The In Vitro Elution Characteristics of Antifungal-loaded PMMA Bone Cement and Calcium Sulfate Bone Substitute

CHRISTOPHER GRIMSRUD, MD, PHD; RAYMOND RAVEN, MD; A.W. FOTHERGILL, MA, MBA; HUBERT T. KIM, MD, PHD

abstract

Full article available online at ORTHOSuperSite.com. Search: 20110627-05

The use of antimicrobial-loaded delivery vehicles, most often as antibiotic beads, is common practice for the treatment of deep musculoskeletal infections. The elution of antibacterial drugs from various bone cements has been extensively studied. However, much less is known about the elution of other antimicrobials from these materials. In particular, the use of this approach for fungal infections has not been well studied despite growing concern about these difficult-to-treat organisms. Voriconazole is a broad-spectrum and highly effective antifungal that has been used in the treatment of resistant fungal pathogens. We examined the in vitro elution characteristics of voriconazole from nonabsorbable polymethylmethacrylate (PMMA) beads and from absorbable calcium sulfate beads. Voriconazole-containing beads were immersed in a 5-mL bath of phosphate-buffered saline at room temperature and placed on an orbital shaker. Eluent samples were collected over the course of 2 weeks. Concentrations of the antifungal drug in solution were measured using high-performance liquid chromatography. To verify biologic activity of the eluted antifungal, collected samples were also tested against control yeasts. We found that samples collected out to 2 weeks contained relatively high voriconazole concentrations and enough active antifungal activity to inhibit growth of the control yeasts. These data demonstrate that voriconazole retains its antifungal activity when mixed into either PMMA or calcium sulfate beads, and elutes out of beads at biologically effective concentrations over a time period of at least 2 weeks. Therefore, incorporation of voriconazole into either absorbable or nonabsorbable beads appears to be a reasonable strategy for the local delivery of a potent, broad-spectrum antifungal agent to an infected wound bed.

Figure: Rate of voriconazole elution. Abbreviation: PMMA, polymethylmethacrylate.
Surgical treatment of orthopedic infections includes debridement of the infection, irrigation of the wound, management of any dead space created by debridement, and delivery of antibiotics to treat the infection. Antibiotic-loaded polymethylmethacrylate (PMMA) bone cement was developed as a way to deliver locally high concentrations of antibiotics to the site of infection and is now commonly used in the treatment of open fractures, osteomyelitis, and infected total joint prosthesis. The antibiotic used in the cement can be tailored to the infecting organism, but the antibiotic must be heat stable to survive the polymerization process of the cement. The bone cement can be fabricated into beads or monoblock spacers to occupy the dead space in treating musculoskeletal infections. Recently, the technique has been modified by using antifungal rather than antibacterial drugs to treat fungal osteomyelitis.

It was previously believed that antifungals such as amphotericin B were heat unstable and therefore could not be used in bone cement due to the exothermic process of polymerization. However, Selmon et al. reported a successful 1-stage exchange total knee arthroplasty for fungal infection using amphotericin B-loaded bone cement. Subsequently, an in vivo study by Marra et al. found high levels of the drug in wound drainage after using amphotericin B-loaded bone cement to successfully treat osteomyelitis due to Candida albicans. Notwithstanding the successful outcome of these case reports, neither of these 2 studies confirmed antifungal activity in the eluent.

Antimicrobial-loaded bone substitute products such as calcium sulfate beads have been used in the treatment of osteomyelitis and may offer advantages when compared with PMMA. Polyethylene methacrylate bone cement is well tolerated, but a second surgical procedure is generally performed to remove the cement after the antibiotic has eluted. Antibiotic-loaded calcium sulfate beads also fill dead space and may elute high concentrations of local antimicrobials. However, calcium sulfate beads are bioabsorbable; therefore, a second procedure to remove them is unnecessary.

Voriconazole is a highly potent broad-spectrum antifungal drug. It has significantly less nephrotoxicity than amphotericin B and is overall better tolerated by patients. It has been effective in treating resistant fungal pathogens when administered systemically. To our knowledge, no studies have investigated the use of antifungal-loaded bone substitute products or voriconazole-loaded vehicles. This article describes the elution characteristics of voriconazole from PMMA and from calcium sulfate beads.

**MATERIALS AND METHODS**

For the in vitro elution, we generally followed the technique of Miclau et al. Palacos antifungal-loaded PMMA bone cement beads (Biomet, Warsaw, Indiana) were prepared by combining voriconazole 200 mg with 3200 mg inert carrier into 10 g of PMMA (one-quarter box powder). One-quarter vial (4.5 mL) of liquid monomer was added to allow the cement to polymerize. Osteoset antifungal-loaded calcium sulfate beads (Wright Medical Technology, Inc, Arlington, Tennessee) were prepared by combining 200 mg voriconazole (plus 3200 mg inert carrier) with one 10-cc package of Osteoset in a 1:10 ratio, by weight.

Analysis

Antifungal drug concentration was determined by high-performance liquid chromatography using standard methodology as described previously and is reported in μg/mL.

**Susceptibility Testing**

Control yeasts were tested to determine minimum inhibitory concentrations to all antifungals evaluated. Methods outlined in National Committee for Clinical Laboratory Standards document M27-A, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, were used.

**RESULTS**

The rate of voriconazole elution from the beads was analyzed (Figure). Initially, both types of beads eluted high concentrations of voriconazole. The rates decreased before 48 hours and remained relatively constant to 2 weeks. The rate of elution was similar from both types of beads.

**DISCUSSION**

The elution of antibacterials such as tobramycin and vancomycin from bone ce-
ments has been well studied. However, few data have been published on the elution of antifungals from bone cements.

Our study shows that the broad-spectrum antifungal voriconazole elutes out of beads made of either resorbable calcium sulfate or nonresorbable PMMA. The levels appear to increase rapidly and then stabilize in the tissue culture dishes. Measurement of the rate of voriconazole elution shows that the beads continue to elute voriconazole even at 2 weeks after implantation. From a clinical point of view, the eluted antifungal will be washed out of the wound by body circulation. The critical factor is the rate of elution. We have shown a relatively constant rate of voriconazole elution at a 2-week time point. It is unknown how much longer the voriconazole will continue to elute from the beads.

Importantly, voriconazole retains biologic activity in the calcium sulfate and PMMA beads. During polymerization, the PMMA beads may become hot, up to 70°C. This does not appear to significantly alter the biologic activity or the ability of the drugs to be detected by high-performance liquid chromatography.

Placement of antibiotic cement at the site of infection allows for the local delivery of locally high concentrations of the antifungal without the side effects of parenteral administration. Relatively few data have been published on the treatment of fungal infections with antifungal beads. The outcomes of treating bacterial infections with surgical debridement and the short-term placement of antibiotic-loaded cement have been significantly better than surgical treatment alone. This technique has become the standard of care in treating bacterial infection of total joint replacements. In these cases, the total joint replacement is removed and a heat stable antibiotic in powder form, usually vancomycin and/or tobramycin, is mixed into the cement. After mixing the powder and liquid monomer, the cement has a doughy phase during which the cement is formed into a spacer that is placed in the cavity left by removal of the prosthesis. The wound is closed and the patient is treated with parenteral antibiotics. If signs of infection have resolved at the end of 6 weeks, a new prosthesis is placed and the patient is rehabilitated.

Fungal osteomyelitis is an uncommon problem but may be a serious threat to life and limb. Generally, patients who develop fungal osteomyelitis or fungal prosthetic joint infections are either immunosuppressed or live in areas such as California’s San Fernando Valley, where a fungus such as coccidioidomycosis is endemic. Treatment of the fungal infection has been limited to standard surgical debridement and parenteral antibiotic therapy, usually with amphotericin B, a particularly toxic drug. The use of amphotericin B-loaded local delivery vehicles has been popularized by 2 recently published case reports. A subsequent in vitro study described antifungal activity of the eluent isolated from amphotericin B-loaded PMMA beads.

Bone graft substitutes such as calcium sulfate and calcium phosphate have provided the orthopedic surgeon with a viable alternative to autogenous bone. These materials effectively mimic the mineral phase of bone and are thus able to provide some structural support and prevent ingrowth of fibrous tissue while facilitating creeping substitution by the host bone. They are also resorbed at a rate similar to the rate of bone formation. The most commonly used antibiotics delivery system for the treatment of deep musculoskeletal infection uses PMMA beads as a drug delivery vehicle. However, the nonbiodegradable nature of the PMMA necessitates a second operation to remove the beads. Several studies have investigated the use of bone graft substitutes impregnated with antibiotics.

The results of our study indicate that a bioactive level of antifungal is present in the eluent of PMMA and calcium sulfate beads loaded with voriconazole. This provides a scientific basis to clinically trial voriconazole-loaded beads in the treatment of resistant fungal osteomyelitis.

REFERENCES


