Hepatic Mucormycosis in a Bone Marrow Transplant Recipient Who Ingested Naturopathic Medicine

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This case report describes a bone marrow transplant recipient in whom hepatic zygomycosis developed after ingestion of multiple naturopathic medicines. *Mucor* was isolated from the patient's liver aspirate and from one of the naturopathic medicines. Arbitrary-primed polymerase chain reaction (PCR) analyses were performed on the *Mucor* isolates from the patient's liver aspirate and from his naturopathic medicine to see if they were genotypically related. *Mucor indicus* was the species identified in both the patient's liver aspirate and the naturopathic medicine. Arbitrary-primed PCR analysis revealed that these isolates were genotypically identical. We conclude that this bone marrow transplant recipient acquired hepatic mucormycosis from ingestion of a naturopathic medicine containing *Mucor*.

Zygomycosis is an infection due to fungi of the class Zygomycetes. *Rhizopus, Absidia,* and *Mucor* are the genera most often implicated in human disease. They are ubiquitous molds found in soil and organic matter worldwide [1]. Infections occur predominantly in patients with malignancy, drug-induced immunosuppression, or chronic illnesses such as diabetes or renal failure [2]. Infection is thought to occur via inhalation, ingestion, or skin inoculation of sporangiospores, which, in the proper host environment, reproduce asexually. Hyphae have a propensity for vasculature and spread locally across tissue planes with thrombosis and necrosis [3]. Dissemination to distant organs, presumably via the bloodstream, can also occur [4].

At least five clinical entities of infection with *Mucor,* including rhinocerebral, pulmonary, cutaneous, gastrointestinal, and disseminated diseases, have been well described [5, 6]. Hepatic infection is usually seen with pulmonary or gastrointestinal infection, although multiple other organs may be involved [4], and is considered part of disseminated disease. In this report we describe a bone marrow transplant recipient in whom isolated hepatic mucormycosis developed after ingestion of several naturopathic medicines. Identical strains of *Mucor* were isolated from the patient's liver aspirate and from one of the naturopathic medicines. We propose that this patient acquired his infection from the naturopathic medicine contaminated with *Mucor*.

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Case Report

A 39-year-old man underwent matched sibling bone marrow transplantation (BMT) in May 1993 because of myelodysplastic syndrome. He received a previously described preparatory regimen for BMT of busulfan, cyclophosphamide, and 14-Gy total-body irradiation [7]. Prophylaxis for graft-vs.-host disease (GVHD) consisted of intravenous cyclosporine and 0.5–1.0 mg/kg·d of methylprednisolone sodium succinate (Solu-Medrol, Upjohn, Kalamazoo, MI). His initial hospital course was uncomplicated. Engraftment, defined as an absolute neutrophil count of $>500/mm^3$, occurred on day 12. Steroid therapy was completely discontinued by July 1993. Beginning in late July 1993, recurrent bouts of mild skin GVHD and gastrointestinal GVHD, documented by upper endoscopy, prompted institution of therapy with prednisone (doses ranging from 10 to 60 mg/d).

Five months after BMT, he began taking multiple oral herbal remedies including purified Reischi mushrooms, ginseng and seaweed extracts, and concentrated *Lactobacillus* bacteria (PB-8; Nutrition Now, Portland, OR). In December 1993 (7 months after BMT), he complained of new right-upper-quadrant pain. His bilirubin level had increased from 2 to 3.8 mg/dL. An abdominal CT revealed three well-circumscribed low-attenuation liver lesions (figure 1). Many hyphae were seen in cloudy yellow fluid obtained by percutaneous fine-needle aspiration of one of these lesions. Fungal growth occurred in <24 hours, and mucor infection was diagnosed within 3 days. Therapy with amphotericin B (1 mg/[kg·d]) was started. Sinus and chest CTs revealed no abnormalities.

A repeated abdominal CT 2 weeks later showed progressive hepatic and renal dysfunction and markedly increased sizes of the hepatic lesions, and his therapy was switched to amphotericin B lipid complex (ABLC [5 mg/(kg·d)]; The Liposome Company, Princeton, NJ) in January 1994. Moderate GVHD of the liver was diagnosed by percutaneous biopsy, and his prednisone dosage was again increased to 60 mg/d. Percutane-

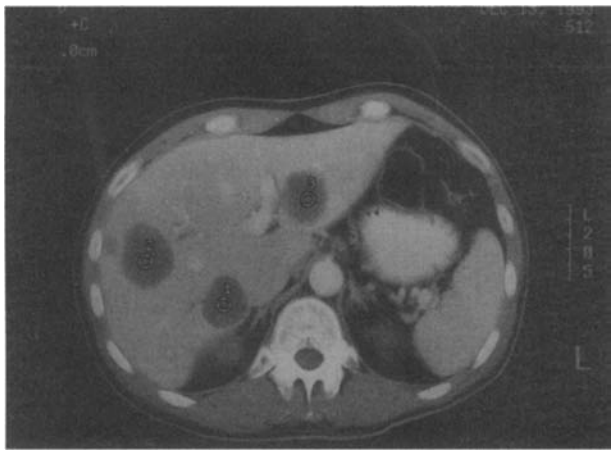

Figure 1. CT of the abdomen of a bone marrow transplant recipient with hepatic mucormycosis; the scan demonstrates three well-circumscribed low-attenuation liver lesions ranging in size (greatest diameter) from 2 to 4 cm.

ous drainage under ultrasound guidance of two of the abscesses was performed on two separate occasions; 100–200 mL of cloudy yellow fluid was removed. Many hyphae characteristic of *Mucor* were again seen, but cultures of the fluid remained negative.

The patient's condition stabilized clinically. Hepatic and renal function improved slowly. Serial CTs performed every 2 weeks revealed that the hepatic lesions remained unchanged. Prednisone therapy was completely discontinued by late March 1994. ABLC treatment was stopped for the first 8 days in April 1994 because of infusion-related fevers and chills, which were the only toxic effects attributed to ABLC therapy.

In early April 1994 (11 months after BMT), he again had right-upper-quadrant pain. An abdominal MRI revealed extension of the most lateral hepatic lesion to the diaphragm and abdominal wall. The lesion was surgically resected. Many hyphae consistent with *Mucor* and moderate GVHD of the liver were again identified. Therapy with ABLC (5 mg/[kg · d]) and prednisone (60 mg/d) was reinstituted. Flucytosine (1,200 mg/d) was added to the therapeutic regimen for additional fungal coverage.

Again the patient's condition improved slowly. The total bilirubin level decreased from a high of 25 mg/dL in January 1994 to 8.5 mg/dL in June 1994, the aspartate aminotransferase level decreased from 298 to 144 mg/dL, and the alkaline phosphatase level decreased from 888 to 346 mg/dL. Serial MRIs through the last follow-up in October 1995 revealed that the size of the hepatic lesions remained unchanged. Abdominal pain has resolved, and his appetite has improved.

Methods

Fungal Isolation and Identification

Pills or capsules from 10 different naturopathic medicines obtained from the patient were rehydrated in sterile water, plated onto potato flake agar, and then incubated at room temperature. Fungi were isolated from four of the 10 medicines. *Aspergillus* species were identified in two medicines, and a *Rhizopus* species was identified in one medicine. These species were not characterized further. Two molds were recovered from one pill of concentrated *Lactobacillus:* a small amount of an *Aspergillus* species and a heavy growth of a fungus with broad nonseptate hyphae resembling *Mucor,* which was subcultured for further testing and designated as fungal isolate 3. There were four control fungal isolates: 1 and 2 were isolated from a patient with rhinocerebral zygomycosis who was treated at the University of Washington (Seattle), 4 was isolated from a fine-needle aspirate of a liver abscess in our patient (culture of this aspirate yielded pure growth of a mold resembling *Mucor*), and 5 was recovered from a patient with an independent case of rhinocerebral zygomycosis. Species identification of isolates 1–5 was also performed at the Fungus Testing Laboratories, University of Texas Health Science Center (San Antonio, TX) by standard mycologic procedures [8].

DNA Extraction and PCR Analysis

Fungal isolates 1–5 were tested by arbitrary-primed PCR analysis. The isolates were grown on slants of potato dextrose agar, scraped into cold fungal extraction media (1% CTAB [cetyltrimethylammonium bromide], 1.4 *M* NaCl, 100 m*M* Tris [pH, 8.0], and 20 m*M* EDTA), sonicated for 15 seconds with a probe sonicator, heated to 55°C for 20 minutes, and centrifuged at 14,000*g* for 5 minutes; the supernatant was extracted with 1 vol of chloroform. After ethanol precipitation, the DNA was purified by means of adherence to glass powder (Geneclean II; Bio 101, La Jolla, CA) as described by the manufacturer. The DNA concentration was estimated by ethidium bromide staining. PCR analysis was performed on two different isolations and with two concentrations of DNA per sample (i.e., 10 ng per tube and 1 ng per tube); in each case the same results were obtained. PCR analysis was carried out in 50-μL volumes as described previously [9], except that one oligonucleotide primer was present per reaction (1 μM concentration) and the cycling conditions were 40 cycles (94°C for 1 minute, 36°C for 1 minute, and 72°C for 1 minute 30 seconds). Ten microliters of the reaction product was run on a 2% agarose gel and stained with ethidium bromide. The following oligonucleotide primers were used in separate reactions: *A*, 5′-AGC CAC TGA GCC AAT TAT TC-3′; *B*, 5′-CGA CCA TGC TCC TCC ATC-3′; and *C*, 5′-CYC TAGA AGG GCY CTG AAA GG-3′.

Results

Species Identification

Isolates 1 and 2 were identified as *Mucor circinelloides,* isolates 3 and 4 were identified as *Mucor indicus,* and isolate 5 was identified as *Mucor plumbeus.*

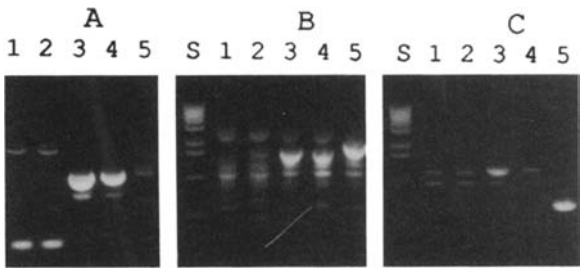

Figure 2. Fingerprints of fungal isolates from naturopathic medicine and from a bone marrow transplant recipient with hepatic mucormycosis that were obtained by arbitrary-primed PCR analysis; the fingerprint of the medicine's isolate was identical to that of the patient's isolate. The products of arbitrary-primed PCR analysis were run on a 2% agarose gel and stained with ethidium bromide. Templates for each reaction were DNA recovered from isolates 1 and 2 (from a patient with rhinocerebral mucormycosis who was treated at the University of Washington, Seattle; the isolates were stored separately for several years at two different mycology laboratories), isolate 3 (from the naturopathic medicine of the patient), isolate 4 (from the liver abscess of the patient), and isolate 5 (from a patient with an independent case of rhinocerebral mucormycosis). The results from testing with three different randomly chosen oligonucleotides (*A*, *B*, and *C* [which were defined in the text]) are shown. *Lane S* (in *B* and *C*), the 1-kb DNA ladder standard (GIBCO-GRL, Gaithersburg, MD).

Arbitrary-Primed PCR Analysis

Arbitrary-primed PCR analysis has been shown to give both strain-specific and species-specific patterns and is a recognized method of differentiating fungal isolates [10, 11]. Arbitrary-primed PCR analysis was used in an attempt to establish whether the strain of *Mucor* from the patient's naturopathic medicine was identical to that from the patient's liver abscess. The five strains of *Mucor* discussed in the Methods section were studied. Figure 2 shows that the fingerprints obtained by arbitrary-primed PCR analysis with three different random oligonucleotide primers were identical for isolates 3 and 4, but these fingerprints differed from those of isolates 1, 2, and 5. As expected, the banding patterns of isolates 1 and 2 were similar, but these banding patterns differed dramatically from that of isolate 5. These results strongly suggest that the isolate from the naturopathic medicine is identical at the DNA level to the isolate from the liver abscess.

Discussion

Zygomycosis is an uncommon infection in bone marrow transplant recipients [12]. Hepatic infection is typically described in association with other organ involvement [2, 4–6]. To our knowledge, this is the first report of isolated hepatic zygomycosis and the second report of human infection due to *M. indicus* [13]. We describe a bone marrow transplant recipient who, several weeks after ingesting a naturopathic medicine containing viable *Mucor,* had multiple hepatic abscesses from which an identical strain of *Mucor* was isolated. The isolates from both the pills and the liver abscess were identified as *M. indicus.* Arbitrary-primed PCR analysis with three different primers revealed identical banding patterns in all three instances, thereby strongly suggesting that the two isolates are genotypically identical. We propose that ingestion of this naturopathic medicine contaminated with *Mucor* was the initial source of our patient's infection.

The phenomenon of fungal persorption was documented in 1969 by Krause et al. [14]; Krause became fungemic after ingesting a concentrated *Candida albicans* slurry. Hepatic infection presumably occurred in our patient by *Mucor* seeding the bloodstream through gastrointestinal mucosa previously damaged by GVHD. Immune dysfunction due to BMT, GVHD, and chronic steroid use are known risk factors for invasive zygomycosis [15]; these factors probably contributed to the ability of the infection to establish itself in the liver of our patient.

In summary this report documents hepatic infection due to *Mucor* in a bone marrow transplant recipient after ingestion of a naturopathic medicine containing this fungus. In addition, it demonstrates the usefulness of arbitrary-primed PCR analysis as an epidemiologic tool.

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