophied in the SM rats. Future studies are needed to clarify the mechanisms of the decrease in MFCV in myopathy.

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