Linezolid penetration into wound tissue of two diabetic patients before and after hyperbaric oxygen therapy

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ABSTRACT

Objective: We describe linezolid tissue penetration in two diabetic patients with lower-extremity ulcers, measured by in vivo microdialysis, before and after hyperbaric oxygen (HBO2) therapy.

Methods: Each diabetic patient received a single orally administered dose of linezolid 600mg within one week of initiating an eight-week HBO2 course for treatment of his or her Wagner Grade 3 lower-extremity wound. A microdialysis catheter was placed at the margin of the wound for collection of extracellular tissue fluid. Blood and tissue samples were collected hourly over the following 12 hours. After completion of HBO2, each patient received a second dose of linezolid 600mg, the microdialysis catheter was reinserted in same location, and blood/tissue samples were recollected for comparison.

Results: Patient 1 completed all eight weeks of HBO2, while Patient 2 completed only five of eight weeks. Based on the 12-hour area under the curve ratio between extracellular tissue fluid and blood, linezolid penetration was 0.474 and 0.479 for Patients 1 and 2, respectively, at the beginning of HBO2. After completing HBO2, penetration improved in both patients to 0.950 and 0.757, respectively.

Conclusion: Tissue concentrations of linezolid at the site of lower extremity ulcers improved following a course of HBO2 in two patients with diabetes.

INTRODUCTION

Treatment of complicated diabetic foot ulcers primarily involves surgical intervention, topical antiseptics and systemic antibiotic therapy if infection is present (1). Linezolid (Zyvox®, Pfizer Inc., New York, N.Y.) is an oxazolidinone antibiotic approved for the treatment of complicated skin and skin structure infections including diabetic foot infections caused by methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus pyogenes or Streptococcus agalactiae. It is used widely to treat infections in this population because of its availability as both intravenous and oral formulations, and its consistent activity against the most common gram-positive pathogens (2). An important characteristic of an effective antibiotic for the treatment of diabetic foot infections is its ability to penetrate to the infection site. This is particularly important since many diabetic patients suffer from peripheral vascular disease, which may limit antibiotic penetration to infected tissues. Studies addressing the tissue penetration of linezolid to the site of infection in diabetics have found disparate results, with reported mean penetration ratios between 0.51 – 1.02 (3, 4).

These studies estimated penetration by collecting tissue specimens at single time points after administration of the dose, a methodology that frequently over- or under-estimates actual concentrations in the extracellular fluid, and which prevents calculation of overall exposure penetration by area under the curve (AUC). The use of in vivo microdialysis has greatly advanced the study of tissue penetration in pharmacology studies because it permits
continuous sampling over a dosing interval and collects only free drug in the extracellular fluid from tissue (5).

The use of hyperbaric oxygen (HBO2) therapy to treat chronic diabetic foot ulcerations has recently received favorable acknowledgement from the Centers for Medicare and Medicaid Services and was recently found to provide a reduction in the number of major amputations associated with these chronically infected wounds (6).

Potential benefits associated with HBO2 include the enhancement of leukocyte-killing activity, bacterial growth suppression in hypoxic tissues, enhancement of antibiotic activity, improvement of tissue repair, and bactericidal effects on anaerobic bacteria (7). Chief effects in diabetic ulcers stem from optimization of the oxygen concentration and the oxygen gradient from wound edge to the hypoxic center, which stimulates fibroblast replication with subsequent collagen deposition.

Importantly, effects on antibiotic penetration to the infected wound have not yet been described, and this may be an additional benefit to the use of HBO2 in diabetic patients. As a basis to justify such a hypothesis, we have described the change in tissue penetration of linezolid as measured by in vivo microdialysis in two patients who received a single oral dose before and after completion of an HBO2 course.

METHODS

Patients

Our hospital’s Investigational Review Board approved the administration of single doses of oral linezolid 600mg and collection of blood and extracellular fluid from tissue by in vivo microdialysis in these patients. Both patients provided written consent to participation prior to linezolid administration. Patients were Type 2 diabetic adults with Wagner Grade 3 diabetic foot ulcers (deeper ulcers with abscess, osteomyelitis or tendonitis extending to those structures) (8) and were candidates to begin a regimen of HBO2 in our hospital’s Wound and Hyperbaric Center.

The hyperbaric protocol used for diabetic foot ulcers in our center involves a two-hour daily hyperbaric treatment administered Monday through Friday for 40 treatment days (eight weeks). The two-hour treatment consists of 10 minutes of compression, 90 minutes of breathing 100% oxygen at 2.2 atmospheres separated by two five-minute air breaks and 10 minutes of decompression.

Study medication

Linezolid 600 mg oral tablets were purchased from the pharmacy department. Linezolid powder for the retrodialysis experiment was supplied by Pfizer Inc. (New York, N.Y.). Each patient received a single dose of oral linezolid 600mg at each sampling visit. The first sampling visit occurred just before or within one week of beginning HBO2, while the second visit occurred within one week after completion of HBO2. Patients were not permitted to eat for one hour before and after the single dose.

Serum and tissue fluid sampling

Prior to administration of linezolid, a CMA 60 microdialysis probe (CMA Microdialysis AB, Solna, Sweden) with a membrane length of 30 mm and molecular cut-off of 20 kDa was inserted into the subcutaneous tissue 10 cm from the margin of the wound via a guidance cannula and advanced toward the wound to avoid spread of infection, if present, to unaffected tissue. A picture of the insertion site was taken with a digital camera so the catheter could be placed in approximately the same location at the post-HBO2 visit.

The microdialysis system was connected and constantly perfused with lactated Ringer’s solution at a flow rate of 2 μL/minute with a CMA 107 microinfusion pump (CMA Microdialysis AB, Solna, Sweden). After a 30-minute baseline priming period, sampling of the interstitial fluid was conducted every hour for the 12 hour dosing interval. Once dialysate sampling was completed, the probe was calibrated by the retrodialysis method over a one-hour interval using a supra-physiologic concentration of linezolid (150 μg/ml) to assess recovery of the antibiotic through the dialysis membrane (5, 9, 10).

In vivo recovery of linezolid via retrodialysis was calculated as follows:

\[
\% \text{Recovery} = 100 - \left( \frac{\text{Concentration}_{\text{dialysate}}}{\text{Concentration}_{\text{perfusate}}} \times 100 \right)
\]

Blood samples were collected simultaneously with microdialysis samples and then immediately centrifuged at 2,000g for 10 minutes to obtain the separated serum fractions. Protein binding studies were conducted on samples collected one hour after the dose by a minimum of three independent tests using Centrifree® Ultrafiltration devices (Millipore Corporation, Billerica, Mass.) with 30 kDa molecular cut-off filters, as per the manufacturer’s package insert.

Linezolid concentrations in serum, dialysate, and ultrafiltrate were assessed by high performance liquid chromatography at the Center for Anti-Infective Research and Development. The lower limit of detection of the assay was 0.2 μg/ml. The interday and intraday coefficient of variability (%CV) was less than 4.4% and 3.2%, respectively.
Pharmacokinetics analyses
The concentration-time profiles of linezolid in serum and extracellular fluid were evaluated visually in both patients. The trapezoidal method was applied to determine the AUC0-12 in serum and extracellular tissue fluid for each patient. The fraction unbound from protein binding studies was applied to the serum concentration data before calculating area under the curve between zero and 12 hours (AUC0-12) (i.e., the dosing interval for linezolid clinically). All microdialysis concentrations were corrected for recovery before AUC calculations as follows:

\[
\text{Concentration}_{\text{tissue}} = 100 \times \left( \frac{\text{Concentration}_{\text{sample}}}{\% \text{ recovery}} \right)
\]

The penetration of linezolid into tissue was calculated by the ratio of AUC_{tissue} / AUC_{serum(free)} for each patient.

RESULTS
Characteristics for both patients at time of sampling and their AUC0-12 results are summarized in Table 1 (above). The concentration-time profiles for total and free corrected linezolid in serum, as well as extracellular tissue fluid concentrations pre- and post-HBO2 are displayed in Figure 1 (Page 14).

Patient 1 was a 62-year-old African-American male with a history of Type 1 diabetes, hypertension, peripheral vascular disease status post-bypass surgery on the right leg, osteomyelitis in the right heel, previous left below-knee amputation, right fifth toe amputation, partial right heel amputation and chronic renal insufficiency. He had received HBO2 intermittently (i.e., three dives) four years prior. On this evaluation, he was prescribed 40 treatments of HBO2 for a non-healing Wagner Grade 3
diabetic foot ulcer at the amputation site of his right fifth toe. Infection was not present. Due to mobility and transportation issues, Patient 1 was scheduled for HBO2 three times per week, but completed all 40 treatments with a positive clinical response on his diabetic foot ulcer.

His first dose of linezolid and sampling was conducted one week after initiation of HBO2 therapy. The penetration of linezolid into his extracellular tissue fluid was 47.4% that of free serum concentrations (Table 1). One week after completion of HBO2, linezolid was readministered. While concentrations in serum were slightly greater than his earlier visit, penetration to the wound site’s extracellular fluid was substantially increased to 95%.

Patient 2 was a 63-year-old, Caucasian female with a history of Type 2 diabetes, congestive heart failure, coronary artery disease status post-coronary artery bypass surgery, breast cancer, gouty arthritis, chronic renal insufficiency, and left foot ulcer with osteomyelitis for approximately five years. On this evaluation, she was prescribed 40 treatments of HBO2 for two non-healing Wagner Grade 3 diabetic foot ulcers with osteomyelitis on her left lateral and plantar foot. Pseudomonas aeruginosa was the infecting pathogen, and she received ciprofloxacin and meropenem to cover this organism.

She received daily HBO2 treatments until Week 5, when she was admitted for wound debridement and
worsening drainage of her open ulcerations. During this admission, her ankle brachial index (ABI) was 0.46 and 0.55, respectively, at her dorsalis pedis artery and posterior tibial artery of the infected limb. As a result, she required revascularization surgery and stopped her HBO2 treatment.

Her first dose of linezolid was administered one week after initiation of HBO2 and penetration was measured as 47.9% (Table 1). Her second linezolid evaluation was done while admitted to the hospital prior to surgery and after only 25 HBO2 sessions. Despite significant arterial occlusive disease in her left lower extremity, linezolid penetration improved to 75.7%.

DISCUSSION
We describe the extracellular tissue fluid penetration of oral linezolid 600mg in two diabetic patients with Wagner Grade 3 ulcers who were undergoing HBO2 therapy. Prior to HBO2, the penetration ratio of linezolid into tissue near the margin of the wound was 0.474 and 0.479 for Patients 1 and 2, respectively.

These observations are not unlike the reported penetration of linezolid into tissue (mean: 0.51; range: 0.183-0.785) of six diabetics undergoing surgical intervention (3). The primary difference between that study and our assessments was the methodology utilized to measure tissue concentrations. Stein and colleagues obtained a single tissue specimen from each patient during surgical interventions at a range of time points over the 12-hour dosing interval, whereas we utilized the in vivo microdialysis procedure to collect extracellular tissue fluid near the margin of the wound continuously over 12 hours. Moreover, in vivo microdialysis permits recovery of only unbound linezolid in the tissues, and thus we utilized free drug-corrected serum concentrations to assess penetration ratios. We also note that our observed tissue penetration ratios in these two diabetic patients with chronic diabetic foot ulcers were lower than ratios reported in healthy volunteers (mean ± SD: 1.4 ± 0.3) and critically ill patients without wound infections (median: 0.896, range: 0.202-1.18), despite similar methodology to collect tissue concentration data (9,10).

A very interesting observation in these patients was the improvement in linezolid tissue penetration after HBO2. Tissue penetration ratios were 0.95 and 0.757, respectively, for patients 1 and 2 after completing eight and five weeks of HBO2. These penetration estimates now more closely resemble the findings in critically ill patients without wound infections (9,10).

As previously mentioned, Patient 2 required revascularization surgery after worsening drainage of her wound and osteomyelitis. It is unknown whether Patient 2’s penetration ratio would have improved more had she completed her fully prescribed HBO2 course, but it is notable that her penetration improved after only five weeks of HBO2 therapy despite needing revascularization surgery.

Increasing antibiotic penetration to the infection site would be a yet-undescribed and paramount benefit of HBO2 for diabetic patients with lower-extremity wounds. Potential physiologic basis for our observations could be a balance of:

1) revascularization of hypoxic tissue after HBO2, which would increase blood flow and antibiotic concentrations in the area of the ulcer (7), and;
2) HBO2’s mobilization of bone marrow-derived endothelial progenitor cells as well as leukocytes to the affected area (11), which could carry an antibiotic like linezolid that has high intracellular concentrations to the site.

These hypotheses require further validation.

A previous study evaluating linezolid concentrations in tissue of 15 diabetic patients identified several clinical and laboratory markers that correlated with linezolid tissue penetration (4). Majcher-Peszynska and colleagues observed significant correlations for penetration with increasing C-reactive protein, body temperature and decreasing serum albumin. C-reactive protein is not routinely measured in our patients; however, both patients had normal (and very similar) tympanic membrane temperatures on the day of sampling, so this could not be evaluated as a potential explanation for penetration changes. Serum albumin was not measured on the day of sampling but was also in the normal range prior to beginning HBO2 for both patients.

Importantly, Majcher-Peszynska and colleagues also noted no correlation between tissue penetration of linezolid and the transcutaneous partial pressure of oxygen (TcPO2). TcPO2 is also not routinely measured in our patients, but the ABI is. Prior to beginning HBO2, Patient 1 had vascular studies that demonstrated hardened, calcified vessels; thus the ABI could not be calculated. Patient 2 had a left-side ABI of 0.55 prior to resampling of linezolid concentrations, and of note, this was increased to 1.08 after her left popliteal atherectomy.

Although we do not have sufficient ABI measurements to postulate any associations with penetration, a previous study by our group comparing healthy volunteers to diabetics demonstrated no association of the ABI and penetration of a different antibiotic,
daptomycin (12). Future studies assessing penetration in diabetic patients should further attempt to quantitate the level of peripheral vascular disease so that an association with tissue penetration, if present, can be explained.

We were not able to characterize linezolid serum pharmacokinetics in both patients, particularly because Patient 1 had a prolonged absorption profile, which occurred during both sampling sessions (Figures 1a and 1b). Although not reported in his medical history, we speculate this patient has diabetic gastroparesis. Nonetheless, his tissue concentration profile mimicked the curve observed in serum.

Patients 1 and 2 also had substantially different protein binding estimates for linezolid. Linezolid protein binding has been typically reported as 30% in studies of healthy volunteers (13), but can be quite variable and generally lower (7% to 15%) in patients (9,10). Patient 1’s protein binding (i.e., 46%) was among the highest we have observed, but his estimated free drug concentrations using these values were consistent with his observed concentrations at the wound site as measured by the microdialysis catheter.

These observations demonstrate the wide variability in linezolid concentration time profiles that can be apparent in the diabetic population with multiple comorbidities. A limitation to our analysis is that penetration was evaluated only after a single oral dose and not at steady-state (two to four doses for linezolid). Penetration may be greater at steady-state when equilibrium with tissue is achieved. Although our methodology did not permit assessment of steady-state exposures, tissue concentrations in both patients post-HBO2 remained above the minimum inhibitory concentrations for most gram-positive bacteria by the end of the dosing interval (2).

In summary, extracellular tissue fluid exposures for oral linezolid were approximately half that of blood in these two diabetic patients with severe lower-extremity wounds prior to HBO2. After treatment with HBO2, an increase in penetration resulted in greatly improved antibiotic tissue concentrations at the site of infection. Further research into the additional pharmacologic benefits of HBO2 on antibiotic therapy is required.

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