

Special Article

Myopathy and neuropathy associated with nucleos(t)ide analog therapy for hepatitis B[☆]

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The development of clevudine as a treatment for hepatitis B was terminated recently because of case reports of myopathy. In each case, the onset of symptoms occurred between 8 and 13 months after the initiation of treatment. Electromyography and muscle biopsy confirmed the presence of myonecrosis. One report also found evidence of mitochondrial toxicity. The delayed onset and the finding of mitochondrial damage are reminiscent of fialuridine toxicity. Telbivudine has also been reported to be associated with myopathy and neuropathy, particularly when used in combination with pegylated interferon. These findings serve as a sober reminder of the lack of data on long-term safety of nucleos(t)ide analogs for hepatitis B, the importance of balancing benefits versus risks before initiating treatment, and the need for more stringent post-marketing surveillance for drug toxicities.

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1. Introduction

On April 20, 2009 Pharmasset Inc. announced that after a discussion with its independent Data Safety Monitoring Board and the Food and Drug Administration (FDA), the company decided to voluntarily terminate its phase III QUASH studies of clevudine for the treatment of chronic hepatitis B virus (HBV) infection [Pharmasset Inc. press release April 20, 2009]. Pharmasset indicated that the company became aware of a number of spontaneous serious adverse event reports and events of special interest in patients receiving clevudine as prescribed therapy for hepatitis B in South Korea. Although it is too early to know the full extent of the

problem, at least 10 cases of myopathy have been reported in the QUASH studies with most cases having an onset of symptoms after roughly 1 year of therapy [personal communications, Michelle Berrey, Pharmasset Inc.]. A much larger number of cases have been reported to Bukwang (the company that markets clevudine in South Korea) from post-marketing surveillance studies as well as spontaneous reports submitted by physicians and consumers in South Korea where clevudine was approved in 2006 [personal communications, Michelle Berrey, Pharmasset Inc.]. While these reports are not available, two publications shed light on the onset, clinical manifestations, reversibility, and possible mechanisms of clevudine myopathy.

2. Clevudine myopathy

2.1. Clinical case reports

In this issue of the Journal, Kim et al. reported two patients developed muscle weakness of the legs after 11 and 12 months of clevudine therapy for hepatitis B

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[1]. Both patients had elevation in creatine kinase (CK) levels, electromyography (EMG) changes of myopathy and muscle biopsy findings of degenerating and necrotic myofibers infiltrated by macrophages. One patient had resolution of symptoms 1 month after clevudine withdrawal while the other patient had improvement in symptoms 2 months after clevudine withdrawal. Electron microscopy of the muscle biopsies of these two patients did not reveal any mitochondrial changes.

In another report, also from Korea, Seok et al. reported 7 patients with severe muscle weakness after 8–13 months of clevudine therapy. None of the patients were taking concomitant medications except for one patient who had been taking colchicine for 1 year [2]. Three patients complained of muscle pain, all 7 patients had symmetric proximal limb weakness and 2 also had weakness of bulbar and neck muscles. Six patients had elevated CK levels (highest 8082 U/L) and 2 of 3 patients tested had elevated lactate levels. EMG showed myopathic changes while nerve conduction studies were normal. Muscle biopsy revealed severe myonecrosis. Electron microscopy showed mitochondrial proliferation and enlarged mitochondria that contained rectangular inclusions. Quantification of mitochondrial DNA content using polymerase chain reaction demonstrated a depletion of mitochondrial DNA in the skeletal muscles. Although all patients regained muscle strength within 16 weeks after discontinuation of clevudine and none had evidence of organ failure, the findings are reminiscent of fialuridine (FIAU) toxicity [3].

These two reports highlight the importance of post-marketing surveillance particularly with drugs that are used for much longer durations than in the pivotal trials. They also raise questions whether there were hints in the preclinical or phase I/II clinical studies that would have prevented this unfortunate outcome.

2.2. Preclinical studies

A study on the preclinical aspects of clevudine, a L-nucleoside 1-(2-fluoro-5-methyl- β -L-arabino-furanosyl)uracil (L-FMAU), found that clevudine was not incorporated into mitochondrial DNA and it was not an inhibitor or a substrate for DNA polymerase γ ; therefore, mitochondrial toxicity was not expected [4]. However, mitochondrial toxicity may arise not only from inhibition of DNA polymerase γ but also from mitochondrial DNA mutations and mitochondrial oxidative stress [5]. Studies of nucleoside reverse transcriptase inhibitor (NRTI) such as azidothymidine (AZT)-associated mitochondrial toxicity highlighted the important role of the intracellular location where the nucleoside is phosphorylated. The enzyme involved in the first step of phosphorylation – thymidine kinase exists in 2 isoforms, one which is located in the cytoplasm (TK1) and the other in the mitochondria (TK2). The

susceptibility of a given tissue to NRTI-induced mitochondrial toxicity is related to the presence of a high concentration of TK2 relative to TK1. One study published in 1996 found that both fialuridine and clevudine are more efficient substrates for TK2 than for TK1 [6]. Another study published in 2008 confirmed that clevudine is preferentially phosphorylated by TK2 [7]. Interestingly, clevudine has been investigated as a positron emission tomography tumor-imaging tracer [8,9]. These studies revealed that phosphorylated clevudine is retained in cells, particularly cells of mitochondrial rich tissues such as heart or muscles, and retention is increased during cellular stress [7]. These findings indicate that long-term use of clevudine may lead to accumulation of clevudine-triphosphate and to mitochondrial toxicity. The lack of mitochondrial changes in Kim et al.'s study may be related to the small number of patients ($n = 2$), timing of the biopsies, or sensitivity of the technique used [1]. By contrast, Seok et al.'s study demonstrated clear evidence of mitochondrial damage based on electron microscopy and polymerase chain reaction [2]. It should be mentioned that initial studies of fialuridine also found that there was no effect on mitochondrial function [10] but subsequent studies indicated otherwise [3,11].

2.3. Clinical studies

A unique feature of clevudine is the lag in viral rebound after treatment is stopped. This has led to questions regarding drug accumulation. Initial studies found that the half-life of clevudine after a single dose was 8–12 h but a subsequent study found that the mean half-life of clevudine measured at steady state, after 12 weeks of treatment, was approximately 70 h [12]. Given that clevudine is administered daily, accumulation of the drug is expected to occur during long-term treatment. It is possible that the half-life of intracellular clevudine-triphosphate may be longer. Accumulation of intracellular clevudine-triphosphate after prolonged administration of clevudine may in part explain the lack of toxicity in earlier trials of shorter (4–24 weeks) duration of treatment [12–17] and the late onset of toxicity (around 12 months). Similarly, initial studies of 2 and 4 week courses of fialuridine did not reveal any safety concerns and side effects were minimal during the first 8 weeks of therapy in the final study [3]. Numbness and tingling in the feet followed by sudden onset of hepatic failure, shock and lactic acidosis manifested after 9.5–13 weeks of treatment.

Using a sensitive radioimmunoassay, Bowsher et al. found that fialuridine had a long elimination half-life of 29.3 h in healthy volunteers instead of the previous estimate of 3–4 h and fialuridine accumulated in genomic DNA of all tissues with the highest concentration in the liver [18,19]. Manifestation of neurotoxicity asso-

ciated with adenine arabinoside monophosphate (ARA-AMP), a drug that was evaluated for the treatment of hepatitis B in the late 1970s and early 1980s, was also delayed. In one study, peripheral neuropathy developed in 1 of 15, 1 of 11 and 5 of 5 patients who received ARA-AMP for 4, 7, and ≥ 8 weeks, respectively [20]. Of note, 3 of these 7 patients had onset of symptoms 1–2 weeks after the withdrawal of therapy. This highlights the importance of continued monitoring of patients in whom clevudine is discontinued.

Elevated CK level is an early indicator of muscle injury but elevated CK may be nonspecific and is often observed after strenuous exercise. In a study of 32 patients who received varying doses of clevudine for 4 weeks, grade 3 or 4 elevation in CK levels (≥ 7 time the upper limit of normal) was recorded in 3 patients [13]. In another study, 3 of 66 patients who received 12 weeks of clevudine had grade 3 or 4 elevation in CK levels compared to 0 of 32 who received placebo [15]. A third study comparing 24 weeks of emtricitabine alone versus emtricitabine plus clevudine found that a similar percentage of patients in both groups had grade 3 or 4 elevation in CK levels, 2% vs. 7% during treatment and 5% vs. 4% during 24 weeks of post-treatment follow-up [14]. Reports of the two pivotal trials of clevudine conducted in Korea in which the drug was administered for 24 weeks did not provide any information on CK levels [16,17]. Despite the lack of reports of clinical myopathy, the elevation in CK levels observed in earlier trials were concerning. Therefore, an algorithm for the management of subjects who develop signs and symptoms of possible skeletal myopathy was incorporated in the phase III QUASH study. It is not clear whether this algorithm led to earlier awareness of clevudine myopathy and termination of the trial before more patients were exposed to the risk.

3. Myopathy/neuropathy associated with other nucleos(t)ide analogs used for treatment of hepatitis B

3.1. Telbivudine

Myopathy and neuropathy have been reported in patients who received telbivudine treatment. In the worldwide phase III GLOBE trial, grade 3 or 4 elevation in CK levels was observed in 88 of 680 (12.9%) patients who received telbivudine and in 28 of 687 (4.1%) patients who received lamivudine for 104 weeks ($p < 0.001$) [21]. Myopathy, characterized by muscle pain and weakness and moderately elevated CK levels during treatment, was reported in 2 patients, both of whom had resolution of symptoms after telbivudine was discontinued. Rhabdomyolysis was not observed in any patient. Preliminary data of 667 patients in the GLOBE trial and another phase III trial in China

(NV-02B-015) who received telbivudine for 3 years found that 9 (1.4%) patients had new onset grade 3 or 4 CK elevation and 9 (1.4%) had myalgia or myositis between weeks 104 and 156 [22].

Telbivudine has also been reported to be associated with peripheral neuropathy, particularly when used in combination with pegylated interferon. Of 3500 patients who had received telbivudine monotherapy in clinical trials, 10 (0.28%) were reported to have peripheral neuropathy compared to 9 of 48 (18.75%) patients who received combination therapy of pegylated interferon and telbivudine [23]. Combination of pegylated interferon and telbivudine was also associated with an earlier onset of peripheral neuropathy: median of 4.5 (range 2–6) months vs. 14 (range 4–25) months and a lower likelihood of improvement 6 of 9 vs. 9 of 10 who received telbivudine monotherapy.

The mechanism(s) of telbivudine associated myopathy or peripheral neuropathy is unknown. To date, there are no reports of EMG, muscle biopsies or nerve conduction studies. *In vitro* studies found that telbivudine at concentrations up to 10-fold maximal clinical exposure had no effect on human hepatocytes, skeletal muscle or neuronal cells and was not associated with mitochondrial toxicity [24]. It is also not clear how interferon increase or accelerate telbivudine-induced neuropathy. Interferon had also been reported to increase the incidence of ARA-AMP induced neuropathy [25].

3.2. Other approved HBV treatments

Data from 3 placebo-controlled trials of lamivudine in patients with HBV mono-infection found that grade 3 or 4 CK elevation was observed in 9% of patients who received lamivudine and in 5% of those who received placebo while myalgia was reported in 14% and 17%, respectively [see package insert, www.fda.gov]. Rhabdomyolysis has been reported in 2 liver transplant patients who received lamivudine. In one case, this occurred after 13 days of lamivudine in a patient with acute exacerbation of chronic hepatitis B and liver failure [26]. In the other case, this occurred 16 h after the first dose of lamivudine in a patient who had just received a liver transplant [27]. The rapidity of onset and the presence of other medical conditions and medications make it difficult to ascertain the causal relationship although re-challenge of the latter case with a single dose of lamivudine 6 days later was reported to lead to recurrence of myoglobinemia and increase in CK levels. Despite many years of marketing experience and extensive use at doses exceeding that for the treatment of hepatitis B, lamivudine has not appeared to be causally associated with myopathy in patients with human immunodeficiency virus (HIV) infection.

Elevation in CK levels has also been observed in patients who received adefovir, tenofovir or entecavir;

however, the incidence is similar to patients who received placebo or active control drug, and overt myopathy or neuropathy has been rarely reported in pivotal trials [see package insert, www.fda.gov].

4. Long-term safety of nucleos(t)ide analog treatment for hepatitis B

The approval of 5 orally administered, well tolerated nucleos(t)ide analogs for hepatitis B in the past 10 years has stimulated enthusiasm for expanding the indications for treatment of hepatitis B. Some experts have argued that treatment should be initiated based on high serum HBV DNA levels alone and that treatment should be continued indefinitely to avoid post-treatment relapse or reactivation of hepatitis B. The withdrawal of clevudine from development is a sober reminder that while the nucleos(t)ide analogs for hepatitis B have excellent safety profiles based on clinical trials of 1-year duration, there is paucity of data on long-term safety of these compounds. Among the approved therapies, lamivudine has the best long-term safety record but it is associated with an unacceptably high rate of drug resistance. Adefovir and tenofovir can cause nephrotoxicity and renal tubular damage and tenofovir has been reported to decrease bone mineral density [see package insert, www.fda.gov] [28,29]. Although these adverse events are rare in clinical trials of 1–2 years duration, nephrotoxicity defined as reproducible increase in serum creatinine by ≥ 0.5 mg/dL was observed in 3% of patients who received adefovir for up to 5 years [30]. As discussed above, telbivudine has been reported to be associated with myopathy and neuropathy. Preclinical studies found that entecavir in doses up to 30 times the approved dose in humans was associated with a wide variety of tumors in rodents [see package insert, www.fda.gov]. These findings raised a lot of concerns at the time entecavir was approved and led Bristol-Myers Squibb to pledge a 10-year follow-up study of up to 10,000 patients receiving entecavir. To date, there have been no serious reports of entecavir-related toxicity but data from the long-term surveillance study are not yet available.

5. Monitoring of toxicities during drug development

The unfortunate situation with clevudine is a reminder of the difficulties in predicting drug-related adverse events. Although each new drug has to be rigorously tested in *in-vitro* and in animal toxicity studies, the ability of preclinical studies to predict toxicities in humans, particularly rare or idiosyncratic events, is low. Preclinical or phase I/II clinical studies may identify safety signals leading to the incorporation of monitoring and management plans in phase III trials that may mitigate

risks or detect toxicity at an early stage. Whether the development of a drug with safety concerns in phase III trials is allowed to continue depends on the balance between the risks (frequency, severity and reversibility of the events) and the potential benefits of the drug (efficacy of the drug, availability of alternative therapies, and prognosis of the disease if untreated). Not all toxicities are evident during phase III trials. In the past 10 years, several drugs have been withdrawn from the market only after these drugs have been used in hundreds of thousands of patients in clinical practice. Establishing a causal relationship between an approved drug and a major adverse event is hampered by the lack of an efficient and comprehensive surveillance program. In most countries, monitoring of drug safety post-marketing is dependent on voluntary reporting by physicians who are often too busy or unfamiliar with the process or who simply could not be bothered. Moreover, an individual physician seeing a patient with a previously unreported adverse event may not associate the event with a drug unless he or she has knowledge of similar events. Thus, under-reporting is a major barrier to assessment of the safety of approved drugs.

Kim and colleagues should be applauded for their vigilance in monitoring their patients, the thorough evaluation, and the prompt reporting of their cases, thereby alerting the hepatitis community and hopefully averting more cases of clevudine myopathy. The decision to terminate the development of clevudine was appropriate given the availability of several alternative therapies that are safer.

6. Conclusion

Substantial progress has been made in the treatment of hepatitis B in the past decade. The availability of well tolerated orally administered nucleos(t)ide analogs has prompted many experts to recommend expansion of treatment to patients with quiescent or minimally active liver disease. The case of clevudine myopathy is a sober reminder that the decision to initiate treatment should balance benefits against risks. Although the drug approval process is rigorous, toxicities may not be detected during preclinical or clinical studies if these events are rare, or if the drugs are used in patient populations that were not included in the clinical trials or for longer durations than in clinical trials. The latter is particularly relevant because most patients with hepatitis B treated with nucleos(t)ide analogs remain on treatment for at least 4–5 years and some are on lifelong treatment.

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