Interaction between warfarin and functional foods derived from *Bacillus subtilis natto*

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Introduction

Thrombotic diseases have been increasing recently and are said to account for approximately 30% of all causes of death among Japanese people. Potassium warfarin (hereinafter referred to as "wf"), a preventive for thrombosis, is the most commonly used oral coumarin anticoagulant and is known to have a number of interactions with foods and other drugs. For example, patients receiving wf must pay attention to the intake of foods and drugs containing vitamin K (hereinafter referred to as "vit K"), because vit K in large doses may cancel the effectiveness of wf. The anticoagulant effect of wf consists of negative action on the carboxylation of vit K and inhibition of the biosynthesis of the vit K-dependent blood coagulation factors in the liver. Drugs containing vit K include, in addition to vit K preparations, enteric nutrients, high-calorie transvenous nutritional infusions and menatetrenone—which is an anti-osteoporosis drug—and unless these drugs are administered, vit K is not delivered in drug form. However, vit K is contained in certain foods in large quantities, especially in natto which contains about 1 mg of vit K_2 per 100 g[1)]. Natto, a traditional Japanese food, has been eaten from old times for the purpose of maintaining good health as it is a good source of B-complex vitamins. In addition, proteases and glycolytic enzymes produced by *Bacillus subtilis natto* have been found to have a variety of bioactivities[2-4)]. According to a national survey of natto consumption conducted in 2003 over the Internet, people who eat natto everyday accounted for 14.6% of respondents while those who have one or more servings of natto every two to three days accounted for 47.0%. The number of servings had also increased in 32.3% of respondents and the reasons cited included nutritional value and health benefits[5)]. While natto has thus been routinely consumed, patients taking wf are instructed to avoid the intake of natto to prevent interactions with vit K[1,6)].

Meanwhile, health foods have been flooding the market due to the health boom. A survey conducted among people 20 years of age or over showed that those with a history of consuming some type of health food accounted for 76% of respondents[7)]. It is therefore possible that patients taking wf are consuming health foods, and as health foods high in vit K

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have been reported to inhibit the effect of wf, this is resulting in the aggravation of conditions[8]. Patients taking wf must accordingly pay close attention to the use of health foods. Health foods containing vit K in relatively large quantities include "nattokinase", "chlorella" and "green juice". It is important to identify the vit K content in health foods by checking ingredient labels to consider interactions with wf[1,6,9].

NKCP® is one of the health foods produced from the culture of *B. subtilis natto*. NKCP® has anticoagulant and thrombolytic effects on human blood and has demonstrated a significant shortening of the euglobulinlysis time and improvement of neck stiffness after ingestion in clinical studies[4,11]. However, some patients taking wf have had concerns about interactions and chemists filling prescriptions have had difficulties determining whether they should recommend the taking of NKCP® as it is derived from *B. subtilis natto*. To help resolve these concerns, a quantitative determination of vit K_2 in NKCP® was performed, as follows.

1. Subject and Method

1.1. Target substance

The sample used was NKCP® extracted from a culture of *B. subtilis natto*. The extraction process included centrifugal separation of a culture of *B. subtilis natto* to remove solids and bacterial bodies, ultrafiltration of the supernatant to remove substances of a molecular weight of 3,000 or less, and vacuum drying. NKCP® has a molecular weight of 34,134 and has been shown in amino acid analysis to be a fragment of Bacillopeptidase F, a protease excreted by *B. subtilis*. Basic studies have demonstrated a thrombolytic effect on artificial thrombosis and a coagulation inhibitory effect on human blood, as well as a coagulation inhibitory effect in rats given enteral administration[4,11].

1.2. Method

Isopropyl alcohol was added to NKCP® to extract vit K_2, which was determined by high-performance liquid chromatography (HPLC). The vit K_2 was then reduced by a reduction column to generate fluorescence and measured by a fluorescence detector. The HPLC conditions are shown in Table 1.

Table 1 Measurement conditions using the HPLC method

Analytical column	Fincpaksil C18 (4.6 × 250 mm)
Reduction column	Platinum black column (4.0 × 10 mm)
Mobile phase	Methanol
Flow rate	0.7 ml./min
Detector	Fluorescence detector (Ex: 320 nm; Em: 430 nm)

2. Results

The results of the measurement of vit K_2 in NKCP® are presented in Table 2. Five of the nine most recent lots were below the detection limit (10 μg/100 g). Concerning these lots, the average vit K_2 content per 100 g of NKCP®, measured assuming the content to be 10 μg/100 g, was 18.9±10.2 μg. The average vit K_2 content in 250 mg of NKCP®, representing the average daily intake of NKCP®, was 0.05 μg or less.

Table 2 vit K_2 content in 100 g of NKCP®

Lot	vit K_2 content (μg)
1	<10
2	<10
3	<10
4	<10
5	30
6	29
7	26
8	35
9	<10
Mean ± SD	18.9±10.2*

(*: <10 was calculated as representing 10 μg)

3. Discussion

The daily requirement for vit K in humans is met by dietary sources such as from green and yellow vegetables (males: 0.065 mg; females: 0.055 mg) and the vit K produced by enteric bacteria[12]. In patients taking wf, a daily intake of 0.2 mg or more of vit K may result in a significant departure from the prothrombin time (international normalised ratio)—which is used for evaluating anticoagulant effect—from the wf treatment range within 14 days, and a daily intake of 1 mg or more of vit K is said to influence the effectiveness of wf[13].

As shown in this study, an average daily intake of 250 mg of NKCP® contains only 0.05 μg or less of vit K_2. This value is below the aforementioned average daily intake from dietary sources, so if taken on a daily basis, NKCP® may not have implications for the effectiveness of wf. Natto can impact upon the effectiveness of wf because it contains vit K_2 in considerable quantities and following ingestion, *B. subtilis natto* continues to produce vit K_2 in the intestine. NKCP®, which is derived from the culture of *B. subtilis natto*, was found to be virtually deprived of vit K_2 as well as bacterial bodies during the production process.

NKCP® must be verified for safety and efficacy as it is used as a health food. Previous studies, including pre-clinical studies, subacute and chronic ingestion studies in humans, have demonstrated its safety[4,11]. This study has confirmed that because it contains an extremely small quantity of vit K_2, NKCP® may not decrease the effectiveness of wf if taken routinely by patients receiving wf.

Conclusion

Generally speaking, patients receiving wf must pay attention to the use of health foods as well as other drugs. The ingredients of health foods suspected of containing vit K or of being derived from *B. subtilis natto* must be examined prior to ingestion, and those containing vit K in large quantities must be avoided. While this study was targeted at NKCP®, it will be important to identify ingredients in health foods as a whole and to scientifically verify their safety and efficacy.

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