Mitochondrial myopathies caused by prolonged use of telbivudine

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Background: Telbivudine is a nucleoside analogue used for the treatment of chronic hepatitis B, but it often develops mitochondrial toxicity leading to symptomatic myopathy. In this study, three patients with telbivudine induced myopathy were enrolled in order to investigate the nature and pathogenesis of mitochondrial toxicity caused by long-term use of telbivudine.

Methods: Clinical features, laboratory findings, muscle pathology, and quantitation of mitochondrial DNA were studied in three patients.

Results: Patients presented with progressive muscle weakness with high serum creatine kinase levels. Light microscopic findings of muscle pathology showed ragged red fibers that reacted strongly with succinate dehydrogenase stain, but negative for cytochrome c oxidase activities. Electron microscopy revealed abnormal mitochondrial accumulation with rod shaped inclusions. The quantitative peroxidase chain reaction showed a depletion of mitochondrial DNA in skeletal muscle of the patients.

Conclusions: Nucleoside analogues including telbivudine are potent inhibitors of viral DNA polymerases. However, they are not specific for viral DNA and can disturb mitochondrial replication at the same time. All nucleotide analogues should be used with close clinical observation in order to avoid development of mitochondrial myopathy.

Key words: Telbivudine; DNA polymerase gamma; Creatine kinase
INTRODUCTION

Hepatitis B virus (HBV) is a widely recognized health problem. Up to 400 million people are affected by HBV globally, and East Asia is an HBV endemic area.1 It is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma.2 The discovery of oral nucleoside/nucleotide analogues was a milestone in the development of chronic hepatitis B (CHB) therapy. They show high rates of clearance in HBV DNA, resulting in improved long-term outcomes for CHB patients. Recently developed CHB drugs show increasingly less side effects. Telbivudine is superior to lamivudine in both its therapeutic response and resistance.3 However, serum creatine kinase (CK) elevation is a frequently observed side effect in patients treated with telbivudine (12.9%).3 The rate of telbivudine-induced myopathy has been reported to range from 0.3 to 5%.3,4 Previous pathological studies have shown that the myopathy associated with nucleoside/nucleotide analogues is mostly due to the mitochondrial toxicity.5,6 However, previous pathological reports on telbivudine-induced myopathy did not clearly reveal the evidence of mitochondrial toxicity.7,8 Here, we showed that the myopathy induced by prolonged use of telbivudine is a mitochondrial myopathy caused by disturbed replication of mitochondrial DNA.

MATERIALS AND METHODS

Subjects

Written informed consent was obtained from all the patients examined for genomic DNA analysis, and this study was approved by ethical review board of Pusan National University Yangsan Hospital (IRB No. 05-2015-086).

Three patients who developed progressive muscle weakness during long-term telbivudine therapy for hepatitis B were included. Clinical data were obtained from their medical records. The information collected from each patient included age at onset, gender, daily dosage and duration of telbivudine therapy before symptom onset, interval between symptom onset and evaluation, and distribution and degree of weakness. Laboratory tests, including serum CK, aspartate aminotransferase, alanine aminotransferase, chest roentgenogram, and electrocardiogram were performed before evaluation and after discontinuation of telbivudine. The nerve conduction studies were performed with standard techniques and electromyography was performed on at least two proximal and distal muscles from the upper and lower extremities.

Muscle biopsy

Muscle biopsy was performed on right tibialis anterior muscle in two and right biceps muscle in one patient under local anesthesia. Flash frozen muscle specimens were stained with series of histochemical stains including hematoxylin and eosin, modified Gomori-Trichrome, reduced nicotinamide adenine dinucleotide tetrazolium reductase succinate dehydrogenase, cytochrome c oxidase (COX), and adenosine triphosphatase at varying pH. Muscle specimens were also prepared for electron microscopic observation. They were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer. After shaking with a mixture of 4% osmium tetroxide, 1.5% lanthanum nitrate and 0.2 M s-collidine for 2-3 h, samples were embedded in epoxy resin. Semi-thin sections of 1 μm-thickness were stained with toluidine blue. Ultrathin sections of 50 nm-thickness were stained with uranyl acetate and lead citrate.

Quantitative polymerase chain reaction (Q-PCR)

For quantification of mitochondrial DNA (mtDNA), Q-PCR was performed using DNA from skeletal muscles of normal control and patients. For the quantification of mitochondrial and nuclear DNA, the COXII gene (mitochondrial) and β-actin gene (nuclear) were selected as target and reference genes, respectively. Genomic DNA was isolated from frozen skeletal muscles as previously described.5 Information on the primer sequences for target and reference amplifications, and compositions and reactions of Q-PCR are available on request. After the 50 cycles, a melting curve analysis (55-95°C) was performed to confirm product specificity. After the equal amplification efficiencies of both genes were confirmed, the comparative cycle threshold method was used to quantify mtDNA and nuclear DNA by a comparison of the control and patients.
RESULTS

Clinical and laboratory findings

Patients’ clinical and laboratory data are summarized in Table 1. The first patient was a 48-year-old female diagnosed as CHB and alcoholic liver cirrhosis 3 years before and started to take 600 mg of telbivudine once daily since then. At 8 months after treatment, a 3 cm sized hepatocellular carcinoma without metastasis was identified and liver transplantation was undergone. She was also taking daily 10 mg of rosuvastatin for 12 months and 40 mg of febuxostat for 3 months, both of which are known to cause myopathy.

The second patient was a 51-year-old female who visited us with progressive muscle weakness predominantly on bilateral legs for 6 months. She had a chronic hepatitis B and had been taking telbivudine 600 mg once daily for 24 months. She also had a past history of glomerulonephritis, which had her kidney transplanted 26 years before. She became dependent on hemodialysis 3 years before because of development of renal failure. She also had been taking 4 mg of lacidipine and 5 mg of nebivolol once daily for the treatment of hypertension. In this patient, sensorimotor polyneuropathy was identified in nerve conduction study, which probably is caused by chronic uremia.

The last patient was a 52-year-old female who suffered from proximal dominant bilateral leg weakness for 11 months. She had been administrated telbivudine 600 mg once daily for 22 months to treat CHB. Eleven months after starting medication of telbivudine, bilateral proximal leg weakness developed. She also had been taking lercanidipine 10 mg, valsartan 80 mg, and torsemide 10 mg for the treatment of hypertension. She also had an IgA nephropathy. Medication for CHB was had been changed to tenofovir 300 mg daily 10 days before muscle biopsy.
Muscle pathology
The muscle pathology showed a myopathic changes indicating mitochondrial toxicity in all patients, including necrotic and regenerating fibers, ragged red fibers, and muscle fibers with loss of COX activities (Fig. 1). Otherwise, no other pathological finding such as inflammatory cellular infiltrates or muscle fibers with disrupted intermyofibrillar networks, rimmed vacuoles, or other abnormal structural changes was observed in none of our patients. On electron microscopy, abnormally shaped mitochondria containing rod shaped inclusions were clustered between myofibrils (Fig. 2).

Quantitation of mtDNA
In patients’ skeletal muscle, mtDNA/nuclear DNA ratio was markedly reduced than normal control in all patients (Fig. 3). These findings indicate a depletion of mtDNA in patients’ skeletal muscle.

DISCUSSION
Nucleoside analogs may inhibit viral reverse transcription through two mechanisms: (i) competitive inhibition for reverse transcriptase and (ii) chain termination of elongating DNA. Nucleoside analogs also inhibit DNA polymerase β and γ by mechanisms similar to those observed with viral reverse transcriptase. DNA polymerase γ is highly sensitive to inhibition by nucleoside analogues, which may lead to mtDNA depletion in skeletal muscle. Telbivudine (β-L-2’-deoxythymidine) is the unmodified β-L enantiomer of the naturally occurring nucleoside thymidine, which inhibits HBV
DNA polymerase by competing with the natural substrate, thymidine 5'-tirphosphate especially.

Evidence of mitochondrial dysfunctions has also been reported with nucleoside analogues such as zidovudine, adefovir, and clevudine.\(^6,9,10\) And our study shows that telbivudine is not an exception from antiviral-induced mitochondrial toxicity. This nucleoside analogue-related cellular toxicity is related to decreased mtDNA content (Fig. 2) and altered mitochondrial function probably by inhibiting mtDNA polymerase as shown in our study.

Although there had been a few reported cases with telbivudine myopathy, either muscle biopsy was not performed or pathology did not provide the direct evidence of mitochondrial dysfunction. Kim et al.\(^7\) reported the telbivudine-induced myopathy of siblings, in whom muscle biopsy was not performed. The duration of telbivudine treatment in their patients was relatively shorter than our series - 9 and 13 months, respectively. This suggests that there may be

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**Fig. 2.** Electron microscopic findings of patient 1 (A, B), 2 (C, D), and 3 (E, F) (scale bar, 2 μm). The rod shaped inclusions are observed within mitochondria (arrows, A). The double-membrane vesicle (long arrowheads) encircling abnormal mitochondria is observed, which indicates the autophagic vesicles (B). Abnormal accumulation of mitochondria is observed in degenerated fibers and between myofibrils (C, E). Mitochondria in degenerated fiber is enlarged and abnormally shaped (black short arrowheads, F).

**Fig. 3.** Quantitative PCR analysis showed mtDNA/nuclear DNA ratio was markedly reduced in comparison to the normal control (12.4% of normal control in patient 1, 18.3% in patient 2, and 3.5% in patient 3). PCR, peroxidase chain reaction; mtDNA, mitochondrial DNA.

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the genetic predisposition to develop telbivudine induced myopathy, Wang et al.\textsuperscript{8} reported telbivudine induced myopathy in a patient with chronic hepatitis. They performed the muscle pathology, but failed to reveal the evidence of mitochondrial myopathy, probably due to the mistimed biopsy.\textsuperscript{8} Recently, overexpression of class 1 major histocompatibility complex, CD4 and CD8 without myositis autoantibodies has been described in telbivudine induced myopathy, and suggested that an immune-mediated mechanism might play a role in the development of nucleoside analogue associated myopathy.\textsuperscript{11}

The treatment for myopathies induced by nucleoside analogues includes withdrawal of causative nucleoside analogues or switching to other kind of nucleoside analogues.

New nucleoside analogue such as entecavir had been chosen as the alternative antiviral treatment for the patients who developed nucleoside analogue induced myopathy, which was supported by the study that entecavir showed no mitochondrial toxicity in vitro.\textsuperscript{12} However, entecavir may also develop another form of myopathy. Yuan et al.\textsuperscript{13} reported a patient with entecavir-associated myopathy, in whom the muscle histopathology showed an inflammatory change, but not mitochondrial toxicity. The patient presented with muscle weakness and high serum CK levels, which improved after cessation of entecavir.

In conclusion, prolonged treatment with telbivudine can cause myopathy, which is manifested as progressive muscle weakness with elevation of serum CK level. Pathogenic mechanism is a mitochondrial toxicity which is probably caused by inhibition of DNA polymerase γ that leads to the depletion of mtDNA. Because any kind of anti-viral agents for the treatment of chronic hepatitis B has a potential risk to develop myopathy, careful clinical observation and regular measurement of serum CK is needed in all patients under long-term treatment with nucleoside analogues.

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