Linezolid-Induced Inhibition of Mitochondrial Protein Synthesis

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Background. Linezolid is a prototype member of the oxazolidinones, is a valuable acquisition in our antibiotic armamentarium because of its excellent activity against drug-resistant, gram-positive pathogens. The mechanism of action is inhibition of bacterial protein synthesis. Optic and/or peripheral neuropathy and lactic acidosis are reported side effects, but the underlying pathophysiological mechanism has not been unravelled.

Methods. We studied mitochondrial ultrastructure, mitochondrial respiratory chain enzyme activity, and mitochondrial DNA (mtDNA) in muscle, liver, and kidney samples obtained from a patient who developed optic neuropathy, encephalopathy, skeletal myopathy, lactic acidosis, and renal failure after prolonged use of linezolid. In addition, we evaluated mtDNA, respiratory chain enzyme activity, and protein amount in muscle and liver samples obtained from experimental animals that received linezolid or placebo.

Results. In the patient, mitochondrial respiratory chain enzyme activity was decreased in affected tissues, without ultrastructural mitochondrial abnormalities and without mutations or depletion of mtDNA. In the experimental animals, linezolid induced a dose- and time-dependent decrease of the activity of respiratory chain complexes containing mtDNA-encoded subunits and a decreased amount of protein of these complexes, whereas the amount of mtDNA was normal.

Conclusion. These results provide direct evidence that linezolid inhibits mitochondrial protein synthesis with potentially severe clinical consequences. Prolonged courses of linezolid should be avoided if alternative treatment options are available.

Linezolid, a prototype member of the oxazolidinones, is a valuable acquisition in our antibiotic armamentarium because of its excellent activity against drug-resistant, gram-positive pathogens and its favorable pharmacokinetics. Linezolid inhibits the initiation phase of bacterial protein synthesis by binding to the 50S ribosomal subunit at its interface with the 30S subunit, thereby preventing the formation of the 70S initiation complex. The most commonly reported adverse effects associated with linezolid are gastrointestinal disturbances, thrombocytopenia, and anemia [1, 2]. In a continuously growing number of patients, optic and/or peripheral neuropathy [1, 3–7] or lactic acidosis [8, 9] have been reported. Although it has been suggested that linezolid may interfere with mitochondrial protein synthesis [9], the underlying pathophysiological mechanism of these latter adverse events has not been unraveled.

In this study, we describe a patient who developed optic neuropathy, encephalopathy, skeletal myopathy, lactic acidosis, and renal failure after a 4-month course of linezolid. Because the clinical and biochemical features in this patient were reminiscent of mitochondrial disorders caused by drugs or hereditary defects in respiratory chain complexes [10], we performed a morphological study of mitochondria, and we measured the activity of the respiratory chain complexes and the amount of mtDNA in organs affected in this patient.
To further investigate any association between linezolid and mitochondrial dysfunction, we administered linezolid to rats and evaluated the activity and amount of protein of the respiratory chain complexes and the amount of mtDNA in various tissue samples obtained from these rats.

**METHODS**

**Clinical records and sample collection.** A 63-year-old woman was admitted to the hospital because of acute, bilateral loss of vision. Four months earlier, a prosthetic joint infection due to methicillin-resistant *Staphylococcus aureus* was treated with linezolid (600 mg administered twice per day) and rifampicin (600 mg administered every day), which she was still taking at the time of hospitalization. Other medications included ciprofloxacin (administered for a secondary wound infection due to *Pseudomonas aeruginosa*), clonidine, bisoprolol, amlodipine, pantoprazol, metoclopramide, lorcazepam, and nadroparine. The patient had a personal history of hypertension, and her family history was unremarkable. At admission to the hospital, the patient’s pupils were dilated with minimal reaction to light. Fundoscopy showed pale optic disks and narrowed retinal vessels. Clinical examination revealed no further abnormalities. The findings of a cerebral CT scan were normal. Laboratory investigation showed a high anion gap metabolic acidosis (bicarbonate, 5 mmol/L; anion gap, 48.4 mmol/L), elevated lactate levels (24.5 mmol/L) with a lactate/pyruvate ratio of 37 (normal ratio, <15), and renal failure. Methanol was not detected in serum samples. On the second day after admission to the hospital, the possibility of linezolid toxicity was considered, and the antibiotic was withdrawn. On the third day, the patient became obtunded and disoriented in time and space. She developed a rapidly progressive flaccid quadriparesis. The findings of sensory and motor nerve conduction studies and needle electromyography were normal, suggestive of a myopathy, rather than a neuropathy. The findings of an MRI of the brain were normal. CSF analysis showed a lactate level of 9.8 mmol/L in liver homogenate. Spectrophotometric assays were performed for the measurement of the activity of complex I (di- 

**Experimental animals.** The studies were performed in 28 male 7-week-old Sprague-Dawley rats (Iffacredo), which received care in accordance with Belgian national guidelines for care and use of laboratory animals. One series of rats received either linezolid (6 rats) administered at a dosage of 125 mg/kg per day (Zyvoxid oral suspension; Pfizer) or saline (6 rats) by gavage in 2 separate doses for 2 weeks [11]. A second series received either linezolid (8 rats) administered at a dosage of 250 mg/kg per day or saline (8 rats) for 4 weeks. Plasma samples were obtained from the tail vein 20 min after gavage at weeks 1 and 2 for the first series and at weeks 2 and 4 for the second series. After 2 and 4 weeks, respectively, the rats were sacrificed, and samples of quadriceps muscle and liver were snap frozen in liquid nitrogen and maintained at −80 °C until analysis.

**Linezolid levels.** Linezolid was assayed by a validated high-performance liquid chromatography method [12] using a Hypersil SODS column (Waters Corporation) and a mobile phase of methanol, water, and phosphoric acid (at a ratio of 30:69:1) with 2 g/L of heptane sulphonic acid added. Serum samples were deproteinated with acetonitrile before assay. Tissue samples were extracted into phosphate buffered saline and then treated as described for serum samples.

**Spectrophotometric assays of catalytic activity of respiratory chain complexes.** Tissue samples (50–100 mg) were homogenized as described elsewhere [13]. Protein content ranged from 1.75–3.20 mg/mL in muscle homogenate and from 6.4–8.4 mg/mL in liver homogenate. Spectrophotometric assays were performed for the measurement of the activity of complex I (dihydroroticinamide adenine dinucleotide and ubiquinone oxidoreductase; rotenone sensitive) [14], complex II (succinate and ubiquinone oxidoreductase; malonate sensitive) [15], complex III (ubiquinone and cytochrome c oxidoreductase; antimycin sensitive) [16], complex IV (cytochrome c oxidase) [17], and citrate synthase [18].

**Blue native PAGE and immunoblotting.** Solubilized skeletal muscle mitochondrial proteins from 2 linezolid-treated rats and 2 control rats underwent blue native PAGE; subsequent staining for catalytic activity of the respiratory chain complexes I, II, IV and V; and immunostaining, as described elsewhere [13]. The signals were generated by a mixture of specific antibodies against selected subunits in the 3 respiratory chain complexes using enhanced chemiluminescence. Skeletal muscle and liver homogenates from the same animals were also subjected to tricine SDS-PAGE and to subsequent immunostaining.
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with specific antibodies against subunits in the 5 complexes. A porin antibody was used as loading control.

Quantitative mtDNA assessment. DNA was extracted from rat and human tissues by the conventional phenol-chloroform method. Relative quantification of total mtDNA to nuclear DNA was measured by a mitochondrial transfer RNA leucine 1/b-2-microglobulin real time PCR quantification method using an ABI Prism 7500 Sequence Detection System (Applied Biosystems). The PCR was performed separately for mtDNA and the reference b-2-microglobulin amplification with the TaqMan Universal PCR Mastermix System, according to the manufacturer’s instructions (Applied Biosystems). The inter- and intra-assay reproducibility was evaluated by repeating the assay 4 times and analyzing the samples in triplicate.

mtDNA analysis. The complete mitochondrial genome in the skeletal muscle biopsy of the patient was analyzed with denaturing high-performance liquid chromatography (Mito-Screen Assay Kit; Transgenomic), as described elsewhere [19], and with Southern blot.

RESULTS

Linezolid levels. The patient’s plasma linezolid concentrations were 11.8 mg/L and 15.5 mg/L 1–2 h after dosing. Normal levels before and after dosing are 5 and 15 mg/L, respectively. In rats given 125 mg/kg of linezolid per day, mean linezolid plasma concentrations (± SEM) were 11.6 ± 2.9 mg/L after 1 week and 10.3 ± 1.3 mg/L after 2 weeks, and the mean linezolid concentration (± SEM) in liver homogenate was 22.8 ± 1.1 mg/kg. In rats given 250 mg/kg of linezolid per day, mean linezolid plasma concentrations (± SEM) were 30.0 ± 4.3 mg/L after 2 weeks and 20.5 ± 1.7 mg/L after 4 weeks, and the mean linezolid concentration (± SEM) in liver homogenate was 31.3 ± 1.0 mg/kg.

Histological examination of the patient samples.
Table. 1. Activity of respiratory chain complexes in tissue samples obtained from a patient with prolonged use of linezolid therapy.

<table>
<thead>
<tr>
<th>Tissue sample</th>
<th>Activity ratio (z score), by complex</th>
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<tr>
<td></td>
<td>I(^{-})/CS</td>
</tr>
<tr>
<td>PBMCs</td>
<td>0.5 (1.26)</td>
</tr>
<tr>
<td>Liver</td>
<td>ND</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.27 (-4.08)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.43 (-3.33)</td>
</tr>
</tbody>
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**NOTE.** To control for mitochondrial mass, activity is expressed as the ratio between the activity of the respiratory chain complex and the activity of citrate synthase. The z score is calculated as the activity ratio for the patient sample minus the mean activity ratio for the control samples divided by the SD for the control samples. When the z score is lower than −1.96 or higher than +1.96, the result for the patient sample is significantly different (P<.05) from the result for the control samples. Results with z scores of less than −1.96, indicative of significantly depressed respiratory chain enzyme activity in the patient tissue sample, are given in bold. CS, citrate synthase; ND, not done.

Histological examination of the liver samples showed macro- and microvesicular steatosis. Morphological examination of kidney samples revealed no pathognomonic changes. Light microscopic examination of muscle samples revealed atrophic muscle fibers without any specific distribution pattern and no ragged red fibers. A slight decrease of subsarcolemmal mitochondria was suspected on histoenzymological examination. Electron microscopic examination, however, showed normal quantities of subsarcolemmal and intermyofibrillar mitochondria (figure 1). In some fibers, mitochondria were enlarged with rarefied cristae. With the exception of a few osmiophilic granules, no paracrystalline inclusions were found.

**Catalytic activity of respiratory chain complexes.** The most prominent findings in the patient’s muscle, kidney, and liver samples were the significantly decreased activity of complexes I and IV, compared with samples from the control population (complex I in liver samples was not measured) (table 1). Citrate synthase activity for the patient sample was in the same range as in the control samples, indicative of a normal mitochondrial mass. The activity of complex II was normal in muscle, kidney, and liver samples. The respiratory chain complex activity in the patient’s PBMCs were normal.

Experimental animals that were given linezolid at a dosage of 125 mg/kg per day for 2 weeks had a mild decrease in the activity of complex IV in muscle and liver samples (figure 2), whereas those that received linezolid at a dosage of 250 mg/kg per day for 4 weeks had a severe decrease in the activity of all respiratory chain complexes except for complex II (figure 2C and 2D).

**Blue native PAGE and SDS-PAGE.** In the skeletal muscle samples from the linezolid-treated rats, the amount of protein of complexes I, III, IV, and V was decreased, compared with the amount in control samples, whereas the amount of protein of complex II did not differ between samples (figure 3A). This was visible directly after electrophoresis because of the presence of Serva Blue G (Serva) and was confirmed by staining for catalytic activity and by immunoblotting of the complexes. In the lanes stained for activity of complex V, the presence of complex V subcomplexes was observed in samples from treated rats but not in control samples.

In skeletal muscle and liver samples from the treated animals, a decrease in the individual subunits from complexes I, III, IV, and V was demonstrated by Western blotting following tricine SDS-PAGE, compared with the results for samples from control rats, whereas the Fp and Ip subunits from complex II and porin were comparable in both groups (figure 3B).

**mtDNA quantification.** The relative amount of total mtDNA versus nuclear DNA (Rq) was not significantly different for the linezolid-treated rats, compared with the control group, for skeletal muscle samples (1.13 ± 0.06 vs. 1.07 ± 0.28; P = .86) and liver samples (1.63 ± 0.26 vs. 2.09 ± 0.28; P = .3). In skeletal muscle samples obtained from the patient, the Rq was not significantly different from the mean Rq for samples from the control group (1.32 vs. 1.42 ± 0.99; z score, −0.10).

**mtDNA analysis.** Denaturing high-performance liquid chromatography did not reveal mutations in the patient’s muscle mtDNA. Southern blot analysis ruled out large size rearrangements (deletions or duplications).

**DISCUSSION**

Mitochondria are the key organelles for energy production in mammalian cells. ATP is synthesized through the oxidative phosphorylation pathway, which is run by a set of 5 multiprotein complexes embedded in the lipid bilayer of the inner mitochondrial membrane. Retina, optic nerve, brain, skeletal muscle, and kidney tissues are highly dependent on oxidative metabolism, and therefore, they experience the greatest involvement in disorders associated with decreased respiratory chain activity. The patient whom we describe developed optic neuropathy, encephalopathy, skeletal myopathy, lactic acidosis, and acute renal failure. The activity of several respiratory chain complexes was significantly decreased in the patient’s skeletal muscles, liver, and kidney (organs affected in the proposita), whereas the patient’s PBMCs demonstrated normal activity.

Each mitochondrion contains several copies of mtDNA and has its own translation apparatus. The mitochondrial genome contains only a limited number of genes. Most of the mitochondrial proteins are encoded by nuclear DNA, translated in the cytoplasm, and subsequently translocated into the mitochondria. Some key subunits of complexes I, III, IV, and V are encoded by mtDNA, whereas complex II consists entirely of nuclearly encoded subunits [20]. Disorders associated with de-
increased intramitochondrial protein synthesis will, therefore, be characterized by a decreased protein amount and decreased biochemical activity of complexes I, III, IV, and V, whereas the protein amount and the biochemical activity of complex II will remain normal. In the affected organs of the patient and in the experimental animals given linezolid, a selective decrease of the activity of complexes I, III, and IV was indeed observed. After separation of the complexes by blue native PAGE and subsequent immunoblotting, we found that the amount of protein in the complexes I, III, and IV was decreased, whereas the amount of protein in complex II was normal. Activity staining in the blue native gel revealed the presence of lower–molecular weight subcomplexes of complex V, a finding seen in conditions associated with decreased intramitochondrial protein synthesis [21]. A decrease of the individual subunits from the complexes I, III, IV, and V was also demonstrated by Western blotting after SDS-PAGE, whereas subunits from complex II were normal. One of the major causes of decreased intramitochondrial protein synthesis is a defect in mtDNA, such as gross rearrangement, deletion, depletion, or tRNA point mutation. In our patient and in the treated rats, however, mtDNA abnormalities were not detected. Taken together, these data indicate that linezolid directly inhibits intramitochondrial protein synthesis and, thus, may disturb cellular energy production in tissues that are highly dependent on oxidative phosphorylation.

Mitochondria are thought to have arisen from an endosymbiotic event between an eubacterium and its host cell. Mitochondrial ribosomes appear to differ extensively from both bacterial ribosomes and mammalian cytoplasmic ribosomes [22]. Nevertheless, obvious similarities between bacterial and mitochondrial ribosomes have been observed [23]. The oxazolidinone antibiotics inhibit bacterial protein synthesis through a mechanism of action that is different from that of other inhibitors of protein synthesis. Linezolid binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, which is an essential component of the bacterial translation process [24]. It is tempting to speculate that linezolid inhibits bacterial and mitochondrial protein translation by a similar mechanism of action.

Because of its high oral bioavailability, linezolid is increasingly used for prolonged outpatient therapy for patients with prosthetic joint infections or osteomyelitis. The patient that we describe developed toxicity after a prolonged course of linezolid.
Figure 3.  

A. Protein samples of skeletal muscle from 2 rats treated with linezolid (250 mg/kg per day for 4 weeks) and from control rats were subjected to blue native PAGE. Electrophoresis in the presence of Serva Blue G is shown in the left panel. Staining for catalytic activity is presented in the middle panel. A complex V subcomplex is observed in samples from the treated animals but not in samples from the control rats (rectangle). Immunoblotting of the complexes is shown in the right panel. The amount of protein and catalytic activity of complexes I, III, IV, and V is lower in samples from the treated animals than it is in samples from the control rats (arrows), whereas the activity of complex II is comparable in both groups.

B. Protein samples of skeletal muscle and liver from treated rats and control rats underwent tricine SDS-PAGE and subsequent immunostaining with specific antibodies against subunits of the 5 complexes. The subunits in the complexes I, III, IV, and V are decreased in the treated animals, compared with in the control rats. The Fp and Ip subunits from complex II were comparable in both groups, as was porin (which was used as a loading control).
therapy. Myelosuppression [2], peripheral and optic neuropathy [3–7], and lactic acidosis [8, 9] have been reported almost exclusively in patients treated for longer than the maximum recommended duration of 28 days. In the experimental animals exposed to linezolid, we found an obvious time- and dose-dependent effect, indicating that cumulative drug dose is important. In addition, individual genetic characteristics may render a patient more vulnerable to the effects of linezolid. In 2 of 3 patients who developed lactate acidosis during long-term linezolid treatment, mtDNA polymorphisms were found in the 16S RNA gene (specifically, in the region homologous to the linezolid-binding site of the bacterial 23S rRNA) [9]. Further, concurrent exposure to drugs affecting the metabolism of linezolid or inhibiting mitochondrial protein synthesis may play a role. Finally, intercurrent illness may increase the requirements for oxidative metabolism and precipitate the clinical expression of this type of side effect, as has been observed for nucleoside analogue reverse-transcriptase inhibitor–associated mitochondrial toxicity. Maintaining a high index of suspicion for mitochondrial toxicity is the key to the diagnosis of this condition. It remains to be determined whether regular ophthalmologic and neurologic evaluation or measurements of lactate levels are useful in predicting linezolid toxicity.

In conclusion, we provide compelling evidence for a causal link between long-term exposure to linezolid and disruption of mitochondrial protein synthesis. First, oxidative phosphorylation was impaired in tissue samples from a patient with long-term exposure to linezolid at therapeutic levels, with a selective involvement of those complexes that are dependent on mitochondrial protein synthesis. Second, hereditary disorders of mitochondrial translation were not detected, thereby excluding preexisting genetic disease as an alternative explanation for the clinical syndrome. Third, the abnormalities of mitochondrial protein synthesis were reproduced in a dose- and time-dependent manner in experimental animals that were given linezolid. We contend that prolonged courses of linezolid therapy should be avoided if alternative treatment options are available.

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References