***Aspergillus* Osteomyelitis in a Child Who Has p67-phox-deficient Chronic Granulomatous Disease**

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Summary: Here we describe *Aspergillus* osteomyelitis of the tibia in a 9-year-old boy who has an autosomal recessive form of chronic granulomatous disease (CGD). The patient showed a p67-phagocyte oxidase (phox) deficiency, which is rare type of CGD in Japan. The initial treatment which consisted of surgical debridement and antibiotic therapy with amphotericin B (AMPH), did not control the infection. *Aspergillus fumigatus* (*A. fumigatus*) pure isolated from drainage fluid and necrotic bone tissue demonstrated less susceptible to antifungal agents, including AMPH, fluconazole and flucytosine. Recombinant interferon gamma was then administrated, and it was effective in controlling the course of severe invasive aspergillosis. This report indicates the use of interferon gamma might be helpful in control for *Aspergillus* osteomyelitis of the tibia in a child with CGD demonstrated p67-phox deficiency refractory to conventional therapy with AMPH.

Key words *Aspergillus* osteomyelitis, chronic granulomatous disease, p67-phox deficiency, interferon gamma, itraconazole

CASE REPORT

A 9-year-old boy presented with an autosomal recessive form of CGD of childhood. He had had recurrent infections since the newborn period; Staphylococcal infections of perineal tissue and acute otitis media were often observed. He had been receiving prophylactic treatment with oral trimethoprim-sulfamethoxazole since he was diagnosed. Immunologic evaluation revealed decreasing oxygen consumption of his neutrophils with normal phagocytosis and chemotaxis. The diagnosis of autosomal recessive CGD was confirmed when he was found to have detection of cytochrome b and an absence of p67-phox of the oxidative pathway.

At the initial examination, the patient developed fever and had a painful swelling without local warmth or erythema at the proximal of the right tibia. Radiographs revealed an osteolytic lesion in the proximal metaphysis of the right tibia (Fig. 1). The

patient was admitted to our hospital at this time with the diagnosis of acute osteomyelitis. Drainage was performed and broad-spectrum antibiotic therapy was initiated.

His laboratory data at admission were as follows: WBC was 14,700/ μ L with 61% polymorphonuclear leukocytes and 4% band forms; hemoglobin concentration, 11.7 g/dL; and platelet count, $65.0 \times 10^4/\mu$ L. The erythrocyte sedimentation rate (ESR) was 49 mm/h and CRP was 10.5 mg/dL. Bacterial cultures of the drainage fluid yielded a mold after 48 hours of incubation and fluconazole was added. It changed to AMPH of initial dose (0.5 mg/kg/day) after *A. fumigatus* was identified. The dose was gradually increased to 1.0 mg/kg. However we had to decrease the dose to 0.65 mg/kg, three times a week, because he complained of high fever, anemia and hypokalemia were noticed in his laboratory findings.

Operative debridements were performed two weeks after admission because clinical response to

Fig. 1. Initial radiograph of the right tibia. Osteolytic lesion (arrow) was noted at the proximal metaphysis.

antifungal treatment was not observed and the culture from the necrotic bone tissue remained positive for *A. fumigatus*. Radiographic examination of the right tibia showed increased bone destruction extending approximately two-thirds of the way down the shaft in seven weeks after administration of the antifungal therapy (Fig. 2), and ESR remained elevated. Bone scintigraphy showed evidence of increased uptake only in the right tibia consistent with an inflammatory lesion.

We started the administration of human interferon gamma (25×10^4 Japan Reference Unit /M², given subcutaneously, three times a week) as destruction of the bone was progressive. After approximately two weeks of adjunct therapy with interferon gamma, the bone destruction on radiographs and his ESR were improved correspondingly. There was remarkable improvement in radiographic findings after eight weeks of the treatment.

In nine weeks after administration of interferon gamma therapy, a painful swelling with local erythema appeared again at the same region of the right tibia, but erythrocyte sedimentation rate and CRP were within normal range. He underwent a second operative debridement of the lesion and purulent

Fig. 2. Before administration of interferon gamma, radiograph showed the deterioration of bone destruction extending approximately two-thirds of the way down the shaft of the tibia after seven weeks of AMPH treatment.

material was not found. The subsequent culture of the drainage fluid was sterile. Therefore it was thought that patients with chronic granulomatous disease had abnormal inflammatory responses that resulted in granuloma formation.

Using a microbroth dilution method [1], minimum inhibitory concentrations (MICs) of *A. fumigatus* isolated from this patient to five antifungal agents were performed; MICs of itraconazole, AMPH and miconazole were 0.78 $\mu\text{g/mL}$, 3.13 $\mu\text{g/mL}$ and 6.25 $\mu\text{g/mL}$, respectively and those of fluconazole and flucytosine were both more than 100 $\mu\text{g/mL}$. AMPH was changed to itraconazole because of good activity to the isolated strain. The treatments of interferon gamma and itraconazole had been continued for one year with good clinical course and there was no adverse reactions.

DISCUSSION

CGD is an uncommon inherited disease characterized by recurrent and life-threatening bacterial and fungal infections that usually begin early in life. This is caused by the abnormality of a superoxide generating system, known as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, present in neutrophils and other phagocytes. It has become apparent that the oxidase is a complex multicomponents enzyme system that includes a heteromeric membrane-bound cytochrome b (cytochrome b558), consisting of a 91-Kd glycoprotein subunit (gp91-phox) and a 22-Kd subunit (p22-phox), and three cytosolic components; two of these are specific for the oxidase, a 47Kd and a 67Kd protein (p47-phox and p67-phox, respectively) [2], and small G-protein from the rac family [3]. The p67-phox-deficient CGD accounts for about 5% in Europe and America, and about 6% in Japan [2,4]. A more mild clinical course among patients with autosomal recessive cytochrome b558 positive type has been reported [5].

In CGD children, chest infections, lymphadenitis and dermatitis were the most frequent infections, and osteomyelitis was seen in 31.3% of the patients [6]. *Staphylococcus aureus* has been the most frequent pathogen and enteric bacilli such as *Klebsiella* species, *Escherichia coli*, *Serratia marcescens* and *Salmonella* spp. also have been recognized as being responsible for frequent and severe infection. A retrospective analysis of the occurrence of bacterial infections in patients who received trimethoprim-sulfamethoxazole as prophylaxis indicate a marked reduction in episodes of bacterial infection and

confirmed that the prognosis for long term survival is better than those suggested in earlier reports [7,8]. In contrast, fungal infections, in particular those caused by *Aspergillus* species, remain frequent and are often fatal [9]. In a review of 245 cases of CGD patients, fungal infections were documented in 20.4% of the patients [10]. *Aspergillus* species infections were most common (78.4%) and the species most common implicated in disseminated aspergillosis is *A. fumigatus*. The vertebrae and ribs were the most common focus of *Aspergillus* osteomyelitis, resulting from contiguous spread from a pulmonary infection in CGD children, and distant long-bone osteomyelitis due to *A. fumigatus* had been rarely described [11,12].

Previous reports on the therapy of *Aspergillus* osteomyelitis recommended that surgical removal of infected tissue, antifungal agents and granulocyte transfusions which could not be proven to be of definite therapeutic benefit [11]. AMPH is the cornerstone of antifungal therapy, however side effects are common and case fatality rates of 50-75% have been reported in patients with *Aspergillus* osteomyelitis of CGD treated with AMPH [13]. The poor results may be due to low penetration of the agent into bone tissue. In addition, *A. fumigatus* commonly demonstrates in vitro resistance to antifungal agents including AMPH [11,14]. We had to decrease the dosage of the agent because of its side effects and the serum concentration of AMPH might not have achieved at the MIC value to *A. fumigatus*. Several reports suggested that itraconazole might be a better therapeutic alternative for invasive aspergillosis because of its high tissue affinity [15,16]. At the time of the discontinuation of AMPH, we began the administration of itraconazole for maintenance therapy and had continued taking the drug for one year without any adverse effects.

The effects of interferon gamma therapy in CGD patients have been reported [17-20]. Completed multicenter, double-blind, placebo-controlled randomized phase III study in 128 CGD patients performed based on in vitro and in vivo studies indicates that interferon gamma can partially correct the metabolic defect in phagocytes and is an effective and well-tolerated treatment on reducing the frequency of serious infections in patients with CGD [21]. The ability of interferon gamma to enhance or prime the oxidase or normal human phagocyte cells in vitro and thereby augment O_2^- production in response to a variety of stimuli has been extensively studied [22], however the precise mechanism of action remains uncertain [23-25]. On the basis of these studies for

interferon gamma therapy, we began to use it for a child in whom AMPH could not prevent progressive osteomyelitis with p67-phox-deficient CGD and it seemed to be effective in controlling the course of invasive aspergillosis. The mechanism of action of interferon gamma in patients of CGD remains unknown and still more evaluations on the efficacy for patients with CGD might be needed.

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