

High plasma concentration of beta-D-glucan after administration of sizofiran for cervical cancer

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Abstract: A 69-year-old woman with a history of cervical cancer was admitted to our hospital for further investigation of abnormal shadows on her chest roentgenogram. Histologic examination of transbronchial lung biopsy specimens revealed epithelioid cell granuloma, and *Mycobacterium intracellulare* was detected in the bronchial lavage fluid. The plasma level of (1→3)-beta-D-glucan was very high, and this elevated level was attributed to administration of sizofiran for treatment of cervical cancer 18 years previously. Therefore, in patients with cervical cancer, it is important to confirm whether or not sizofiran has been administered before measuring (1→3)-beta-D-glucan levels.

Keywords: (1→3)-beta-D-glucan, cervical cancer, *Mycobacterium intracellulare*, sizofiran

Introduction

(1→3)-beta-D-glucan is a characteristic cell wall component of almost all fungi. The measurement of the plasma concentration of this glucan is clinically useful when screening for invasive fungal infections or fungal febrile episodes.¹ In addition, this glucan is known to stimulate humoral and cell mediated immunity in humans, and has antitumor effects.² High plasma concentrations of (1→3)-beta-D-glucan may occur even in the absence of a fungal infection because of the administration of sizofiran, an antitumor (1→3)-beta-D-glucan preparation (SPG; Kaken Pharmaceuticals, Tokyo, Japan). We report an extremely rare case of a patient with elevated plasma concentrations of (1→3)-beta-D-glucan 18 years after she was treated with sizofiran for cervical cancer.

Case report

A 69-year-old woman was admitted to our hospital in November 2007 for further examination of abnormal shadows in both lungs. She was a nonsmoker and had no history of occupational exposure. She had undergone hysterectomy in 1990 and was administered SPG for six months thereafter. Physical examination revealed that she had no skin lesions or neurologic abnormalities. There was no peripheral lymphadenopathy in the cervical, axillary, or inguinal region. Fine crackles were audible in the middle lung fields bilaterally.

The white blood cell count was $6.2 \times 10^9/L$, with 56.6% neutrophils, 34.3% lymphocytes, 5.2% monocytes, 3.2% eosinophils, and 0.7% basophils. The level of C-reactive protein was normal. Her fasting blood glucose level was 149 mg/dL and Hb_{A1c} was 6.8%. The serum concentration of immunoglobulin G (IgG) was elevated

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to 2034 mg/dL (normal, 837–1825 mg/dL), and the serum IgM and IgA levels were within the normal range. The level of (1→3)-beta-D-glucan was elevated to 260 pg/mL (cutoff value, 11 pg/mL) and the plasma endotoxin level was normal. *Aspergillus* antigens (Platelia® *Aspergillus* EIA; Bio-Rad, Marnes-la-Coquette, France) and *Candida* antigens (Cand-Tec; Ramco Laboratories, Inc., Houston, TX.) were absent. There was no liver and kidney dysfunction. A chest roentgenogram showed infiltrative shadows in both lungs (Figure 1). Chest computed tomography revealed centrilobular nodules and patchy infiltrative shadows. Bronchiectasis was observed in the upper lobes of both lungs and in the middle lobe of the right lung (Figure 2). There was no radiographic evidence of infection, purulence, or tumors of the abdominal or pelvic organs. The areas of intense fluorodeoxyglucose activity detected by ¹⁸FDG-positron emission tomography coincided with the infiltrative shadows detected by computed tomography. Histologic examination of transbronchial lung biopsy specimens obtained from the right upper lobe revealed epithelioid cell granuloma (Figure 3). *Mycobacterium intracellulare* was detected in the bronchial lavage fluid, but *Aspergillus* spp, *Candida* spp, and *Pneumocystis jiroveci* were not detected. There was no evidence of systemic fungal infection in this patient. Therefore, the patient was diagnosed with pulmonary *M. intracellulare* infection. However, the cause of the high plasma concentration of (1→3)-beta-D-glucan remained unknown. Because the possibility of



Figure 1 Chest roentgenogram showing infiltrative shadows in both lungs.

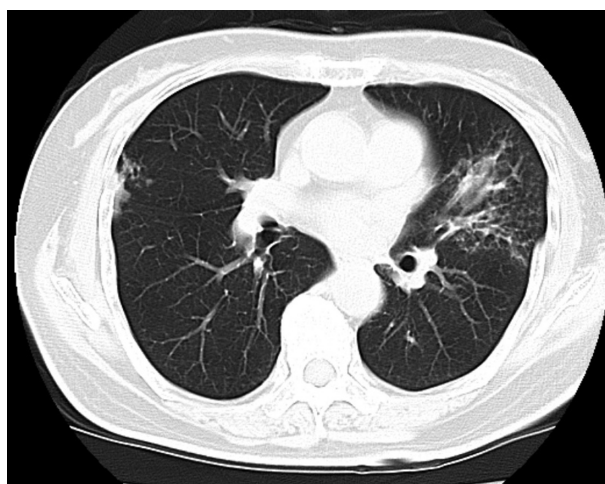
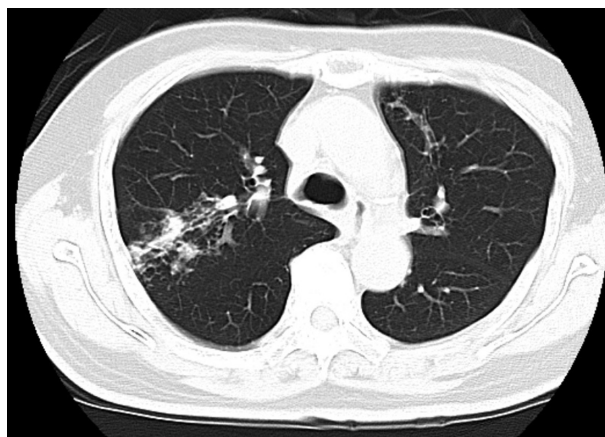


Figure 2 Chest computed tomography showing centrilobular nodules and patchy infiltrative shadows and bronchiectasis in the upper lobes of both lungs and the middle lobe of the right lung.

deep-seated mycosis could not be ruled out, the patient was treated with oral voriconazole for three months. However, the plasma concentration of (1→3)-beta-D-glucan remained high; by February 2009, it had increased from 160 pg/mL to 410 pg/mL (Figure 4). Because it was unlikely that any other factor was responsible, we concluded that the high (1→3)-beta-D-glucan level was caused by intramuscular injections of 40 mg SPG every week for six months, which had been administered for the treatment of cervical cancer 18 years previously.

Discussion

The plasma concentration of (1→3)-beta-D-glucan, a characteristic cell wall component of almost all fungi and absent in bacteria, is widely used in Japan as an indicator of fungal infection. In 2004, the United States Food and Drug Administration recognized that measurement of this glucan is useful for the detection of fungal infection.

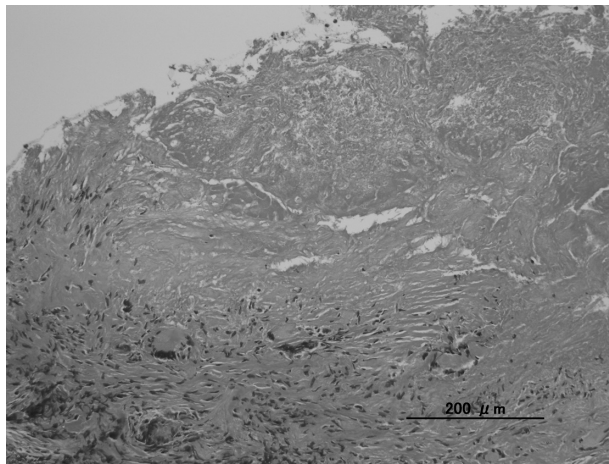


Figure 3 Histologic examination of hematoxylin-eosin stained transbronchial lung biopsy specimens obtained from the right upper lobe revealed caseous necrosis with lymphocytes, epithelioid granuloma, and Langhans' giant cells.

Factor G in the lysate from the Japanese horseshoe crab (*Tachypleus tridentatus*) reacts with (1→3)-beta-D-glucan [3]. Obayashi et al reported that the determination of plasma (1→3)-beta-D-glucan concentration for the detection of fungal infection with a cutoff value of 20 pg/mL has a sensitivity of 90% and a specificity of 100%.¹ In Japan, three test kits are currently available for the measurement of plasma glucan levels, ie, the FUNGITEC G Test MK (FUNGITEC-MK), beta-Glucan Test Wako (Wako), and beta-Glucan Test Maruha (Maruha). The FUNGITEC-MK kit, which involves alkaline pretreatment of the sample before measurement, is widely used in Japan because it is highly sensitive and enables easy handling of many samples. In our hospital, we measure glucan levels using the Wako kit, which shows an overall agreement rate of nearly 90% with the FUNGITEC-MK kit for fungal infection.⁴ However, in cases where (1→3)-beta-D-glucan preparations, such as SPG and lentinan, have been administered, alkaline pretreatment of the sample (FUNGITEC-MK kit) yields drastically higher beta-D-glucan values than the values obtained by dilution and heat pretreatment (Wako and Maruha kits).⁵ SPG and lentinan have a triple helical conformation that is denatured into a single helical structure under alkaline conditions.^{6,7} Most of the (1→3)-beta-D-glucan in the blood of patients with deep-seated mycosis is in the single helical conformation, which reacts more strongly with factor G than the triple helical conformation of beta-D-glucan.⁵ In December 2008, the level of (1→3)-beta-D-glucan in our patient was found to be 3240 pg/mL (cutoff value, 20 pg/mL) with the FUNGITEC-MK kit and 124 pg/mL (cutoff value, 11 pg/mL) with the Maruha kit, whereas the glucan level was found to be 268 pg/mL with the Wako kit.

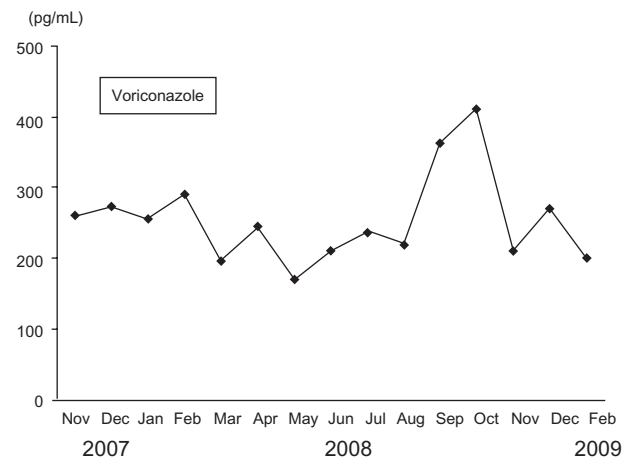


Figure 4 Plasma concentration of beta-D-glucan between November 2007 and February 2009.

These results suggested that the administered SPG remained in the triple helical conformation, and reacted strongly with factor G after alkaline treatment.

Some clinical conditions interfere with the results obtained using the Wako kit. False positive reactions have been reported in patients receiving human immunoglobulin products, which contain substances that react with factor G.⁸ These substances include drugs containing (1→3)-beta-D-glucan, for example, lentinan and SPG,^{9,10} the cellulose membrane used for hemodialysis,¹¹ and glucan-containing gauze used in surgery.¹² However, in the case of our patient, there was no history of the use of glucan-containing gauze, intake of Chinese medicines,¹³ supplements, over the counter medicines for intestinal disorders containing (1→3)-beta-D-glucan, or administration of blood products, such as albumin and globulin.

Lentinan is clinically used as a biologic response modifier in the treatment of gastric cancer,⁹ and SPG is used in combination with radiotherapy to improve the local response to radiation treatment for cervical cancer.¹⁰ SPG is isolated from culture media in which the basidiomycete *Schizophyllum commune* Fries is grown. It is a true glucan (molecular weight approximately 450,000) consisting of repeating units of beta-1,3-D-glycopyranosyl residues.¹⁴

It has been reported that SPG, upon entry into the body, is transported to the liver, spleen, and mesenteric lymph nodes almost immediately, and its concentration in the blood decreases.¹⁵ In rats, SPG present in the liver is degraded over a period of six months to SPG-like substances, the molecular weight of which is lower than that of SPG. Furthermore, SPG in the spleen and mesenteric lymph nodes is metabolized at a

much slower rate than in the liver. Finally, SPG is excreted in the urine, mainly in the form of metabolites with a molecular weight < 10000.¹⁶ Hase et al studied the clearance of SPG in humans, and found that 30 days after a single intramuscular injection of 20 or 40 mg SPG, the blood SPG concentration was in the order of tens of nanograms per milliliter.¹⁷ Ishizuka et al reported that lentinan (total dose 400 mg) and SPG (total dose 280 mg) can be detected in the body at three years and 350 days, respectively, after the last intramuscular injection.⁵ The biologic half life of (1→3)-beta-D-glucan in humans remains unknown. However, from these reports, it can be concluded that the administration of larger doses of SPG results in elevation of the plasma concentration of (1→3)-beta-D-glucan for longer periods of time.

Our patient was diagnosed with *Mycobacterium avium* complex pulmonary disease. The specificities of Cand-Tec and Platelia® *Aspergillus* EIA used to detect fungal infection were 76.9% and 87.5%, respectively, while their respective sensitivities were 93.9% and 78.6%.^{18,19} However, deep-seated mycosis cannot be ruled out only on the basis of laboratory data obtained using these kits. In our patient, there was no evidence of fungal infection, including clinical symptoms, radiographic findings, and laboratory data, except for the elevated plasma concentration of (1→3)-beta-D-glucan. Moreover, oral administration of voriconazole, an antifungal agent that acts against a wide variety of yeasts and molds,²⁰ for three months did not decrease the plasma (1→3)-beta-D-glucan concentration. We concluded that intramuscular administration of SPG for treatment of cervical cancer 18 years previously had caused the increased beta-D-glucan levels. Thus far, there have been no reports of long-term elevation of blood (1→3)-beta-D-glucan concentrations. The maximum blood concentration of (1→3)-beta-D-glucan in patients with deep-seated mycosis is in the order of several nanograms per milliliter. Thus, we estimated that in our patient, the total amount of (1→3)-beta-D-glucan in circulating blood would be in the order of micrograms.⁵ The cause of the high level of (1→3)-beta-D-glucan in our patient may have been continuous administration of SPG for long periods of time, resulting in the accumulation of hundreds of milligrams of (1→3)-beta-D-glucan.

(1→3)-beta-D-glucan remains in the peripheral blood for many years and interferes with blood (1→3)-beta-D-glucan measurement. Therefore, in patients with cervical cancer, it is important to confirm whether or not SPG has

been previously administered before measuring (1→3)-beta-D-glucan levels.

Disclosure

The authors report no conflicts of interest in this work.

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