Objective Measurements of Upper and Lower Motor Neuron Loss in Amyotrophic Lateral Sclerosis

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One of the major problems in treating patients with amyotrophic lateral sclerosis is how to measure the therapeutic effects

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive disease with an average surviving time of 3 to 5 years[1,2]. The hallmarks of the pathological changes are loss of lower motor neurons in the spinal cord and upper motor neurons(UMN) in the motor cortex[3]. However, structural MRI studies of the spinal cord and the brain often fail to show any changes. Due to lack of objective and quantitative markers for upper and lower motor neuron losses, all clinical trials use clinical measures to monitor the disease progression and therapeutic effects. However, these clinical measures may not reliably assess the upper and lower motor neuron losses. For example, two clinical trials of Riluzole in ALS used survival time and changes in functional status as the primary efficacy outcomes[4,5]. In other clinical trials, the Tuft Quantitative Neurological Examination was used to measure effectiveness[6]. There is no surrogate marker for diagnosing ALS, monitoring disease progression or checking therapeutic effects. Therefore, physiological and objective methods would be extremely valuable in evaluating the function of lower motor neurons and upper motor neurons in ALS, identifying disease process and monitoring the effects of potential therapeutic agents.

Motor unit number estimate (MUNE) physiologically assesses lower motor neuron loss

Structural MRI studies of the spinal cord have revealed little change at the anterior horn where lower motor neurons are located. However, a physiological motor unit number estimate (MUNE), provides an objective and quantitative way to measure lower motor neuron loss[7,8].

MUNE can potentially play an important role in monitoring the lower motor neuron loss during a clinical trial[7,8]. It provides a direct way to follow the courses of the disease longitudinally. There are five different techniques used in MUNE including the incremental[9], statistical methods[10], spike-triggered average[11], F-wave[12] and multiple-point stimulation[7,8]. Although they differ regarding mechanisms, they all are based on the same principles. First, the maximal amplitude of a muscle response to an electrical stimulation is obtained. Then, the average amplitude of a single motor unit is estimated. The number of motor units is then obtained by dividing the maximal amplitude of the muscle by the average amplitude of a single motor unit. Most techniques yield similar motor unit numbers (about 200-300) in the thenar muscle of normal subjects[7].

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a. The incremental technique

In 1971, McComas et al.[9] described a method for counting motor units. They first obtained a maxim compound motor action potential (CMAP) from extensor digitorum brevis (EDB) by stimulating the deep peroneal nerve at the ankle. Then, they started from subthreshold stimulation levels and gradually increased stimulus intensity until a quantal response was seen. The quantal response represented the first motor unit activated. They further increased stimulus intensity to record more quantal increases. They recorded up to 11 discrete increments with each increment represent the addition of one more motor unit. The estimated number of motor units was obtained by dividing the amplitude of the maximum CMAP by the average quantal responses. Using the incremental MUNE technique, researchers found that the motor unit counts of the median, ulnar and radial-innervated muscles in young subjects averaged between 250 and 420[13,14,15].

b. Multiple point stimulation

In this technique, the nerve is stimulated at very low intensities to elicit a quantal response[16]. The stimulating electrode is then moved along the nerve and the process repeated. For median and ulnar innervated intrinsic hand muscles, up to 20 morphologically distinct units can usually be recorded. From this sample, mean amplitude is calculated, and this value is divided into the maximum response to yield the MUNE.

c. F-wave technique

This technique is based on the fact that when motor nerves are stimulated, a small minority (approximately 2%) of neurons will generate a recurrent response, or F wave[17]. Submaximal stimulation increases the probability of the F wave being composed of a single unit response[12]. Usually more than 10 such responses can be identified after 300 stimuli. These responses are averaged to yield an average single motor unit response, which is then divided into the maximum CMAP to give the MUNE.

d. Spike-triggered averaging

In this technique, the subject voluntarily activates the muscle of interest at a low level[11]. The response to a single motor unit is recorded with either a concentric or single fiber EMG needle, and the electrode is positioned so that only that single motor unit response is recorded and surface potentials are recorded simultaneously. The single motor unit spike triggers to time lock the surface recorded potentials to obtain an average surface response. By altering needle position to record from different motor units, 10 to 20 surface motor unit potentials can be generated, and average motor unit amplitude calculated. MUNE is obtained by dividing the average single unit response into the maximum CMAP.

e. Statistical method

In this method[10], the entire response range of a muscle is first evaluated by applying threshold to supramaximal stimuli. A few response ranges are chosen from the stimulus-response curve, and a constant intensity stimulus producing a response within a given range is presented repeatedly. There will be a population of motor units with thresholds near the stimulus intensity. These units will fire variably with each stimulus, resulting in a response with a variability that can be calculated. Under certain assumptions, this variability directly estimates the size of the variably firing motor units. By investigating 3-4 different stimulus intensities, estimates of the motor unit size at each intensity are obtained and averaged to produce a single motor unit potential amplitude. This amplitude can be divided into the maximum CMAP response to yield the MUNE.

Each of the above five techniques has its advantages and disadvantages[7,8]. However, these different techniques yield similar motor unit numbers (about 200-300) in the thenar muscle of normal subjects[7,8].

Researchers have used these different MUNE techniques to study ALS extensively. Using the multiple point stimulation method, Carleton and Brown[18] found that MUNE was reduced by more than 80% in the thenar and hypothenar muscles of ALS patients, with motor unit sizes increased up to 600%. Because of the compensatory increase of motor unit size, compound muscle action potential amplitudes did not decrease until MUNE dropped below 10% of normal values.

MUNE is also useful in following the rates of motor unit dropout in ALS patients. Using repeated incremental studies, Dantes and McComas[19] showed that motor

unit dropout occurred more rapidly early in the disease. Using multiple point stimulation technique, Arasaki and Tamaki[20] also demonstrated that motor unit numbers dropped by 70% during the first year after the diagnosis.

MUNE is more sensitive than any other measure in detecting the progression of ALS in clinical trials

Most clinical trials to this date have used quantitative muscle testing or manual muscle testing to measure the force and monitor the progression of the disease[21]. However, Bromberg et al.[22] found that isometric strength in elbow flexors and biceps did not correlate with MUNE in these muscles. Although motor unit dropped out rapidly during the first year of the disease[20], muscle strength in ALS declined almost linearly during most of the course of the disease[23]. The most likely explanation is that the early rapid decline in motor unit numbers is compensated by an increase in force generated by the remaining motor units (reinnervation and increased firing rates).

MUNE, therefore, has obvious advantages over other commonly used measures in evaluating disease progression and monitoring therapeutic effects in clinical trials. It can document the motor unit loss in the minimally affected muscles. Two studies[24,25] have demonstrated that MUNE is more sensitive in measuring disease progression than other commonly used measures such as compound motor action potential amplitude, handgrip, strength testing and vital capacity.

Transcranial magnetic stimulation can assess upper motor neuron loss by measuring cortical muscle representation areas in the motor cortex

MRI studies fail to reveal any changes in the motor cortex where upper motor neurons are located. A new physiological technique, transcranial magnetic stimulation (TMS), provides an objective and quantitative method to assess the function of upper motor neurons.

"Cortical muscle representation area" refers to an area on the motor cortex occupied by all of the upper motor neurons that innervate a specific muscle. Upper motor neurons that innervate a single muscle are organized into small clusters in the motor cortex. These clusters are spatially separated into multiple foci[26].

Researchers have used TMS to perform cortical mapping to investigate the cortical representation of muscles in humans and demonstrated that cortical muscle representation areas in the motor cortex are plastic, not fixed. In cortical mapping using TMS, the MEP amplitude is plotted as a function of the position of the stimulation coil on the scalp. Using this technique, Cohen et al.[27] showed that in amputees, cortical muscle representation areas for muscles ipsilateral to the stump are larger than for the muscles contralateral to the stump, suggesting that cortical motor neurons controlling the muscles distal to the stump are redirected to control those proximal to the stump. Pascual-Leone et al.[28] demonstrated that in proficient Braille readers, the first dorsal interosseous muscle has larger presentation area than the blind controls. These findings indicate that motor neuronal reorganization takes place at the cortical level and that mapping technique using transcranial magnetic stimulation can detect these changes.

Cortical muscle representation areas reduce as ALS progresses

Carvalho et al.[29] performed cortical mapping using transcranial magnetic stimulation about once every three month in 11 ALS patients for about a year. Every patient was taking Riluzole. They found that the cortical representing area of the abductor digiti minimi reduced by 25% over a year. They also calculated the map volume by multiplying the area of each grid element by the MEP amplitude at that point and summing all grid elements. They found that the map volume reduced by 47%. However, other TMS parameters, including central motor conduction time and motor threshold, remained unchanged. These findings suggest that cortical mapping using transcranial magnetic stimulation is a sensitive technique in detecting UMN loss in ALS patients and can potentially serve as a surrogate marker to monitor the disease progression and checking the therapeutic effects in clinical trials. Unfortunately, they[29] did not study the changes of the cortical muscle representation in ALS patients who were not on Riluzole.

Interneurons in the motor cortex are affected in ALS

In addition to UMN, cortical interneurons are also affected in ALS. Nihei et al.[30] examined patterns of neuronal degeneration in the motor cortex of ALS patients using non-phosphorylated neurofilament immunoreactivity as histochemical marker for UMN and NADPH-diaphorase and paraalbumin as markers for cortical interneurons. They demonstrated that both UMN and cortical interneurons are affected in ALS patients. However, this histological study did not determine if degeneration of UMN or interneurons is the initiating event. The exact mechanisms by which cortical interneurons are affected remain unknown.

Paired-pulse TMS is useful in evaluating the dysfunction of cortical interneurons in ALS

The function of the cortical interneurons can be assessed using a paired-pulse TMS paradigm. In this paradigm[31], two pulses are given through the same coil. The first, or conditioning, stimulus is set to a subthreshold intensity and evokes no EMG responses in muscles at rest. The second, or test, stimulus is suprathreshold and evokes an EMG response at rest. With intervals of 1 to 6 ms between the stimuli, the test responses are slightly suppressed by the presence of the conditioning pulse. Because the conditioning stimulus produces no responses, inhibition is thought to be due to the inhibitory activity of the interneurons in the motor cortex.

Using a paired conditioning-test TMS paradigm, Ziemann et al.[32] found that intracortical inhibition in ALS patients is less than in an aged-matched control group. They[32] suggested that that selective abnormality of intracortical inhibition in ALS patients is compatible with an impaired function of inhibitory interneurons.

Riluzole partially corrects the dysfunction of the cortical interneurons in ALS

Using paired-pulse TMS paradigm, Stefan et al.[33] have shown that Riluzole partially corrected the impaired intracortical inhibition in ALS patients. They tested 13 ALS patients at the baseline, and then over one year at 3-month intervals after Riluzole therapy was started. All of these patients had disease duration of less than one year. They showed that at baseline, the intracortical inhibition was reduced in ALS patients as compared with normal controls. Thus, Riluzole partially normalized the intracortical inhibition. In two patients treated with Riluzole, the initially normal intracortical inhibition decreased after five months. An increase of Riluzole dose from 100 mg per day to 150 mg increased and normalized intracortical inhibition. Their study, therefore, shows that the dysfunction of interneurons is reversible, at least in the early stage of ALS[33]. Furthermore, paired-pulse transcranial magnetic stimulation is useful in assessing the intracortical inhibition.

Riluzole interacts with glutamate-mediated neurotransmission at multiple pre-and post-synaptic sites[34]. It is the only drug currently approved by FDA for treating ALS patients.The antiglutaminergic property of Riluzole therefore removes excessive glutaminergic excitation of the upper motor neurons. However, two important questions remain unanswered: first, we do not know if the dysfunctions become irreversible as ALS progresses; second, we do not know if antiglutaminergic properties of Riluzole will slow down the progression of the disease by reducing the rate of the loss of UMN. Longitudinal studies using transcranial magnetic stimulation to assess intracortical inhibition will shed some lights on these questions.

FUTURE DIRECTIONS

This is a very exciting time for clinicians and researchers working in the area of amyotrophic lateral sclerosis. With rapid progress in the basic research, many hypotheses about the pathogenesis of amyotrophic lateral sclerosis have been proposed[1]. Consequently, many potential agents have been proven to slow down the disease progression in animal models for amyotrophic lateral sclerosis. For example, there is an increasing evidence that inflammation may play a role in ALS. There is upregulation of cyclo-oxygenase 2 in the SOD1 mouse model and inhibitors of COX-2 prolonged survival in the mouse model. Yasojima et al.[35] demonstrated a seven-fold increase in COX-2 messenger RNA in

the spinal cord of patients with ALS whereas no increase was found in healthy controls or patients with Alzheimer's disease, Parkinson's disease or stroke. A microglial marker was increased two-fold, again suggesting activation of inflammatory pathways. Human trials using COX-2 inhibitors such as minocycline or rofecoxib are underway or being planned. Objective methods to measure upper and lower motor neuron loss, such as MUNE or cortical mapping, can potentially play crucial roles in these therapeutic trials. They are potential surrogate markers for diagnosing amyotrophic lateral sclerosis, monitoring disease progression and checking therapeutic effects.

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