Motor Neuron Involvement in a Patient with Long-term Corticosteroid Administration

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Abstract

An asthmatic patient with corticosteroid treatment for 45 years presented with slowly progressive limb muscle atrophy. His muscle symptoms were involved in four limbs and tongue, and deep tendon reflexes were exaggerated. Biopsied muscle pathology indicated the presence of neurogenic muscular atrophy in combination with corticosteroid myopathy. Furthermore, 8-hydroxy-deoxyguanosine (8-OH-dG) was prominently increased in mitochondrial and nuclear DNA. An aerobic exercise test demonstrated remarkable serum lactate elevation, which was attenuated by the administration of coenzyme Q10. These findings are consistent with the assumption that long-term corticosteroid administration potentially induces not only myopathy but also motor neuron involvement as in mitochondrial diseases. (Internal Medicine 42: 862-866, 2003)

Key words: asthma, corticosteroid myopathy, denervation, neurogenic muscular atrophy, mitochondria

Introduction

Corticosteroid myopathy is a well-known side effect of long-term corticosteroid administration. Although the cause is not precisely known, it appears that the major action of corticosteroid is to induce muscle protein catabolism. This effect is prominent in type 2B (fast-twitch) fibers, resulting in the fiber atrophy (1). It has been generally accepted that corticosteroid does not interfere with the neuronal system. However, there are a few reports discordant with such a notion; fibrillation motor unit potentials (1) and delayed nerve conduction velocities (2) are observed under corticosteroid treatment. Following the large-dose corticosteroid treatment in status asthmatics, acute muscle symptoms often occur involving asymmetric paresis of varying degrees of one or more limbs, occasionally with tetraplegia (3–5). Because many patients developed these symptoms after receiving mechanical ventilation, several contributory factors other than corticosteroid itself have also been considered, such as muscle relaxants or consequent immobilization (3–5). However, because some patients develop these symptoms without mechanical ventilation (4, 5), corticosteroid-induced muscular damage should be considered at least in those patients. Although EMG usually reveals myopathic changes, some of them exhibit neurogenic muscular atrophies called asthmatic amyotrophy (5). Those previous findings suggest that corticosteroid administration potentially damages the motor neuronal system. However, the pathophysiological mechanism remains to be elucidated.

In the present study, we report an asthmatic patient administered with corticosteroid for 45 years, who presented with upper and lower motor neuron symptoms, compatible with amyotrophic lateral sclerosis. His biopsied muscle demonstrated neurogenic muscular atrophy in addition to the findings of corticosteroid myopathy. Furthermore, the biopsied specimen revealed remarkable oxidative damage to mitochondrial DNA.

Case Report

A 65-year old Japanese man with a history of asthma since early childhood presented in September 2000 with progressive weakness in both legs. There was no family history of neuromuscular or respiratory diseases. His asthma had been successfully controlled with betamethazone (0.25–0.5 mg/day) since the age of 20. In 1984, an elevation of serum creatine kinase was found by his local medical practitioner. In 1998, he complained of difficulty climbing stairs and get-
Motor Neuron Damage in Steroid Therapy

Figure 1. Representative profile of needle electromyography recorded in biceps brachii. Renervation potentials were detected in all muscles examined.

nting out of chairs. Neurologic examination revealed mild tongue atrophy without fasciculation. Bilateral muscle wasting and weakness were evident in four extremities, particularly in arms and thighs. Deep tendon reflex, including jaw jerk were moderately exaggerated, but there was no pathologic reflexes including Babinski, Chaddock and Marie-Foix signs. Sensation was normal. Laboratory findings were normal except for mild elevation of serum concentration of creatine kinase (624 IU/L, normal 36-175) and percent creatinuria (15, normal <6). Nerve conduction studies were normal in four extremities. Electromyography revealed chronic neurogenic changes in masseter and bilateral limb muscles including biceps brachii, triceps, interossei, quadriceps, and tibialis anterior. Motor unit potentials showed high amplitude (5-8 mV) and long duration (12-20 msec), but not low amplitude and short duration (Fig. 1). Fibrillation potentials and positive sharp waves were not detected. Brain and neck MRI were normal. A left quadriceps biopsy was performed.

Materials and Methods

Materials
The muscle specimen was quick frozen and stored in liquid nitrogen until use. A small portion of each specimen were fixed with 2.5% glutaraldehyde and processed for electron microscopy. Cryosections (6 μm) were stained with hematoxylin-eosin, modified Gomori’s trichrome, cytochrome c oxidase, NADH-TR, myosin ATPase (pH 4.6), and PAS, according to routine procedures.

Aerobic exercise test
An aerobic exercise test was performed with a bicycle ergometer at 15 watts for 15 minutes (6). Serum lactate and pyruvate concentrations were assayed at 0, 5, 10, 15, 20, and 30 minutes after the exercise. At the exercise test, his asthma was well controlled and arterial blood gas analyses confirmed the aerobic state. Normal range was defined as mean ±SD of 32 controls (20 normal controls and 12 disease controls including bronchial asthma and various neuromuscular disorders).

High-performance liquid chromatography with electrochemical detection (HPLC-EC)
After isolating mitochondria and nuclei from muscle samples (10), each DNA was isolated and enzymatically hydrolyzed. 8-OH-dG and deoxy-guanosine (dG) were assayed by HPLC-EC, as reported elsewhere (7-9).

Mitochondrial enzymatic assays
Individual respiratory chain complexes (I–IV) were assayed in mitochondrial fractions of biopsied skeletal muscles, as reported elsewhere (8–10). 50–100 μg mitochondrial protein was required for each assay, and all reactions were performed at 25°C. Normal values were obtained from 11 control subjects at joint surgery (aged from 25 to 80 years, mean 43.3 years).

Mitochondrial DNA (mtDNA) analyses
We analyzed mtDNA deletion with Southern blotting and PCR as reported elsewhere (11). For Southern blotting, we used a probe for human mtDNA (nt 3153–3551). For PCR analysis, we designed a pair of primers to detect common mtDNA deletions; 5’-GTACTGAACTACGAGTACAC-3’ (nt.7897–nt.7917) and 5’-ATTCGAGTCCTATAGGCGCTT-3’ (nt.13593–nt.13613). The PCR was performed according to the previous reports with minor modifications (11). Each PCR product was subcloned and sequenced. A positive control sample was obtained from a patient with chronic progressive external ophthalmoplegia (CPEO; 9).

Results

Histologic and electron microscopic findings
The muscle specimens revealed small group atrophy, angulated fibers (Fig. 2A), and lobulated fibers (Fig. 2B, asterisks). Myosin ATPase staining revealed that the atrophic fibers were preferentially type 2B fibers (Fig. 2C, arrows). Glycogen was accumulated in subsarcolemmal regions of lobulated fibers, and in some of these atrophic fibers (Fig. 2D, arrows). Ragged-red fibers were not observed in modified Gomori-Trichrome staining. The lobulated fibers, found in type 1 fibers, had many proliferative mitochondria on electron microscopy (Fig. 2E), but these mitochondria showed preserved structures.

Aerobic exercise on bicycle ergometer
The aerobic exercise test demonstrated abnormal serum lactate elevation (Fig. 3). Administration with Coenzyme Q10 (60 and 120 mg/day) for 4 weeks reduced the serum lactate levels.

HPLC-EC analysis of 8-OH-dG
We previously reported that mitochondrial damage is
Figure 2. Muscle specimen from a quadriceps femoris specimen of the patient with long-term corticosteroid administration. A. Small group atrophy and fatty invasion are seen. HE stain, ×180. B. Small group atrophy and lobulated fibers (asterisks) are seen. NADH-tetrazolium reductase stain, ×180. C. Preferential atrophy of type 2B fibers (arrows). Myosin ATPase stain, ×180. D. Preferential accumulation of glycogen in type 2 fibers (arrows). PAS stain, ×180. E. Proliferative mitochondria with a normal structure are seen in an electron micrograph. Bar=1 μm.

associated with a marked increase in 8-OH-dG in skeletal muscles from patients with chronic corticosteroid administration as well as mitochondrial encephalomyopathies (7–9). The molar ratios of 8-OH-dG/dG in mitochondrial DNA and nuclear DNA were prominently elevated to 14.2% and 7.6%, respectively, since we previously reported that the ratios in normal subjects were 0.211±0.071 (mean±SD) % and 0.121±0.068% in mitochondrial DNA and nuclear DNA, respectively (8).

**Mitochondrial enzymatic analysis**

The enzymatic activities of respiratory chain were as follows; complex I 104.9 nmol/min/mg of mitochondrial protein (normal 76.5±39.3, mean±SD), complex II 43.7 nmol/min/mg (55.7±16.7), complex III 9.1 nmol/min/mg (20.9±9.0), complex IV 26.1 nmol/min/mg (34.3±11.3). The complex III activity was mildly decreased.

**Mitochondrial DNA analysis**

PCR analysis demonstrated an extraband at 739 bp, corresponding to the long mtDNA deletion, as in a case with CPEO (data not shown). However, no such deletions were detected in all 10 samples from age-matched control subjects under the present PCR conditions. The deleted mtDNAs were not detected by Southern blotting (data not shown).

**Discussion**

In the present case, signs of lower motor neuron degeneration were evident by clinical, electrophysiological and pathological examination. Hyperreflexia was observed in four extremities, however, upper motor neuron signs were equivocal since there were not pathologic reflexes. Therefore, this case could not be diagnosed as definite amyotrophic lateral sclerosis (ALS) according to the ALS criteria (12). Furthermore, the present patient showed slowly progressive muscular atrophy during the course of corticosteroid administration for 45 years. In fact, clinical disability has not been aggravated for the last two years. The progression of clinical symptoms is slow compared to typical ALS (13). The muscle biopsy demonstrated type 2B fiber atrophy, and preferential accumulation of glycogen in type 2 fibers, compatible with corticosteroid myopathy. The type 2
fibers are markedly atrophied and occasionally grouped, indicating the presence of neurogenic fiber atrophy. These pathologic findings are suggestive of a combination of corticosteroid myopathy and neurogenic atrophy.

The present study revealed mitochondrial dysfunction in skeletal muscle by enzyme assays of respiratory chain and aerobic exercise test despite an increase in the number of mitochondria in lobulated fibers. PCR analysis demonstrated a long mitochondrial DNA deletion. In addition, coenzyme Q<sub>10</sub> treatment ameliorated the impaired exercise tolerance. These mitochondrial changes are reminiscent of mitochondrial encephalomyopathies, however, the mitochondrial changes are not specific to mitochondrial encephalomyopathies but are observed in skeletal muscles of other neuromuscular disorders and even normal aging (14). In the case of skeletal muscle under corticosteroid treatment, reduced mitochondrial functions and morphological alterations such as enlargement, aggregation, and ragged-red fibers have been reported (15–17). Recently, we reported that chronic corticosteroid administration significantly induced mitochondrial dysfunction associated with oxidative damage in skeletal muscle (8). The molar ratio of 8-OH-dG/dG was prominently increased in corticosteroid-treated skeletal muscles and this increase was similar to that observed in skeletal muscles of patients with mitochondrial diseases (7). Furthermore, we found two patients with long-term corticosteroid therapy, who exhibited ocularskeletal symptoms similar to those in a mitochondrial disease, chronic progressive external ophthalmoplegia (9). These findings suggest that long-term corticosteroid administration potentially induces oxidative stress-mediated mitochondrial damage in skeletal muscles as observed in mitochondrial diseases.

In patients with mitochondrial diseases, oxidative damage seems to be closely related to mitochondrial dysfunction and it plays an important role in the development of muscular damage (7). Recently, we reported that mitochondrial diseases harbor unique apoptosis-related changes with overproduction of reactive oxygen species (18). It is well known that mitochondrial abnormalities exist not only in skeletal muscle but also in various organs including the nervous system, eye, heart, and endocrine system; these are called mitochondrial cytopathies. Particularly, neurogenic muscle atrophy and peripheral neuropathy are often observed and seem to result from mitochondrial dysfunction in the corresponding motor neurons (19, 20). On the other hand, morphological (21), functional (22) and genetic (23) abnormalities have been reported in mitochondria of motor neurons from patients with amyotrophic lateral sclerosis. We could not rule out the possibility that atypical ALS independently occurred in this patient. However, the present observations as well as those previous reports suggest that long-term corticosteroid administration potentially induces mitochondrial dysfunction not only in skeletal muscle but also in motor neurons, resulting in myopathy and denervated muscle atrophy in some patients.

References


MITSUI et al

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