Original Article

Determination of β -D-glucan and endotoxin levels in Kampo extractsHajime Nakae,¹ Gaku Takahashi,² Ryo Sato,² Shigehiro Shibata,² and Shigeatsu Endo²¹Department of Emergency and Critical Care Medicine, Akita University Graduate School of Medicine, Akita; and²Department of Critical Care Medicine, School of Medicine, Iwate Medical University, Morioka, Japan

Aims: Kampo medicine is based on the use of established formulations combining natural extracts with no “brand-name” products or corresponding “generic” formulation. Due to differences in manufacturing practices, products of different pharmaceutical companies may contain different concentrations of β -D-glucan and endotoxins. The aim of this study was to compare the concentrations of β -D-glucan and endotoxins in five Kampo extracts from four pharmaceutical companies.

Methods: Packages of Kampo extracts were dissolved in distilled water. β -D-Glucan and endotoxin concentrations were measured using high-sensitivity kinetic turbidimetric Limulus assay.

Results: All Kampo extracts examined in this study were found to contain detectable concentrations of β -D-glucan and endotoxins. Significant differences in the concentration of β -D-glucan and endotoxins ($P = 0.0024$ and $P = 0.0013$, respectively) were observed between products of different pharmaceutical companies.

Conclusions: High β -D-glucan and endotoxin contents were detected in Kampo extracts, with a large variability in the concentrations of both β -D-glucan and endotoxins among extracts from different pharmaceutical companies.

Key words: Endotoxins, Japanese traditional herbal medicine, Kampo formulations, Limulus assay, β -D-glucans

INTRODUCTION

AS EVIDENCED BY a growing number of recent studies, there is increasing interest in comparing the clinical characteristics of generic and brand-name drugs.¹ Extracts used in Kampo medicine do not contain any generic drugs, as every pharmaceutical company uses different manufacturing parameters in the production of crude drugs, with great variability in factors such as production area, batch size, and formulation components. Therefore, it is unclear whether Kampo extracts exhibit bioequivalence.^{2–4} We previously reported a significant variability in the total antioxidant capacity measured in Kampo extracts produced by different pharmaceutical companies.^{5,6}

β -D-Glucans (BDG) are naturally occurring polysaccharides found in the cell walls of yeast, fungi, cereal plants, and some bacteria. They exhibit a variety of biological and immunopharmacological activities.^{7–9} Endotoxins (ETX),

including lipopolysaccharides, are cell wall constituents of gram-negative bacteria. Both BDG and ETX are recognized as common environmental contaminants, found in materials and substances such as tap water, food, and gauze.^{10–13}

Kampo extracts are combinations of several crude natural materials, so there is a possibility of contamination with detectable amounts of BDG. However, thus far, no study has compared the relative concentrations of BDG and ETX in Kampo extracts.¹⁴

In this study, we compared the concentrations of BDG and ETX in Kampo extracts produced by pharmaceutical companies and assessed whether the Kampo extract compositions differed between the manufacturers.

METHODS

THE CONCENTRATIONS OF BDG and ETX in five different Kampo extracts (Hangeshashinto, Goreisan, Maoto, Rikkunshito, and Inchinkoto) produced by four pharmaceutical companies (Group A, B, C, and D) were examined. These Kampo extracts comprised several crude drugs mixed in specific ratios (Table 1).

The Kampo extracts used in this study were produced by the following four Japanese pharmaceutical companies:

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Table 1. Weight ratios of crude drugs comprising Kampo extracts

A. Hangekobokuto				
Crude drug, g	Group A	Group B	Group C	Group D
Pinellia tuber	6.0	6.0	6.0	6.0
Hoelen	5.0	5.0	5.0	5.0
Magnolia bark	3.0	3.0	3.0	3.0
Perilla herb	2.0	2.0	2.0	2.0
Ginger rhizome	1.0	1.3	1.0	1.0
Mass of one packet, g	2.5	3.0	2.0	1.0
B. Goreisan				
Crude drug, g	Group A	Group B	Group C	Group D
Alisma rhizome	4.0	5.0	6.0	6.0
Chuling	3.0	3.0	4.5	4.5
<i>Atractylodes lancea</i> rhizome	3.0	not included	not included	4.5
<i>Atractylodes</i> rhizome	not included	3.0	4.5	not included
Hoelen	3.0	3.0	4.5	4.5
Cinnamon bark	1.5	2.0	2.5	3.0
Mass of one packet, g	2.5	3.0	2.0	2.5
C. Maoto				
Crude drug, g	Group A	Group B	Group C	Group D
Ephedra herb	5.0	5.0	5.0	5.0
Apricot kernel	5.0	5.0	5.0	5.0
Cinnamon bark	4.0	4.0	4.0	4.0
Glycyrrhiza root	2.0	2.0	2.0	2.0
Ginger rhizome	1.5	1.5	1.5	1.5
Mass of one packet, g	2.5	3.0	2.0	1.5
D. Rikkunshito				
Crude drug, g	Group A	Group B	Group C	Group D
Ginseng root	4.0	4.0	4.0	4.0
Pinellia tuber	4.0	4.0	4.0	4.0
<i>Atractylodes lancea</i> rhizome	4.0	not included	not included	not included
<i>Atractylodes</i> rhizome	not included	4.0	4.0	4.0
Hoelen	4.0	4.0	4.0	4.0
Citrus unshiu peel	2.0	2.0	2.0	2.0
Jujube fruit	2.0	2.0	2.0	2.0
Glycyrrhiza root	1.0	1.0	1.0	1.0
Ginger rhizome	0.5	0.5	0.5	0.5
Mass of one packet, g	2.5	2.0	3.0	2.5
E. Inchinkoto				
Crude drug, g	Group A	Group B	Group C	Group D
<i>Artemisia capillaris</i> spike	4.0	4.0	4.0	4.0
Gardenia fruit	3.0	3.0	3.0	3.0
Rhubarb rhizome	1.0	1.0	1.0	1.0
Mass of one packet, g	2.5	3.0	2.0	1.0

Tsumura & Co., Tokyo; Kracie Pharmaceutical, Ltd., Tokyo; Kotaro Pharmaceutical Co., Ltd., Osaka; and OHSUGI Pharmaceutical Co., Ltd., Osaka.

One package of each Kampo extract was dissolved in distilled water (Otsuka distilled water; Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan). Ten milliliters of distilled water was used for dissolving 1 g of Kampo extract. All measurements were carried out immediately after the extract was completely dissolved. The mass of each Kampo extract packet is shown in Table 1. The concentrations of BDG and ETX were measured using high-sensitivity kinetic turbidimetric Limulus tests (β -glucan test and endotoxin single test, respectively) from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) by using an MT-500 Toxinometer (Wako Pure Chemical Industries, Ltd.).^{15–17}

Data are expressed as medians with the range of values. Statistical evaluations were carried out using the Kruskal–Wallis rank test. A *P*-value lower than 0.05 was considered statistically significant.

RESULTS

Measurements of BDG concentration in dissolved Kampo extracts

THE BDG WAS not detected in 25 mL distilled water, which was set to the control. The median BDG concentrations in Kampo extracts of the four groups were as follows: Group A, 6.00 μ g/mL (1.99–11.28 μ g/mL); Group B, 11.43 μ g/mL (1.87–27.00 μ g/mL); Group C, 1,113.00 μ g/mL (274.00–4,066.00 μ g/mL); and Group D, 1,314.00 μ g/mL (274.00–2,695.00 μ g/mL), as presented in Table 2. A significant difference was observed in BDG concentrations between the four groups (*P* = 0.0024).

Considering the individual Kampo extracts, the median concentration of BDG in solutions of the extracts were as follows: 670.50 μ g/mL (6.88–4,066.00 μ g/mL) in Hangeshashinto, 811.22 μ g/mL (6.00–2,247.00 μ g/mL) in

Table 2. Concentrations of β -D-glucans in solutions of Kampo extracts

Group	Median (minimum–maximum), μ g/mL
Control	Not detected
A	6.00 (1.99–11.28)
B	11.43 (1.87–27.00)
C	1,113.00 (274.00–4,066.00)
D	1,314.00 (274.00–2,695.00)

Table 3. Endotoxin levels in solutions of Kampo extracts

Group	Median (minimum–maximum), μ g/mL
Control	Not detected
A	0.64 (0.34–2.50)
B	12.43 (1.87–27.32)
C	44.50 (4.20–1,945.00)
D	2,034.00 (174.00–3,543.00)

Goreisan, 558.08 μ g/mL (2.65–2,695.00 μ g/mL) in Maoto, 143.69 μ g/mL (11.28–973.00 μ g/mL) in Rikkunshito, and 138.00 μ g/mL (1.87–637.00 μ g/mL) that in Inchinkoto extracts (Table 2). No significant difference was observed in BDG content between the five Kampo extracts (*P* = 0.6475).

Measurements of ETX concentration in dissolved Kampo extracts

Endotoxins were not detected in 25 mL distilled water, which was set to the control. The median ETX concentrations in Kampo extracts of the four groups were as follows: Group A, 0.64 μ g/mL (0.34–2.50 μ g/mL); Group B, 12.43 μ g/mL (1.87–27.32 μ g/mL); Group C, 44.50 μ g/mL (4.20–1,945.00 μ g/mL); and Group D, 2,034.00 μ g/mL (174.00–3,543.00 μ g/mL) (Table 3). A significant difference was observed between the four groups (*P* = 0.0013).

The median ETX concentrations in extract solutions were as follows: 986.16 μ g/mL (0.64–2,265.00 μ g/mL) in Hangeshashinto, 25.75 μ g/mL (0.45–3,543.00 μ g/mL) in Goreisan, 426.83 μ g/mL (2.50–2,034.00 μ g/mL) in Maoto, 8.79 μ g/mL (0.34–174.00 μ g/mL) in Rikkunshito, and 23.19 μ g/mL (1.73–420.00 μ g/mL) in Inchinkoto extract solutions (Table 2). No significant difference in median ETX concentration was observed between the five Kampo extracts (*P* = 0.8442).

DISCUSSION

β -d-Glucan concentration is a useful diagnostic marker for detecting invasive fungal infections. Due to its ability to activate the immune system, BDG is known as a biological response modifier and has been used in the treatment of cancer and infectious diseases.^{7,8} All Kampo extract samples examined in this study contained a high concentration of BDG. Aketagawa *et al.* detected gelation, caused by activation of limulus factor G, in the fractions containing BDG with molecular weights (MWs) exceeding 6,800; the

gelation reaction intensity progressively increased with increasing MW.¹⁸ Although the high-MW BDG do not undergo direct absorption following ingestion, they can stimulate the intestinal mucosa and activate the intestinal immune response.

A significant difference in the BDG concentrations was observed between the Kampo extracts produced by different pharmaceutical companies, possibly due to variations in the crude components of the extracts. The Japanese Pharmacopoeia precisely defines all herbal species used in the preparation of Kampo drugs. Nonetheless, as Kampo components are natural products and mixtures of various chemical substances, some variation in quality can exist. Among other possible causes of variability, differences in plant ingredients, processing techniques, and preservation conditions can affect the quality of the final product. It is unclear whether the differences in BDG concentration among the products of different pharmaceutical companies would be reflected in the presence or absence of beneficial effects in a clinical setting. Further study is necessary to determine the clinical relevance of the differences in BDG concentrations in Kampo extracts.

All samples of the Kampo extracts examined in this study were found to have detectable concentrations of ETX. As ETX can originate from many bacterial strains in the environment, contamination of crude drugs or the water used for irrigation of component herbs can occur.¹³ Additionally, the elimination of ETX from crude drugs is difficult because the toxin persists as a stable substance even if the bacteria are destroyed by heat sterilization.¹⁹ Due to the widespread presence of ETX as a contaminant and its possible biological relevance, studies quantifying ETX in natural health products are important for establishing product safety.

Endotoxin molecules were shown to self-associate in water, forming structures that vary in size, forming dimers (15–16 kDa) to large micelles (10^4 – 10^5 kDa). Endotoxins present in large associations may remain unabsorbed from the gastrointestinal tract following ingestion, as long as the barrier function of the intestine is intact. Endotoxins are key toxic mediators of the body responses that comprise septic shock. Conversely, ETX was found to activate the biological defenses of the body and exhibit antitumor activities.¹⁰ Some Kampo extracts were shown to inhibit proinflammatory cytokines.²⁰ Animal studies carried out by Sakaguchi *et al.* have shown the effectiveness of the Shosaikoto Kampo extract in protecting rats against the lethal effects of ETX through the regulation of heme metabolism in septic shock.²¹ A significant variation in ETX level was reported between the Kampo extracts produced by different pharmaceutical companies. This may reflect the use of an individual crude drug component in multiple products by a single pharmaceutical company.

As Kampo extracts are composed of several crude drugs, their overall observable medical activity involves multiple mechanisms of action. Furthermore, the effects of Kampo medicines are difficult to quantify and analyze using scientific approaches. For these reasons, it would seem difficult to apply these traditional treatment approaches in emergency medicine. Nevertheless, use of Kampo extracts is gradually increasing in clinical settings.^{22–24} However, it should be examined whether and how different concentrations of BDG and ETX in Kampo extracts affect patients. In further studies, which are currently underway, the mechanisms of action underlying the biological effects of BDG and ETX in herbal preparations will be determined.

CONCLUSIONS

THE CONCENTRATIONS OF BDG and ETX in Kampo extracts produced by pharmaceutical companies were compared. High BDG and ETX content was detected in Kampo extracts, with a large variability in the concentrations of both BDG and ETX among extracts from different pharmaceutical companies.

CONFLICT OF INTEREST

D.R. NAKAE HAS received lecture fees from Tsumura & Co. in the past. However, the company had no role in the decision to publish or in the preparation of this manuscript. Other authors declare no competing interests.

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