Interference of (1→3)-β-D-glucan Administration in the Measurement of Plasma (1→3)-β-D-glucan

Yasuo ISHIZUKA, Hiroki TSUKADA and Fumitake GEJYO

Abstract

Objective Blood (1→3)-β-D-glucan (βG) measurement is widely used as an effective sero-diagnostic method for deep-seated mycosis. Antitumor βG (lentinan, schizophyllan) administration is known as one of the false-positive factors of blood βG measurement. To understand the influence of administered βG preparation to βG measurement in blood, we compared the interfering effect of βG administration in different βG measuring methods.

Methods βG concentration in plasma was measured by three different methods.

Materials βG concentration was measured in plasma of 18 samples of 7 cases with βG administration and 86 samples without βG administration. The period after last βG administration was three days to three years.

Results In the cases for which βG was administered, blood βG level drastically increased using the method which employs alkaline pretreatment. Even in the cases for which βG was administered three years previously; βG value measured by alkaline pretreatment was significantly high. Thus, interference of βG administration in blood βG measurement continued for years after the last administration.

Conclusion Disparity in βG values measured by different methods for βG administered cases is due to differences among sample pretreatment methods. Conformation of administered βG seemed to be transformed into a sensitive form to factor G by alkaline pretreatment. Especially in the case of the alkaline pretreatment method, βG administration disturbance was much stronger than for dilution-heating pretreatment. Therefore, in suspected cases, it is important to pay attention to βG administration during the previous few years.

Key words: (1→3)-β-D-glucan, deep-seated mycoses, false-positive reaction, lentinan, schizophyllan

Introduction

As an auxiliary sero-diagnostic method for deep-seated mycosis, the measurement of (1→3)-β-D-glucan (βG) concentration in blood is used widely in Japan (1–3). Since βG, which is the common cell wall component of fungi, is the target of measurement, it is impossible to specify a species of infectious fungi. However, βG measurement in blood is effective as the screening test for fungal infection, because the result can be derived in a short time period, and many species of pathogenic fungi have βG as a cell wall component. Furthermore, βG is measured quantitatively (rather than qualitatively), the βG value reflects the extent of illness and it is useful for the judgment of cure effect (2, 4). All the βG measurement methods utilize βG sensitive blood coagulation cascade reaction of horseshoe crab (5). However, methods differ in terms of sample pretreatment and/or principle of final enzyme activity measurement.

Antitumor βG preparation (lentinan and schizophyllan) is known to be a false-positive factor in βG measurement. This treatment has a direct influence on the βG concentration in blood, because the preparation is, in fact, βG itself, and administration is intra-muscular. To understand the influence of administered βG preparation on βG measurement in blood, βG concentration in the cases with βG administration was measured by three different methods. We found that administered βG preparation interfered with the βG measurement for several years, and the degree of interference differed among βG measuring methods.

Materials and Methods

Materials

We measured βG concentration in 18 plasma samples from 7 cases with prior βG administration (Table 1), and in 86 samples from 38 cases without prior βG administration. In all cases with βG administration, no signs of deep-seated mycoses and no signs of liver function failure were observed. In all 7 βG administered cases, hemodialysis with
There is no indication of deep-seated mycoses in all cases. **SPG: schizophyllan, LNT: lentinan.

cellulose membrane and intravenous administration of plasma fraction preparation such as albumin or globulin were not done within a week before blood sampling. Surgical operation within two weeks before blood sampling was not carried out. Schizophyllan was used in 6 cases and lentinan in a single case. The longest period after the last administration of βG preparation was 3 years.

Methods of βG measurement

We used three types of βG measurement kits, according to the manufacturer’s instructions, Dilution and Heating treatment—Chromogenic Endpoint method (6) (DH-CEP method, β-Glucan Test MARUHA, Maruha Corporation, Tokyo), Dilution and Heating treatment—Turbidimetric Kinetic method (2, 7) (DH-TK method, β-Glucan Test WAKO, Wako Purechemical Industries, Ltd., Osaka), and Alkaline treatment—Chromogenic Kinetic method (3) (Alk-CK method, Fungitec G Test MK, Seikagaku Corporation, Tokyo) (Table 2). All of the kits utilize βG sensitive blood coagulation cascade reaction of horseshoe crab.

To confirm differing influences of the sample pretreatment methods, we measured the alkaline treated samples using the main reaction reagent of the DH-CEP method. Alkaline treated plasma was diluted more than four times and measured using the chromogenic endpoint method.

Results

In all of the cases with prior βG administration, the plasma βG concentration was above the cutoff value (Table 3). A much higher value was obtained for the Alk-CK method than the other methods. Although deep-seated mycosis was not observed in any of the cases, and 3 years had elapsed after the last βG administration in the longest case, blood βG concentration was high in all βG administered cases. βG administration affected the blood βG concentration for long periods after the last βG administration (Fig. 1).

The correlation of βG concentration obtained by the three methods is shown in Fig. 2. The DH-CEP and DH-TK methods, in which dilution and heating methods were employed for sample pretreatment, showed a good correlation in cases with or without βG administration. In the cases with βG administration, the Alk-CK method showed as much as 100 to 1,000 times the βG concentration than for the DH-CEP and DH-TK methods. In the cases without βG administration, the Alk-CK method showed higher βG concentrations than the

### Table 2. Summary of Three (1→3)-β-D-glucan Measurement Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample pretreatment</th>
<th>Enzyme activity measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH-CEP</td>
<td>Dilution and Heating treatment (6)</td>
<td>Chromogenic Endpoint assay (6)</td>
</tr>
<tr>
<td></td>
<td>10 fold dilution with 0.01% Triton X-100, 4 mM NaIO₄, 2 mM NaOH Incubation at 75°C for 15 minutes</td>
<td>Released 1LD⁺ from synthetic substrate is coupled DEA⁻². Coupled chromogen is measured by absorption at 730 nm–650 nm.</td>
</tr>
<tr>
<td>DH-TK</td>
<td>Dilution and Heating treatment (2, 7)</td>
<td>Turbidimetric Kinetic assay (2)</td>
</tr>
<tr>
<td></td>
<td>10 fold dilution with 0.2% Triton X-100, 0.002% polymixin B Incubation at 70°C for 10 minutes</td>
<td>Turbidity caused by coagulin gel formation is measured continuously.</td>
</tr>
<tr>
<td>Alk-CK</td>
<td>Alkaline treatment (3)</td>
<td>Chromogenic Kinetic assay (3)</td>
</tr>
<tr>
<td></td>
<td>5 fold dilution with 150 mM KOH, 300 mM KCl, 0.1% phenylpyrene Incubation at 37°C for 10 minutes</td>
<td>Released pNA⁻³ from synthetic substrate is measured continuously. by absorption at 405 nm–492 nm.</td>
</tr>
</tbody>
</table>

DH-CEP and DH-TK methods, but the difference among the three methods was much less than for the βG administered cases, and a good correlation was observed between Alk-CK and DH-CEP methods. In the cases without βG administration, 30 samples from 10 cases were above cut-off value in DH-CEP method; all 10 cases were proven deep-seated mycoses or deep-seated mycoses suspected cases.

The correlation between alkaline pretreatment and dilution-heating pretreatment measured by the main reaction reagent of chromogenic endpoint method is shown in Fig. 3. In the cases without βG administration, βG concentration was not changed by the sample pretreatment method. In all cases with βG administration, βG concentration was drastically elevated by alkaline pretreatment.

**Discussion**

To clarify the interfering effect of prior βG administration on blood βG concentration, we compared blood βG concentration levels measured by three different methods in cases both with and without prior βG administration. In all the cases with βG administration, blood βG concentration was significantly elevated, with no signs of deep-seated mycosis. In the cases with βG administration, hemodialysis with celllose membrane, intravenous administration of plasma fraction preparation and surgical operation, which are known as false-positive factors of βG measurement, were not done before blood sampling. The abnormally high βG concentration was not due to these false-positive factors. We found that βG administered up to three years previously still remains in circulating blood and affects the blood βG measurement. Blood βG which originate in βG administration seemed to be cleared more slowly than that originate in fungal infection. In all the cases with prior βG administration, no signs of liver function failure were observed. Since βG is metabolized mainly in liver (8), long-term existence of administered βG was not caused by the metabolic disorder of βG in liver.

In all the cases with prior βG administration, βG concentration measured by Alk-CK method was drastically higher than for the DH-CEP and DH-TK methods. Such a difference was not observed in the cases without βG administration. A typical reaction pattern in the cases with βG administration is that βG concentration determined by the Alk-CK method is drastically higher than those determined by the DH-CEP or DH-TK methods. In the cases without βG administration, βG concentration showed a good correlation with βG administration, hemodialysis with celllose membrane, intravenous administration of plasma fraction preparation and surgical operation, which are known as false-positive factors of βG measurement, were not done before blood sampling. The abnormally high βG concentration was not due to these false-positive factors. We found that βG administered up to three years previously still remains in circulating blood and affects the blood βG measurement. Blood βG which originate in βG administration seemed to be cleared more slowly than that originate in fungal infection. In all the cases with prior βG administration, no signs of liver function failure were observed. Since βG is metabolized mainly in liver (8), long-term existence of administered βG was not caused by the metabolic disorder of βG in liver.

In all the cases with prior βG administration, βG concentration measured by Alk-CK method was drastically higher than for the DH-CEP and DH-TK methods. Such a difference was not observed in the cases without βG administration. A typical reaction pattern in the cases with βG administration is that βG concentration determined by the Alk-CK method is drastically higher than those determined by the DH-CEP or DH-TK methods. In the cases without βG administration, βG concentration showed a good correlation with βG administration, hemodialysis with celllose membrane, intravenous administration of plasma fraction preparation and surgical operation, which are known as false-positive factors of βG measurement, were not done before blood sampling. The abnormally high βG concentration was not due to these false-positive factors. We found that βG administered up to three years previously still remains in circulating blood and affects the blood βG measurement. Blood βG which originate in βG administration seemed to be cleared more slowly than that originate in fungal infection. In all the cases with prior βG administration, no signs of liver function failure were observed. Since βG is metabolized mainly in liver (8), long-term existence of administered βG was not caused by the metabolic disorder of βG in liver.

To determine βG concentration in plasma or serum,
sample pretreatment is necessary to eliminate the interference of blood component. Since the Alk-CK method, that employs alkaline treatment as sample pretreatment, showed high $\beta$G concentrations, we assumed that different sample pretreatment methods resulted in the disparities in measured $\beta$G concentrations for the different methods for $\beta$G administered cases. We compared $\beta$G concentrations measured using chromogenic endpoint reagent with alkaline or dilution-heating pretreatment. In the cases with prior $\beta$G administration, $\beta$G concentration measured by alkaline treatment showed significantly higher values than for the dilution and heating pretreatment. In the cases without $\beta$G administration, $\beta$G concentration was not changed by sample pretreatment method. We conclude that the high $\beta$G concentration by Alk-CK method in $\beta$G administered cases is due to the alkaline treatment.

$\beta$G concentration measured by the alkaline pretreatment method showed drastically higher values than those of the dilution-heating pretreatment in the cases with $\beta$G administration. If abnormally high $\beta$G concentrations judged from clinical symptoms were observed, we could estimate whether $\beta$G had been administered or not, by measuring $\beta$G using different sample pretreatment methods, even in the cases in which $\beta$G administration in the past was uncertain.

All the methods for $\beta$G measurement utilize $\beta$G sensitive factor G of horseshoe crab (5). Factor G activating activity of $\beta$G depends on conformation of $\beta$G, single-helical or random-coiled conformer exhibits stronger factor G activating activity than a triple-helical conformer (11, 12). Most $\beta$G in

Figure 2. Correlation of plasma (1 $\beta$-3)-$\beta$-D-glucan ($\beta$G) concentration for three methods. □: $\beta$G administered cases, ◊: cases without $\beta$G administration. Dotted line shows cut-off value of each method. DH-CEP: Dilution and heating treatment—Chromogenic endpoint method, Alk-CK: Alkaline treatment—Chromogenic kinetic method, DH-TK: Dilution and heating treatment—turbidimetric kinetic method.

Figure 3. Correlation of plasma (1 $\beta$-3)-$\beta$-D-glucan ($\beta$G) concentration between two sample pretreatment methods. Plasma $\beta$G concentration was measured with chromogenic endpoint reaction reagent after alkaline or dilution heating pretreatment. □: $\beta$G administered cases, ◊: cases without $\beta$G administration.
aqueous solution of lentinan or schizophyllan preparation exhibits triple-helical conformer; it is converted to single-helical conformer under alkaline conditions (11, 12). If administered βG remained as triple-helical conformer in blood for long periods, converted to single-helical conformer and displayed stronger activity to factor G after alkaline treatment, the typical reaction pattern in βG administered cases should be appropriate.

In the cases without prior βG administration, a large difference in the βG concentration among the different sample pretreatment methods was not observed. Since βG concentration in deep-seated mycosis is of an order of ng/ml, even in the highest cases, it is very difficult to determine conformation of βG in blood. Alkaline pretreatment did not activate factor G activating activity of βG in the cases without βG administration. This suggests that most of the βG in the blood of deep-seated mycosis has single-helical conformer, which displays strong activity to factor G.

Blood concentration of $^3$H labeled lentinan after intra-muscular administration in mouse, rat and dog declines in a di-phasic manner, with a half-life below three hours and over months. Investigation of long period clearance of βG administered, the typical reaction pattern in blood for long periods, converted to single-helical conformer /TypeOne0 which displays strong activity to factor G.

βG concentration in deep-seated mycoses responds to cure effect and declines in a few months, if the cure effect is successful (2, 4). βG in blood as a result of prior βG administration might be different in terms of clearance rate from that originated in fungal infection. Since blood βG concentration in the cases with deep-seated mycoses is several ng/ml in the highest cases, the total amount of βG in circulating blood would be on the order of μg. A normal dose of lentinan is 2 mg per week and schizophyllan is 40 mg per week. Since they are administered continuously for long periods, the totals amount to hundreds of mg. Different clearance rates for βG originating from βG administration and fungal infection may be due to differences in the total amount of βG present in the whole body.

Clearance rates of different conformers of schizophyllan in mouse depend on its conformation; single-helical conformer clears more rapidly than triple-helical conformer (15). Long-term presence of administered lentinan or schizophyllan may be due to their triple-helical conformer.

Administered βG preparation is present for years in peripheral blood and interferes with blood βG measurement. βG preparation other than oral administration is only in the forms of schizophyllan and lentinan in Japan, and susceptible diseases are only stomach cancer and uterocervical cancer. If βG is measured in the cases with these diseases, it is important to confirm whether βG preparation had been administered or not over long periods.

References