〈Original Article〉

Significant 1, 3-β-D-glucan content in a penicillin G potassium product

Jun Suzuki[1], Shuji Hatakeyama[1,2,*], Yuji Morisawa[1] and Toshiyuki Yamada[3]

[1] Division of Infectious Diseases, Jichi Medical University Hospital, 3311–1 Yakushiji, Shimotsuke-shi, Tochigi 329–0498, Japan
[2] Division of General Internal Medicine, Jichi Medical University Hospital, 3311–1 Yakushiji, Shimotsuke-shi, Tochigi 329–0498, Japan
[3] Department of Clinical Laboratory Medicine, Jichi Medical University, 3311–1 Yakushiji, Shimotsuke-shi, Tochigi 329–0498, Japan

(Received for publication December 4, 2017)

β-D-Glucan (BDG) is a fungal cell wall component and a useful marker in diagnosing invasive fungal infections. However, false-positive BDG test results occasionally limit its utility. We recently treated a patient whose plasma BDG levels were elevated during administration of penicillin G potassium despite the absence of fungal infections. We therefore investigated the BDG contents of different penicillin G potassium products available in Japan. BDG levels were measured using two different assays. An injectable penicillin G potassium product, the only product approved for clinical use in Japan, was found to contain significant amounts of BDG in all batches and lots tested. According to the Fungitec G test MK II "Nissui" (cut-off value, 20 pg/mL) and β-glucan test Wako (cut-off value, 11 pg/mL) assays, BDG levels in 10,000 units/mL solutions of this agent (5 lots) ranged from 79.4 to 192.9 pg/mL and 16.9 to 34.5 pg/mL, respectively. The two other penicillin G potassium products tested, which are used for cell culture, did not contain significant amounts of BDG. The amounts of BDG contained in the injectable product are probably sufficient to cause false-positive test results in patients receiving clinical doses (e.g., 24 million units per day). Further clinical studies are needed to confirm this result.

*Corresponding author, Tel: +81–285–58–7498, Fax: +81–285–40–5160, E-mail: shatake-tky@umin.ac.jp

Introduction

The fungal cell wall component 1,3-β-D-glucan (BDG) is frequently used in clinical settings to diagnose invasive fungal infections. However, positive BDG test results associated with non-fungal etiology (clinical false-positive results) occasionally limit the utility of the test. False-positive BDG results have been reported with several causes, including hemodialysis with cellulose membrane, transfusion of blood products, and the use of wound gauze[1,2,3]. An antibiotic, intravenous amoxicillin-clavulanic acid (not available in Japan), has also been recognized as a causative agent of false-positive BDG test results[4]. Previous studies have demonstrated that several other antibiotics are reactive to the BDG assay *in vitro*, but practical administration of the contaminated drugs is thought to have a small or unknown influence on plasma BDG concentrations[5,6].

Regarding penicillin G potassium, it has been reported in two studies that no significant levels of BDG were detected[5,6]. However, we recently treated a patient whose plasma BDG levels were elevated during administration of intravenous penicillin G potassium despite not having any fungal infections or other known causes of false-positive results. The patient's plasma BDG levels (20 pg/mL is the cut-off to be considered positive) were elevated from 8.5 pg/mL before starting penicillin G potassium at 24 million units (MU) per day to above 75.0 pg/mL 14 days after administration of the drug (a case report is in preparation).

Therefore, we hypothesized that the penicillin G potassium product used in Japan might be associated with false-positive BDG results. In this study, we measured the BDG levels of three penicillin G potassium products available in Japan.

Materials and Methods

Penicillin G potassium products tested

We purchased three different penicillin G potassium products: penicillin G potassium 1 MU for injection (Meiji Seika Pharma Co., Ltd., Tokyo, Japan), penicillin G potassium salt 5 g (1,530 units/mg) (Nacalai Tesque, Inc., Kyoto, Japan), and benzylpenicillin potassium 5 g (1,430 units/mg) (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Penicillin G potassium 1 MU for injection (Meiji Seika Pharma) is the only penicillin G potassium product licensed for use in humans in Japan, and five different lots (lot numbers 840, 930, 932, 934, and 942) were examined in this study. Single lots of penicillin G potassium manufactured by Nacalai Tesque (lot number M6N1379) and Wako Pure Chemical Industries (lot number LKK2659), both of which are used for cell culture, were also tested for comparison. Each penicillin G potassium product was diluted with 0.9% saline, and solutions with a final concentration of 10,000 units/mL were used for BDG measurements.

BDG assay

We measured BDG in the penicillin products using two different assays, the Fungitec G test MK II "Nissui" (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and the *β*-glucan test Wako (Wako Pure Chemical Industries, Ltd.), according to the manufacturers' instructions. Glucan-free equipment and saline were used. The detection ranges and the cut-off values for positivity were 4.0–500.0 pg/mL and 20.0 pg/mL, respectively, for the Fungitec G test MK II "Nissui" assay and 6.0–600.0 pg/mL and 11.0 pg/mL, respectively, for the *β*-glucan test Wako assay. All samples were measured in triplicate using the Fungitec G test MK II "Nissui" and measured singly using the *β*-glucan test Wako. For the former assay, the means of the triplicate measurements are shown in the results. When the test results were negative according to the Fungitec G-test MK II "Nissui", the *β*-glucan test Wako was not performed.

Results

Regarding the penicillin G potassium 1 MU for injection (Meiji Seika Pharma Co., Ltd.), the mean (±standard deviation) BDG levels of triplicate 10,000 units/mL solutions of lot numbers 840, 930, 932, 934, and 942 were 79.4 (±4.0), 192.9 (±12.0), 187.0 (±26.0), 156.6 (±1.9), and 146.3 (±9.8) pg/mL, respectively, according to the Fungitec G test MK II "Nissui" assay. High levels of BDG were also detected using the *β*-glucan test Wako assay, with values of 16.9, 34.5, 30.1, 24.5, and 20.3 pg/mL, respectively (Table 1). In contrast, BDG concentrations in the penicil-

Table 1. *β*-D-Glucan levels measured by two assays in penicillin G potassium products

Manufacturer	Lot number[1]	Expiration date	β-D-glucan concentration in 10,000 units/mL of penicillin G potassium solution[2]	
			Fungitec G test MK II "Nissui" (pg/mL)	β-glucan test Wako (pg/mL)
Meiji Seika Pharma (1 million units/vial)	840	Oct. 2017	**79.4**	**16.9**
	930	Mar. 2019	**192.9**	**34.5**
	932	May 2019	**187.0**	**30.1**
	934	May 2019	**156.6**	**24.5**
	942	Jul. 2019	**146.3**	**20.3**
Nacalai Tesque	M6N1379	-	<4.0	NT
Wako Pure Chemical	LKK2659	-	5.8	NT
0.9% Saline	-	-	<4.0	NT

NT: not tested.
[1] Products with lot numbers 900–999 are from different batches as those with lot numbers 800–899.
[2] Values above the positive cut-off for each assay are shown in bold. Cut-off values for positivity were 20.0 pg/mL and 11.0 pg/mL for the Fungitec G test MK II "Nissui" and *β*-glucan test Wako, respectively.

lin G potassium products for cell culture manufactured by Nacalai Tesque and Wako Pure Chemical Industries were below the cut-off value (Table 1).

Discussion

The penicillin G potassium for injection that is available in Japan contained significant amounts of BDG, regardless of the batch or lot numbers of the product. Amounts of BDG in 1 MU (1 vial) penicillin G potassium were estimated to be 7,940–19,290 pg and 1,690–3,450 pg as measured by Fungitec G test MK II "Nissui" and β-glucan test Wako, respectively, indicating some variation in BDG concentrations across batches and lots. For adult patients, penicillin G potassium is generally administered intravenously as 18–24 MU per day. To date, there are few reports on the kinetics of plasma BDG. With that in mind, based on the estimated half-life of BDG in humans (median, 20 h; range, 3–181 h)[1] and the estimated volume of distribution in rabbits[7], it is postulated that the clinical use of this drug may lead to significant elevations (false positives) in plasma BDG levels in humans. However, further kinetic studies of BDG in humans are needed to confirm this estimate.

To the best of our knowledge, there are no other studies showing significant BDG contamination in penicillin G potassium products. Marty *et al.* measured BDG levels in 44 intravenous antibiotics and demonstrated that BDG was not detected in a penicillin G product (manufactured by Baxter, USA; lot number, LN044859) using the Glucatell assay[5]. In a study conducted by Liss *et al.* that investigated BDG contents in 35 antimicrobial agents, another penicillin G product (Infectopharm, Germany; batch number, B011401,1) did not contain significant levels of BDG according to two different BDG tests[6].

The BDG-positive penicillin G potassium product (Meiji Seika Pharma) in this study is produced by fed-batch fermentation of *Penicillium* species, the same technique adopted in the products manufactured by Nacalai Tesque (tested in this study) and Baxter (tested in the study by Marty *et al.*[5]). Because significant amounts of BDG were not contained in the latter two penicillin G products, BDG contamination may depend on the manufacturing processes of specific manufacturers. Contamination with *Aspergillus* galactomannan seems to have been eliminated from non-generic piperacillin-tazobactam[8], which had been described as a cause of false-positive results in patients tested using the *Aspergillus* galactomannan assay. Likewise, as Liss *et al.* have also pointed out[6], it is probably important for manufacturers to take measures to reduce the risk of BDG contamination in their products.

The penicillin G potassium for injection used in Japan was found to contain a significant amount of BDG. Although this amount is probably enough to cause false-positive test results in patients receiving clinical doses of this agent, further clinical (*in vivo*) studies are needed to confirm these results.

Acknowledgments

We would like to thank Tomoyuki Yamazaki (Nissui Pharmaceutical Co., Ltd.) for BDG testing support. We would like to thank Dr. Takehito Yamamoto (The Education Center for Clinical Pharmacy, Graduate School of Pharmaceutical Sciences, The University of Tokyo) for estimating the kinetics of BDG in humans.

Conflict of Interest

The authors state that they have no conflict of interest (COI).

References

1) Kanda H, Kubo K, Hamasaki K, *et al.*: Influence of various hemodialysis membranes on the plasma (1→3)-*β*-D-glucan level. Kidney Int. 2001; 60: 319–23.
2) Kanamori H, Kanemitsu K, Miyasaka T, *et al.*: Measurement of (1–3)-beta-D-glucan derived from different gauze types. Tohoku J Exp Med. 2009; 217: 117–21.
3) Barton C, Vigor K, Scott R, *et al.*: Beta-glucan contamination of pharmaceutical products: How much should we accept? Cancer Immunol Immunother. 2016; 65: 1289–301.
4) Mennink-Kersten MA, Warris A, Verweij PE: 1,3-beta-D-glucan in patients receiving intravenous amoxicillin-clavulanic acid. N Engl J Med. 2006; 354: 2834–5.
5) Marty FM, Lowry CM, Lempitski SJ, Kubiak DW, Finkelman MA, Baden LR: Reactivity of (1→3)-*β*-D-glucan assay with commonly used intravenous antimicrobials. Antimicrob Agents Chemother. 2006; 50: 3450–3.
6) Liss B, Cornely OA, Hoffmann D, Dimitriou V, Wisplinghoff H: 1,3-*β*-D-Glucan contamination of common antimicrobials. J Antimicrob Chemother. 2016; 71: 913–5.
7) Yoshida M, Roth RI, Grunfeld C, Feingold KR, Levin J: Soluble (1→3)-beta-D-glucan purified from *Candida albicans*: Biologic effects and distribution in blood and organs in rabbits. J Lab Clin Med. 1996; 128: 103–14.
8) Mikulska M, Furfaro E, Del Bono V, *et al.*: Piperacillin/tazobactam (Tazocin) seems to be no longer responsible for false-positive results of the galactomannan assay. J Antimicrob Chemother. 2016; 67: 1746–8.