Does Linezolid Cause Lactic Acidosis by Inhibiting Mitochondrial Protein Synthesis?

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Linezolid, an oxazolidinone antibiotic, inhibits bacterial protein synthesis by binding to 23S ribosomal RNA (rRNA). We studied 3 patients who experienced lactic acidosis while receiving linezolid therapy. The toxicity may have been caused by linezolid binding to mitochondrial 16S rRNA. Genetic polymorphisms may have contributed to the toxicity in 2 patients.

Linezolid (Zyvox; Pfizer) is the first commercially available oxazolidinone antibiotic agent and is effective against gram-positive organisms, including bacterial strains that are resistant to methicillin and vancomycin [1, 2]. Lactic acidosis associated with linezolid treatment was first reported by Apodaca and Rakita in 2003 [3], but the mechanisms of toxicity have not been studied. Here, we describe clinical and molecular genetic features of the patient from the original study [3] and 2 additional patients who developed lactic acidosis while receiving linezolid therapy. We hypothesize that linezolid-associated lactic acidosis is due to inhibition of mitochondrial protein synthesis that is analogous to the mechanism of aminoglycoside-induced deafness [4].

Patient 1. A 52-year-old woman, described elsewhere [3], developed disseminated Nocardia oitidis-caviarum infection in the brain, kidneys, and adrenal glands. The organism was detected in bronchoalveolar lavage fluid and in a mediastinal biopsy specimen. After developing adverse effects from trimethoprim-sulfamethoxazole therapy, the patient was given linezolid and clarithromycin. During week 12 of antibiotic treatment, the patient developed nausea, vomiting, and myelosuppression. She was noted to have lactic acidosis (lactate level, 9.9 mmol/L; normal level, 0.5–2.2 mmol/L), which normalized 10 days after discontinuation of therapy with both antibiotics. Linezolid therapy was restarted, but at day 14 of antibiotic therapy, the patient’s lactate level was elevated to 6.5 mmol/L. Linezolid therapy was then stopped, and the serum lactate level returned to normal 2 weeks later.

Patient 2. A 49-year-old woman received an allogenic bone marrow transplant for acute myelocytic leukemia. She experienced multiple relapses of leukemia. Four years after transplantation, the patient started salvage chemotherapy with a high dose of cytarabine followed by 2 cycles of vincristine, cytarabine, and carboplatin therapy. She tolerated chemotherapy; however, her postchemotherapy clinical course was complicated by life-threatening polymicrobial sepsis; isolates included Mycobacterium abscessus, vancomycin-resistant enterococcus, and Candida glabrata.

In August 2003, at age 53 years, the patient was hospitalized, and her condition was treated with multiple antibiotics. Linezolid therapy was started on day 37 of hospitalization. After nearly 3 months of hospitalization, the patient was discharged to an embolus in the setting of paroxysmal atrial fibrillation. Five days after linezolid therapy was stopped, the patient’s lactic acidosis resolved.

In January 2004, the patient was readmitted to the hospital for recurrent disseminated M. abscessus infection. The serum lactic acid level was normal. She was given imipenem, azithromycin, and cefoxitin—antibiotics selected on the basis of the limited sensitivities of the M. abscessus isolate. The patient ex-
Pseudomonas aeruginosa. The infection was treated with linezolid, ertapenem, and ciprofloxin. After 40 days of receiving linezolid therapy, the patient’s serum bicarbonate level was normal; however, 1 week later, she experienced nausea, vomiting, abdominal discomfort, and an altered mental status. She was admitted to the hospital and was found to have metabolic acidosis with a low serum bicarbonate level (13 mmol/L) and marked anemia (hemoglobin level, 7.6 g/dL). Her condition was treated with linezolid and intravenous administration of fluids; however, the patient’s mental status worsened over 3–4 days, and linezolid therapy was discontinued. The findings of upper and lower endoscopy and stool guaiac tests were normal. CT of the brain showed normal findings. The patient received packed RBC transfusions. On day 7 of hospitalization, her platelet count decreased to 8000 platelets/mm³, and her venous lactate level was 18.4 mmol/L. Three days later, the patient died. Postmortem bone marrow examination and flow cytometry studies demonstrated a B-cell type monoclonal lymphoproliferative disorder.

**Methods.** The patient studies followed the ethical standards of the Helsinki Declaration of 1975, as revised in 1983. Consent for collection of blood samples was obtained with a protocol approved by a Columbia University institutional review board.

Screening for polymorphisms in mitochondrial 12S and 16S rRNA was performed by sequencing PCR fragments amplified from blood DNA. Oligonucleotide primers are listed in table 1. Tag polymerase and buffers from Roche were used for the PCR reaction. PCR conditions were 35 cycles at 94°C for 1 min, 56°C for 1.75 min, and 72°C for 1.75 min, followed by 1 cycle at 72°C for 5 min. PCR was performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems [ABI]). DNA sequencing of PCR products was performed using the BigDye Terminator kit and an ABI PRISM 310 system (ABI).

To check the frequency of the polymorphisms among 100 control subjects, restriction fragment–length polymorphism assays were performed. To identify the G3010A polymorphism, we used the restriction enzyme Bci I (0.5 μL, 5 units; New England Biolabs) to digest a 1074-bp PCR product (with primers 2251F1 and 3325R1); wild-type DNA was cut twice, producing 2 bands, of lengths 754, 224, and 96 bp, whereas fragments containing the G3010A polymorphism produced 2 bands, of lengths 978 and 96 bp. To identify the A2706G polymorphism, we used the restriction enzyme Nla III (New England Biolabs) to digest a 237-bp PCR product (with primers POL2706Up and POL2706Lo), which, in the presence of the polymorphism, produced 2 bands, of lengths 121 and 116 bp, but did not cut the wild-type fragment. The statistical significance of the observed prevalence of the polymorphisms among the patients was assessed by χ² analyses.

**Results.** Sequencing the mitochondrial 12S and 16S rRNA genes in all 3 patients revealed a homoplasmic A2706G polymorphism (according to numbering used in [5], A1036G in 16S rRNA) in patient 1, a homoplasmic G3010A polymorphism (according to [5], G1340A in 16S rRNA) in patient 2, and no polymorphisms in patient 3. Both polymorphisms are in the mitochondrial 16S rRNA gene and have been described previously [6, 7]. In addition, we identified the A2706G polymorphism in 38 (38%) of 101 control subjects and the G3010A polymorphism in 38 (38%) of 100 control subjects. The frequency of the polymorphisms among patients was not statistically significant, compared with the frequency among controls (P = .87, for A2706G; P = .46, for G3010A).

**Discussion.** Apodaca and Rakita [3] described a patient who developed lactic acidosis after 11 weeks of linezolid ther-

Table 1. Oligonucleotide primers used to amplify and sequence the mitochondrial 12S and 16S rRNA genes and for restriction fragment–length polymorphism analyses.

<table>
<thead>
<tr>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tr>
<td>595F1, 5′-CTCAAAAGCATACATACCTGAAA-3′</td>
<td>735R1, 5′-TAGATTAGGGTTCAACTGGAACGGG-3′</td>
</tr>
<tr>
<td>752F1, 5′-CAAGGCATCAACGCGAGCAGA-3′</td>
<td>1180R1, 5′-ACCGCAGCGTCCTTTGATTTTAAGCTGTGCTG-3′</td>
</tr>
<tr>
<td>1250F1, 5′-CAAGCCCTATATCCGCGACCCT-3′</td>
<td>1567R1, 5′-TGTACGATTGTCCTCCTC-3′</td>
</tr>
<tr>
<td>1482F1, 5′-ACCAGCCGTACCTCTCTTAAGATATCTCAGG-3′</td>
<td>1803R1, 5′-TTTCATCTTTCCCTTTCG-3′</td>
</tr>
<tr>
<td>1690F1, 5′-CCAAGCGTGCTCTACTTACAAGAC-3′</td>
<td>2010R1, 5′-ACCCAGCCGTGGTATGTGTG-3′</td>
</tr>
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<td>1759F1, 5′-TGAAACGTGCGCCTAGATG-3′</td>
<td>2501R1, 5′-GTAAAGCTGCGCTAGTGTG-3′</td>
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<td>2251F1, 5′-ACTCTCTACACCAAGATTGGA-3′</td>
<td>3000R1, 5′-TGATCCCAATCGGATGCTG-3′</td>
</tr>
<tr>
<td>2762F1, 5′-CTAACAAAAACCAAGGCTCTT-3′</td>
<td>3325R1, 5′-GGAGGTTGGGGCCATGATG-3′</td>
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<tr>
<td>POL2706Up, 5′-ACCGTGCAAGGTAGCATAC-3′</td>
<td>POL2706Lo, 5′-GCCCCAACCGAAATTTTA-3′</td>
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apy. Here, we describe 2 additional patients who received linezolid therapy for >6 weeks and developed lactic acidosis. In addition to lactic acidosis, myelosuppression and optic and peripheral neuropathy have been reported as toxic effects of linezolid, particularly with a treatment course longer than the 28-day period approved by the US Food and Drug Administration [8, 9]. For clinicians, it is important to be aware of these toxicities when giving linezolid to patients for prolonged periods, which is necessary for treatment of osteomyelitis or infections due to mycobacteria or other slow-growing species. The mechanisms of these toxicities have not been determined.

As a therapeutic agent, linezolid inhibits bacterial protein synthesis [10] by binding to the 50S ribosomal subunit and preventing the formation of the initiation complex, which requires interactions with tRNA\(^{\text{Met}}\), mRNA, and the 30S ribosomal subunit [11]. Linezolid interacts with the 50S ribosomal subunit at or near the binding sites of other antibiotics. In fact, linezolid is a competitive inhibitor of chloramphenicol and lincomycin [12], because all 3 antibiotics bind to domain V of bacterial 23S rRNA, at the peptidyl transferase center that catalyzes peptide-bond formation and that has a conserved equivalent structure in the mammalian mitochondrial 16S RNA of the large ribosomal subunit [13, 14]. Although these antibiotics bind to the same region of the 23S rRNA, their mechanisms of action differ. In contrast to linezolid, chloramphenicol and lincomycin inhibit peptide-bond formation, and there is no cross-resistance between linezolid and these other 2 antibiotics [1, 11, 12]. Not surprisingly, genetic polymorphisms in domain V of the bacterial 23S rRNA have been associated with linezolid resistance [15, 16].

Given the similarities between the conserved domains of rRNAs in bacterial and human mitochondrial ribosomes, it is plausible that linezolid binds to similar sites on the RNA of the large ribosomal subunits of the mitochondrial and bacterial ribosomes. Thus, it is likely that linezolid causes mitochondrial toxicity by inhibiting protein synthesis. Furthermore, mtDNA polymorphisms in the 16S RNA gene, such as those we identified in patients 1 and 2, may confer genetic susceptibility to linezolid toxicity. In fact, there is a well-established precedent of linking a mitochondrial polymorphism to antibiotic-induced mitochondrial toxicity. The A1555G mutation in the mitochondrial 12S RNA of the small ribosomal subunit confers susceptibility to aminoglycoside antibiotic–induced hearing loss as well as nonsyndromic sensorineural hearing loss [4, 17]. Aminoglycosides inhibit bacterial protein synthesis by binding to a highly conserved region of 16S rRNA, which is analogous to the human 12S rRNA gene [4]. The A1555G mtDNA mutation alters the ribosomal structure, allowing frequent aminoglycoside access to its ribosome-binding site.

Another potential pathogenic link between an antibiotic and an mtDNA mutation has been described in a patient with an mtDNA mutation that causes Leber hereditary optic neuropathy [18]. The patient had acute vision loss while taking erythromycin. Erythromycin binds to the 50S ribosomal subunit and inhibits bacterial protein synthesis by blocking the peptidyl transferase exit tunnel [19]. Luca et al. [18] hypothesized that the drug may impair mitochondrial respiration by inhibiting mitochondrial translation. In fact, they demonstrated that, under cell culture conditions that require mitochondrial respiration for growth, erythromycin inhibited proliferation of cells containing the patient’s mtDNA [18]. Furthermore, erythromycin impaired mitochondrial translation in vitro.

To our knowledge, no other study has yet examined the mechanism of linezolid-associated lactic acidosis. Although the findings of the present study are not statistically significant, because of the small sample size, 2 of the 3 patients showed polymorphisms in the mitochondrial 16S rRNA, specifically in the region homologous to the linezolid-binding site located at domain V of the bacterial 23S RNA. Patient 3 did not harbor any polymorphisms in the 12S or 16S rRNA genes; therefore, mtDNA polymorphisms may not be necessary for linezolid to induce lactic acidosis. Nevertheless, by altering the structure of the mitochondrial ribosome, polymorphisms in the 16S rRNA gene could predispose patients to linezolid toxicity. In support of this hypothesis, we note that both nucleotides substitutions are located on the L7L12-stalk side [13], a functional domain of the ribosome [20, 21]. The G1340A polymorphism (G3010A, according to [5]) in the large ribosomal subunit is close to the alpha-sarcin-ricin stem-loop, a universally conserved structure that interacts with initiation, elongation, and termination factors during protein synthesis [22]. By contrast, the A1036G polymorphism (A2706G, according to [5]) is among the few segments of the 16S RNA that is not shielded by mitochondrial proteins and therefore may interact frequently with molecules like linezolid.

On the basis of our findings, we hypothesize that lactic acidosis and possibly other toxicities associated with prolonged linezolid treatment are caused by inhibition of mitochondrial protein synthesis. Confirmation of our hypothesis may come from an investigation of additional patients who develop linezolid-associated complications and from cell-culture and animal-model laboratory studies.

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References