

Mitochondrial dysfunction in Kennedy's disease: a new pharmacological target?

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Background: Mitochondrial impairment and elevated oxidative stress have been implicated in the pathogenesis of Kennedy's disease. However, there is still no study describing the mitochondrial nutrient management in patients with Kennedy's disease.

Methods: We assessed the clinical and electrophysiological features in a patient with Kennedy's disease. This patient was diagnosed by genetic analysis. We also measured the plasma 8-hydroxydeoxyguanosine (8-OHdG) levels of the patient and his family members using commercial enzyme-linked immunosorbent assay (ELISA). Treatment with intravenous L-carnitine (2 g/day) for the patient was started on admission, followed by two weeks.

Results: Routine laboratory tests revealed a severe elevation of creatine kinase (CK) (606.5 U/L; normal value: 15-170 U/L). Sequencing of the first exon of androgen receptor (AR) revealed an increased number of CAG repeat (50; the normal range from 10-36) in the patient. Plasma 8-OHdG level in the patient was relatively elevated (34.68±1.01 ng/mL) compared with the female carriers and non-carriers. Two weeks after L-carnitine treatment, we observed a reduction of approximately 40% in CK level (391 U/L) in the patient.

Conclusions: Oxidative stress resulting from mitochondrial dysfunction could be involved in Kennedy's disease. Targeting the mitochondrial dysfunction in Kennedy's disease may have significant therapeutic potential.

Keywords: Kennedy's disease; spinal and bulbar muscle atrophy; mitochondrial dysfunction; oxidative stress

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Introduction

Kennedy's disease, also known as spinal and bulbar muscular atrophy (SBMA), is a rare inherited lower motor neuron disease characterized by bulbar and proximal limb muscular atrophy. It is caused by a CAG-repeat expansion, encoding a polyglutamine tract, in the first exon of the androgen receptor (AR) gene on the X-chromosome (1). The number of CAG repeat range from 10 to 36 in normal population, while in Kennedy's disease the expanded repeat ranges from 38 to 62 (2). To date, the abnormal expansion of CAG repeat has been identified to cause nine neurodegenerative diseases including SBMA, Huntington's disease (HD),

dentatorubral-pallidolusian atrophy (DRPLA) and six forms of spinocerebellar ataxia (3). Although the causative gene varies with each disease, these polyglutamine disorders share common pathways of molecular pathogenesis, such as accumulation of abnormal proteins, transcriptional dysregulation and disruption of axonal transport (3,4). Additionally, several lines of evidence suggest that mitochondrial impairment and elevated oxidative stress are also implicated in the pathogenesis of Kennedy's disease (5,6). As we know, progression of Kennedy's disease is slow and the management focuses on preventing complications of the disease. Therefore, targeting mitochondrial dysfunction in Kennedy's disease may have significant therapeutic potential.

Here, we assessed oxidative stress status in a 51-year-old case who diagnosed with Kennedy's disease. In particular, we investigated whether the treatment of mitochondrial nutrient (L-carnitine) could improve the disease progression.

Materials and methods

Patients

In our study, a 51-year-old man presented to our outpatient clinic with a 10-year history of weakness of proximal lower limbs, as well as with mild swallowing difficulty. His weakness gradually increased and then involved upper limbs about 5-year ago. He also noticed some hand tremor and fasciculation in his bilateral face. His cousin was diagnosed with "Kennedy's disease" 2-year ago. For genetic analysis, we also recruited eight family members from this pedigree, including three males (III-2; III-4; IV-3) and five females (II-1; II-2; III-3; IV-1; IV-2), across three generations (*Figure 1A*). All subjects gave informed consent for participation in the investigation. A detailed medical history of the patient was obtained and medical documentation was confirmed. Subsequently, the patient underwent standardized neurologic examination and biologic tests, including serum creatine kinase (CK), cholesterol and hormones level. Electromyography (EMG) was also performed on one distal and one proximal muscle of at least one lower and upper.

Treatment with intravenous L-carnitine (2 g/day) for the patient was started on admission. To assess efficacy, detailed neurologic examination and routine laboratory tests were checked at 2 weeks.

Genetic analysis and plasma 8-hydroxydeoxyguanosine (8-OHdG) measurement

Genomic DNA was acquired from peripheral blood cells of the patient and other family members using conventional techniques. Polymerase chain reaction (PCR) amplification of CAG repeats in exon 1 of the AR gene was performed using a forward primer (5'-TCCAGAACTGTTCAGAGCGTGC-3') and a reverse primer (5'-TGGCCTC GCTCAGGATGTCTTTAAG-3'). The detailed PCR conditions and measurement of the CAG repeat have been previously described elsewhere (1).

Blood samples were collected at our outpatient clinic with fasting. Plasma was separated by centrifugation

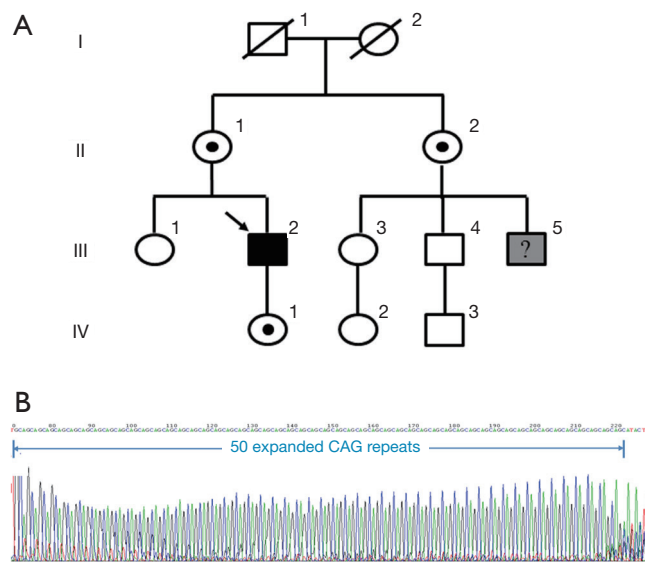


Figure 1 (A) Pedigree of the family. Black square indicates the patient (arrow); black dot indicates the female carrier; the cousin of patient shown with grey color is suspected of having Kennedy's disease; (B) DNA sequencing of the patient reveals 50 CAG repeats in the first exon of the androgen receptor gene.

at 3,000 rpm for 5 min and kept frozen at -80°C until assayed for 8-OHdG. The plasma 8-OHdG levels of each member was measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Santa Cruz Biotechnology, Inc. Dallas, Texas, USA) according to the manufacturer's protocol. All samples were assayed in triplicate.

Results

Neurologic examination revealed a mildly nasal quality and tongue atrophy. Proximal muscles of the upper and lower limbs were moderately atrophic. Muscle strength as measured with manual muscle tests of the left upper limb was grade 4⁺/5, and those of proximal lower limb was grade 4⁺/5. Sex hormones, thyroid function, rheumatologic antibodies, routine blood and serum biochemistries were normal except for a severe elevation of CK (606.5 U/L; normal value: 15-170 U/L). EMG revealed high amplitude potential, reduced interference and prolonged duration suggesting neurogenic damages.

The CAG repeat number was assessed in the patient as well as eight relatives within the pedigree. Sequencing of the first exon of AR revealed an increased number of CAG repeat

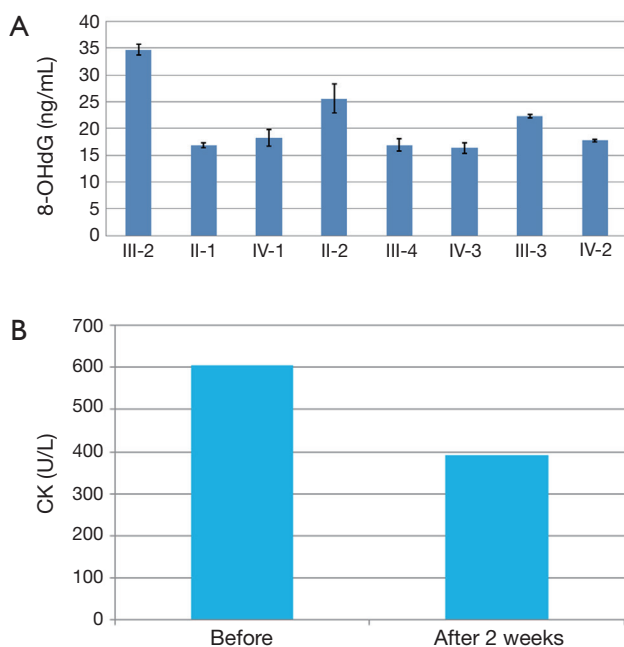


Figure 2 (A) Oxidative stress status was measured by the plasma levels of 8-OHdG in this pedigree (three times for each sample), with the patient (III-2) showing relatively elevated 8-OHdG level than other family members; (B) changes in the creatine kinase (CK) levels before, after 2 weeks treatment. 8-OHdG, 8-hydroxydeoxyguanosine.

(50; the normal range from 10-36) in the case (Figure 1B). We also detected three heterozygous female carriers in this family (II-1; II-2; IV-1) (Figure 1A). Plasma 8-OHdG level in the patient was relatively elevated (34.68 ± 1.01 ng/mL) compared with the female carriers and non-carriers (Figure 2A).

Two weeks after L-carnitine treatment, subjective feeling of muscle weakness was ameliorated to a significant extent. But we observed no prominent recovery of muscle strength of limbs. Follow-up biochemistries test revealed a reduction of approximately 40% in CK level (391 U/L) (Figure 2B).

Discussion

Kennedy's disease, caused by the expansion of CAG repeat within the AR gene, has been found to be involved in a number of complex and not necessarily independent pathogenesis (4). It is now widely accepted that accumulation of polyglutamine-expanded AR is central to the pathogenesis of Kennedy's disease. Both the structure of the polyglutamine-expanded AR and its interactions with other proteins are altered relative to the normal AR. The major histopathological hallmark of Kennedy's disease is

loss of lower motor neurons in the spinal cord as well as in the brainstem motor nuclei. Androgen-dependent nuclear accumulation of the pathogenic polyglutamine-expanded AR appears to play a significant role in the neuronal dysfunction and eventual neuronal death (7). Except for nuclear accumulation, polyglutamine-expanded AR is shown to induce the depolarization of the mitochondrial membrane directly through abnormal associations with mitochondria. It also suppresses the transcription of mitochondrial proteins indirectly through alterations in the expression of gene that regulate mitochondrial biogenesis (6). These likely contribute to mitochondrial dysfunction and resulted in increased reactive oxygen species (ROS) generation. 8-OHdG, produced by the reaction between ROS and guanine residues in DNA, is considered to be an indicator of oxidative stress and mitochondrial dysfunction. Increased plasma 8-OHdG level in the case may directly result from the mitochondrial dysfunction-elicited oxidative stress, further raised the awareness of perturbed mitochondrial function in the pathogenesis of Kennedy's disease. Meanwhile, oxidative stress exacerbates the mitochondrial function and restricts ATP production, eventually contributing to neuronal death.

L-carnitine is an important mitochondrial nutrient which acts as a carrier for long chain fatty acids across the mitochondrial membrane. It is necessary for the normal mitochondrial oxidation of fatty acids and subsequent ATP production. Besides, L-carnitine also exerts an effective antioxidant role and may protect tissues against oxidative damage in mitochondrial disorders (8). L-carnitine transports across the blood-brain barrier by an organic cation/carnitine transporter (OCTN2) and accumulates in neurons (9). Thus L-carnitine supplementation may enhance the mitochondrial function of neurons, by the activation of mitochondrial biogenesis and the amelioration of oxidative stress damage. The observed reduction in CK level may present effective treatment of L-carnitine in Kennedy's disease.

Conclusions

To date, no therapy has proved to be effective on patients with Kennedy's disease in clinical trials with hormonal therapy showing only limited improvement in certain disease features (10). Consequently, the mitochondrion-specific antioxidant property of L-carnitine possibly represents a new approach for targeting the rescue of mitochondrial dysfunction in the treatment of Kennedy's disease.

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Disclosure: The authors declare no conflict of interest.

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